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# Guideline on the Development and Manufacture of Synthetic Peptides

Draft

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# Guideline on the Development and Manufacture of Synthetic Peptides

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# Executive summary

This guideline addresses specific aspects regarding the manufacturing process, characterisation, specifications and analytical control for synthetic peptides which are not covered in the Guideline on the Chemistry of Active Substances (EMA/454576/2016) or Chemistry of Active Substances for Veterinary Medicinal Products (EMA/CVMP/QWP/707366/2017). It also contains requirements and considerations related to conjugation, to medicinal product development, to synthetic peptide development using biological peptides as European reference medicinal product, and to clinical trial applications (human products only).

# 1. Introduction (background)

This guideline has been prepared in accordance with the structure agreed for the quality part of the dossier for human medicinal products (Format ICH-CTD). The subheadings have been included for the sake of clarity.

# 2. Scope

The purpose of this guideline is to set out the type of information required for the development, manufacture and control of synthetic peptides (existing or new chemical entities) used in a medicinal product.

Synthetic peptides are at the interface of small molecules and proteins and, from a quality point of view, specific considerations apply to this class of therapeutics.

Synthetic peptides are fully or partially (i.e. depending on their size[1]) excluded from the scope of ICH Q3A/B (VICH GL10/GL11), ICH Q6A/B (VICH GL39/GL40) and ICH M7 (EMA/CVMP/SWP/377245/2016). This guideline addresses those specific aspects regarding the manufacturing process (solid phase peptide synthesis, fragment condensation), characterisation, specifications and analytical control for synthetic peptides which are not covered in the Guideline on the Chemistry of Active Substances (EMA/454576/2016) and Chemistry of Active Substances for Veterinary Medicinal Products (EMA/CVMP/QWP/707366/2017), and is to be considered complementary to the latter guidelines.

This guideline is not applicable to biological and biotechnological products manufactured by recombinant technologies, radiopharmaceuticals and radiolabelled products containing peptides.

For synthetic peptides used in radiopharmaceuticals, the principles of this guideline apply regarding synthesis and starting materials. However, for other aspects reference is made to the guideline on radiopharmaceuticals and to the applicable monographs in Ph. Eur. for radiopharmaceuticals.

Whilst veterinary products are outside the scope of ICH guidelines there are no corresponding VICH guidelines on certain topics. Nevertheless, the principles outlined in these ICH guidelines may also be relevant to veterinary products to facilitate flexibility and to allow the applicant the option of using different approaches to product development.

[1] Tetrapeptides and below will be considered as small molecules to which the quoted (V)ICH guidelines apply

# 3. Legal basis and relevant guidelines

This guideline should be read in conjunction with the introduction and general principles of Annex I to Directive 2001/83/EC as amended for human medicinal products and Annex II of Regulation (EU) 2019/6 as amended for veterinary medicinal products, and all other relevant EU and (V)ICH guidelines. These include, but are not limited to:

Guideline on the Chemistry of Active Substances (EMA/454576/2016) and Chemistry of Active Substances for Veterinary Medicinal Products (EMA/CVMP/QWP/707366/2017)

EU GMP Part II: Basic Requirements for Active Substances used as Starting Materials

ICH Q2 Validation of analytical procedures (veterinary VICH GL1 and GL2)

ICH Q3A Impurities in new drug substances (veterinary VICH GL10)

ICH Q3B Impurities in new drug products (veterinary VICH GL11)

ICH Q3C Residual solvents (veterinary VICH GL18)

ICH Q3D Elemental impurities CHMP/ICH/353369/2013 (veterinary Reflection paper EMA/CVMP/QWP/153641/2018)

ICH Q6A Specifications – Test Procedure and Acceptance Criteria for New Drug Substances and New Drug Products – Chemical Substances (veterinary VICH GL39)

ICH Q9 Quality risk management

ICH Q11 guideline on development and manufacture of drug substances (chemical entities and biotechnological/ biological entities)

ICH Q13 Continuous manufacturing of drug substances and drug products

ICH M7 Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk (veterinary EMA/CVMP/SWP/377245/2016)

Investigation of Chiral Active Substances 3CC29a, EMEA/CVMP/128/95

Note for Guidance on Minimizing the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Human and Veterinary Medicinal Products - EMA/410/01

Ph. Eur. Monograph 2902 'Precursors for radiopharmaceutical preparations'

Reflection paper on statistical methodology for the comparative assessment of quality attributes in drug development - EMA/CHMP/138502/2017

# 4. Active substance

## *4.1. General Information 3.2.S.1*

### 4.1.1. Nomenclature 3.2.S.1.1

No additional requirements.

### 4.1.2. Structure 3.2.S.1.2

Letter codes may be used for the primary structure of the active substance, i.e. 3-letter amino acid codes for the natural amino acids. If also unnatural amino acids or substituents are part of the

structure and shown with letter codes, the used codes have to be accompanied with a legend. In case of peptide chains, the N-terminus and the C-terminus of the chain have to be clearly indicated.

In case the molecule only contains amino acids with the natural L-configuration (eventually in conjunction with unnatural amino acids without chiral center), a structural formula with indication of the chiral centers is not needed (i.e. the 3-letter codes are sufficient). If the molecule contains amino acids with both D- and L-configuration (eventually in conjunction with unnatural amino acids without chiral center), a chemical formula with clear indication of the chiral centers and their configuration is necessary. In the latter case, a structure consisting of 3-letter codes only preceded by the appropriate D- or L- letters for unnatural amino acid may be helpful (in addition to the structural formula) for further use throughout the dossier.

Full chemical structure of non-peptidic side chains and linkers is expected.

### 4.1.3. General Properties 3.2.S.1.3

Relevant general properties of the peptide in question should be listed. In most cases, water content, isoelectric point, pH of a solution of the peptide, optical rotation, biological activity and solubility in different media would be expected. Hygroscopicity needs to be indicated, e.g. with moisture sorption isotherms, or reference could be made to 3.2.S.3.1 where such information could be provided more in detail.

Most peptides are amorphic powders, therefore melting point and polymorphic form are generally not applicable.

The counter ion needs to be indicated, if relevant, and whether it is present in a stoichiometric or non-stoichiometric ratio.

## *4.2. Manufacture 3.2.S.2*

### 4.2.1. Manufacturer(s) 3.2.S.2.1

No additional requirements.

### 4.2.2. Description of Manufacturing Process and Process Controls 3.2.S.2.2

**Schematic representation of the manufacturing process**

For the graphical presentations of the synthetic process(es) for peptides, it is considered acceptable to replace chemical structures by (three) letter codes in the reaction schemes to improve legibility, especially in case of longer peptide sequences. Letter codes for unnatural amino acids and substituents should be accompanied with a legend.

**Sequential procedural narrative**

The sequential procedural narrative should describe each step in the manufacturing process. During peptide synthesis the same standardized steps may be used several times, e.g. in Solid Phase Peptide Synthesis (SPPS), the peptide sequence is built up on a solid support by repeated cycles of deprotection, washing and coupling steps. These standardized steps with their associated Proven Acceptable Ranges (PARs) need not be described in detail each time they are used, provided clear descriptions of the used conditions (e.g. reagents, solvents, reaction times, …) are given, and provided it is clearly indicated where these steps are used in the overall manufacturing process. The final deprotection step should be described in detail, including any use of scavengers and other reagents, in

case of which a discussion of their genotoxic potential should be included in 3.2.S.3.2. Amounts can be described as weights/volumes or equivalents.

Splitting or combining of sub-batches/multiple cycles may be performed at different stages during manufacturing, e.g. based on equipment capacity or operational efficiency in SPPS. The criteria applied in the decision on splitting or pooling of sub-batches and/or multiple cycles should be provided, along with an adequate justification for the selected approach. Moreover, material traceability from the synthesis steps through the final drug substance is expected and S.2.2 should contain an unambiguous definition of the commercial batch size (range).

Hybrid manufacturing approaches i.e. manufacturing of peptide fragments by solid phase peptide synthesis followed by fragment condensation (liquid phase synthesis) have been used in the past and may become more popular e.g. for large scale production. For the manufacturing of the peptide fragments it is referred to the section above. The fragment condensation steps should be described in adequate detail including the purification steps.

When continuous manufacturing approaches are intended, the requirements of ICH Q13 on the description of the manufacturing process should be considered.

In case two drug substance manufacturing processes will be used in parallel (e.g. solid phase synthesis and a hybrid process), results from comparability studies on drug substance and drug product level should be provided.

**Reprocessing, recovery and rework**

The terms should be used and understood as defined in ICH Q7.

Synthetic peptides are generally purified using chromatographic techniques, often starting from a relatively complex crude intermediate. It is recognized that the crude peptide typically contains pre- and post-eluting structurally related impurities. It is acceptable to perform repeated purification steps of these side-fractions resulting in eluate meeting the purity requirements of the main fractions. When routine repurification is carried out this is not considered reprocessing but part of the regular manufacturing process and should be justified accordingly. Clear description of the repurification procedure and the criteria for deciding when it can be performed should be provided (see also ‘4.2.3. Control of Critical Steps and Intermediates 3.2.S.2.4’ below). The routine repurification process of the side fractions (if used) should be part of the manufacturing process qualification/validation. Likewise, if repetition of the coupling reaction is part of the routine manufacturing process, it is not considered reprocessing.

Appropriate measures to prevent cross-contamination due to the successive purification of different peptides using the same column should be in place, as required by GMP.

**Lyophilisation**

Lyophilisation of synthetic peptides is considered common practice. Lyophilisation process parameters should be described.

## 4.2.3. Control of Materials 3.2.S.2.3

**Active Substance (AS) Starting Material(s)**

The requirements of ICH Q11 and its associated Q&A in relation to the selection of starting materials are relevant to synthetic peptides. The name and address of all starting material manufacturers should be provided. The addition of manufacturers for the starting materials needs to be approved by a variation according to European legislation. Information, in the form of flowcharts, indicating the

synthetic process(es) of all starting materials including details of reagents, solvents and catalysts used, should be provided, followed by a criticality assessment of which starting material impurities may have an impact on the impurity profile of the peptide.

*Amino acids*

Protected amino acid derivatives (with terminal and side-chain protection as relevant) are generally acceptable as starting materials in the manufacturing process of synthetic peptides. Nevertheless, a justification on the designation of starting materials needs to be provided. For D-amino acid and unnatural amino acid derivatives, more detailed information regarding their manufacture (e.g., precursors and used reagents) and impurity profile is required than for standard L-amino acid derivatives.

In justified cases, short peptide segments such as protected dipeptide building blocks, may be acceptable as starting materials. Examples are dipeptides containing glycine or other dipeptides whose use will reduce the formation of diketopiperazine by-products compared to consecutive couplings of the individual amino acid derivatives. Other examples include Dmb-Glycine and pseudoproline-dipeptides which can be used to minimize aggregation during peptide assembly. However, polypeptide segments that undergo further modification (e.g. cyclisation or conjugation) are generally not acceptable as starting materials but are considered intermediates.

More complex peptides could be acceptable as starting materials in duly justified cases (e.g. in fragment condensation manufacturing processes). Companies are recommended to go for scientific advice to discuss their proposal well in advance.

Quality attributes for amino acid derivatives used as starting materials for synthetic peptides generally include: appearance, identification, related impurities, other impurities and assay. For the protected amino acid derivatives used as starting materials during peptide synthesis several typical related impurities may be present, these include: enantiomeric and diastereomeric impurities, (partially) unprotected amino acids, dipeptides and β-alanyl impurities. Other impurities may include e.g. residual solvents, water content and elemental impurities. Since the impurities of the amino acid derivatives, which can react like the parent compound during coupling, can accumulate in the final drug substance, the relevant impurities should be adequately controlled and limited in the starting material specifications. The impurity profiles of the starting materials and their potential impact on the quality of the final drug substance should be investigated during manufacturing process development. This should include a fate and purge assessment of the impurities that may be formed downstream in the manufacturing process.

Amino acids from human or animal origin should be avoided where possible. If used, Ph. Eur. chapter 5.2.8 on 'Minimizing the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Medicinal Products,' and the 'Note for Guidance on Minimizing the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Human and Veterinary Medicinal Products' (EMA/410/01) should be considered and TSE safety should be addressed.

Peptide synthesis resins preloaded with the first amino acid of the peptide sequence (through a linker) are also considered starting materials. However, the unloaded solid support resin itself is not considered a starting material as it is not incorporated as a significant structural fragment into the structure of the drug substance.

For solid support resins preloaded with amino acids, quality attributes related to the (chiral) purity and potential impurities of the loaded amino acid derivative are recommended. For the resin itself, reference is made to section 'Other materials used in the manufacturing process'.

*Non-peptidic structural moieties*

Conjugation and other derivatisations of peptides are commonly used. In these cases, sometimes complex structures are added to the peptide sequence. The classification of these materials will be handled on a case-by-case basis and early interaction (scientific advice) with the regulatory agencies is recommended. However, also for starting materials of non-peptide structural moieties (e.g. PEG-chains), compliance with the requirements as laid down in ICHQ11 and its associated Q&A is expected and its selection as starting material should be justified. For instance, sufficient subsequent chemical transformation steps after the starting material should be performed under GMP.

**Other materials used in the manufacturing process**

A list of all other reagents, such as resins, solvents and chromatographic materials used in the manufacturing process of a synthetic peptide should be provided. Adequate specifications for all materials should be laid down considering their role in the process but covering as a minimum identity as well as purity and/or assay where applicable.

The solid support resin is a key component of the SPPS process, typical quality attributes of the resin include: appearance, identification, cross-linking, swelling volume, mesh size and loading.

## 4.2.4. Control of Critical Steps and Intermediates 3.2.S.2.4

The criticality of the manufacturing steps for peptides made by solid phase synthesis should be evaluated during development according to the principles described in ICH Q9–Q11. In-process controls should be defined. The control of critical steps can be achieved by a combination of analytical tests and process control. During SPPS critical steps could include, e.g., 9-fluorenylmethoxycarbonyl (Fmoc) deprotection, control of washing steps, coupling or capping reaction monitoring, control of cleavage steps and drying steps. The most common test for the monitoring of coupling, capping and deprotection reactions is the Kaiser test, which is a colorimetric test based on the reaction of ninhydrin with primary amines.

During peptide purification by preparative chromatography, individually collected fractions are usually combined into a pool of fractions. The pooling strategy should be defined and acceptance criteria for the purity of individual fractions and the main pool should be stated. These criteria for purity usually include overall purity and criteria for individual impurities. In case secondary purification is proposed in the manufacturing process, adequate requirements for side-fractions that are allowed to undergo such purification, and the conditions thereof, should be defined. It should be stated which fractions are discarded. Filtration and lyophilisation steps should also be adequately controlled.

Intermediates as defined in the manufacturing process are tested before use in the next stage of the manufacturing process. The methods used for in-process control and/or intermediate testing should be described and confirmation of validation provided where applicable.

The ICH Q7 definition of intermediate should be considered, i.e., "A material produced during steps of the processing of an API that undergoes further molecular change or purification before it becomes an API. Intermediates may or may not be isolated." For all intermediates, justified specifications should be presented. Only intermediates complying with specifications may be pooled.

For the crude peptide after cleavage and deprotection adequate and justified specifications should be presented.

For solution-phase synthesis or fragment condensation processes, other requirements may apply for control of intermediates compared to SPPS.

### 4.2.5. Process Validation and/or Evaluation 3.2.S.2.5

No additional requirements (i.e. process validation data would normally not be expected in the dossier).

### 4.2.6. Manufacturing Process Development 3.2.S.2.6

It is acknowledged that there are general aspects of peptide synthesis where established knowledge and manufacturing experience may be extrapolated between different processes. Also, the starting materials and their properties are mostly well-known. If justified, manufacturers may make reference to prior knowledge for general aspects of the manufacturing process (e.g. choice of resin or coupling reagents). However, it is expected that substance specific aspects such as e.g. reaction times, temperatures and molar equivalents are addressed in the development section. If in-house knowledge from related products is referred to, the data and source should be identified as appropriate and differentiated from product-specific data. A discussion of how the prior knowledge data is to be used should be integrated with the relevant product-specific data to provide an overall understanding of product development and control. If prior knowledge from scientific papers is quoted, copies of the paper should be provided as appropriate.

## *4.3. Characterisation 3.2.S.3*

### 4.3.1. Elucidation of Structure and other Characteristics 3.2.S.3.1

The structure of the peptide should be confirmed by analytical data, this includes the primary, secondary, tertiary and quaternary structure where relevant. Mass spectrometry (MS) is a powerful analytical tool for the structure elucidation of peptides. Variants of the MS technique can be used to determine the molecular mass of a peptide and to confirm its amino acid sequence. Typical representative spectra and interpretation of the fragmentation data, including assignments and tables with theoretical and observed mass values, should be provided.

Amino acid analysis as described in the Ph. Eur. general chapter 2.2.56 usually complements the characterisation of synthetic peptides.

Elemental analysis may be used in view of structure confirmation.

NMR spectrometry is described in Ph. Eur. general chapter 2.2.64, 'Peptide Identification by Nuclear Magnetic Resonance Spectrometry.' However, the scope described in that general chapter is qualitative and consists of comparing the NMR spectrum of a test sample with that of a reference sample acquired under identical conditions. Furthermore, the scope is restricted to one-dimensional NMR spectrometry. NMR experiments are recommended to be part of the characterisation studies. One- and two-dimensional techniques should be used to assign the structure by means of $^{1}H$, $^{13}C$ and $^{15}N$ NMR data where relevant.

NMR can be used for

- determination of the number and types of proton nuclei;
- determination of the peptide sequence;
- identification of amino acids;
- assignment of carbon atoms;
- assignment of nitrogen atoms;
- secondary and tertiary structure elucidation.

Chiral gas chromatography (GC) is often used to identify and quantify the enantiomers of the different amino acids after acid hydrolysis. As the hydrolysis is known to induce some level of racemisation, it is carried out in deuterated hydrochloric acid, yielding deuterated amino acids if the racemisation takes place at this stage; deuterated and non-deuterated amino acid residues are detected separately by a mass spectrometric detector placed in tandem with the chiral GC system. Enantiomeric purity can be controlled by several means during the manufacture of a synthetic peptide. However, it should be justified that it is sufficient to perform the test on enantiomeric purity as a characterisation test and that no routine release control is required.

Ultraviolet (UV) and infrared (IR) spectroscopy are part of the standard characterisation programme. Additional information on the secondary structure can be gathered from these techniques.

Circular dichroism (CD) spectroscopy can be used to determine the absorption, e.g. in the presence of chromophores such as tryptophan, tyrosine, phenylalanine, disulfide bonds and peptide bonds. It measures differences in absorbance between left and right circularly polarized light and hence asymmetric properties of the chromophores. Changes in the structure and hence aromatic environments result in different CD spectra. Near-UV CD spectroscopy determines the tertiary structure due to asymmetric environments of tryptophan, tyrosine, phenylalanine and disulfide.

Far-UV CD spectroscopy determines the secondary structure due to asymmetric environments of the peptide.

Usually, no biological assay is required for the routine release of synthetic peptides. Nevertheless, biological assays can serve as additional tools for the characterisation of synthetic peptides.

Peptide mapping may be applicable for longer peptides. Ph. Eur. General chapter 2.2.55, 'Peptide Mapping', should be considered.

Peptides can also be characterized by Thioflavin T (ThT) dye assays and intrinsic tryptophan fluorescence to investigate whether a peptide can form fibrillary aggregates.

Where relevant disulfide bridge confirmation should be part of the characterisation studies.

**Evidence of chemical structure**

The information will normally include such evidence as:

List of characterisation techniques used for synthetic peptides (example table):

| Test | Analytical technique |
|---|---|
| Molecular mass | MS, LC-MS |
| Amino acid sequence confirmation | LC-MS/MS of intact molecule<br>LC-MS of enzymatically treated material (peptide mapping for long peptides) |
| Enantiomeric purity | Chiral GC-MS |
| Identity of potential counter ions | RP-HPLC and Ion Chromatography |
| Extinction coefficient | UV spectroscopy |
| Secondary structure | Far-UV circular dichroism (CD) spectroscopy<br>FT-IR spectroscopy |
| Tertiary structure* | Near UV CD spectroscopy |

| Test | Analytical technique |
|---|---|
| Quaternary structure / association state* | Composition gradient multiangle light scattering (CG-MALS) |
| Biological characterisation | Cell-based and other biological assays |

* In rare cases, tertiary structures or the association state (e.g. in the form of oligomers) may be relevant and should be addressed on a case by case basis

The relevance of the eventual or possible isomers regarding biological/pharmacological activity should be discussed (for veterinary products see Investigation of Chiral Active Substances 3CC29a, EMEA/CVMP/128/95).

**Physico-chemical Characteristics**

Physicochemical characterisation of the drug substance could include solubility and hygroscopicity studies, determination of the isoelectric point (pI) and thermogravimetric studies. The morphology may be examined by powder X-ray diffraction (PXRD), differential scanning calorimetry (DSC) and light microscopy.

## 4.3.2. Impurities 3.2.S.3.2

Purity is one of the most important critical quality attributes (CQAs) for synthetic peptides. Impurities are often categorized as either peptide-related impurities or non-peptide impurities. Peptide-related impurities contain structural elements of the synthetic peptide sequence. Non-peptide impurities include process reagents and their potential by-products, residual solvents, elemental impurities and potential genotoxic impurities.

Peptide-related impurities may originate from different sources:

- starting materials;
- formation during the manufacturing process;
- resulting from degradation during the manufacturing process or during storage.

The levels of certain related substances may be the result of combined contributions from these sources.

Related substances resulting from starting materials

Impurities present in the starting materials may cause the formation of peptide-related impurities. Examples of such impurities include incorrect enantiomers, β-Ala residues, dipeptides, single amino acid derivatives in dipeptides and amino acids with incorrect or without protecting groups. These impurities may be incorporated in the sequence during the assembly of the peptide by SPPS. In addition, with the exception of glycine, all natural amino acids constituting synthetic peptides are optically active with at least one stereogenic centre each. The presence of isomeric impurities in these starting materials can lead to the formation of stereoisomers of the final peptide.

Narrow acceptance criteria for those impurities should be set for each starting material used in the manufacture of the peptide.

Related substances formed during the manufacturing process

Related substances designated as process impurities may be a result of undesired or incomplete reactions during SPPS or cleavage.

*Stereoisomers*

In addition to starting materials, the manufacturing process can be a source of stereoisomers formation, for example by epimerisation. Racemisation of amino acids during the manufacturing process is possible. Hence, in theory, a multitude of different diastereoisomers of the target peptide can be formed. However, SPPS conditions should be optimised to ensure minimized racemisation. Amino acid derivatives with urethane-type protecting groups (e.g. Fmoc) are rather resistant to oxazolone formation which leads to racemisation at the stereocentre. Additionally, additives and bases used in the manufacturing process also suppress the potential racemisation of utilized amino acids.

*Deletion sequences and truncated sequences*

Deletion sequences are peptides with one or several amino acids missing. Their formation is often prevented by repetition of the coupling procedure to complete the coupling reaction and systematic acetylation to cap unreacted coupling sites. The acetylation procedure results in acetylated peptide fragments (truncated sequences).

*Insertion sequences*

If a particular amino acid is coupled more than once during one coupling step, an insertion sequence is generated. Insertion sequences may occur as the result of premature deprotection during prolonged coupling reactions or incomplete removal of excess amino acid derivative prior to the next deprotection step.

*Related substances formed during cleavage*

Certain side-chain protecting groups are released as reactive cationic species during cleavage. These can react with the nucleophilic side chains of sensitive amino acids (e.g. Trp, Tyr, Met). To prevent the formation of related substances through such side reactions, suitable scavengers are added to the cleavage mixture. Potential impurities could also result from incomplete cleavage of the amino acid side-chain protecting groups.

Related substances resulting from degradation during the manufacturing process or during storage

Degradation products of synthetic peptides may also occur as process impurities. Their content may increase during storage. Generally, the following pathways can contribute to the formation of degradation products of synthetic peptides:

- oxidation;
- hydrolysis;
- deamidation;
- diketopiperazine and pyroglutamic acid formation;
- β-elimination;
- condensation and formation of dehydropeptides;
- disulfide cleavage/exchange.

Aggregation may occur for synthetic peptides and could potentially be related to safety issues, including immunogenicity and should therefore be investigated.

Possible routes of degradation should be discussed - see section 3.2.S.7.1.

Analytical methods

Highly specific analytical methods (with appropriate limits of detection (LOD) and limits of quantitation (LOQ) used to detect each of the likely impurities considered above, or other related impurities, the exact identities of which may be unknown, should be described. Copies of relevant chromatograms should be provided. A summary should be given on the nature and levels of the actual impurities detected in the batch samples of the material.

Process-related impurities

Non-peptide impurities include process reagents, by-products, residual solvents and elemental impurities. The solid phase synthesis process requires extensive washing of the resin with solvents. Reagents and solvents used for the coupling steps are washed with incremental quantities of a suitable solvent. Nevertheless, for all reagents and solvents used in the manufacturing process, the depletion should be addressed in the dossier by either data and/or risk analysis. Any residuals of reagents and/or solvents should either comply with ICH M7 / EMA/CVMP/SWP/377245/2016 (if genotoxic), or – if not genotoxic - ICH Q3C/VICH GL18 thresholds, or (in absence of ICHQ3C/VICH GL18 thresholds), be toxicologically qualified.

Synthetic peptides themselves and peptide-related impurities are not within the scope of ICH M7 / EMA/CVMP/SWP/377245/2016).

## *4.4. Control of the Active Substance 3.2.S.4*

### 4.4.1. Specification 3.2.S.4.1

The active substance specification should be provided.

Typical specification tests included as an attribute in the specification are as follows (non-exhaustive list):

- appearance (+ appearance of solution if relevant);
- identification;
- amino acid analysis;
- purity (total impurities; individual impurities (unspecified/specified);
- aggregates / oligomers by SEC-HPLC (if relevant);
- assay/content; e.g. by HPLC or elemental analysis;
- acetic acid content/counter-ion content*;
- (residual) TFA content*;
- pH of solution;
- water content;
- mass balance;
- residual solvents;
- elemental impurities (e.g. in case of use of metal catalyst);
- bacterial endotoxins;
- microbiological purity.

The acceptance criteria laid down in the drug substance specification are identical with the limits that apply for stability studies.

Synthetic peptides are excluded from the scope of ICH Guideline Q3A/VICH GL10, 'Impurities in New Drug Substances', and consequently the limits laid down in this guideline are not applicable. Specific thresholds for peptide-related impurities are defined in the Ph. Eur. According to the Ph. Eur. general monograph 'Substances for Pharmaceutical Use', peptide-related impurities should be reported above 0.1%, identified above 0.5% and qualified above 1.0%.

* The type of counter ion can affect the biological and physicochemical properties of the peptide and related final formulations. For synthetic peptides, usually acetate is used as counter ion, however, other counter-ions are also possible (e.g. trifluoroacetic acid or TFA). The type of counter ion should be justified, and the amount of counter ions should be controlled in the drug substance specification with an upper and lower limit.

## 4.4.2. Analytical Procedures 3.2.S.4.2

**Analytical Development**

Ph. Eur. general chapters applicable to peptides (e.g. 2.2.55 Peptide mapping, 2.2.56. Amino acid analysis, 2.5.34 Acetic acid in synthetic peptides, 2.2.64 Peptide identification by NMR) and the "EDQM Technical guide for the elaboration of monographs on synthetic peptides and recombinant DNA proteins" may be also helpful for the development of the analytical methods.

The development of analytical procedures to control the quality of peptides, specifically the identity and the peptide-related impurities, could be a challenge due to the complexity of the structure of these molecules and the risk of co-eluting impurities.

*Identification*

The evidence of chemical structure should be discussed under Section 3.2.S.3.1. For the identification of the peptide as part of the specification and release, use of at least two orthogonal methods is recommended. Identification by mass, relative retention time (RRT), LC-MS, LC-MS/MS, peptide mapping, bioactivity, amino acid analysis or NMR are considered appropriate.

The applicant has to ensure that the proposed identification test or combination of tests is suitable to unambiguously confirm the sequence of the peptide.

*Purity*

The suitability of analytical procedures to detect and quantify impurities discussed under Section 3.2.S.3.2 should be demonstrated. At least, the analytical methods used for the control of purity should be suitable to fulfil the requirement for the Ph. Eur. reporting threshold of 0.1% for synthetic peptides.

If one analytical method for detection and quantification of all the peptide-related impurities is not appropriate to separate all peaks, additional independent method(s) may be needed. The combination of methods may enable measurement of structurally related impurities measured in drug substance. Stability indicating properties and mass balance of the method(s) need to be studied.

Control of diastereomers may require the development of specific chiral methods.

*Changes of the analytical methods during development*

The level of detail of the commercial analytical procedures used for testing peptides should be described in the dossier in such a way that they can be repeated by an Official Medicines Control Laboratory.

If different methods have been used to generate data during development, a brief description and comparison of data should be included in appropriate place in the dossier.

### 4.4.3. Validation of Analytical Procedures 3.2.S.4.3

The analytical procedures used for the control of the drug substance, including the analytical procedures used during the development of the peptide, should be fully validated. In general, the validation of analytical tests concerning the active substance should be performed according to the requirements of the current Guidelines (ICH Q2, VICH GL1 and GL2).

In case the quality of the peptide is covered by a monograph in the Ph. Eur. and the methods of the monograph are used, it is not necessary to present the validation of these analytical procedures in the dossier. In case the peptide is described in the Ph. Eur. monograph but the company applies its own methods, then (cross)validation data is needed in the dossier.

### 4.4.4. Batch Analyses 3.2.S.4.4

This section should summarize the batch analysis data for all the peptide batches described in the dossier.

Apart from the analytical determination, for each batch the following information should be provided: date of manufacture, batch size and number, scale (laboratory/pilot/commercial), route of synthesis (commercial or not), place of manufacture, use of batches.

As recommended in other guidelines, presentation of this information in tabular form is recommended for improved clarity. Usually, early development batches are tested using a slightly different specification. The differences in the results obtained in the batches used in earlier development and pilot/commercial batches should be explained and justified.

The improvement in the analytical methods during development of the peptide could lead to the observation of new impurities in pilot/commercial batches. In those cases, comparison of the batch analysis data should be performed, and the impact on the quality of the drug substance and or clinical/preclinical data should be discussed.

### 4.4.5. Justification of Specification 3.2.S.4.5

The proposed specification should be supported by batch data from preclinical, clinical and production scale batches combined with an adequate understanding of the manufacturing process of the peptide.

The limits applied for peptide-related impurities should be based on the general monograph of the European Pharmacopoeia 'Substances for Pharmaceutical Use (2034)'. In case that the limit for identified or unidentified impurities is above the prescribed Ph. Eur. qualification threshold, qualification of these impurities is expected. Nevertheless, the acceptance criteria for the peptide-related impurities and total impurities should not only be based on the qualified limit but on data obtained from the batches used to support the quality of the drug substance.

Grouping of impurities which can be analytically separated (e.g. pre- and post-eluting groups) is not recommended, and can only be accepted in duly justified cases, based on demonstrated analytic efforts.

In case of a very complex impurity profile or where two or more impurities are very similar, it may not be technically feasible to obtain peak separation. In such cases it may be necessary to set a limit for a combination of unresolved peaks. In this case, thresholds should be applied for the combination of peaks. For qualification, the impurity profile of the batches used in the toxicological studies should be taken into account.

The assay and how it is calculated should be clearly defined. The assay limits are typically expressed in terms of the counter-ion free, anhydrous substance, unless otherwise justified. The limits for assay are typically asymmetric, the upper limit being 100 per cent + the permitted assay reproducibility, and the lower limit being 100 per cent – (the permitted assay reproducibility + the maximum permitted level of impurity).

The absence of a biological assay should be justified.

## *4.5. Reference Standards or Materials 3.2.S.5*

Peptides are often very hygroscopic powders, therefore appropriate precautions against moisture uptake by the reference standard during storage and during analysis should be taken when relevant.

The origin of the reference standards should be briefly indicated (e.g. batch synthesised according to the commercial process). If a 2-tiered system is used (primary reference standard and working reference standard) the preparation and qualification strategy should be briefly explained, and the characterisation results obtained for the reference standard batches, the approach to periodically requalify the reference standards, as well as the approach that will be followed to qualify future batches of reference standards, including the measures that will be taken to prevent drift in peptide content, should be presented.

If reference standards are used for certain impurities, a short description on how these were prepared and characterised/qualified for use should be provided.

## *4.6. Container Closure System 3.2.S.6*

The container closure system should be suitable, considering the substance properties, storage conditions and use: e.g. for hygroscopic powders, appropriate desiccant should be included. Alternatively, storage under inert atmosphere could be considered.

## *4.7. Stability 3.2.S.7*

### 4.7.1. Stability Summary and Conclusions 3.2.S.7.1

The principles outlined in EMA's and (V)ICH scientific guidelines on the stability of drug substances should be followed with regards to aspects such as the types of studies conducted, protocols used, selection of batches, container closure system and storage conditions.

The choice of test conditions applied during stability storage (temperature and humidity) should be justified. Generally, to prevent or minimize degradation, peptides are stored under refrigerated (5°C ± 3°C) or freeze conditions (-20°C ± 5°C), but the use of higher temperatures/humidities are also expected not only to address short term excursions, but to obtain a comprehensive overview of the degradation pathways of the drug substance; these data might be especially important for the development of the drug product.

The potential degradation pathways of the peptide should be discussed taking into account the amino acids composition and sequence: e.g. oxidation of Cys and Met residues, deamidation, hydrolysis, β-

Asp-containing sequences. Forced degradation studies are foreseen to evaluate both, the degradation of the peptide and the ability of the analytical procedures to detect the degradation.

For hygroscopic powders, it is expected that water content should be part of the stability protocols.

Aggregation may also occur for synthetic peptides and could potentially be related to safety issues, including immunogenicity and should therefore be investigated during stability.

The retest period and storage conditions should be justified following EMA's and (V)ICH scientific guidelines on the stability of drug substances.

Variability in stability testing results should be avoided by establishing appropriate handling procedures during analytical testing.

### 4.7.2. Post-approval Stability Protocol and Stability Commitment 3.2.S.7.2

General principles outlined in EMA's and (V)ICH scientific guidelines should be followed.

### 4.7.3. Stability Data 3.2.S.7.3

In some cases, the analytical procedures used during stability, especially during earlier development stages, are not identical to the analytical procedures which are part of the drug substance specification. The changes between these methods and the commercial analytical methods should be justified, and the impact on generated stability results to be discussed.

## *4.8. Conjugation*

Conjugation has emerged as a popular mechanism to alter or enhance the properties of peptide drug candidates. Conjugation to poly(ethylene glycol) (PEG), lipids and proteins has been used as a half-life extension strategy. Conjugation can also be used to deliver a cytotoxic payload or imaging agent to specific cell types targeted by the peptide.

However, there is added complexity with respect to the characterisation and control of these conjugates. The control of the unconjugated peptide which is usually classified as an intermediate is essential. Adequate specifications and control methods should be established for these intermediates. In cases where no intermediate is isolated these approaches should be justified and an adequate control strategy should be developed (see also 4.2.3).

The underlying conjugation chemistry should be described in the manufacturing process development section. Conjugatable versus non-conjugatable impurities should be identified by means of a risk analysis and the incorporation into the target molecule should be investigated. Purging of process-related impurities from the conjugation process should be investigated.

An additional quality attribute for conjugated peptides is the amount of the free unconjugated peptide and the free form of the conjugate moiety (e.g. free PEG/linker). Di-PEGylation or multi-PEGylation (or other conjugation moieties) may also occur and should be adequately controlled.

The choice of the starting material of the conjugation component needs to be justified according to ICH Q11, 'Questions & Answers: Selection and Justification of Starting Materials for the Manufacture of Drug Substances'. It has to be assured that all steps of the intermediate synthesis starting from the defined starting material are performed under good manufacturing practice (GMP). Consequently, e.g. the activation of the suitable PEG starting material is considered a part of the manufacturing process and an activated PEG derivative (e.g. in the form of an N-hydroxysuccinimide (NHS) ester) may not be suitable as starting material and is considered to be an intermediate itself.

Full information should be provided in Section 3.2.S.2.2 of Module 3, including flowchart, process description with all process steps, raw materials and manufacturing process controls.

In numerous development programmes, polymers or other conjugation moieties are coupled to the peptide via a chemical linker. The points mentioned above are also applicable for such chemical linkers, especially for the selection of suitable starting materials and the control of the impurity profile. The critical attributes should be evaluated and a justification for the specification attributes should be provided. The basic principles of ICH M7/EMA/CVMP/SWP/377245/2016 regarding a mutagenic impurities assessment should be considered for chemical linkers and conjugation moieties.

In many cases, the conjugation moiety and the linkers are manufactured by a different manufacturer than the synthetic peptide. In the case of multiple suppliers of the conjugation moiety and/or linker, for each supplier separate documentation is expected, and a compiled specification for the conjugation moiety should be elaborated by the manufacturer of the peptide-conjugate.

Peptide-conjugated material from all suppliers of the conjugation moiety and/or linker should be manufactured and batch analysis and stability data should be generated.

It is recommended to consider the legal framework for cases where a New Active Substance status is claimed and an unconjugated or differently conjugated product is already approved.

Conjugation-specific aspects regarding the SPC and labelling may be discussed with the Competent Authorities prior to submission.

# 5. Medicinal Product Considerations

The quality target product profile (QTPP) relates to quality, safety and efficacy, considering e.g. the route of administration, dosage form, bioavailability, strength and stability of a medicinal product containing a synthetic peptide as active substance.

(V)ICH Guidelines ICH Q3B (VICH GL11) and ICH Q6A (VICH GL39) are not or only partly applicable to synthetic peptides. The thresholds for impurities as defined in the general monograph of the Ph. Eur. 'Substances for Pharmaceutical Use' may also be applicable to the resulting medicinal products. Limits should be justified on a case-by-case basis considering the batch analysis history and qualification data.

Synthetic peptides are included in the scope of ICH Q3D 'Guideline for Elemental Impurities' (Reflection paper EMA/CVMP/QWP/153641/2018 for veterinary products), thus the requirements laid down in this guideline are applicable for medicinal products containing synthetic peptides as active substances. Also the risk considerations and requirements for nitrosamine impurities are applicable to synthetic peptide active substances that are used in finished products for human use.

Potential interactions of the peptide with the excipients present in the formulation and leachables that could result from manufacturing materials and packaging materials such as stoppers should be evaluated during pharmaceutical development.

If the mode of action is based on the primary structure and the content (quantity) of the peptide only, no potency assay is needed for release and stability testing of the finished product. Applicants are encouraged to give more details on the possible (absence of) 3-D (secondary) structure, e.g. based on NMR and FTIR, as well as computation investigations. Additionally, experiments on the 3-D structure stability characteristics in the formulation, with techniques such as CD or others are recommended as characterisation data, to justify the omission of such analysis in the routine control strategy.

Furthermore, where relevant, formulation development should address the aggregation propensity and the nature of the aggregates formed, especially under stress conditions including terminal sterilisation (see below), in which case the immunogenicity risk should be discussed.

Most of the medicinal product formulations containing synthetic peptides as active substance are for parenteral use. The principles for the choice of sterilisation process for finished products and containers are presented in the form of decision trees in the 'Guideline on the Sterilisation of the Medicinal Product, Active Substance, Excipient and Primary Container' are also relevant for synthetic peptides. Terminal sterilisation provides the highest sterility assurance level, thus this should be the method of choice unless demonstrated unsuitable.

A combination of sterile filtration, pre-sterilised container closure system and aseptic processing is only acceptable if the applicant demonstrates by data that the use of a terminal steam sterilisation process under the least stressful conditions ($F_0 \geq 8$ minutes) causes significant degradation. In case of moderate degradation, exceeding the qualification threshold is not a valid argument in itself to reject terminal sterilisation. Formulation optimisation efforts (e.g. pH, buffer system, osmolality), and choice of container closure system should be made during pharmaceutical development in view of enabling terminal sterilisation.

If synthetic peptide drug products in development show moderate degradation towards heat stress, feasibility of terminal sterilisation should be addressed from early-development onwards. At that point, assay loss and increase in impurities/degradations products at levels that would not be observed with aseptic processing, may still be qualified in toxicological and pivotal clinical studies, including those impurities that exceed the qualification threshold. Such studies should address the physicochemical properties, biological activity, and if relevant the immunogenicity risk of the product after terminal sterilisation. All of this with due consideration of the potential issues that may occur during formulation development (e.g. pH and buffering range) and further upscaling towards the commercial-scale terminal sterilisation process. To this extent, timely availability of stability indicating analytical methods with good resolution of peaks and good mass balance, are a pre-requisite. If needed complementary/orthogonal methods should be established to detect and quantify difficult to detect impurities.

Thresholds for peptide-related impurities as defined in the Ph. Eur. general monograph 'Substances for Pharmaceutical Use', also apply to finished products: peptide-related impurities should be reported above 0.1%, identified above 0.5% and qualified above 1.0%. If aggregation/oligomerisation occurs during finished product manufacture and/or storage, aggregates/oligomers should be included in the finished product release and stability specification, unless otherwise justified.

Manufacturing processes should take into account any special characteristics such as hygroscopicity of (lyophilised) active substance, as well as any temperature and/or light sensitivity of the active substance, as relevant.

If correction factors are applied during dispensing (e.g. based on assay, purity, moisture content, residual solvent content, and/or salt content of active substance) to achieve a specific declared (labelled) amount of active in the formulation, these have to be described in the dossier.

The label claim strategy should be conclusively described and justified, including (where relevant) calculation of active substance assay, any correction factors applied during dispensing, any in-process controls for assay adjustment during drug product manufacturing, and assay calculation for release- and stability testing. Any changes in label claim strategy during development have to be described in detail and justified carefully, to ensure that the dose definition used in clinical trial(s) can be bridged unequivocally to the proposed commercial product with label claim as per the SmPC/labelling.

For medicinal products where European product-specific guidance on the demonstration of the bioequivalence has been published the generic product should comply with the quality requirements described therein e.g. for comparability studies.

Additional characteristics for complex finished product dosage forms should be considered on a case-by-case basis.

# 6. Synthetic Peptide Development Programmes Using a Biological Medicinal Product as a European Reference Medicinal Product (human products only)

The European legislation for medicinal products clearly differentiates between biological medicinal products ('biologicals') and chemically derived molecules.

The quality of biological medicines is tightly controlled and specific guidelines and regulations with additional requirements apply. The level of information that needs to be submitted in the dossier is more extensive than for chemically derived molecules.

A biosimilar is a biological medicine highly similar to another biological medicine already approved in the EU (called 'reference medicine') in terms of structure, biological activity and efficacy, safety and immunogenicity profile.

A biosimilar is not regarded as a generic of a biological medicine. This is mostly because the natural variability and more complex manufacturing of biological medicines do not allow an exact replication of the molecular micro-heterogeneity.

Most biological medicines in current clinical use contain active substances made of proteins. Some smaller proteins can be produced either by using a biological manufacturing process or through chemical synthesis (e.g. solid phase synthesis). Typically, structural heterogeneity and post-translational modifications are not relevant for these molecules.

The biosimilar regulatory pathway is not possible for chemically synthesised peptides since these fall outside the definition of a biological substance.

Nevertheless, the basic principles to demonstrate biosimilarity – high similarity in terms of structure, biological activity and efficacy, safety and immunogenicity profile – should be considered for synthetic peptide development programmes using a biological medicinal product as a European Reference Medicinal Product. (Reference to: 'Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: quality issues (revision 1)).

Analytical comparability testing, comprising physicochemical (structural) and biological (functional) assays and conventional analytical testing, forms the basis of the demonstration of comparability.

A broad panel of analytical methods (see section 4.1.3 Characterisation 3.2.S.3) to demonstrate the comparability between the recombinant reference product and the synthetic version is required for the side-by-side comparability studies. It is important to note that the reference product used in the comparability studies should be sourced from the European market.

The applicants will need to fully quantify all differences in peptides produced by chemical synthesis and peptides produced by recombinant technology and demonstrate that both products are comparable. The applicants should consider what analytical tests might be used to confirm comparability and to define and justify, prior to conducting these studies, the acceptance range to conclude comparability. Any observed differences from the reference medicinal product should be evaluated and justified.

The primary structure should be confirmed by, e.g., MS, peptide mapping with MS/MS, capillary isoelectric focusing (IEF), western blotting, etc. Higher-order structures should be addressed by CD analysis, Fourier transform infrared (FTIR) spectroscopy, 2D NMR spectrometry, fluorescence spectroscopy and DSC as appropriate. For certain peptides the oligomeric state should be investigated.

Characterisation of purity should be addressed using an orthogonal approach, i.e. size-based, charge-based and hydrophobicity-based separation techniques. It is the responsibility of the applicant to demonstrate that the purity methods are suitable to cover the complete impurity profile of the peptide or whether additional purity testing with additional supplementary methods is necessary. Aggregation propensity should also be investigated by suitable techniques detecting fibrillary aggregates such as Thioflavin T (ThT) assay. When differences in the impurity profiles are observed it should be demonstrated that the impurities in the synthetic peptide not present in the biological reference product are qualified and do not raise concerns regarding immunogenicity.

Regarding the assessment of impurity related immunogenicity, experience has shown that immunogenicity of peptides is of lesser concern than that of proteins due to their size. Furthermore, changes or modifications (e.g. deamidations) of a small number of amino acids are not noticeably immunogenic. If the total amount of peptide-related impurities does not exceed the respective amount of peptide-related impurities of the originator product, this is not considered as a concern even if a given peptide-related impurity is not present in the originator preparation. In case a novel type of impurity occurs, i.e. differing from the drug substance in more than a few amino acid modifications, this novel impurity should be reduced as far as possible since reliable prediction of immunogenicity is not feasible.

In-silico prediction of immunogenicity, e.g. based on predicted binding to T-cell receptors (TCR), or in-vitro tests of T-cell activation are not considered useful since also T-cell independent immune responses are described (e.g. heparin-induced thrombocytopenia (HIT)). Mainly intended for vaccine development, their predictive value for impurities appears to be low.

In general, these synthetic peptides have to comply with the requirements of the Ph. Eur. General monograph 'Substances for Pharmaceutical Use'. This monograph allows an identification threshold of 0.5%. However, for comparability purposes a full evaluation of the impurity profile also covering impurities lower than 0.5% is expected. A limit of quantification (LOQ) of 0.1% for HPLC purity testing is required.

Comparative forced degradation studies are also recommended and the suitability of the analytical purity methods to fully characterize the impurity profiles of both products should be demonstrated.

Process-related impurities from the cell construct (e.g. host cell protein (HCP), DNA) or resulting from the manufacturing process (e.g. antibiotics and other media components) do not need to be part of the comparability studies. Clearance of reagents, residual solvents, elemental impurities and potential genotoxic impurities for the synthetic peptides should be addressed as described above under 4.1.3.

Functional assays (e.g. cell based assays using appropriate cell lines) should be developed and used in the comparability studies. It depends on the mechanism of action which additional functional assays may be needed to demonstrate similarity (e.g. binding kinetics).

The absence of a biological assay in the release specifications for drug substance and drug product should be appropriately justified, e.g. by commercial-scale batch biological assay data and, in addition, by appropriate characterisation of higher-order structure by physicochemical testing.

The analytical methods used in the comparability exercise should be suitable, sufficiently qualified and/or validated and sensitive to detect potential differences between both products. In the case that

statistical models are used to demonstrate comparability they should be adequately described and justified.

Batches preferably from the commercial process should be used for the side-by-side analyses. The number of batches used in the comparability studies should be adequately justified. (Reference to: 'Reflection paper on statistical methodology for the comparative assessment of quality attributes in drug development' - EMA/CHMP/138502/2017)

Comparative stability studies can be useful for detecting potential differences in the stability profile of the peptides manufactured either by chemical synthesis or by recombinant techniques. Stability and shelf-life claims cannot be derived from the reference product without their own data.

# 7. Requirements for Clinical Trial Applications (human products only)

The requirements for peptides intended to be used in the course of clinical studies are evolving depending on the stage of development, with increasing expectations going towards Phase 3 and in preparation of MAA. The main focus should be on the safety of the synthetic peptide, especially in the early stages of development.

It is acknowledged that most peptides will be manufactured by solid phase supported synthesis hence a platform similar to other peptides will be used; nevertheless, details regarding the type of resin used, as well as coupling agents and the use of capping will be expected.

With regard to starting materials of the active substance, it is expected that from an early-stage, individual impurities will be monitored in the amino acid building blocks in order to allow understanding and control of the impurity profile of the final peptide. Setting of limits for certain impurities may be expected for later development.

Similar expectations also apply to isolated intermediates (e.g. crude peptide after cleavage from the resin) and critical purification steps (e.g. preparative chromatography on the crude peptide).

The changes introduced during manufacturing process development should be described in terms of potential impact on the quality of the active substance; particular attention should be paid to differences in impurity profile compared to preclinical batches used for qualification of impurities.

The novel peptide should be fully characterised in terms of primary structure; particular attention should be paid to its potential for aggregation from as early as possible in the development in order to avoid problems during formulation of the drug product. The propensity toward racemisation should also be investigated.

Impurities in the peptide active substances should be identified in the course of development. Peptide-related impurities above the threshold of 1.0%, should be identified and qualified in preclinical studies. Orthogonal/complementary analytical procedures should be employed also in the early development stages in order to minimise the risk of co-elution of impurities and to adequately characterise the impurity profile of the synthetic peptide; if it can be shown in the course of development that one analytical procedure is sufficient to control all impurities, the other(s) could be omitted.

With regard to the stability studies to be conducted on the synthetic peptide, it is essential that stability-indicating analytical procedures are employed given that peptides tend to be thermally labile hence degradation should be detected at inappropriate storage conditions.