EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

2 May 2024

Submission of comments on 'Guideline on the Development and Manufacture of Synthetic Peptides '

Comments from:

Name of organisation or individual
EFPIA

Please note that these comments and the identity of the sender will be published unless a specific justified objection is received.

When completed, this form should be sent to the European Medicines Agency electronically, in Word format (not PDF).

Official address Domenico Scarlattilaan 6 ● 1083 HS Amsterdam ● The Netherlands
Address for visits and deliveries Refer to www.ema.europa.eu/how-to-find-us
Send us a question Go to www.ema.europa.eu/contact **Telephone** +31 (0)88 781 6000

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1. General comments

Stakeholder number *(To be completed by the Agency)*	General comment (if any)	Outcome (if applicable) *(To be completed by the Agency)*
	immunogenicity is mentioned >10 times in the document, and assessment of this aspect is requested in multiple situations, but the std method for predictions (in silico and in vitro) are described as not 'useful' (ln775 to 778). If no 'useful' methods for assessments are available why is this requested? Could you please provide some guidance on how to assess.	
	Please clarify the scope of the guideline for example would Heptapeptide such as Vedotin used in ADC as a payload be within the scope and considered as peptides intermediate.	
	The guideline states that the 1% qualification from the Pharm Eur guidance is applied in the clinical phase. Current industry practice is to justify higher limits during clinical studies based on duration and exposure.	
	Biological activity is referenced throughout as being expected (S.1.3/S.3.1), not required (S.4.4, line 348) or justifiably removed (S.4.5). Is this appropriate starting point for a synthetic molecule?	
	Aggregation/oligomers, quaternary structure and oligomeric state are discussed interchangeably throughout and could be made clearer. Wording of 'if relevant' for testing could be more specific in these instances also.	
	Clarify if all sections of this Guideline apply only to Marketing Authorization applications, with the exception of section 7, which applies only to the clinical study phases. This should clearly be stated in the document.	
	For radiopharmaceuticals. the Ph. Eur. Monograph “Chemical Precursors for Radiopharmaceutical Preparations” (EP2902) needs to be referred to/ reflected for peptides as chemical precursors used in the manufacturing of radiopharmaceuticals (kits and ready-to-use products). Clear distinctions to be made between API and chemical precursor for radiopharmaceutical manufacturing are expected to come in this upcoming guideline on peptides, considering the specificities of manufacturing of radiopharmaceuticals.	

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	Consequently, the EP2902 defines and fully covers the quality characteristics for the peptides used as chemical precursor in the manufacturing of radiopharmaceuticals.	
	The guidance is very specific, listing, for example, the use of specific techniques. It is suggested to add the following sentence: "others approaches can be considered if fully justified".	
	Many topics are discussed under medical considerations (point 5) and no longer following the CTD structure. It is suggested to follow CTD format throughout.	
	To prevent inclusion of GMP information in the marketing authorisation dossier, peptide specific GMP guidance could be addressed in a separate section of this guideline or by referencing to appropriate GMP guidelines. To avoid repeated guidance, reference to ICH Q7A can be made for general GMP practice, common with small molecules. The reference to the appropriate GMP principles in the legal section of the guideline and in text is either Part II of the EU GMP guide or ICH Q7A.	
	Consider alignment with the principles of Technical Guide for the elaboration of monographs on synthetic peptides and rDNA proteins - section 6 where EDQM describes synthetic peptides as being small, typically below 5,000 Da with chemical structures that do not occur naturally in proteins or peptides.	
	It is noted that the legend of the draft guidance can be completed with following relevant guidance: ICHQ12 - ICHQ14 **-** EMA/CHMP/QWP/545525/2017/R2 - ICHQ7 – Technical Guide for the elaboration of monographs on synthetic peptides and rDNA proteins – Requirements to the chemical and pharmaceutical quality documentation concerning investigational medicinal products in clinical trials EMA/CHMP/QWP/545525/2017 Rev 1 - Ph. Eur. Monograph 'Substances for pharmaceutical use - Manufacture of the finished dosage form (human) - Ph. Eur. Monograph for AA analysis (Ph Eur 2.2.64) and Peptide mapping (Ph Eur 2.2.55)- EMA/CHMP/QWP/245074/2015 - Guideline on Active Substance Master File procedure CHMP/QWP/227/02 Rev 4/ Corr., 108 EMEA/CVMP/134/02 Rev 4/ Corr. 109 • Guideline on the Summary	

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	of Requirements for the Active substance in the Quality Part of the Dossier CHMP/QWP/297/97 Rev 1 corr., EMEA/CVMP/1069/02 - ICH guideline Q8 (R2) on pharmaceutical development CHMP/ICH/167068/04.	
	Additional clarity should be provided for the use of recombinant technologies to manufacture peptides that would traditionally fall into the general category of 'synthetic peptides' (as opposed to biological products, which are explicitly called out in the introduction).	
	It is proposed to connect solid phase peptide synthesis more meaningfully as a platform technology with clearer and more succinct connection to leveraging prior knowledge. While this is addressed in 3.2.S.2.6, it remains very high level.	
	The scope should specify whether these guidelines are for early and/or late phase programs, especially in terms of phase-appropriate characterisation of the drug substance where the current wording is too prescriptive. If certain sections are meant for early or late stage, they should be explicitly called out	

2. Specific comments on text

Line number(s) of the relevant text *(e.g. Lines 20-23)*	Stakeholder number *(To be completed by the Agency)*	Comment and rationale; proposed changes *(If changes to the wording are suggested, they should be highlighted using 'track changes')*	Outcome *(To be completed by the Agency)*
Line 12 (Keywords)		**Comment:** Original text:“Solid phase synthesis,” “liquid phase synthesis” **Proposed change (if any):** We recommend revising these keywords to state “peptide synthesis” instead of “synthesis.” “Solid phase peptide synthesis,” “liquid phase peptide synthesis”	
64, 67		**Comment:** It is not entirely clear if both products in development and marketed products are in scope for the guideline. Recommend clarifying the exact scope.	
68		**Comment:** : Clarify that guideline is not to be applied retrospectively for approved dossier contents, but for post approval changes and new MAAs only	
72-73		**Comment:** The text in lines 72-73 introduces some unclarity as to when ICH guidelines should be followed. E.g. ICH Q6 B does not apply to any synthetic peptides, even when tetrapeptides or smaller. Recommend addressing this unclarity.	
74-79		**Comment:** Liquid phase approaches, enzymatic approaches neither in nor out of scope. Clarification would be beneficial especially because LPPS is mentioned in some sections of the guideline	
80-84		**Comment:** To be clarified what are the sections of this guideline applicable to radiopharmaceuticals (harmonize the radiopharmaceuticals products type as per the definitions stated in ENVI-PR-753470). **Proposed change (if any):**	
89		**Comment:** Add reference to ICH Q7 **Proposed change (if any):** ICH Q7 Good manufacturing practice for active pharmaceutical ingredients	

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122/123/128 and 151		**Comment: i.e** 3-letter amino acid codes for the natural amino acids" **Proposed change (if any):** Single letter code can be used as well in peptide that is entirely composed of proteinogenic or native amino acids.	
Line 121		**Comment:** Include clarification on requirements for nomenclature as for cyclic peptides	
137		**Comment:** In relatively small peptides, biological activity may not be a relevant property to discuss in this section. Also, If activity is based on the primary structure of the peptide only (i.e. evidence that no secondary structure is present), a biological characterisation does not provide additional benefit. **Proposed change (if any):** Suggestion that biological activity is changed from "in most cases" to "where appropriate", consistent with the discussion under 3.2.S.3.1. what extend of data is expected? Which non-clinical/in vitro data should be referenced?	
136-138, 348-349, 557		There appear to be contradictory statements about the need for an assay for biological activity. In 4.1.3. General Properties 3.2.S.1.3, it is stated that "... biological activity... would be expected." In contrast, in line 348-349, the following is stated: "Usually, no biological assay is required for the routine release of synthetic peptides,". Furthermore, in line 557 it is stated that "The absence of a biological assay should be justified". It is recommended to resolve these seemingly contradictory statements.	
136,138 and 462		3.2.S.1.3: General properties: Use the established terminology from existing guidelines such as EMA/454576/2016 and remove quality attributes such as water content, pH of the solution, biological activity from section 3.2.S.3.2.. These tests are listed in 3.2.S.4.1.	
137		**Comment:** Optical rotation testing should be optional as other chiral amino acid analysis can be performed. **Proposed change (if any):** Optical rotation (optional)	
158-159		**Comment:** "Proven acceptable ranges" are only relevant in late phase development. **Proposed change (if any):**	

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163		**Comment:** Sentence is hard to follow. **Proposed change (if any):** Propose change to "…which should be included in the discussion in 3.2.S.3.2".	
168		**Comment:** Material traceability is a GMP consideration and is always expected **Proposed change (if any):** This line should be removed (leaving the information on the definition of the batch size). Additionally, the information on criteria for batch splitting/pooling would be better captured under 3.2.S.2.6 rather than 3.2.S.2.2.	
171-173		**Comment:** The manufacture of peptide fragments can occur using either SPPS or LPPS processes and should not be listed as SPPS only. **Proposed change (if any):** "	
175		**Comment:** A statement that fragment condensation and liquid phase synthesis should follow the considerations of the Guideline on the Chemistry of Active Substances would aid clarity. The discussion in this section otherwise refers to SPPS. **Proposed change (if any):**	
179		**Comment:** Per ICH Q5E, comparability should be evaluated at the point most likely to detect the change. Use of alternate process could be in early part of the synthesis with no impact to downstream operations. In such cases, full DS/DP data for comparability would not be necessary to demonstrate comparability: **Proposed change:** "...comparability studies on drug substance ~~and drug product~~ level <u>as appropriate</u>, should be provided" or "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 <u>in some cases</u> drug product level should be provided."	

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185-186		**Comment:** Side fractions may be combined with the main fraction even if they do not comply with the specifications of the main fraction. The combined fractions need to comply with the set specifications. If every side-fraction has to comply with the specification of the main fraction this has an impact on the yield and ultimately on sustainability of the process.	
185		Quality requirements of purification side fractions is discussed in more detail later and is unclear in this section. Recommend removing quality requirement as key message is that repeat purification is not considered re-processing.	
Line 196		**Comment:** Propose to rename this section “Drug Substance Isolation” to consider other API isolation techniques beside lyophilization, e.g. precipitation, spray drying **Proposed change (if any):**	
Lines 200-256		**Comment:** It is not clear, why the choice of API SM should not follow the rules of ICH Q11, i.e. control of identity and chiral purity by characterization with adequate analytical methods and a certain number of bond formation/bond breaking steps. Many peptides indeed undergo few modifications after cleavage from solid support, where indeed AA building blocks seem to be adequate API SMs. Though there are also numerous cases where the intermediates undergo several purifications and liquid chemistry steps between cleavage from solid support and final API. In such cases it will a much more constructive to define such an intermediate as API SM, if criteria above can be fulfilled. In case of cyclic peptides, it is the only way to confirm the AA sequence on a linear intermediate. **Proposed change (if any):**	

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Lines 204-207		**Comment:** Original text: "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, […]." We believe this should be explicitly waived for protected natural amino acids. **Proposed change (if any):** "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, […]. <u>This does not apply for natural amino acids.</u>"	
211		This is not aligned with ICH Q11 Q&A, #5.6 which states: "An applicant generally need not justify the use of a commercially available chemical as a starting material, whereas a custom synthesised chemical proposed as a starting material should be justified in accordance with the ICH Q11 general principles." and "In some cases, a chemical that does not meet the definition of a commercially available chemical (e.g., it does not have a non-pharmaceutical use) but is simple enough in structure may be accepted as a starting material (e.g., protected natural amino acids)." Recommend aligning with ICH Q11 Q&A.	
215-221		**Comment:** Recommend including the well characterized protected tri and tetrapeptide building blocks in the description of the short peptide segments **Proposed change (if any):** In justified cases, short peptide segments such as protected di, tri and tetrapeptide building blocks, may be acceptable as starting materials	

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215-217		In accordance with ICH Q11 Q&A's "An applicant generally need not justify the use of a commercially available chemical as a starting material, whereas a custom synthesised chemical proposed as a starting material should be justified in accordance with the ICH Q11 general principles.". It is suggested to add text to the guideline to reflect that commercially available chemicals are generally acceptable as starting materials without further justification.	
219-224		**Comment:** Consider rewording that polypeptide segments that undergo further modifications (e.g. cyclization) are generally not acceptable as starting materials. The sentence which follows that more complex peptides could be acceptable as starting materials (e.g. in fragmentation cases) could contradict the previous statement, especially since cyclization is typically done in solution. Furthermore, it should be clarified what is meant by “conjugation”. **Proposed change (if any):** “Precursor materials for polypeptides and longer peptide sequences could be considered as a starting material with appropriate justification.” Alternatively, cases where more complex peptides could be acceptable should be expanded for clarity.	
242 - 243		**Comment:** Please specify the supportive data for justification of pre-loaded resins as starting materials.	
253-255		Experience has shown that EU regulators apply different interpretations of Q11 with regards to PEG and lipid derivatives. Recommend including in the guideline a statement that coupling pre-cursor of a PEG or lipid conjugate is an acceptable API SM designation.	
255-256		**Comment:** Focus only on number of chemical transformation not meaningful. **Proposed change:** The applicant should include the chemical transformation which impact the impurity profile of the drug substance.	

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268		**Comment:** The Kaiser test mentioned as most common test is not necessarily required for the final control strategy of the SPPS process but used during development to establish respective targets for critical process parameter. As such coupling, capping and deprotection can be monitored via those process parameter (i.e. prior knowledge approach).	
265-270		**Comment:** Original text: "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." We believe that only critical IPCs need to be defined in this section if they are part of the solid phase peptide synthesis (SPPS) section or the purification. **Proposed change (if any):** We recommend simplifying the discussion to highlight this point.	
271-271		**Comment**: Narrow scope - Kaiser test **Proposed change**: Include other tests and reference to PAT techniques like refractive index measurement.	
273-275		**Comment:** Narrow scope. **Proposed change**: Extend to include continuous chromatography approaches with automated side fraction recycling.	
279		Suggest replacing "filtration and lyophilisation" with "filtration and drying." in order to reflect that other drying methods can also be relevant.	

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Lines 273-279		**Comment:** Original text: "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." Only intermediates complying with specifications may be pooled. Intermediates purity should be defined independently of pooling, only the combined pooled fractions should have to pass specifications. This can be assessed using a test-pool. **Proposed change (if any):** Intermediates purity should be defined independently of pooling, only the combined pooled fractions should have to pass specifications. This can be assessed using a test-pool.	
275 -276		**Comment:** Criteria for individual impurities are not always necessary in early-phase development. More important in such cases is understanding of criticality of specific impurities. **Proposed change (if any):**	
285		**Comment:** Explicitly requiring specifications for all intermediates is not in line with current practice for chemical drug substances and will likely lead to confusion over the definition of intermediates. **Proposed change (if any):** Suggestion "In general, justified specifications should be presented for intermediates".	

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287-288		**Comment:** There are concerns with this portion of the text, because the variability of crude overall purity as well as the variability of the content in each of the many impurities in the crude are usually too large to derive meaningful specification for the crude peptide. In addition, requesting specification also suggests that the crude peptide should become a regulatory intermediate, with proper release prior to purification. Crude peptide after cleavage and deprotection may not always be an intermediate that is released.	
Lines 313-314		**Comment:** Peptide mapping, accurate mass, MS techniques utilized to characterize the structure of synthetic peptides, plus GMP controls are in place to ensure peptide structure. Lines 313-314 discuss Amino Acid Analysis (AAA) as complementary analysis but is not listed in the table below.	
Lines 316-322		**Comment:** NMR is missing from the Evidence of Chemical Structure table starting on Line 357 and should be added there for alignment with this section.	
330-334		**Comment**: narrow scope to chiral gas chromatography. **Proposed change**: allow alternatives chromatographic techniques.	
Lines 346-347		**Comment:** When NMR is utilized as outlined in 316-322, Far UV CD and FTIR should not be necessary as they are "lower resolution techniques" **Proposed change (if any):**	
Line 352		**Comment:** Other methods besides Thioflavin T assay test should be considered to investigate aggregation, e.g. fibrillary aggregates.	
308, 339, 646 and 717		Use for secondary, tertiary and quaternary structure, general and simple terminology such as <u>high-order</u> structure.	
348-349		**Comment:** See misalignment with verbiage for S.4.5 (line 557). Here it is stated that a bioassay is usually not required for routine release, while in the justification of specification section it is stated that absence of a bioassay needs to be justified.	

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350 – 351		**Comment:** A definition of «longer peptide» should be provided and testing flexibility considered. **Proposed change (if any):** Suggest replacing with "Peptide mapping may be applicable based on cleavage site(s) in the primary structure." Also, consider peptide sequencing via MS/MS as alternative of peptide mapping depending on molecule size and amino acid composition.	
348		**Comment:** biological activity is described as a tool for characterisation of synthetic peptides. **Proposed change (if any):** update wording to be clear that as a test in isolation it does not characterise structure but could be supportive of other techniques. Suggest removing from table.	
358		**Comment:** "tertiary structures or the association state (e.g. in the form of oligomers) may be relevant" – what makes them relevant: presence of quat structure, change in structure in batches or stability, change in activity as a function of structure? Is quaternary structure / association state determined by CG-MALS for anything more than simple associative complexes? Is this appropriate for DS? **Proposed change (if any):** change wording of 'quaternary structure / association state' or remove CG-MALS as exemplar technique.	
357-358		Biological characterisation may not be justified depending on peptide size, secondary/tertiary structure aspects and analytical procedure capabilities. Recommend removing or further clarifying within text that it is required for greater or equal to 40 amino acids to align with US FDA guidance.	
357/Example table		**Comment:** Inclusion of additional analytical techniques (e.g. enantiomeric purity after partial or complete enzymatic digestion).	
360		**Comment:** Discussion of biological activities of isomers required, which extend of data is expected? Which non-clinical/in vitro data should be referenced? Suggest restricting data generation to relevant isomers. Is this referencing isomers as an impurity or as an API with an undefined isomer ratio? What supporting data/risk assessment is required as part of a discussion on their relevant re biological/pharmacological activity?	

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368		**Comment:** It should be clarified that identification of impurities is performed at a later stage. **Proposed change (if any):**	
388		**Comment:** This line should specify that it especially applies to SPPS, considering that other manufacturing processes may utilise intermediate controls. Also, Use of the wording "narrow acceptance criteria" is too vague and would recommend use of "justified acceptance criteria". **Proposed change (if any): e.g**. "Appropriate (narrow in the case of SPPS)…".	
395, 399, 827 and several other lines in the draft document		It is proposed not to use the term "racemisation", because it describes a 1:1 mixture of two enantiomers, which is not the case for peptides with multiple stereogenic centers. Consider using "epimerisation" where relevant. Ref. Duengo S et al. Epimerisation in Peptide Synthesis. Molecules 2023, 28(24), 8017; https://doi.org/10.3390/molecules28248017	
Lines 417-430		**Comment:** It is suggested to complete the list of potential degradation pathways by including cyclic imide formation (aspartamide).	
432		**Comment:** Requirement of full peak resolution may not be feasible for every impurity despite exhaustive method development. Statement may be understood as contradictive to other guideline sections (e.g. line 547 ff.).	

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431-435		Per reference guidance for active substances, EMA/454576/2016, the structure of the impurities is provided in Section 3.2.S.3.2. The analytical method for routine analysis of impurities is detailed in Section 3.2.S.4.2 – Copies of relevant chromatograms should be provided only when relevant …	
451		Use of the word 'Typical specification tests' doesn't align with the concept of developing a holistic control strategy where CQAs are identified and justified, and the specification tests are then set to control these where needed. Consider changing to 'Specification tests should be included to ensure safety and efficacy and may include the follow (non-exhaustive list)….'	
451-467		Comment: Several of the specification tests listed here may not be valuable/necessary to demonstrate the quality of the API. Add if applicable to the proposed tests. Furthermore, this section should reflect that only stability-indicating parameters may be included in stability studies. "The acceptance criteria laid down in the drug substance specification are identical with the limits that apply for stability studies <u>(while non-stability indicating parameters may be omitted from these studies)."</u>	
463		**Comment:** A mass balance specification relies upon results from several other critical quality attributes and is therefore not value added when the contributing specifications for those CQAs are set appropriately. Mass balance is an attribute that should be assessed during method validation during well controlled experiments. Calculating mass balance on batch release and stability is of little value if each individual CQA is satisfied. Recommend deleting mass balance from list of typical specification tests.	
460		Recommend removing "TFA content" – it is dependent on the process and reagents used. Suggest replacing with "residual solvents / reagents / acids / bases (when relevant)".	

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455		Amino acid analysis (AAA) is part of characterisation testing and important during analytical development. Ph. Eur. general chapters, applicable to peptides (eg, 2.2.55 Peptide mapping, 2.2.56. Amino Acid Analysis, and the "EDQM Technical guide for the elaboration of monographs on synthetic peptides and recombinant DNA proteins") are helpful for the development of these analytical methods. Although AAA and Mass balance were commonly used as a routine identification test and assay/purity calculation previously, these tests have been gradually replaced by other techniques, such as HPLC and MS. Also, mass balance specification relies upon results from several other critical quality attributes and is therefore not value added when the contributing specifications for those CQAs are set appropriately. Calculating mass balance on batch release and stability is of little value if each individual CQA is satisfied. Recommend deleting mass balance from list of typical specification tests. Aligned with ICH Q14, reduced testing can be justified with appropriate scientific justification or alternative testing based on risk analysis.	
457/8		Replace the specific instrument type 'HPLC' with broader technique 'LC'.	
459 and 475-479		**Comment:** The counter ion identification should be adequate and the content requirements should be removed. Also, added language to specify a zwitterion may be appropriate. Recommend removing "acetic acid content" – it is dependent on the process and counter ion. Suggest replacing with: "counter-ion content". Where acetic acid is not a true counterion, but a process related impurity, the acceptance criteria should be based on batch data and allowable levels. No lower limit should be necessary	

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495		Aligned with ICH Q14, reduced sequence analysis testing during routine batch release can be justified based on risk analysis. The amino acid sequence of a drug substance can be confirmed for example by Tandem Mass Spectrometric (MS/MS) analysis of the reference standard during characterisation. The combination of MS and UHPLC for routine identification confirmation can be an appropriate control strategy for synthetic peptides manufactured by a GMP-controlled process.	
501 - 502		**Comment:** Depending on the size of the peptide, separation of all peptide related impurities with one method is not feasible. The requirement to develop additional methods for routine testing should be limited based on development data and respective safety considerations.	
505		**Comment:** The requirement to develop additional methods for routine testing of diastereomers should be limited based on development data and respective safety considerations. Control should be limited to relevant diastereomers.	
502/544		Line 544 Highlights to separate 'all peaks'. Line 544 Indicates grouping of peaks not recommended unless justified and based on demonstrated analytic effort. It is not feasible to expect that all peaks must be separated. And what is the threshold for effort required to justify not resolving these? **Proposed change (if any):**"Grouping of impurities (pre- and post-eluting groups) can be accepted when scientifically justified and may be informed by prior knowledge."	
513		**Comment:** Method validation during development should follow a stage-based approach. For early clinical phases, full validation of methods should not be performed. Qualification of method should be sufficient. Recommend revising accordingly.	
541-543		The variability in the potential impact on the efficacy and safety of the product should also be considered when setting the acceptance criteria based upon a limited number of clinical batches (Peng, D.; Bercu, J.; Subashi, A. K.; Yu. L. X. "Patient-Centric Specification: Regulatory & Pharma Industry Progress", ISPE, September/October 2019). Recommend deleting or revising the statement accordingly.	

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557		Suggest adding "If the mode of action is based on the primary structure and the content (quantity) of the peptide only, no potency assay is needed".	
575		The use of Prior Knowledge to establish a DS retest date should be added to the guidance. For the use of Prior Knowledge stability data, see: Hedegaard, S. F. et al "Leveraging Prior Knowledge to Support Early Phase Clinical Trial Applications: Regulatory CMC Considerations and Case Studies", Org. Process Res. Dev. 2023, 27, 784−787.	
552		**Comment:** In addition to the described calculation for specifications limits, results of batch analyses should also be taken into account to derive the assay specification.	
559 - 560		**Comment:** Moisture uptake is not necessarily critical for the analytical result, if the precise water content prior analysis is known. Alternatively, a dissolved reference substance may be used.	
585		**Comment:** Forced degradation studies are typically conducted during analytical method development to understand the stability indicating of the method and the degradation pathway. It is not part of the stability protocol and not sure if it should be discussed in the stability section. **Proposed change (if any):**	
590 - 591		**Comment:** It may be challenging to implement a test for aggregation control on drug substance level. Instead, aggregation can be controlled on drug product level for ready to use dosage forms e.g. solution for injection.	
599-602		This statement is not specific to synthetic peptides. Recommend considering replacing with a reference to existing guideline.	
603-644		**Comment:** It is not clear whether conjugation with a metal-free chelator to form the precursor for radio-ligand therapy is in scope of this chapter. The concerns raised above with regard to definition of API SM are brought forward in this case again. **Proposed change (if any):**	

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620-626		Experience has shown that EU regulators apply different interpretations of Q11 with regards to PEG and lipid derivatives. Recommend including in the guideline a statement that coupling pre-cursor of a PEG or lipid conjugate is an acceptable API SM designation (e.g., carboxylic acid derivatives).	
623		**Comment:** Several PEG-NHS esters are commercially available with good stability and quality control. Instead of outright restriction in its use as starting material, advised to consider in a case-by-case basis. **Proposed change (if any):**	
639-640		**Comment:** Depending on the number of manufacturers (e.g., 2 per moiety), and depending on the number of batches to be put on stability (up to 3?) this could result in a lot of stability programs. While it is acknowledged that quality needs to be understood and monitored at the stages where this is best possible based on analytical method capability, contemporaneous concepts should be allowed to complement stability studies, and where justified to reduce the number of batches and the duration of stability studies. Specifically, statistical predictive stability models should be allowed and recommended in the guideline. **Proposed change:** 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. Statistical predictive stability models can be used to complement stability studies, and where justified, to reduce the number of batches and the duration of stability studies.	
643-644		**Comment:** Use of abbreviation should be aligned (SmPC vs SPC), see also line 712	
639-640		This is an overly broad and conservative statement. A scientifically driven, risk-based approach toward determining comparability of new conjugate API SM suppliers is proposed.	
677-683		**Comment:** A clear definition of “significant“ and “moderate degradation”, together with some examples would be helpful.	

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675-676		Prior knowledge should also be an acceptable justification. Recommend: "Terminal sterilisation provides the highest sterility assurance level; thus, this should be the method of choice unless demonstrated unsuitable or with Prior Knowledge justification."	
688-692		**Comment:** There is little clarity among finished product developers what type of measured should be taken and what levels of degradation can be accepted. While specific numbers on e.g., assay losses may be too prescriptive, it may be helpful to provide guidance on other aspects, like e.g., whether overages should be added during manufacture to enable a certain active substance content in the finished product. **Proposed change:** 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. While it is reasonable to modify pH conditions and buffer concentrations, it is typically not expected that overages be added during manufacture to compensate for assay losses during terminal sterilization.	
687-689		**Comment:** There are important concerns related to the requirement to use heat sterilization unless demonstrated unsuitable, as it significantly complexifies the toxicological and clinical development. The final process is fixed for the phase 3 clinical study. In case a terminal sterilization is implemented at this stage and results in an increase of degradation product(s), it may invalidate the toxicological studies and delay the phase 3 study. In addition, toxicological studies are usually not performed with the human drug product presentation (e.g., pre-filled syringe). The degradation pattern can be highly linked to the drug product presentation and to the DP sterilization process (as well as batch size).	
685		Late clinical and commercial formulation studies are not commonly carried out in 'early development' hence feasibility for terminal sterilisation is unlikely to be addressed at this point in development.	
671-676		**Comment:** Could aseptic techniques in lieu of terminal sterilization manufacturing facility be used throughout the manufacturing and the DS is tested against sterility?	

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662		**Comment:** Inclusion of bioassay in the section feels absolute? What would a package of work look like to show no potency assay is required? **Proposed change (if any):** add text to clarify that confidence in the activity of the sequence and its relation structure would mean no potency testing required.	
736-737		**Comment:** Guidance should be provided which regulatory route / legal basis could be followed for synthetic peptides using an EU reference product (synthetic or biological) (e.g., hybrid application under Article 10(3) of Directive 2001/83/EC).	
775-778		**Comment:** There may be a place for non-clinical immunogenicity assessment in vitro. Reference to HIT on Line 777 is out of place. HIT is a very particular situation, the immunogenic mechanisms of which are still matter of debate and cannot be considered an ADA response in the same way as the response to a peptide, for instance, because the antibodies recognize the structurally rearranged PF4 upon binding to heparin, so it's actually closer to autoimmunity than ADA. Recommend deleting the HIT example reference for correctness. **Proposed change (if any):** 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.	
769-771		This is not agreed. New impurities not present in the reference product need to be justified.	
806 [Suggested additional paragraph at the end of section 6]		The following new paragraph is proposed: “Depending on the residual uncertainty following the quality comparability tests outlined above, clinical trials should be considered to establish the same efficacy, safety and tolerability profile compared to the reference product.”	
807-808		Recommend adding reference to “Guideline on the requirements to the chemical and pharmaceutical quality documentation concerning investigational medicinal products in clinical trials”.	
817		**Comment:** Clarification should be included that this is when amino acids are SMs (which is not always the case) **Proposed change (if any):** "…monitored (e.g. in the amino acid building blocks where applicable)…"	

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816-819		**Comment:** Setting of limits for certain impurities may be expected for later development. *Examples of impurities where limits could be set at a later stage of development could be water content, content of counter-ion, etc.* **Proposed change:** From experience there is different understanding between regulators and industry on what CQAs need limits at given stages of development. An example of acceptable approaches could be helpful.	
822-824		This is already addressed in "Guideline on the requirements to the chemical and pharmaceutical quality documentation concerning investigational medicinal products in clinical trials". Recommend deleting or revising to "Significant changes in the manufacturing process, which may impact on quality, should be discussed; particular attention should be paid to differences in impurity profile compared to preclinical batches used for qualification of impurities."	
827-828		**Comment:** It should be clarified that this information is not required for inclusion in the IMPD until later development.	
829-834		**Comment:** Ph Eur 2034 is applicable at the time of registration. Applying these requirements to development phases is not appropriate. There is not a strong reason to expect that a peptide related impurity will be more potent/toxic than the active peptide substance. A large database of toxicology data supports the safety of normal impurities (e.g. not GTIs) up to 1 mg/day. Applying modified Haber's Law to provide conservative adjustment for less-than-lifetime exposure due to intermittent dosing could be considered appropriate and is much more conservative than risk-based assessment based on dietary intake of peptides. In addition, molecular weight adjustments could be considered to assess the risk. Recommend qualifying Line 829-830 specifying it applies at registration. **Proposed change (if any):**	

Please add more rows if needed.