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Guideline on the Development and Manufacture of Synthetic Peptides

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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 investigational medicinal products (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 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 or precursors, this guideline applies 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.

The guideline is applicable to Marketing Authorisation Applications and post-authorisation procedures; specific considerations related to Investigational Medicinal Products are provided in section 7 of this guideline and applicants are advised to liaise with the relevant National Competent Authorities (NCAs) responsible for the approval and supervision of clinical trials.

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 Q1 A-F Stability testing of new drug substances and drug products – Scientific guidelines (veterinary VICH GL3-5, GL45, GL51 and GL58)

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 (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 Q7 Good Manufacturing Practice for Active Pharmaceutical Ingredients

ICH Q8 Pharmaceutical development

ICH Q9 Quality risk management

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

ICH Q12 guideline on technical and regulatory considerations for pharmaceutical product lifecycle management

ICH Q13 Continuous manufacturing of drug substances and drug products

ICH Q14 Analytical procedure development

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. General Monograph 2034 'Substances for Pharmaceutical Use'

Ph. Eur. General Monograph 20255 'Peptide Mapping'

Ph. Eur. General Monograph 20256 'Amino Acid Analysis'

Guideline on summary of requirements for active substance in the quality part of the dossier (CHMP/QWP/297/97, EMEA/CVMP/1069/02)

Guideline on Radiopharmaceuticals (EMEA/CHMP/QWP/306970/2007)

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

Guideline on manufacture of the finished dosage form (EMA/CHMP/QWP/245074/2015)

Guideline on manufacture of the veterinary finished dosage form (EMA/CVMP/QWP/798401/2015)

Guideline on the sterilisation of the medicinal product, active substance, excipient and primary container (EMA/CHMP/CVMP/QWP/850374/2015)

Guideline on the requirements to the chemical and pharmaceutical quality documentation concerning investigational medicinal products in clinical trials (EMA/CHMP/QWP/545525/2017)

Commission Implementing Regulation (EU) 2025/2091 laying down good manufacturing practice for veterinary medicinal products

Commission Implementing Regulation (EU) 2025/2154 laying down good manufacturing practice for active substances used as starting materials in veterinary medicinal products

4. Active substance

4.1. General Information 3.2.S.1

4.1.1. Nomenclature 3.2.S.1.1

No additional requirements than for other chemical active substances.

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, according to IUPAC nomenclature. 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. Modifications on the terminals needs to be shown in the structure.

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. Describing the peptide sequence followed by the designation, nature of cyclisation and the positions involved in the cyclisation, if applicable, is acceptable.

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 described, such as, water content, isoelectric point, pH of a solution of the peptide, optical rotation, solubility in different media over the physiological pH range and mechanism of action. 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. In the event that there is no counter ion, the free base should be indicated.

4.2. Manufacture 3.2.S.2

4.2.1. Manufacturer(s) 3.2.S.2.1

No additional requirements than for other chemical active substances.

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 standardised 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, coupling and capping steps. These standardised steps need not be described in detail each time they are used, provided clear descriptions of the used conditions (e.g. reagents, solvents and relevant process parameters as applicable) are given, and provided it is clearly indicated where these steps are used in the overall manufacturing process. The final cleavage/deprotection step should be described in detail, including any use of scavengers and other reagents. Amounts can be described as weights/volumes or equivalents.

Splitting of batches or combining of sub-batches that are produced in multiple cycles of the same manufacturing steps may be performed at different stages during manufacturing to facilitate the process, e.g. based on equipment capacity or operational efficiency in SPPS. The justification for splitting or pooling of (sub-)batches should be provided. Moreover, material traceability from the synthesis steps through the final active substance is expected according to GMP and S.2.2 should contain an unambiguous definition of the commercial batch size (range).

Hybrid manufacturing approaches e.g., manufacturing of peptide fragments by solid phase peptide synthesis followed by fragment condensation (solution 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 by SPPS method it is referred to the section above. Fragment condensation and solution phase synthesis generally should follow the considerations of the Guideline on the Chemistry of Active Substances.

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

In case more than one active substance manufacturing process will be used in parallel (e.g. solid phase synthesis and a hybrid process), results from comparability studies on active substance should be provided. It should be demonstrated that active substance from both processes show no significant differences in relevant quality characteristics. For a synthetic peptide, biological (functional) assays are

not required when data are provided indicating that the peptide will show consistent higher order structure within investigated batches. For complex peptides comparability data for finished product batches manufactured with each process variant should be provided. For other peptides, justification may be provided for the absence of finished product data from both processes.

Synthetic peptides are generally purified using chromatographic techniques, often starting from a relatively complex crude intermediate. As complete separation of the desired peptide and structurally related pre- and post-eluting impurities is not always achieved, it is common to collect fractions and pool the main fractions with highest purity for further processing. In order to improve the yield, it is acceptable to perform repeated purification steps of the side-fractions with lower purity before combining them with the main fraction. Generally, separate sets of acceptance criteria are established for pooling of main fractions and side-fractions. A clear description of these successive purification and 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). When routine repurification is carried out, this is not considered reprocessing but part of the regular manufacturing process and should be justified. Likewise, if repetition of the coupling reaction is part of the routine manufacturing process, it is not considered reprocessing. The routine repurification process of the side fractions (if used) should be part of the manufacturing process qualification/validation.

Furthermore, filtration methods may be utilised to purify peptides by removing smaller contaminants or to concentrate peptide solutions or fractions.

Purification conditions, including any filtration step, should be described in adequate level of detail.

Isolation

Lyophilisation of synthetic peptides is considered common practice. Alternative isolation methods, such as precipitation, crystallisation, and spray drying are also used. Relevant process parameters for the isolation operations should be described.

Reprocessing, recovery and rework

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

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.

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. A justification on the designation of starting materials needs to be provided, as well as the name and address of all starting material manufacturers. 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 and peptide fragments

Protected natural L-amino acid derivatives (with terminal and side-chain protection as relevant) are generally acceptable as starting materials in the manufacturing process of synthetic peptides. In justified cases, short peptide fragments 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 minimise aggregation during peptide assembly. However, polypeptide fragments that undergo a limited number of further modifications under GMP (e.g. cyclisation or conjugation to non-peptide structural moieties such as PEG-chains) are generally not acceptable as starting materials but are considered intermediates.

Longer peptide fragments could be acceptable as starting materials in duly justified cases (e.g. in fragment condensation manufacturing processes). Companies are recommended to request scientific advice to discuss their proposal 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 specification attributes 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 have an impact on the impurity profile of the active 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 active substance should be investigated during manufacturing process development. This should include a fate and purge assessment of the impurities that may be formed along 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. Relevant supporting information should be presented in section 3.2.A.2 for each source of amino acid derivatives.

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 active substance.

For solid support resins preloaded with amino acids, the control of the stereoisomers and other potential impurities, if applicable, is expected. For the resin itself, reference is made to section 'Other materials used in the manufacturing process' below.

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 non-peptide structural moieties (e.g. PEG-chains with or without linkers), compliance with the requirements as defined 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 introduction should be performed under GMP. Please also refer to section 4.8 for further details.

Other materials used in the manufacturing process

A list of all other reagents, such as solid phase 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 may include: appearance, identification, 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/or process controls. During synthesis critical steps typically include 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. Other equivalent alternative tests are Chloranil test and TNBS test.

During peptide purification by preparative chromatography, individually collected fractions are usually combined into a pool of fractions. The pooling strategy should be defined and justified. Acceptance criteria for the purity of individual fractions and/or the main pool should be stated. These criteria for purity usually include overall purity and individual impurities and should be appropriately justified. In case repurification 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.

In this context, it should be stated which fractions are discarded. Continuous chromatography approaches with automated side fraction recycling may also be used, if adequately described. Filtration and drying 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." The end product of SPPS is the peptide bound to the resin. Even though this is by definition an intermediate, it is normally not subject for intermediate testing as the peptide is bound to the resin and cannot be tested without chemical modification. The crude peptide after cleavage and deprotection is typically considered to be an intermediate. For all intermediates, 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 than for other chemical active substances.

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, and higher order structure (secondary, tertiary and quaternary) where relevant.

Secondary structure is the local arrangement of a peptide's backbone stabilised mainly by hydrogen bonds. The tertiary structure is the three-dimensional folding of a peptide resulting from interactions between side chains. The quaternary structure refers to the specific, defined arrangement of two or more peptide subunits that assemble into a single functional complex. Oligomers are associations of a few peptide molecules (often 2–10) that may be transient or undefined in structure.

If the absence of higher order structure of the peptide is evident from literature and the absence is additionally confirmed with the data gathered during development, the additional evaluation is not required during characterisation. 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.

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 can be used for determination of the number and types of proton nuclei and of the peptide sequence, for identification of amino acids, for assignment of carbon and nitrogen atoms, and for secondary and tertiary structure elucidation.

One- and two-dimensional techniques may be used for characterisation studies e.g. to provide proof of specific structure motives including secondary and tertiary structure elements by means of ^{1}H and ^{13}C NMR or other heteronucleii data where relevant. Representative spectra and relevant interpretation should be provided.

Chiral gas chromatography (GC) is often used to identify and quantify the enantiomers of the different amino acids after acid hydrolysis. As hydrolysis is known to induce some level of epimerisation, it is

carried out in deuterated hydrochloric acid, yielding deuterated amino acids if the epimerisation 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. Other chromatographic techniques, such as e.g. LC analysis (using reference standards of the most potential epimer peptide impurities) may also be used. Enantiomeric purity can be controlled by several means during the manufacture of a synthetic peptide. However, it should be justified e.g. by means of a risk analysis or prior knowledge on the mechanisms of epimerisation during peptide manufacturing processes 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 may be part of the characterisation programme. Additional information on higher order structures can be gathered from these techniques.

Circular dichroism (CD) spectroscopy can be used to provide information on secondary and tertiary structure elements by the absorption of polarised light.

Peptide mapping may be applicable for longer peptides based on cleavage site(s) in the primary structure in cases where the MS/MS sequencing data may be difficult. Ph. Eur. general chapter 2.2.55, 'Peptide Mapping', should be considered.

Aggregation propensity of a peptide may be evaluated by a suitable technique, e.g. Thioflavin T (ThT) dye assays and intrinsic tryptophan fluorescence to investigate whether a peptide can form fibrillary aggregates, if relevant. Other analytical methods may be used case by case.

Where relevant characterisation of disulfide bridges and cyclic structures should be performed.

Biological assays serve as additional tools for the characterisation of synthetic peptides. In particular, when the mechanism of action is linked to the higher order structure and the latter cannot be sufficiently characterised by physicochemical tests, a biological assay should be included in the characterisation exercise.

Evidence of chemical structure

The information will normally include such evidence as:

List of characterisation techniques used for synthetic peptides (non-exhaustive example table, not all techniques may be relevant):

Test	Analytical technique
Molecular mass	MS, LC-MS
Amino acid sequence confirmation	LC-MS/MS of intact molecule LC-MS of enzymatically treated material (peptide mapping for long peptides) NMR Edman degradation
Amino acid composition	Amino acid analysis
Enantiomeric purity	Chiral GC-MS LC Other chiral chromatographic techniques Enantiomeric purity after partial or complete enzymatic digestion
Identity of potential counter ions	LC Ion chromatography
Extinction coefficient	UV spectroscopy
Secondary structure	Far-UV circular dichroism (CD) spectroscopy FT-IR spectroscopy NMR
Tertiary structure*	Near UV CD spectroscopy NMR
Quaternary structure / association state*	Different types of interactions observed in quaternary structures require specific methods suitable for the characterisation
Biological characterisation	Cell-based and other biological assays

* In rare cases, tertiary/quaternary 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 using appropriate analytical methods if quality, safety or efficacy could be affected.

Physico-chemical Characteristics

Physicochemical characterisation of the active substance could include solubility and hygroscopicity studies, determination or calculation of the isoelectric point (pI) and thermogravimetric studies. The morphology may be examined by light microscopy while amorphous state may be examined by X-ray powder diffraction (XRPD) and differential scanning calorimetry (DSC) or other techniques, as relevant.

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 categorised 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 mutagenic 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/diastereomers, incorrect amino acids (e.g. Ile in Leu and vice versa), 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. 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.

Suitably justified acceptance criteria for those impurities should be set for each starting material used in the manufacture of the peptide based on carry over and final peptide control strategy.

Related substances formed during the manufacturing process

Related substances designated as process impurities may be a result of undesired or incomplete reactions during SPPS, LPPS, fragment condensation, cleavage and other synthetic steps.

Stereoisomers

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

Deletion sequences and truncated sequences

Deletion sequences are peptides with one or several amino acids missing either by incomplete coupling or deprotection reactions. Their formation is often prevented by repetition of the coupling procedure to complete the coupling reaction and systematic acetylation to cap unreacted coupling sites during SPPS. 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 the presence of free amino acid in the coupled protected amino acid, 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;
- isomerisation;
- deamidation;
- cyclic imide formation (aspartamide);
- diketopiperazine and pyroglutamic acid formation;
- β-elimination;
- condensation and formation of dehydropeptides;
- disulfide cleavage/exchange.
- acetylation

The potential degradation pathways of the peptide should be discussed taking into account the amino acids composition and sequence.

High molecular weight (HMW) impurities in synthetic peptides are undesired by-products with a molecular mass higher than that of the target peptide, typically formed during synthesis or storage due to side reactions or incomplete purification. They often consist of peptide aggregates, dimers, oligomers, or branched products that form through covalent or noncovalent linkages. They should be investigated using suitable analytical techniques.

Aggregation may occur for synthetic peptides and should be investigated. Aggregates are larger, often disordered assemblies of many peptide molecules, typically formed through uncontrolled or spontaneous self-association.

Analytical methods

Highly specific analytical methods with appropriate lower range limit used to detect 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.

Non-peptide impurities

Non-peptide impurities include process reagents, by-products, residual solvents, elemental impurities and nitrosamines. 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 purge 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 mutagenic), or – if not mutagenic - ICH Q3A/VICH GL10 or ICH Q3C/VICH GL18 thresholds, or (in absence of ICHQ3C/VICH GL18 thresholds), be toxicologically qualified. In addition, the basic principles of ICH M7 regarding mutagenic impurities assessment should be considered for non-peptide impurities.

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.

Specification tests should be included to ensure quality, safety and efficacy and may include the following (non-exhaustive list):

- appearance (+ appearance of solution if relevant);
- identification;
- purity (total impurities; individual impurities (unspecified/specified);
- high molecular weight impurities* (e.g., aggregates / oligomers) by SEC-LC or other techniques (if relevant);
- assay/content; e.g. by LC, elemental analysis, amino acid analysis, nitrogen analysis by Kjeldahl, or qNMR;
- counter-ion identity and content, e.g. acetic acid, if relevant**;
- residual ion content (TFA);
- water content;
- residual solvents;
- elemental impurities (e.g. in case of use of metal catalysts);
- bacterial endotoxins;
- microbiological purity.

*A specification parameter only denoted "high molecular weight impurities" is not acceptable; the impurities should be specified (e.g. dimers, trimers, oligomers, aggregates) to indicate that the identities of the impurities are known.

** 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. trifluoroacetate or chloride). The type of counter ion should be defined, and the amount of counter ions should be controlled in the active substance specification with a justified upper limit.

Other tests for process-related impurities should considered to be included in the specification, if relevant. An example is residual fluoride when HF is used during deprotection in the manufacturing process.

The acceptance criteria laid down in the active substance specification also apply for stability studies (while non-stability indicating parameters may be omitted from these 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%.

4.4.2. Analytical Procedures 3.2.S.4.2

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.

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, should take into account 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, 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.

Assay

A test for Assay (e.g., based on a chromatographic method usually the same as for purity) using a reference standard should be considered. Other tests like elemental analysis, amino acid analysis, nitrogen analysis by Kjeldahl, or qNMR may be used.

Impurities

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 impurities 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 impurities, additional independent method(s) may be needed. When co-eluting impurities are observed as one peak the qualification threshold of 1.0% applies unless otherwise justified. Stability indicating properties and mass balance of the method(s) need to be studied.

Control of diastereomers may require the development of specific methods.

Changes of the analytical methods during development

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

No additional requirements than for other chemical active substances.

4.4.4. Batch Analyses 3.2.S.4.4

The improvement in the analytical methods during development of the peptide could lead to newly observed 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 active substance and or clinical/preclinical data should be discussed by the applicant.

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 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 active 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. Limits of assays determined by LC are expressed in terms of the counter-ion free, anhydrous substance, unless otherwise justified.

Generally, a biological assay is not required for the release and stability testing of synthetic peptides. In certain cases, considering the mechanism of action and characterisation studies (as described in section 4.3.1.) performed during development, a biological assay may be considered necessary for routine control.

4.5. Reference Standards or Materials 3.2.S.5

The origin of the reference standards should be briefly indicated (i.e. whether they have been synthesised according to the commercial process or another 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.

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. Where appropriate, the moisture content should be confirmed prior to use. Alternatively, a dissolved reference substance may be used.

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. For hygroscopic powders, appropriate desiccant or storage under inert atmosphere could be considered if relevant.

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 minimise degradation, peptides are stored under refrigerated (5°C ± 3°C) or frozen conditions (-20°C ± 5°C), but the use of higher temperatures/humidities are also expected to address short term excursions.

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.

Formation of high molecular weight impurities may also occur for synthetic peptides and should therefore be investigated during stability, when applicable to the active substance.

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

No additional requirements than for other chemical active substances.

4.8. Conjugation

Conjugation has emerged as a popular mechanism with the aim 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, this can lead to 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 level of information on characterisation studies depends on the complexity of the conjugation moiety and may be different for e.g. a polydisperse polymer compared to a fatty acid used for conjugation.

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. Process-related impurities from the conjugation process should be considered as part of the control strategy of the drug substance.

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 as well as non-site specific PEGylation products (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 may thus be 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. When adequately justified a risk-based approach may be used for stability testing e.g. reducing the number of batches or testing intervals etc.

Conjugation-specific aspects regarding the SmPC 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 peptide-related impurities as defined in the general monograph of the Ph. Eur. 'Substances for Pharmaceutical Use' also apply 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) higher order structures, e.g. based on NMR and FTIR, as well as computation investigations. Additionally, experiments on higher order 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.

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 that the use of a terminal sterilisation process under the least stressful conditions (achieving the required SAL $\leq 10^{-6}$) causes significant degradation. In case of 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 finished products in development show degradation towards heat stress, the feasibility of terminal sterilisation should be taken into account from early-development onwards. In this way, 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. Potential issues that may occur during formulation development (e.g. pH

and buffering range) and further upscaling towards the commercial-scale terminal sterilisation process should be taken into account. 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.

Furthermore, where relevant, formulation development should address the formation of high molecular weight impurities. Aggregation propensity and the nature of the aggregates formed, especially under stress conditions including terminal sterilisation should be discussed. Where relevant the associated immunogenicity risk should be addressed.

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 resulting in high molecular weight impurities, these should be characterised and 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 finished 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.

In line with the principles of the guideline on SmPC, the strength of the finished product should be defined with respect to the mass of peptide base (not including salt or counter-ion).

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.

In view of the natural variability and more complex manufacturing of biological medicines which do not allow an exact replication of the molecular micro-heterogeneity, a specific regulatory pathway (biosimilar) was introduced.

Most biological medicines in current clinical use contain active substances made of proteins. Some smaller proteins (i.e. peptides) 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 manufactured by chemical synthesis.

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. The design of the comparability protocol should be developed in a product-specific manner considering the relevant quality attributes.

A broad panel of analytical methods (see section 4.3 Characterisation 3.2.S.3) to demonstrate the comparability between the recombinant reference product and the synthetic version is required for the comparability studies. The finished product should be tested on or near release and at the end of the proposed shelf life, with reference product being tested at different time points prior to expiry, after aging under conditions consistent with the labelled storage conditions. 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 suitable analytical techniques. Higher-order structures should be addressed by CD analysis, Fourier transform infrared (FTIR) spectroscopy, 2D NMR spectrometry, fluorescence spectroscopy and DSC or other suitable analytical techniques, if justified and appropriate. For certain peptides the oligomeric state should be investigated (please refer to the table with examples of characterisation techniques in section 4.3.1).

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 if relevant. 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 do not raise concerns regarding immunogenicity, and are qualified if above 1.0%.

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. In case a novel type of impurity occurs, this novel impurity should be reduced as far as possible.

In-silico prediction of immunogenicity, e.g. based on predicted binding to T-cell receptors (TCR), or in-vitro tests of T-cell activation may provide relevant information on immunogenicity potential of peptides impurities.

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 peptide-related impurity profile at levels 0.1-0.5% is expected and the levels of each peptide-related impurity should be compared to their corresponding levels in reference product. A quantification limit (QL) of 0.1% for LC purity testing is required.

Comparative forced degradation studies are also recommended including shear/mechanical stress, and the suitability of the analytical purity methods to fully characterise the impurity profiles of both products should be demonstrated e.g. for one exemplary batch.

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 mutagenic 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 comparability (e.g. binding kinetics).

The absence of a biological assay in the release specifications for active substance and finished 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 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).

Stability and shelf-life claims cannot be derived from the reference product without their own data.

In case of observed differences following the quality comparability tests outlined above, the observed differences should be justified with regard to their potential impact on safety and efficacy. Additional tests and studies (including non-clinical studies and/or clinical trials) may be required to address residual uncertainty.

7. Requirements for Investigational Medicinal Products (human products only)

The following section covers only those quality aspects that are specific to Investigational Medicinal Products having synthetic peptides as active substance. It is intended to be complementary to the EMA IMPD guideline (see reference list in chapter 3), i.e. for all other quality-related issues not specifically addressed hereafter, guidance laid down in IMPD guideline should be followed. In line with the aforementioned IMPD guideline, it is reiterated that, when compiling the quality part of the IMPD for phase II and phase III clinical studies, the larger and longer exposure of patients to the product have to be taken into account compared to phase I clinical studies. Also, based on the diversity of products to be used in the different phases of clinical trials (differences such as duration of treatment, maximum daily dose, severity of clinical indication) the requirements defined in this guideline can only be of an illustrative nature and cannot be expected to present an exhaustive list. The main focus should be on the safety of the IMP containing the synthetic peptide, especially in the early stages of development.

It is acknowledged that most peptides will be manufactured by solid phase supported synthesis; 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 or other building blocks in order to allow understanding and control of the impurity profile of the final peptide. Setting of limits for certain impurities based on criticality assessment is only expected for later development (phase II/III).

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 peptide should be fully characterised in terms of primary structure. Particular attention should be paid to its potential for the formation of high molecular weight impurities (e.g. aggregates) in order to avoid problems during formulation of the finished product. In justified cases, a risk assessment regarding tendency towards the formation of high molecular weight impurities based on prior knowledge may be acceptable for phase I studies. For phase II/III studies, this should be substantiated by experimental data. The propensity toward epimerisation should also be investigated. A similar approach as for the formation of high molecular weight impurities may be followed for epimerisation.

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. These impurities should also be qualified in preclinical studies. Orthogonal/complementary analytical procedures/detection techniques (e.g. mass spectroscopy) 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.