EUROPEAN MEDICINES AGENCY
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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
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An agency of the European Union

23 April 2026
EMA/66185/2026

Overview of comments received on draft guideline on the development and manufacture of Synthetic Peptides

(EMA/CHMP/CVMP/QWP/387541/2023)

Name of organisation or individual	General or Specific comment	Line no. from	Line no. to	Comment and rationale	Proposed changes / recommendation	Outcome	Outcome (comment)
EFPIA	General comment	0	0	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.		Comment noted	It is considered that the discussion on immunogenicity should not be discussed in a quality guideline. Further discussions may be covered by future Agency work.
EFPIA	General comment	0	0	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.		Comment noted	General principles as outlined in the guideline are applicable to peptide intermediates.
EFPIA	General comment	0	0	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.		Comment noted	Applicability of the guideline has been clarified in the scope.
EFPIA	General comment	0	0	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?		Accepted	Relevant amendments have been made to align the requirements among sections.
EFPIA	General comment	0	0	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.		Accepted	Relevant amendments have been made throughout the text for improved clarity.
EFPIA	General comment	0	0	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.		Accepted	This has been clarified in the scope section of the guideline.
EFPIA	General comment	0	0	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).		Not accepted	For radiopharmaceuticals, reference to the relevant Guideline (i.e., EMEA/CHMP/QWP/306970/2007) on Radiopharmaceuticals is made. For synthetic peptides used in radiopharmaceuticals or precursors, the synthetic peptides guideline applies only regarding synthesis and starting materials.
EFPIA	General comment	0	0	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. Consequently, the EP2902 defines and fully covers the quality characteristics for the peptides used as chemical precursor in the manufacturing of radiopharmaceuticals.		Comment noted	For radiopharmaceuticals, reference to the relevant Guideline (i.e., EMEA/CHMP/QWP/306970/2007) on Radiopharmaceuticals is made. For synthetic peptides used in radiopharmaceuticals or precursors, the synthetic peptides guideline applies only regarding synthesis and starting materials.
EFPIA	General comment	0	0	The guidance is very specific, listing, for example, the use of specific techniques.		Comment noted	Specific techniques are provided in most cases as examples and are not meant to be prescriptive.
EFPIA	General comment	0	0	It is suggested to add the following sentence: "others approaches can be considered if fully justified".		Comment noted	A specific text line has not be indicated by the commenter.
EFPIA	General comment	0	0	Many topics are discussed under medical considerations (point 5) and no longer following the CTD structure. It is suggested to follow CTD format throughout.		Comment noted	

EFPIA	General comment	0	0	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.		Accepted	Reference to GMP guidance has been included in section 3.
EFPIA	General comment	0	0	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.		Comment noted	Synthetic in this context is to be understood as made by chemical synthesis rather than not naturally occurring.
EFPIA	General comment	0	0	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 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.		Accepted	Section 3 has been amended accordingly.
EFPIA	General comment	0	0	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).		Not accepted	Peptides manufactured by recombinant technologies are not in the scope of the guideline
EFPIA	General comment	0	0	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.		Not accepted	No further amendments are deemed necessary.
EFPIA	General comment	0	0	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		Accepted	Please refer to the amended scope of the guideline.
Medicines for Europe	General comment	0	0	Medicines for Europe welcomes the publication of the Draft Guideline on the Development and Manufacture of Synthetic Peptides. Peptides represent a link between products derived from biotechnology and small molecular chemical compounds - a specific circumstance which poses several analytical and regulatory considerations. Having in mind the diverse nature of these molecules, we have provided detailed commentary on various aspects of synthetic peptide development and manufacturing, some of which being: peptide size and structure, characterization techniques, impurity qualification, conjugates, overall quality control considerations, and regulatory alignment. We look forward to receiving further information on the next steps in this process of guideline development.		Comment noted	N/A
Viatris	General comment	0	0	Viatris appreciates the European Medicines Agency's (EMA's) development of guidance on the type of information required for the development, manufacture, and control of synthetic peptides used in medicinal products. The draft guideline supplements the limited guidance previously available for synthetic peptides, which gives manufacturers more certainty on agency expectations.		Comment noted	N/A
BCN Peptides	General comment	0	0	In the proposed guideline there is no differentiation on the requirements needed for existing drug substances and for new drug substances. This differentiation is needed in some of the sections. Also there are some topics that, in our opinion are out of the API manufacturing scope and, therefore they should be removed from this guideline. Such topics are those that are related to drug product development, and depends on the conditions used for this development.		Not accepted	No amendment is deemed necessary.
PolyPeptide Group	General comment	0	0	The guideline on synthetic peptides is very welcome. It is well written, informative and address many important subjects for synthetic peptides. PolyPeptide's comments are intended to make the guideline as clear and crisp as possible.		Comment noted	N/A

EUCOPE	General comment	0	0	We commend the Agency for issuing the draft guideline, and for seeking input from developers; we appreciate the 6-month consultation window. The draft represents a comprehensive paper with welcome guidance on a number of aspects regarding the development of synthetic peptides such as impurity limits and Regulatory Starting Materials (RSMs) management. In fact, reading through the paper a question related to the depth of details requested for RSMs arises. The level of details requested makes the task of meeting these requirements more onerous by the (typically) large amount of RSMs. Our suggestion is to soften language to say that the tests noted in the guideline could be used as 'some' of the attributes which could be probed, but the list of tests should be ultimately decided on a case-bycase basis. Doing all the tests on all the RSMs would be onerous and not add quality value. As large synthetic molecules such as peptides and oligonucleotides are fully or partially excluded from the scope of ICH Q3B (Impurities in New Drug Products) and ICH Q11 (Development and Manufacture of Drug Substances) and given that the EMA has proposed draft guidances for both peptides and oligonucleotides, it is important that the proposed guidelines explicitly define the scope of where conjugated molecules containing both moieties would reside. Examples of areas within draft guidance where additional clarity for conjugated products could be provided are listed below.		Comment noted	N/A
Bachem AG	General comment	0	0	"Active substance" and "drug substance" are used throughout the guideline. This should be harmonized according to European nomenclature.		Accepted	Relevant amendments have been made
EDQM	General comment	0	0	The current guideline draft envisages it to be applicable to pentapeptides and above, while tetrapeptides and below are to be considered as small molecules. This is not in line with the requirements of general monograph Substances for pharmaceutical use (2034) which states requirements for reporting, identification and qualification thresholds of impurities in all peptides, regardless of their size, obtained by chemical synthesis. These limits constitute the basis for limits included in individual Ph. Eur. monographs for synthetic peptides.		Accepted	Relevant amendments have been made
Granzer Regulatory Consulting & Services GmbH	General comment	0	0	In-line with the scope of the guidance, this document is predominantly focussed on MAA submissions, where only limited guidance has been provided for clinical development. Therefore, it would be useful if the guidance document was expanded to encompass and amalgamate (section-by-section) the IMPD guidance to help better understand the Agency's expectation during Phase 1, 2 and 3.		Comment noted	Section 7 of the guideline is intended to complement existing guidance, i.e. Guideline on the requirements to the chemical and pharmaceutical quality documentation concerning investigational medicinal products in clinical trials (EMA/CHMP/QWP/545525/2017)
Granzer Regulatory Consulting & Services GmbH	General comment	0	0	It would be useful for the Drug Product section to be organised as per Drug Substance section, i.e., section-by-section.		Comment noted.	N/A
Granzer Regulatory Consulting & Services GmbH	General comment	0	0	It would be useful for the guidance to include a glossary of terms – It is noteworthy that the USP has a guidance document '<1503 Quality Attributes of Synthetic Peptide Drug Substances>' which includes definitions of such terms. Ideally, alignment and/or harmonisation with the latter document would be very useful for both industry and the regulatory authorities.		Comment noted.	Consistency with the mentioned USP guidance document has been verified. No need for further amendment has been identified.
Granzer Regulatory Consulting & Services GmbH	General comment	0	0	The term 'No additional requirements' is mentioned in numerous sections. This should be replaced with a suitable reference.		Accepted	Relevant amendments have been made
NMEU-Nuclear Medicine Europe	General comment	0	0	In alignment with the Concept Paper on the Establishment of a Guideline on the Development and Manufacture of Synthetic Peptides commented by NMEU In Dec 2022, NMEU considers that there is a specific CMC guideline for radiopharmaceuticals that covers their quality requirements. Synthetic peptides may be used in the manufacture of Radiopharmaceuticals (kits and ready-to-use products) and are considered as chemical precursors. Therefore, NMEU has proposed using monograph EP2902 as a basis for defining the specifications for synthetic peptides used in radiopharmaceuticals. As such, when referring to the specifications, it should be exempted from this proposed EMA guideline with a cross-reference to the EP2902., (cfr section 2 scope (lines 82-83)) Monograph EP2902 is appropriate to refer to because radiopharmaceuticals are microdose/ low dose products, that are usually not part of 'conventional' treatment regimen administrated daily, and therefore their impurity profile is less relevant from a toxicity perspectives. Impurities may be carried over in situations where peptides are not fully labelled but the impurity levels are still within the thresholds even if there is no further purification. As a conclusion, NMEU, Ph. Eur. Monograph "Chemical Precursors for Radiopharmaceutical Preparations" (EP2902) needs to be referred to/ reflected in the for peptides as chemical precursors used in the manufacturing of radiopharmaceuticals ((kits and ready-to-use products).		Accepted	For radiopharmaceuticals, reference to the relevant Guideline (i.e., EMEA/CHMP/QWP/306970/2007) on Radiopharmaceuticals is made. For synthetic peptides used in radiopharmaceuticals or precursors, the synthetic peptides guideline applies only regarding synthesis and starting materials.
NMEU-Nuclear Medicine Europe	General comment	0	0	The EP2902 defines and fully covers the quality characteristics for the peptides used as chemical precursor or API in the manufacturing and preparation of radiopharmaceuticals.		Accepted	For radiopharmaceuticals, reference to the relevant Guideline (i.e., EMEA/CHMP/QWP/306970/2007) on Radiopharmaceuticals is made. For synthetic peptides used in radiopharmaceuticals or precursors, the synthetic peptides guideline applies only regarding synthesis and starting materials.

NMEU-Nuclear Medicine Europe	General comment	0	0	It should be clarified throughout the document which requirement is expected at which state of the development process. A statement like "….applies for registration phase and commercial phases, and differences may be applied during clinical development…." is advisable.		Accepted	Relevant amendements have been made
NMEU-Nuclear Medicine Europe	General comment	0	0	The understanding of NMEU is that all sections of this Guideline apply only to Marketing Authorization application, with the exception of section 7, which applies only to the clinical study phase. This should clearly stated in the document.		Accepted	Relevant amendements have been made
NMEU-Nuclear Medicine Europe	General comment	0	0	A clear definition of peptide (e.g. based on IUPAC nomenclature) and "non-peptidic" constituents should be included. (Same comment for the lines 121-134 and 253.), specifically regarding limits applicability of this guideline for peptides and Biologicals		Comment noted.	A definition is not considered necessary for this guideline, however it is agreed that IUPAC definition of peptide applies.
NMEU-Nuclear Medicine Europe	General comment	0	0	Information from suppliers is often not available as the suppliers are not willing to share sensitive information especially on the process, starting materials, starting materials supplier, critical process parameters and manufacturing process development, due to confidential intellectual property or 'know-how' of the manufacturer of the active substance (ASM) to be protected. Note: Supplier generally follow "Guideline on Active substance Master file Procedure" only share the applicant's part of the ASMF to the Applicant and submit the restricted part of the ASMF to Agency directly. The restricted part of the ASMF can be accessed via letter of access. (refer to Lines 148– 304:)		Comment noted	N/A
Aspen Oss B.V.	General comment	0	0	Emphasis is placed in the guideline on peptides made by solid phase synthesis. Liquid phase peptide synthesis should be added.		Comment noted.	Liquid phase peptide synthesis is in the scope of the guideline.
AstraZeneca	General comment	0	0	Immunogenicity is mentioned >10 times in the document, and assessment of this aspect is requested in multiple situations, but the standard method for predictions (in silico and in vitro) are described as not 'useful' (ln775 to 778). If no 'useful' methods for assessments are mentioned, why is this requested without guidance on how to assess?		Comment noted	It is considered that the discussion on immunogenicity should not be discussed in a quality guideline. Further discussions may be covered by future Agency work.
AstraZeneca	General comment	0	0	Please clarify the scope of the guidelines, would Heptapeptide such as Vedotin used in ADC as a payload be within the scope, and is it considered as a peptide intermediate?		Comment noted	General principles as outlined in the guideline are applicable to the peptide intermediate
AstraZeneca	General comment	0	0	The guideline states that the 1% qualification from the Pharm Eur guidance is applied in the clinical phase. Should this apply to early clinical studies? Industry practice allows justifying higher limits in early phase clinical trials.		Comment noted	Please refer to the scope for the applicability of the guideline
AstraZeneca	General comment	0	0	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). This seems as an excessive testing requirement that is not appropriate starting point for a synthetic molecule?		Accepted	Relevant amendments have been made
AstraZeneca	General comment	0	0	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.		Accepted	Relevant amendments have been made
Bachem AG	General comment	0	0	We wonder whether and how the scope of the future guidance document could include or comment on GMP requirements specifically for the CMC of synthetic peptides, as nothing is mentioned in this draft version. GMP requirements could be more clearly delineated in contrast to the documentation requirements for a registration dossier, since fulfilling GMP requirements is a prerequisite to any registration process and the GMP requirements are described in a separate set of legal texts, e.g. EU GMP guide. We would very much welcome additional explanations in the scope. Background: The guideline addresses several GMP requirements that are not part of a registration dossier. Examples for these GMP requirements see "References" below. References 2.4.2. Manufacture 3.2.S.2 Lines 169-170 ("material traceability") Lines 194-195 ("prevent crosscontamination, using same column") Lines 280-281 ("intermediates to be tested 14b efore use") 2.4.5. Reference Standards or Materials 3.2.S.5 Lines 562-567 ("…the approach to periodically requalify the reference standards, etc.") 2.4.7 Stability 3.2.S.7 Lines 594-595 ("Variability in stability testing results should be avoided…")		Comment noted	N/A
EFPIA	Specific comment	12	12	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"		Not accepted	No amendment is deemed necessary

Granzer Regulatory Consulting & Services GmbH	Specific comment	55	61	It should be stated at the outset that this guideline addresses specific aspects regarding the manufacturing process, characterisation, specifications and analytical control for synthetic peptides which are not covered in the guidance documents listed in Section 3.	'This guideline addresses specific aspects regarding the manufacturing process, characterisation, specifications and analytical control for synthetic peptides which are not covered in the guidance documents listed in Section 3.' (It is noteworthy that the Guideline on the Chemistry of Active Substances (EMA/454576/2016) or Guidance on the Chemistry of Active Substances for Veterinary Medicinal Products (EMA/CVMP/QWP/707366/2017) is also listed in Section 3).	Comment noted	This guideline should be seen as complementary to the guidelines on the Chemistry of Active Substances (EMA/454576/2016) and Chemistry of Active Substances for Veterinary Medicinal Products (EMA/CVMP/QWP/707366/2017) and should be read in conjuction with guidelines as stated in section 3.
EFPIA	Specific comment	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.		Accepted	Relevant amendments have been made
AstraZeneca	Specific comment	64	64	It is not clear if both products in clinical development and marketed products are in scope for the guideline.	Recommend clarifying the exact scope.	Accepted	Relevant amendments have been made
PolyPeptide Group	Specific comment	66	66	Please clarify in chapter 2 the scope of chapter 4. Since chapter 4 follows the CTD section numbering for the drug substance, it indicates that the guideline covers information that should be included in the CTD documents in a registration application for a peptide drug substance. However, the guideline in some cases includes statement that are GMP instructions and normally not part of a registration application, see for example comments below regarding lines 165-170, 190, 194, 278-279, 286. It is proposed that such GMP statements are clarified or removed.		Comment noted	No amendment is deemed necessary.
AstraZeneca	Specific comment	67	67	It is not clear if both products in clinical development and marketed products are in scope for the guideline.	Recommend clarifying the exact scope.	Accepted	Relevant amendments have been made.
Viatris	Specific comment	67	69	The purpose of the draft guideline is "set out the type of information required for the developme		Comment noted	Please refer to section 6 for guidance on the development of synthetic peptides with reference to a biological product. The guideline does not provide guidance on the development of biosimilars.
Granzer Regulatory Consulting & Services GmbH	Specific comment	67	71	It would be beneficial for the scope of the guidance be expanded to also include specific peptide-based molecules, e.g., peptide aptamers.		Not accepted	No amendment is deemed necessary
EFPIA	Specific comment	68	68	Comment: : Clarify that guideline is not to be applied retrospectively for approved dossier contents, but for post approval changes and new MAAs only		Accepted	No amendment is deemed necessary. However it is confirmed that the guideline is not intended for retrospective application.
Sun Pharma	Specific comment	70	73	Tetrapeptides and below will be considered as small molecules to which the quoted (V)ICH guidelines apply, however higher side number of amino acid limit for peptide definition is not given, for example till 40 amino acid it will be considered as peptide and more than 40 amino acid will be considred as protien	Requesting agency to include clarity regarding higher side amino acid number for peptide consideration	Not accepted	For the applicability of this guideline the manufacturing method should be considered rather than number of amino acids.
Viatris	General comment	70	79	The draft guideline states that synthetic peptides are "at the interface of small molecules and proteins" and are fully or partially (i.e., depending on their size) excluded from the scope of certain ICH guidelines. The draft guideline implies that the size of the peptide dictates guideline applicability but creates uncertainty for which drug substances are within scope due to a lack of specificity around size. We recommend that EMA further define size parameters in the scope of the draft guideline by either the number of amino acids or mass of applicable peptide products. For example, US FDA's Guidance for Industry ANDAs for Certain Highly Purified Synthetic Peptide Drug Products That Refer to Listed Drugs of rDNA Origin (May 2021), defines peptides as "any alpha amino acid polymer composed of 40 or fewer amino acids," and EDQM's Technical guide for the elaboration of monographs on synthetic peptides and recombinant DNA proteins (Rev. 2, 2018), states that synthetic peptides are "typically below 5000 Da."		Not accepted	For the applicability of this guideline the manufacturing method should be considered rather than number of amino acids.
EFPIA	Specific comment	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.		Accepted	Relevant amendments have been made

BCN Peptides	Specific comment	72	72	Line 72 (and foot note) Remove the "(i.e. depending on their size1)" includding the foot note. Please note that up to date there is no differentiation between small peptides (tetrapeptides or below) and other peptides. It is considered that all peptides need to be regulated under the same framework. Note that current Ph. Eur 2034 differentiates the reporting, identification and qualification thresholds for active substances (treated as small moleucles) from the thresholds applied to peptides obtained by chemical synthesis. If a differentiation wants to be included, it should apply only to dipeptides, since in this case, there is no peptide chain (only one amide bond).	Synthetic peptides are fully or partially excluded from the scope	Accepted	Relevant amendments have been made
Aspen Oss B.V.	Specific comment	72	72	72 (footnote) Is there a toxicological reason why a tetrapeptide requires much stricter specifications for impurities than a pentapeptide? The ICH guidelines exempt peptides in general and do not distinct on the basis of the peptide length. The assumption that short peptides may be manufactured with lower impurity levels is incorrect for two reasons. First, impurities in the peptide API often originate from impurities in the starting amino acid derivatives and the strict impurity levels in the API for tetrapeptides and below would require specifications for individual impurities in amino acid derivatives <=0.10%, which are levels that are generally not guaranteed by the manufacturers of amino acid derivatives. Second, tetrapeptides and below are generally not purified by a powerful chromatographic purification (unlike longer peptides), which makes attaining and guaranteeing such strict specifications in the API even less feasible.	Delete footnote and adapt line 72 accordingly.	Accepted	Relevant amendments have been made
Medicines for Europe	Specific comment	72	79	72-79 And footnote Peptides of smaller sizes (< 10 AA) are usually highly pure and less likely to generate severe adverse immune events, therefore ICH guidelines may be more appropriate to apply to them [1]. In addition, smaller peptides usually do not possess secondary or other higher order structures, since for the formation of stable α-helix 2–3 turns of the helix are required, with 3.6-residue-per-turn repeat, corresponding to at least 10 amino acids of length [2]. Similar is also true for β-sheets, where one segment of sheet consists of at least 4 amino acids, with two segments separated by 4+2n amino acid residue [3]. Additionally, the methods, normally used for the detection of aggregates (i.e., SEC, AUC, AF4), are not suitable for detection of smaller size peptide aggregates, since the differences in sizes (monomers vs dimers vs trimers etc.) are too small to be detected. Therefore, we propose that this guideline addresses only the synthetic polypeptides, consisting of at least 10 amino acids or more, for smaller peptides ICH guidelines should be applicable. References: [1] Wu et al. Building parity between brand and generic peptide products: Regulatory and scientific considerations for quality of synthetic peptides. Int J Pharm. 2017 Feb 25;518(1-2):320-334. [2] Levels of Protein Organization, accessed 12 February 2024, https://comis.med.uvm.edu/VIC/coursefiles/MD540/MD540-Protein_Organization_10400_574581210/Protein-org/Protein_Organization3.html [3] Secondary Structure: β-Pleated Sheet, accessed 12 February 2024, https://chem.libretexts.org/Bookshelves/Biological_Chemistry/Supplemental_Modules_%28Biological_Chemistry%29/Proteins/Protein_Structure/Secondary_Structure%3A_-Pleated_Sheet	"This guideline addresses those specific aspects regarding the manufacturing process (solid phase peptide synthesis, fragment condensation), characterisation, specifications and analytical control for synthetic peptides, consisting of 10 amino acids or more, 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." Proposed change of the footnote 1: "Peptides with less than 10 amino acids will be considered as small molecules to which the quoted (V)ICH guidelines apply"	Not accepted	No amendment is deemed necessary
NMEU-Nuclear Medicine Europe	General comment	72	72	Comment related to the footnote (1): It should be clearly stated that for radiopharmaceuticals and peptides used for radiopharmaceuticals, the quality thresholds and specifications defined in Ph. Eur. 2902 apply, even if the peptide is below 4 aminoacids. NMEU welcomes the clear threshold between small molecules and peptides as currently stated (considering tetrapeptides and below as small molecules). However, a clear definition of peptidic and "non-peptidic" constituents should also be included. Same comment for the lines 121-134 and 253.		Comment noted	N/A
EFPIA	Specific comment	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		Comment noted	Liquid phase peptide (solution phase) synthesis is in the scope of the guideline.
EFPIA	Specific comment	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):		Comment noted	Please refer to the scope section of the guideline.

NMEU-Nuclear Medicine Europe	Specific comment	81	81	Comment: “radiophamaceuticals and radiolabelled products containing peptides” NMEU indicates the need to harmonize the definition of radiopharmaceuticals in alignment with the new EC regulations (see ENVI-PR-753470_EN) NMEU jointly with EANM have proposed amendments to the current revision of the EU legislation (regulation/directive) .This review of the definition have been submitted to the Parliament rapporteurs and respective shadow rapporteurs. The purpose is to align this proposal with this radiopharmaceutical guidelines Proposed definitions are: ‘radiopharmaceutical’ means any medicinal product that, when ready for use, contains a radioactive component and that is intended to treat or diagnose a disease, including detection and/or quantification of a target for treatment. This includes ready-to-use dosage forms, as well as kits for radiopharmaceutical preparation. Radiopharmaceuticals include: - “Radionuclide radiopharmaceuticals” which means the radionuclide or its salt is the active substance, or - “Complex radiopharmaceuticals” which means the radionuclide is bound to or within a carrier molecule to achieve targeted accumulation. The term radiopharmaceutical does not include: · Radionuclides or formulations thereof which are used solely for radiolabelling purposes Medical devices which include radioactive materials (e.g., implants, microparticles or other articles similar to an implant with radioactive material), or radiation sources thereof; -Radionuclide generator’ means a system incorporating a fixed parent radionuclide from which a daughter radionuclide is produced. The daughter radionuclide is used either as medicinal product (i.e., radionuclide radiopharmaceutical), or as radionuclide for radiolabelling purposes; -Kit for radiopharmaceutical preparation’ means a pre-formulated medicinal product containing all ingredients required to directly prepare a radiopharmaceutical with the exception of the radionuclide.; -‘preparation of a radiopharmaceutical from a kit (kit-radiolabelling)’ means the combination of sterile components for reconstitution and radiolabelling according to the instructions outlined in the Summary of Product Characteristics (SmPC) including simplified quality control methods. This preparation of a radiopharmaceutical is distinct from “manufacturing” as the specification of the final radiopharmaceutical product has been developed and tested by the kit manufacturer and is ensured by the instructions for reconstitution and radiolabelling of the kit. Furthermore, it should also be clarified from which stage on a process validation of the peptide production is expected in Section 4.2.5	This guideline is not applicable to biological and biotechnological products manufactured by recombinant technologies, radiopharmaceuticals (including cold kits for radiolabeling) and radiolabelled products containing peptides.	Not accepted	No amendment is deemed necessary.
NMEU-Nuclear Medicine Europe	Specific comment	83	84	For the sake of clarity, the sections, for which this guideline apply for radiopharmaceuticals should be spelled out in the text	.. the principles of this guideline apply regarding synthesis and starting materials. (sections: 4.2.2. Description of Manufacturing Process and Process Controls 3.2.S.2.2) and starting materials (section 4.2.3. Control of Materials 3.2.S.2.)	Not accepted	No amendment is deemed necessary
NMEU-Nuclear Medicine Europe	Specific comment	84	84	larification also about required EP monographs (peptide specific EP monographs available and the general EP 2902)	..... applicable monographs in Ph. Eur. for radiopharmaceuticals (EP Monograph 2902 and the peptide specific monographs)	Comment noted	N/A
EFPIA	Specific comment	89	89	Comment: Add reference to ICH Q7. Proposed change (if any): ICH Q7 Good manufacturing practice for active pharmaceutical ingredients		Accepted	Relevant amendments have been made
EDQM	Specific comment	89	89	The list of documents includes Ph. Eur. general monograph Chemical precursors for radiopharmaceutical preparations (2902) (the word ‘chemical’ is missing from the title on the list), while it does not include the general monograph Substances for pharmaceutical use (2034) nor a reference to individual Ph. Eur. monographs on synthetic peptides.	It is proposed to correct the title of Ph. Eur. monograph 2902 Chemical precursors for radiopharmaceutical preparations and to add the monograph Substances for pharmaceutical use (2034) to the list. Collective reference to the Ph. Eur. monographs on synthetic peptides could also be considered.	Accepted	Relevant amendments have been made
Granzer Regulatory Consulting & Services GmbH	Specific comment	89	116	The list of guidelines should also include ‘Guideline on the requirements to the chemical and pharmaceutical quality documentation concerning investigational medicinal products in clinical trials (EMA/CHMP/QWP/545525/2017 Rev. 2)’.		Accepted	Relevant amendments have been made
Bachem AG	Specific comment	108	109	Reference to ICH Q14 to be added.	ICH Q14 Analytical procedure development - Scientific guideline	Accepted	Relevant amendments have been made
BCN Peptides	Specific comment	117	117	Addition of reference to Ph. Eur monograph 2034, since this monograph refers to peptides obtained by chemical synthesis	Ph. Eur monograph 2034-Substances for Pharmaceutical use	Accepted	Relevant amendments have been made

PolyPeptide Group	Specific comment	121	121	Reference to IUPAC nomenclature guideline is missing	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 and symbolism for amino acids and peptides.	Accepted	Amended accordingly
EFPIA	Specific comment	121	121	Comment: Include clarification on requirements for nomenclature as for cyclic peptides		Comment noted.	No additional clarification is deemed necessary since too specific.
EFPIA	Specific comment	122	151	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.		Not accepted	3-letter amino acid codes are expected for ease of reading
NMEU-Nuclear Medicine Europe	Specific comment	122	125	A clear definition of peptide (e.g. based on IUPAC nomenclature) and "non-peptidic" constituents should be included. Any peptide shall be represented with its structural formula (up to a certain aminoacid chain length) including the chiral indicators (R, S). The 3-letter code can be used as complementary communication tool.	The Nomeclature of peptides and non-peptidic" constituents should be clearly defined based on IUPAC Nomeclature. In addition, the 3-letter code can be used./ Letter codes may be used for the primary structure of the active substance, i.e. 3-letter amino acid.....	Not accepted	No amendment is deemed necessary as IUPAC nomenclature is already covered by the guideline on the Chemistry of active substance
EUCOPE	Specific comment	125	125	Add: modifications on the terminals needs to be shown in the structure. Background: modifications at C- or N-terminal of a synthetic peptide are often desired because of their potential therapeutic properties.		Accepted	Relevant amendments have been made
AstraZeneca	Specific comment	128	128	INN for larger (>20 mer) peptide drug substances usually use single letter IUPAC code (Pure & Appi. Chem., Vol. 56, No. 5, pp. 595—624, 1984.).	The text states only 3-letter code, please include that single letter option is also applicable to align with IUPAC.	Not accepted	3-letter amino acid codes are expected for ease of reading
BioNTech SE	Specific comment	131	134	We suggest providing guidance on the naming of cyclic peptides as this is a common modification. Describing the peptide sequence (3 letter code) followed by the designation, nature of cyclization and the positions involved in the cyclisation is acceptable e.g. for a lactam bridge in between amino acids x and y in the sequence: AAA1-AAA2-…-AAAx (Cyclic; lactam AAAy-AAAz).	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 (3 letter code) followed by the designation, nature of cyclization and the positions involved in the cyclisation is acceptable, e.g. for a lactam bridge in between amino acids x and y in the sequence: AAA1-AAA2-…-AAAx (Cyclic; lactam AAAy-AAAz). Full chemical structure of non-peptidic side chains and linkers is expected.	Accepted	Proposed amendments have been included.
PolyPeptide Group	Specific comment	133	133	No guidance on how to describe cyclic configuration is given.	Insert the following: For cyclic peptides, the cyclic linkage should be represented according to IUPAC nomenclature.	Not accepted	No amendment is deemed necessary as IUPAC nomenclature is already covered by the guideline on the Chemistry of active substance
United States Pharmacopeia l	Specific comment	136	137	Are the results of the listed techniques expected to be included in the dossier and not as routine release test for the substance? This should be clearly specified.		Comment noted.	Please refer to the Guideline on the Chemistry of Active Substance.

BCN Peptides	Specific comment	136	140	For peptides range for the water content is always accepted. Additionally the water content generally increase along stability studies since lyophilized peptides are hygroscopic. So water content should not be included as a general property. This parameter is already included under test. The pH of a solution of the peptide may be different from batch to batch. This property is not considered relevant for the ASMF, since it depends on the solvent, concentration, etc used for this test (it is not a standard test). Biological activity is understood to be studied during drug product development. Once the structure of the active substance is defined , its activity in terms of bioassay is not considered to be relevant for the ASMF. It is noted that biological activity has been never requested during the ASMF assessment of synthetic peptides. A Short biological activity description as includded under "Definition" in the Ph. Eur monographs published for Peptides could be added. It is important not confusing this with biological assay testing) Lyophilized Peptides are known to be hygroscopic. However, the relevance of specific hygroscopic studies is not understood.	Relevant general properties of the peptide in question should be listed. In most cases, isoelectric point, optical rotation, and solubility in different media would be expected.Hygroscopicity needs to be indicated Short biological activity description as included under "Definition" in the Ph. Eur monographs published for Peptides could be added. [This sentence could be moved to the end of this section. After line 144]	Accepted	Relevant amendments have been made to clarify that the listed general properties are meant to provide examples
Almac Sciences	Specific comment	136	138	Are chemical characterisation tests to determine primary and secondary structure e.g. MS-MS sequencing and CD-spectroscopy acceptable in lieu of biological activity testing?		Comment noted	N/A
Bachem AG	Specific comment	136	137	• Water content: typically specified and discussed in S.4.5. Since many peptides are hygroscopic, the water uptake is more characteristic than a snapshot after manufacture. The point "water content" should therefore be eliminated. • Isoelectric point: The determination of the isoelectric point bears uncertainties and may be affected with significant systematic errors, therefore it should not be reflected as general property. Further, isoelectric point is of more importance for proteins than for peptides, especially for their electrophoretic separation. Nevertheless, the isoelectric point influences membrane permeability and thus the absorption of peptides which may be of relevance for designing the composition of certain drug product formulations. Such an information, however, is not relevant for a drug substance. • Optical rotation should be stated more general than optical activity since: optical rotation is a non-specific, outdated test which is considered of limited scientific value for the characterization of peptides. It should not be listed as "expected" for peptide drug substances. [R. Vergote, C. Burvenich, B. De Spiegeleer, Quality specifications for peptide drugs: A regulatory-pharmaceutical approach, November 2009, Journal of Peptide Science]. Circular dichroism is an alternative technique for the optical activity characterization providing more relevant analytical data for peptides. • Biological activity: it is acknowledged that qualitative bioassays could be informative tests in certain cases. However, they should not be generally listed as "expected" in this chapter but limited to cases where it is informative.	Relevant general properties of the peptide in question should be listed. In most cases, pH of a solution of the peptide, solubility in different media and relevant optical activity data would be expected. Where appropriate, e.g., in cases where higher-order structure formation is anticipated or known, information on biological activity of the peptide should be provided.	Accepted	Relevant amendments have been made to clarify that the listed general properties are meant to provide examples
EFPIA	Specific comment	136	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. Lines: 136-138, 348-349, 557		Accepted	Relevant amendments have been made
EFPIA	Specific comment	136	136	Comment on lines 136, 138, 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.		Accepted	Relevant amendments have been made to clarify that the listed general properties are meant to provide examples
EFPIA	Specific comment	137	137	Comment: Optical rotation testing should be optional as other chiral amino acid analysis can be performed. Proposed change (if any): Optical rotation (optional)		Accepted	Relevant amendments have been made to clarify that the listed general properties are meant to provide examples
Medicines for Europe	Specific comment	137	137	The request for biological activity is mandatory for new molecular entities, innovative. As for generic, Biological activity of short (<50 AA) peptides is a function of primary (sequence) and secondary structure. Once the sequence and secondary structure proven to be identical to those of innovative DS, the biological activity is expected to be identical as well, thus not required for generics.	Biological activity (unless duly compared and justified)	Comment noted	Relevant text has been amended to clarify that it refer to mechanism of action
AstraZeneca	Specific comment	137	137	biological activity specified in S.1.3 as 'expected'.	remove from this section entirely	Accepted	Relevant amendments have been made
EFPIA	Specific comment	137	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?		Comment noted	Relevant text has been amended to clarify that it refer to mechanism of action

Bachem AG	Specific comment	138	138	With reference to: "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". Hygroscopicity is also investigated during ICH stress testing. A reference to the stability section may be appropriate as well.	Hygroscopicity needs to be indicated, e.g. with moisture sorption isotherms, or references could be made to 3.2.S.3.1 or 3.2.S.7 where such information could be provided more in detail.	Not accepted	No amendment is deemed necessary
Granzer Regulatory Consulting & Services GmbH	Specific comment	141	142	It is unclear whether the characterization data on crystallinity will be expected in order to prove the amorphous state and thus to justify not providing data on melting point / polymorphic forms etc. (see also 365-367).	Most peptides are amorphic powders which should be supported by suitable data. In case synthetic peptides are presented in crystalline form then additional characterization data should be provided e.g. melting point and polymorphic form.	Not accepted	No amendment to the text is considered necessary since it is considered case-specific.
BCN Peptides	Specific comment	143	144	Clarifications are added	The counter ion needs to be indicated. In general, the counter ion is present in a non stechecmiometric ratio. In the event that there is no counterion, the free base should be indicated.	Accepted	Amended accordingly
BioNTech SE	Specific comment	143	144	We suggest providing precise guidance on expectation regarding the counter ion ratio to peptide determination. Counter Ion ratio vs. assay allows establishment of the stoechiometric ratio.	The counter ion needs to be indicated, if relevant, and whether it is present in a stoichiometric or non-stoichiometric ratio. Counter ion(s) content (e.g. w/w) should be measured and expressed as counter ion(s) ratio vs assay.	Not accepted	No amendment is deeemed necessary
NMEU-Nuclear Medicine Europe	Specific comment	143	144	Protonation state of peptide and counterion (amine / ammonium and free acid / anion) is not always clear, especially in early phase development. Salt forming agents (except the intended counterion) may as well be dealt with asimpurities of the API. The counterion content should be part of the specifications, but not regarded as an impurity	The counter ion shall be indicated, if relevant, and whether it is present in a stoichiometric or non-stoichiometric ratio.	Not accepted	No amendment is deeemed necessary
Medicines for Europe	Specific comment	143	144	Lines 143–144+ Lines 477–479 In our opinion, counter ions should be considered ions, when present in drug substance in a significant amount (it could be stoichiometric or non-stoichiometric ratio, since the amount can depend on pH), and can as such have a significant impact on peptide's properties. They should therefore be specified with upper and lower limit. However, when ions are present in traces (in non-stoichiometric ratio) they do not significantly impact the peptide's structure and properties. In this case they should be considered as residual ions and therefore controlled only with an upper limit. This approach is consistent with the general principles of quality control, where trace impurities are typically specified with an upper limit. Therefore, we suggest a change to the text – it should be emphasized, that the ions present in drug substance in a significant amount (whether it be stoichiometric or non-stoichiometric) should be considered as counter-ions. On the other hand, ions present in traces (always a non-stoichiometric ratio) should be considered as residual ions. Comment on upper and lower limit for counter-ion is posted below (on lines 477–479).	The counter ion needs to be indicated, if relevant (i.e., when present in significant amounts), and whether it is present in a stoichiometric or non-stoichiometric ratio.	Not accepted	No amendment is deeemed necessary
Medicines for Europe	Specific comment	143	144	Lines 143–144+ Lines 477–479 In our opinion, counter ions should be considered ions, when present in drug substance in a significant amount (it could be stoichiometric or non-stoichiometric ratio, since the amount can depend on pH), and can as such have a significant impact on peptide's properties. They should therefore be specified with upper and lower limit. However, when ions are present in traces (in non-stoichiometric ratio) they do not significantly impact the peptide's structure and properties. In this case they should be considered as residual ions and therefore controlled only with an upper limit. This approach is consistent with the general principles of quality control, where trace impurities are typically specified with an upper limit. Therefore, we suggest a change to the text – it should be mentioned, that when an ion is classified as counter-ion, its amount should be controlled with an upper and lower limit. However, when an ion is considered a residual ion, it should be controlled only with an upper limit. Comment classification when an ion should be considered as counter-ion is posted above (on lines 143–144).	The type of counter ion should be justified, and the amount of counter ions should be controlled in the drug substance specification with an upper and lower limit. The amount of residual ions should be controlled in the drug substance specification only with an upper limit.	Not accepted	No amendment is deeemed necessary
Aspen Oss B.V.	Specific comment	156	157	A similar standardized protocol may also be applied in Liquid Phase Peptide Synthesis (LPPS).	If desired, we can write a proposal for such a protocol.	Commetn noted	N/A

NMEU-Nuclear Medicine Europe	Specific comment	157	158	Capping step should be mentioned as well	...deprotection, washing and coupling and capping steps	Accepted	Amended accordingly
EFPIA	Specific comment	158	159	Comment: “Proven acceptable ranges” are only relevant in late phase development. Proposed change (if any):		Accepted	Relevant amendments have been made
PolyPeptide Group	Specific comment	158	160	It is stated that "These standardized steps with their associated Proven Acceptable Ranges (PARs) need not to be described in detail each time they are used, provided clear descriptions of the used conditions…". It is not understood why PARs are mentioned in this sentence specifically. Process steps in SPPS may have established PARs or may not have depending on their criticality etc. The same applies for all steps in the processes (cleavage, purification etc.). It is proposed to remove the reference to PARs to avoid the interpretation that all process steps in SPPS must have established PARs.	These standardized steps need not to be described in detail each time they are used, provided clear descriptions of the used conditions…	Accepted	Relevant amendments have been made
NMEU-Nuclear Medicine Europe	Specific comment	158	159	Proven acceptable ranges” are typically only relevant in late phase development.	These standardized steps with their associated Proven Acceptable Ranges (PARs) need not be described in detail each time they are used, provided clear descriptions of the used conditions (e.g. reagenprots, solvents, reaction times, …) are given, and provided only for the late stage of development.	Accepted	Relevant amendments have been made
Bachem AG	Specific comment	158	159	With reference to: “These standardized steps with their associated Proven Acceptable Ranges (PARs) need not be described in detail each time they are used”. In line with EMA/454576/2016 and EMA/CVMP/QWP/707366/2017 it is recommended not to mention PARs but rather use the more unspecific language of the cited guidelines to avoid confusion.	These standardized steps with their ranges need not be described in detail each time they are used	Accepted	Relevant amendments have been made
Bachem AG	Specific comment	160	161	With reference to: “provided clear descriptions of the used conditions (e.g. reagents, solvents, reaction times, …) are given, and provided it is clearly indicated where these steps are used in the overall manufacturing process”. Reaction times are just one process parameter out of several and might be - in contrast to used reagents and solvents - not even relevant for API quality. Therefore, a more general language is recommended.	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	Accepted	Amended accordingly
United States Pharmacopeia l	Specific comment	161	162	It is suggested to change to “The final deprotection/cleavage step should be described in detail…” to better indicate the manufacturing step.	The final deprotection/cleavage step should be described in detail...	Accepted	Relevant amendments have been made
NMEU-Nuclear Medicine Europe	Specific comment	161	162	It is not quite clear whether cleavage from solid support is meant indeed (which is typically the last deprotection step.)	The final deprotection and cleavage steps should be described in detail, including any use of scavengers and other reagents…	Accepted	Relevant amendments have been made
Bachem AG	Specific comment	162	163	With reference to: 'The final deprotection step should be described in detail, including any use of scavengers and other reagents, in case of which a discussion of their genotoxic potential should be included in 3.2.S.3.2.' The final cleavage should be better defined. It is not understood why the Guideline singles out the cleavage reagents in terms of genotoxic potential here. This applies to all reagents used in the process and should be only covered under the paragraph of 4.3.2 of the present Guideline for clarity.	The cleavage and side-chain deprotection step should be described in detail	Accepted	Amended accordingly
EFPIA	Specific comment	163	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".		Comment noted	Relevant text has been deleted

PolyPeptide Group	Specific comment	165	170	Changes are proposed to the section: "Splitting or combining of sub-batches/multiple cycles may be performed at different stages during manufacturing, e.g. based on equipment capacity or operational efficiency in SPPS. The criteria applied in the decision on splitting or pooling of sub-batches and/or multiple cycles should be provided, along with an adequate justification for the selected approach. Moreover, material traceability from the synthesis steps through the final drug substance is expected and S.2.2 should contain an unambiguous definition of the commercial batch size (range)." The text indicated in Italics in the above excerpt is proposed to be removed. It is good that the guideline recognize that splitting and combining of batches can be done for, for example, capacity and efficiency reasons. However, the reasons for splitting and combining may be complex, situation dependent and is related to the production equipment, scale etc. Thus, it will be difficult to describe the criteria for splitting/combining in the regulatory application. Procedures and criteria regarding splitting and combining should (of course) follow GMP requirements and be covered in GMP audits rather than in the regulatory application. Likewise, material tracebility is a fundamental GMP requirement and there is no need include this in a regulatory guideline.	Splitting or combining of sub-batches/multiple cycles may be performed at different stages during manufacturing, e.g. based on equipment capacity or operational efficiency in SPPS. S.2.2 should contain an unambiguous definition of the commercial batch size (range).	Accepted	Relevant text has been deleted
Bachem AG	Specific comment	165	168	With reference to: “Splitting or combining of sub-batches/multiple cycles may be performed at different stages during manufacturing, e.g. based on equipment capacity or operational efficiency in SPPS. The criteria applied in the decision on splitting or pooling of sub-batches and/or multiple cycles should be provided, along with an adequate justification for the selected approach.” Definition for batch/sub-batch needs to be given in the Guideline	RMX: 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.	Accepted	Relevant amendments have been made
BCN Peptides	Specific comment	166	168	The criteria for splitting or pooling falls in the GMP System. It is not considered information to be included in the dossier. Please delete these lines	...based on equipment capacity or operational efficiency in SPPS.	Accepted	Relevant text has been deleted
EFPIA	Specific comment	168	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.		Accepted	Amendments have been made to clarify that it's a GMP aspect
EUCOPE	Specific comment	168	168	Clarify what is meant by “material traceability” in the context of the process description in 3.2.S.2.2 or if this information is a GMP expectation with information to be contained in executed batch records.		Accepted	Amendments have been made to clarify that it's a GMP aspect
Bachem AG	Specific comment	169	170	With reference to: “Moreover, material traceability from the synthesis steps through the final drug substance is expected and S.2.2 should contain an unambiguous definition of the commercial batch size (range).” Material traceability is covered by GMP and should be applicable for all kinds of drug substances, i.e., it's nothing specific to peptides. It is recommended to omit this part for clarity that it is not required to describe this in the dossier.	Moreover, S.2.2 should contain an unambiguous definition of the commercial batch size (range).	Accepted	Amendments have been made to clarify that it's a GMP aspect
EFPIA	Specific comment	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):		Accepted	Amendments have been made to clarify that the case described is an example and does not exclude other hybrid approaches
NMEU-Nuclear Medicine Europe	Specific comment	171	172	“Liquid phase synthesis” is the use of a soluble tag. Proposal: suggest replacement of "Liquid phase synthesis" by solution phase chemistry	..followed by fragment condensation (solution phase chemistry) have been used… RMX: solution phase synthesis	Accepted	Relevant amendments have been made
EFPIA	Specific comment	175	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):	RMX: For the manufacturing of the peptide fragments by SPPS method it is referred to the section above, fragment condensation and liquid phase synthesis generally should follow the considerations of the Guideline on the Chemistry of Active Substances.	Accepted	Relevant amendments have been made

Granzer Regulatory Consulting & Services GmbH	Specific comment	178	180	It is stated that: 'In case two drug substance manufacturing processes will be used in parallel (e.g. solid phase synthesis and a hybrid process)'. Can it be clarified if this means two drug substance manufacturing processes can be submitted by the same and/or different manufacturers?		Comment noted	Not within the scope of the guideline
Granzer Regulatory Consulting & Services GmbH	Specific comment	178	180	It is stated that: 'In case two drug substance manufacturing processes will be used in parallel (e.g. solid phase synthesis and a hybrid process)'. Can it be clarified if >2 drug substance manufacturing processes cam be used?		Accepted	Relevant amendments have been made
Granzer Regulatory Consulting & Services GmbH	Specific comment	178	180	It is stated that 'results from comparability studies on drug substance and drug product level should be provided.' Can it be clarified what tests/example of tests would be expected to be conducted.		Comment noted	Not within the scope of the guideline
Granzer Regulatory Consulting & Services GmbH	Specific comment	178	180	It is stated that 'results from comparability studies on drug substance and drug product level should be provided.' Can it also be clarified what section this data should be presented in, e.g., 3.2.S.4.4 (Batch Analysis)?		Comment noted	Not within the scope of the guideline
ProPharma	Specific comment	178	180	Please consider to add that for a synthetic peptide, functional comparability tests are not required when data are provided indicating that the peptide will show consistent conformational structure within investigated batches.	For a synthetic peptide, functional comparability tests are not required when data are provided indicating that the peptide will show consistent conformational structure (in relevant media) within investigated batches.	Accepted	Relevant amendments have been made
BicycleTx Limited	Specific comment	179	179	Recommend to remove reference to 'comparability' as synthetic peptides are outside the scope of ICH Q5E. Instead product from both processes should be shown to have no significant differences.	Results from studies on drug substance showing no significant differences between processes	Partially accepted	Relevant amendments have been made
BicycleTx Limited	Specific comment	179	179	Data from drug product should not be mandated, instead justification should be provided for not providing drug product data from both processes.	Delete 'and drug product level'. Add: Justification should be provided for the absence of drug product data from both processes.	Accepted	Relevant amendmemt have been made.
EFPIA	Specific comment	179	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 as appropriate, 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 in some cases drug product level should be provided."		Accepted	Relevant amendmemt have been made.
United States Pharmacopeia l	Specific comment	181	182	It is suggested to remove the title and line 182 since the following paragraph is not relevant to the title. The purification phase, part of the Downstream process, is part of the process and as detailed in the proposed guidance. It should be part of the process qualification/validation. Reprocessing, recovery and rework could occur in those cases as per EMA/454576/2016, ICH Q7 (Ref 8) or EU GMP Part II (Ref 9).	Remove the title and line 182	Partly Accepted	Relevant amendments have been made
Granzer Regulatory Consulting & Services GmbH	Specific comment	181	195	The current paragraph does not align and read well in terms of the definitions for 'Reprocessing, Recovery and Rework' in ICH Q7. It is recommended that the paragraph is broken-down to consider the above terms as sub-headings with regard to synthetic peptides.		Accepted	Relevant amendments have been made

Bachem AG	Specific comment	183	193	With reference to: "Synthetic peptides are generally purified using chromatographic techniques, often starting from a relatively complex crude intermediate. It is recognized that the crude peptide typically contains pre- and post-eluting structurally related impurities. It is acceptable to perform repeated purification steps of these side-fractions resulting in eluate meeting the purity requirements of the main fractions. When routine repurification is carried out this is not considered reprocessing but part of the regular manufacturing process and should be justified accordingly. Clear description of the repurification procedure and the criteria for deciding when it can be performed should be provided (see also '4.2.3. Control of Critical Steps and Intermediates 3.2.S.2.4' below). The routine repurification process of the side fractions (if used) should be part of the manufacturing process qualification/validation. Likewise, if repetition of the coupling reaction is part of the routine manufacturing process, it is not considered reprocessing." The section is named "Reprocessing, recovery and rework" and should therefore be about reprocessing and not purification techniques. We recommend adding in the "sequential procedural narrative", between lines 170 and 171 the text about purification techniques which is currently in lines 183-186 and lines mid 188-mid 190.	New text to be added between lines 170 and 171: "Synthetic peptides are generally purified using chromatographic techniques, often starting from a relatively complex crude intermediate. It is recognized that the crude peptide typically contains pre- and post-eluting structurally related impurities. A clear description of the repurification procedure and the criteria for deciding when it can be performed should be provided (see also '4.2.3. Control of Critical Steps and Intermediates 3.2.S.2.4' below)." Text to replace the one in lines 183-193: It is acceptable to perform repeated purification steps of these side-fractions resulting in eluate meeting the purity requirements of the main fractions. When routine repurification is carried out this is not considered reprocessing but part of the regular manufacturing process and should be justified accordingly. The routine repurification process of the side fractions (if used) should be part of the manufacturing process qualification/validation. Likewise, if repetition of the coupling reaction is part of the routine manufacturing process, it is not considered reprocessing.	Accepted	Relevant amendments have been made
EFPIA	Specific comment	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.		Accepted	Relevant amendments have been made
EFPIA	Specific comment	185	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.		Accepted	Relevant amendments have been made
AstraZeneca	Specific comment	185	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.	Accepted	Relevant amendments have been made
PolyPeptide Group	Specific comment	190	191	It is stated that "The routine repurification process of the side fractions (if used) should be part of the manufacturing process qualification/validation." The entire manufacturing process is subjected for process validation. The purpose of this guideline is not to outline process validation requirements and it is unclear why process validation is referred to only here and not for other parts of the manufacturing process. Thus, it is proposed to remove the reference to process validation.	Delete sentence "The routine repurification process of the side fractions (if used) should be part of the manufacturing process qualification/validation."	Not accepted	N/A
PolyPeptide Group	Specific comment	194	194	Measures to prevent cross-contamination is fundamental in GMP. No need to state this in a regulatory guideline.	Delete sentence "Appropriate measures to prevent cross contamination due to successive purification ofn different peptide using the same column should be in place, as required by GMP."	Not accepted	It is explicitely stated that it is required by GMP
Regeneron Pharmaceuticals, Inc.	Specific comment	194	195	The guideline states that appropriate measures to prevent cross-contamination due to successive purification of different peptides using the same column should be in place, as required by GMP. However, the guideline does not provide further detail on whether this information should be incorporated into a CTA or MA submission. The guideline should clarify that information regarding the processes for preventing cross-contamination does not need to be described in Module 3 of the CTD. Instead, the guideline should require that applicants confirm that such cross-contamination prevention measures are described in the Quality Management System (QMS). By allowing an applicant to confirm the QMS accurately and appropriately describes cross-contamination prevention, the Agency will reduce duplicative regulatory information for both the regulator and the sponsor.	Revise line 195 to add: 'These measures do not need to be detailed in Module 3, rather the applicant should confirm that they are described in the Quality Management System'.	Not accepted	It is explicitely stated that required by GMP

PolyPeptide Group	Specific comment	196	198	There are other isolation tehniques than lyophilization used for peptides, e.g. crystallization and spray drying. It is proposed to change the word lyophilization to isolation (in heading and in text) to recognize that the are other techniques.	"Isolation The technique used for isolation including process parameters should be described. "	Accepted	Relevant amendments have been made
Granzer Regulatory Consulting & Services GmbH	Specific comment	196	198	It would be useful if the level of detail expected for lyophilisation process parameters be explained.		Accepted	Relevant amendments have been made
Bachem AG	Specific comment	196	198	With reference to: "Lyophilisation Lyophilisation of synthetic peptides is considered common practice. Lyophilisation process parameters should be described." The lyophilisation section (lines 196-198) should be included at the end of the "sequential procedural narrative". A minimum set of necessary parameters should be indicated (e.g. end pressure and end temperature set point or range). Only the final lyophilization is meaningful	To be added between lines 175 and 176: Lyophilisation of synthetic peptides is considered common practice. Final lyophilisation process parameters should be described (e.g. end pressure and end temperature set point or range).	Partially accepted	Relevant amendments have been made to clarify that relevant process parameters should be described.
EFPIA	Specific comment	196	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):		Accepted	Relevant amendments have been made
BioNTech SE	Specific comment	197	198	We suggest including other typical isolation methods for peptides such as precipitation, crystallization and spray drying and include a section similar to the lyophilization to clarify the Agency's expectations.	Lyophilisation of synthetic peptides is considered common practice. Alternative isolation methods such as precipitation, crystallization and spray drying of synthetic peptides are also used. Respective process parameters should be described.	Accepted	Relevant amendments have been made
Aspen Oss B.V.	Specific comment	197	197	Only relevant process parameters should be described.	Relevant lyophilisation process parameters should be described, e.g. temperature.	Accepted	Relevant amendments have been made
NMEU-Nuclear Medicine Europe	Specific comment	199	199	It must be clearly stated if this section applies as a whole also for peptides used in radiopharmaceuticals (and if so, this should be stated in lines 83-84. See comment 2 from scope section)		Comment noted	This point addressed in the scope of the guideline.
Granzer Regulatory Consulting & Services GmbH	Specific comment	199	263	In-line with ICH Q11, it would be useful for a decision tree/flow diagram to be included an Appendix in-line with the textual guidance.		Comment noted	No amendment is deemed necessary
BicycleTx Limited	Specific comment	200	207	As a general comment, the requirements here appears beyond the expectations of ICH Q11. As an alterative, ICH Q11 should be referenced for RSM requirements with additional information what substances (e.g. protected amino acids) may be considered appropriate RSMs for synthetic peptides.	n/a	Comment noted	No amendment is deemed necessary
EFPIA	Specific comment	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):		Accepted	Relevant amendments have been made
Granzer Regulatory Consulting & Services GmbH	Specific comment	201	207	Could EMA clarify if a CoA for the active substance starting materials should be provided.		Comment noted	General requirements applicable to all active substance starting materials do not need explicit mention in the guideline.

BCN Peptides	Specific comment	202	204	For the manufacture of Peptides, typically, the protected amino acids are defined as the starting materials. Most of them are commercially available, and according to ICH Q11 an applicant generally does not need to justify the use of a commercially available chemical as a starting material. A pragmatic approach should be applied when thinking in any potential change of manufacturer of the APISM. In these cases, the requirements to be assessed for this change (such as the manufacturing process(IPC)) can not be evaluated because the manufacturing process of the commercially available protected amino acids is confidential and impossible to assess as required by the variations regulation. Additionally, the impact of the manufacturing process of the protected amino acids in the quality of the API has been assessed as negligible, if the quality attributes required for any potential new manufacturer are exactly the same as those required for the declared manufacturers. If all the manufacturers and/ or manufacturing processes of APISM for peptides should be assessed, a large number of batches of the concerned peptide would need to be manufactured for the original assessment. Taking as an example the manufacture of a nonapeptide with at least 3 different suppliers for each protected amino acid, 54 batches of the API would be needed before the DMF submission (9 amino acids x 3 suppliers x 2 batches required for assessment=54 batches)], which is excessive and nonsensical. Additionally, current variations regulation classifies the change in the manufacturer of a starting material different if the proposed manufacturer is from the same pharmaceutical group as currently approved or not. Considering the complexity of peptides, the low risk identified when the specifications are the same, and the large amount starting materials used in each process, it is important to allow a pragmatic approach in order to avoid shortage of the API.	The name and address of all starting material manufacturers should be provided. The addition of manufacturers for the starting materials needs to be approved by a variation according to European legislation. However, current legislation does not include the scenario applicable to peptides, where, in the event that the starting materials are commercially available, and the specifications of the starting material are the same as those already approved, it could be notified under "B1a1a like" classified as (IA), regardless the proposed manufacturer is from the currently approved manufacturer or not. In these circumstances, a practical approach is acceptable without the need of manufacture of 2 batches with material from the new manufacturer.	Not accepted	Already described in the variations guidelines
piCHEM Forschungs- und Entwicklungs GmbH	Specific comment	202	207	In accordance with ICHQ11 it should be defined, that the requirements to define the name and address of the manufacturer as well the synthetic route is to be presented for the "non-commercial" starting materials (see also comment to line 211-214)		Comment noted	N/A
piCHEM Forschungs- und Entwicklungs GmbH	Specific comment	202	250	It is not clear how much information on starting materials has to be provided in which stage of development (clincial phases) In small scale synthesis used for personnalized medicine only limited information on manufacture of commercial starting material is accessable for peptide manufacturer.		Comment noted	Please refer to the scope of the guideline and to section 7) Requirements for Clinical trial applications (human products only)
BCN Peptides	Specific comment	204	207	It is understood that such detailed flow charts are easy to obtain for custom synthesis starting materials, but it is not the case for commercially available materials where this information is not generally disclosed by the starting materials manufacturers. Definition included in ICH Q11 should be considered: "An applicant generally need not justify the use of a commercially available chemical as a starting material. A commercially available chemical is usually one that is sold as a commodity in a preexisting, non-pharmaceutical market in addition to its proposed use as starting material. Chemicals produced by custom syntheses are not considered to be commercially available. If a chemical from a custom synthesis is proposed as a starting material, it should be justified in accordance with the general principles for the selection of starting materials outlined above in Section 5.1.1." Finally, it is noted that if the initial proposal is set as a requirement, guidances and regulations should be available for the manufacture of protected amino acids.	Except for commercial available (acording to ICH Q11 definition) protected amino acids, 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,	Not accepted	No amendment is deemed necessary. This information is considered necessary for commercially available protected amino acids as well.
NMEU-Nuclear Medicine Europe	Specific comment	204	207	information from suppliers is often not available as the suppliers are not willing to share sensitive information especially on the process	…Available information indicating the synthetic processes of relevant starting materials (including details of reagents, solvents and catalysist used) should be provided, followed by….	Not accepted	No amendment is deemed necessary. This information is considered necessary for commercially available protected amino acids as well.
EFPIA	Specific comment	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, […]. This does not apply for natural amino acids."		Not accepted	No amendment is deemed necessary. This information is considered necessary for commercially available protected amino acids as well.

Bachem AG	Specific comment	206	207	With reference to: "… should be provided, followed by a criticality assessment of which starting material impurities may have an impact on the impurity profile of the peptide." For sake of clarity the section on criticality assessment needs to be rephrased	…should be provided. Based on the amount of the relevant impurities in the Starting Material, the criticality on the impurity profile of the Drug Substance should be discussed in the peptide specific risk assessment.	Not accepted	No amendment is deemed necessary. S.2.3 should be presented including relevant and justified specification of each starting material. Critical impurities should be part of the specification.
Granzer Regulatory Consulting & Services GmbH	Specific comment	207	207	The information and data requirements stated on all starting materials (e.g. also commercially available protected amino acid building blocks) is questionable.	For any starting material that is not commercially available (ref to ICH Q11 and Q/A) a flowchart should be presented indicating the synthetic process(es) including details of reagents, solvents and catalysts used. For commercially available starting materials the flow diagram is not necessarily required, however the basic information on the starting material and the control strategy should be provided as described in the ICH Q11 and Q/A. For any starting material a criticality assessment is expected of which starting material impurities may have an impact on the impurity profile of the peptide.	Not accepted	No amendment is deemed necessary. This information is considered necessary for commercially available protected amino acids as well.
United States Pharmacopeia l	Specific comment	208	208	It is suggested to change to "Protected amino acid derivatives and peptide fragments" to better describe the type of starting materials which could be used and identified in the synthesis.	Protected amino acid derivatives and peptide fragments	Not accepted	Peptide fragments are covered in the following paragraph
BCN Peptides	Specific comment	208	208	Aligned with title in line 249	Amino acids and Peptidic structural moieties	Accepted	Relevant amendments have been made
PolyPeptide Group	Specific comment	208	208	Certain linkers will donate a minor structural element to the peptide drug substance. For example, a Ramage linker will donate a nitrogen atom to the peptide. It is suggested to clarify that such donation of a minor structural element, does not mean that the donator shall be classified as a starting material.	Insert somewhere in the section: Raw materials that donate only a minor structural element, e.g. one atom, to the peptide are not defined as starting materials.	Not accepted	No amendment is deemed necessary.
EUCOPE	Specific comment	208	214	Comment: "Protected amino acid derivatives (with terminal and side-chain protection as relevant) are generally acceptable as starting materials in the manufacturing process of synthetic peptides. Nevertheless, a justification on the designation of starting materials needs to be provided." The 'justification' that is described as needed to be provided seems to be redundant as it is self-evident and called out explicitly that derivatised amino acids are generally acceptable; it would appear that a justification, as requested, does not add value. The justification would only be required should one decide NOT to designate a given amino acid derivative as a starting material. Proposed change (if any): "A justification on the designation of starting materials needs to be provided if a given amino acid derivative is not designated as a starting material."		Accepted	Relevant amendments have been made
BioNTech SE	Specific comment	209	214	We suggest providing more detailed information only for unnatural amino acid derivatives as D-amino acids are generally achieved by simple chiral resolution of racemic amino acids and are generally commercially available.	Protected amino acid derivatives (with terminal and side-chain protection as relevant) are generally acceptable as starting materials in the manufacturing process of synthetic peptides. Nevertheless, a justification on the designation of starting materials needs to be provided. For unnatural amino acid derivatives, more detailed information regarding their manufacture (e.g., precursors and used reagents) and impurity profile is required than for standard L-amino acid or D-amino acid derivatives.	Accepted	Relevant amendments have been made

EFPIA	Specific comment	211	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.		Comment noted	N/A
BCN Peptides	Specific comment	211	214	Same rationale as for lines 204-207	For non commercially available D-amino acid and unnatural amino acid derivatives, more detailed information regarding their manufacture (e.g., precursors and used reagents) and impurity profile is required than for commercially available amino acid derivatives.	Accepted	Relevant amendments have been made
piCHEM Forschungs- und Entwicklungs GmbH	Specific comment	211	214	"For D-amino acids and unnatural amino acid derivatives, more detailed information is required regarding manufacture and impurity profile than forr standard L-amino acid derivatives."" The reason for this higher requirement is not given, nor it is understandable. Also, it is controversial to lines 204-207, where it is stated, that for all starting materials, synthesis chart, reagents and impurity profile should be provided.		Accepted	Relevant amendments have been made
piCHEM Forschungs- und Entwicklungs GmbH	Specific comment	211	214	"In accordance with ICHQ11 a decicion tree is available in annex 1 to serve as a pictoral exemplification to apply all ICHQ11 general principles for the selection and justification of a starting material. No additional requirements are considered to be appropriate as especially in SPPS not related to the Compare also ICHQ11 Q&A: What is the difference between a commercially available chemical and a custom synthesized chemical? (5.6) ICH Q11 states that “a commercially available chemical is usually one that is sold as a commodity in a pre-existing, non-pharmaceutical market in addition to its proposed use as starting material.” A definition of “custom synthesized chemical” was not provided in ICH Q11, but a custom synthesized chemical is generally understood to be one that is made specifically to a drug substance manufacturer’s requirement, either in-house or externally, or available for purchase but where the only use is for pharmaceutical manufacture. The reference to “non-pharmaceutical market” in the ICH Q11 description of commercially available chemicals is intended to preclude purchased intermediates from being claimed as commercially available chemicals. ICH Q11 makes an important distinction between commercially available chemicals and custom synthesized chemicals. An applicant generally need not justify the use of a commercially available chemical as a starting material, whereas a custom synthesized chemical proposed as a starting material should be justified in accordance with the ICH Q11 general principle. "		Accepted	Relevant amendments have been made
BicycleTx Limited	Specific comment	212	213	Lines 204-205 already specify that manufacturing information is required for RSMs, so it is unnecessary to request it again	Delete text: their manufacture (e.g., precursors and used reagents) and	Accepted	Relevant amendments have been made
EFPIA	Specific comment	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		Accepted	The guideline provides some examples of peptide segments, which are not meant to define a limit in terms of the acceptable number of amino acids.
EFPIA	Specific comment	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.		Comment noted	N/A
United States Pharmacopeia l	Specific comment	215	216	Peptide fragment should not be limited to dipeptide only.	We suggest adding tripeptide or tetrapeptide.	Comment noted	The guideline provides some examples of peptide segments, which are not meant to define a limit in terms of the acceptable number of amino acids.

Bachem AG	Specific comment	215	218	With reference to: "In justified cases, short peptide segments such as protected dipeptide building blocks, may be acceptable as starting materials. Examples are dipeptides containing glycine or other dipeptides whose use will reduce the formation of diketopiperazine by-products compared to consecutive couplings of the individual amino acid derivatives." Explanation on the meaning of short peptide segments should be more generally illustrated by referring to tri- and tetra-peptides, as well	In justified cases, short peptide segments such as e.g. protected di-, tri- or tetra-peptide building blocks, may be acceptable as starting materials. Examples are dipeptides containing glycine or other short peptides segments whose use will reduce the formation of diketopiperazine by-products compared to consecutive couplings of the individual amino acid derivatives.	Accepted	The guideline provides some examples of peptide segments, which are not meant to define a limit in terms of the acceptable number of amino acids.
EFPIA	Specific comment	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.		Accepted	Relevant amendments have been made
Mitul kumar Pa	Specific comment	219	221	We request agency to include more clarity for selection of polypeptide segments which do not undergo further modification like cyclisation and usage of such polypeptide segment is for betterment on delete sequence generation due to sterically hindered amino acids coupling and folding due to growing length of peptide chain on the resin.	clarity to be given for selection of polypeptide segments which do not undergo further modification like cyclisation and usage of such polypeptide segment is for betterment on delete sequence generation due to sterically hindered amino acids coupling and folding due to growing length of peptide chain on the resin	Accepted	Relevant amendments have been made
BioNTech SE	Specific comment	222	224	We suggest being more precise regarding peptide segments not undergoing further modifications: We recommend that it is acceptable to consider commercially available protected synthetic peptides of up to six amino acids in length as starting materials.	More complex peptides, such as commercially available protected synthetic peptides of up to six amino acids in length, could be acceptable as starting materials in duly justified cases (e.g. in fragment condensation manufacturing processes). Companies are recommended to go for scientific advice to discuss their proposal well in advance.	Comment noted	The guideline provides some examples of peptide segments, which are not meant to define a limit in terms of the acceptable number of amino acids.
NMEU-Nuclear Medicine Europe	Specific comment	222	224	It is not clear whether a scientific advice on starting materials is recommended in any case or only in cases of selection of non-standard amino acids as SMs. More clarity is needed regarding the definition of complex peptides and which compounds should be regarded as starting materials in such a case.		Comment noted	The relevant text has been amendment to provide further clarity
United States Pharmacopeia l	Specific comment	225	226	In the peptide synthesis, the Assay may not be relevant to the synthesis since they are normally used in excess and the purity is normally very high. Moreover, all other relevant impurities (i.e. water, residual solvents, elemental impurities) have to be part of the proposed specifications if relevant.	It is suggested to revise to "Quality attributes for amino acid derivatives used as starting materials for synthetic peptides generally include: appearance, identification, related impurities (including total impurities), other impurities.".	Comment noted	The text provides sufficient level of flexibility
BCN Peptides	Specific comment	225	230	Usually the quality attributes provided by the Supplier CoA include more parameters than those considered as critical Quality attributes to be included in the specifications applied in-house. In the context of this guidelines, it is important to talk only about the specifications that will appear in the DMF as regulatory specifications for the Starting Materials. When excess of the amino acid materials are used for the coupling reactions, the assay of the material is not a relevant Quality parameter. Water content is not considered as an impurity Elemental impurities and residual solvents do not need to be included in the Quality Control parametres to be monitored on a routine basis. Typically, elemental impurities used in the starting materials are assessed in the risk assessment for elemental impurities. On the other hand, the risk of having any residual solvent used in the manufacture of the starting material in the final API is considered to be negligible given the multiple steps of the process from the incorporation of the starting material until the final API is obtained.	Quality attributes for amino acid derivatives used as starting materials for synthetic peptides generally include: appearance, identification and related impurities. 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. Since the impurities of the amino acid derivatives....	Accepted	Relevant amendments have been made

EUCOPE	Specific comment	225	229	Comment: "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". The suggested quality attributes for 'general' inclusion are probably not meant to be exhaustive and it might be useful to have further clarifications/examples. A suggestion would be to state that the attributes should be assessed on a case-by-case basis and the ones provided in the text are just some examples.		Accepted	Relevant amendments have been made
Granzer Regulatory Consulting & Services GmbH	Specific comment	225	236	Regarding the Quality Attributes for amino acids, it would be useful for this guidance to define these attributes as sub-headings. It is noteworthy that the USP has a guidance document '<1504> Quality Attributes of Starting Materials for the Chemical Synthesis of Therapeutic Peptides' which defines these respective attributes. Ideally, alignment and/or harmonisation with the latter document would be very useful for both industry and the regulatory authorities.		Comment noted	N/A
piCHEM Forschungs- und Entwicklungs GmbH	Specific comment	225	236	It should be discussed that in SPPS the peptide chain is assembled on a solid support, enabling non reactive and non-peptide process related impurities to be washed off. Therefore these impurities are not of major concern and only impurities that might be incorporated as a significant structural fragment like enantiomers need to be controlled. It is not clear to which extent the regulation should be applied for Synthetic peptides in development and used in clinical trials. As in early development the synthesis strategy and thus the starting materials are not defined, this chapter might not be fully applicable to Synthetic peptides used in clinical trials.		Comment noted	Please refer to section 7) Requirements for Clinical trial applications (human products only)
Aspen Oss B.V.	Specific comment	226	226	Appearance is generally not considered a critical quality attribute, but a descriptive property.	delete appearance	Not accepted	Sufficient level of flexibility is provided
United States Pharmacopeial	Specific comment	230	230	Water can be a component of the protected amino acid but not an impurity.	Remove water	Accepted	Relevant amendments have been made
PolyPeptide Group	Specific comment	230	231	It is stated that impurities in the starting materials can react and accumulate in the drug substance. The word accumulate could be interpreted as that the concentration could increase. Rewording is suggested.	Since the impurities of the amino acid derivatives can react like the parent compound during coupling, they can form peptide impurities in the final drug substance,	Accepted	Relevant amendments have been made
United States Pharmacopeial	Specific comment	231	231	"accumulate" could be interpretated as there is no purification step.	It is suggested to revise to "…can have an impact in the impurity profile of the drug substance".	Accepted	Relevant amendments have been made
Bachem AG	Specific comment	233	234	With reference to: "The impurity profiles of the starting materials and their potential impact on the quality of the final drug substance should be investigated during manufacturing process development." The sentence needs to be rephrased to align with lines 206-207	Relevant impurities of the starting materials and their potential impact on the impurity profile of the final drug substance should be investigated during manufacturing process development.	Comment noted	N/A
Bachem AG	Specific comment	234	236	With reference to: "This should include a fate and purge assessment of the impurities that may be formed downstream in the manufacturing process." The sentence is not clear. What exactly are "impurities that may be formed downstream"? The term "downstream" may be misinterpreted in the context of peptide manufacturing. A clarification from EMA side would be highly appreciated, otherwise the sentence should be amended	This should include a fate and purge assessment of the relevant impurities that may be formed along the manufacturing process.	Accepted	Relevant amendments have been made
Regeneron Pharmaceuticals, Inc.	Specific comment	237	241	The guideline should clarify whether supporting information for amino acids of human or animal origin be provided in Section 3.2.A.2. Although the guideline does provide some clarity on Agency expectations, it does not discuss how or whether this information should be presented for review. Agency expectations for the information to be presented in 3.2.A.2, in accordance with the relevant ICH guideline, would ensure alignment across industry for what is needed and how it should be presented in the dossier.	Revise Line 241 to add: Relevant supporting information should be presented in 3.2.A.2 in accordance with ICH M4Q, "Common technical document for the registration of pharmaceuticals for human use- quality Scientific guideline."	Accepted	Relevant amendments have been made
EFPIA	Specific comment	242	243	Comment: Please specify the supportive data for justification of pre-loaded resins as starting materials.		Comment noted	Please refer to ICH Q11
Bachem AG	Specific comment	246	247	WIth reference to: "For solid support resins preloaded with amino acids, quality attributes related to the (chiral) purity and potential impurities of the loaded amino acid derivative are recommended." The sentence needs to be rephrased to align with lines 206-207. The control of chiral purity should be given more general as control of stereo isomers. The quality of the amino acid derivative prior the loading step may also be involved in the control strategy.	For solid support resins preloaded with amino acids, the control of the stereoisomers, if applicable, is recommended, or of the relevant quality attributes of the amino acid derivate prior to loading should be controlled.	Partially accepted	Relevant amendments have been made

EFPIA	Specific comment	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.		Not accepted	No amendment is deemed necessary
Bachem AG	Specific comment	253	256	With reference to: “However, also for starting materials of non-peptide structural moieties (e.g. PEG-chains), compliance with the requirements as laid down in ICHQ11 and its associated Q&A is expected and its selection as starting material should be justified. For instance, sufficient subsequent chemical transformation steps after the starting material should be performed under GMP.” “PEG” is a special case which often concerns a late-stage conjugation and would then be an example for an intermediate, and not a starting material. We therefore suggest using a more typical starting material, e.g. “fatty acid”. We would like to add to the last sentence "and/or purification steps". Non peptidic structural moieties are not always implemented during but often also at the end of the synthesis without further chemical transformations. In these cases, purification steps ensure the quality of the final product.	However, also for starting materials of non-peptide structural moieties (e.g. fatty acid-chains), compliance with the requirements as laid down in ICHQ11 and its associated Q&A is expected and its selection as starting material should be justified. For instance, sufficient subsequent chemical transformation steps, and/or purification steps after the starting material should be performed under GMP.	Partially accepted	Relevant amendments have been made
EFPIA	Specific comment	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.		Not accepted	No amendment is deemed necessary
Medicines for Europe	Specific comment	255	256	Ambiguous statement, not defining number of steps is understood as it follows the ICHQ11 however, examples can be useful. Specifically referring to the conjugated moiety or any chemical transformation in the molecule, e.g.: fatty side-chain added at the last step (intermediate or starting material); multiple amino acids coupled at the side-chain.	Sufficient subsequent chemical transformation steps after the starting material should be performed under GMP (e.g., …)	Not accepted	No amendment is deemed necessary
Bachem AG	Specific comment	258	258	With ref. to: “A list of all other reagents, such as resins, solvents and chromatographic materials used in the manufacturing process of a synthetic peptide should be provided.” For "resins" it should be clarified that “SPPS resins” are meant. Stationary LC phases are typically considered as part of the purification equipment. They are typically controlled via column life cycle as part of the GMP system and therefore should rather be declared in S.2.2 than S.2.3.	A list of all other raw materials, such as unloaded solid support resins, solvents and reagents used in the manufacturing process of a synthetic peptide should be provided.	Accepted	Relevant amendments have been made
EUCOPE	Specific comment	260	260	Editorial: suggest replacing "laid down" with "defined" or "established"		Accepted	Relevant amendements have been made
Bachem AG	Specific comment	262	263	With ref. to: “The solid support resin is a key component of the SPPS process, typical quality attributes of the resin include: appearance, identification, cross-linking, swelling volume, mesh size and loading.” ID testing of an SPPS resin is not meaningful due to limited possibilities at peptide manufacturers. Furthermore, cross-linking and mesh size are typically no quality-relevant parameters in peptide manufacture but rather impact the overall yield. The same is true for the very commonly reported swelling volume. It is therefore recommended to either limit the list to appearance and loading or to leave room for providing the parameters that are indeed relevant in view of drug substance quality.	The solid support resin is a key component of the SPPS process, attributes of the resin may include: appearance, swelling volume and loading.	Accepted	Relevant amendements have been made
United States Pharmacopeial	Specific comment	263	263	It is suggested to change to “include: appearance, identification, cross-linking, and/or swelling volume, mesh size and loading.” to avoid redundance.		Accepted	Relevant amendements have been made
BCN Peptides	Specific comment	263	263	Usually the quality attributes provided by the Supplier CoA include more parameters than those considered as critical Quality attributes to be included in the specifications applied in-house. In the context of this guideline, it is important to talk only about the specifications that will appear in the DMF as regulatory specifications for the solid support. The cross linking is tipically given in the definition of the resin, but not as a parameter in the specifications. So it is better to delete this from the guideline in order to avoid confusions on the fact that the cross linking should be part of the specifications. The cross-linking is assessed by the swelling volume, so we suggest to include only the swelling volume, since it is more representative of the test performed.	The solid support resin is a key component of the SPPS process, typical quality attributes of the resin include: appearance, identification, swelling volume, mesh size and loading.	Accepted	Relevant amendments have been made
Granzer Regulatory Consulting & Services GmbH	Specific comment	264	272	It is stated that: ‘The control of critical steps can be achieved by a combination of analytical tests and process control.’ It would be useful if the level of detail expected for analytical methods and analytical method validation could be outlined.		Comment noted	Not within the scope of the guideline

EFPIA	Specific comment	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.		Comment noted	N/A
EFPIA	Specific comment	268	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).		Comment noted	N/A
PolyPeptide Group	Specific comment	268	270	The sentence states which SPPS steps that "could" be defined as critical. There is no reason to include this sentence, and there is a risk that it is viewed as a requirement to define these exact steps as critical. Criticality shall be determined by the applicant for each unique process (which is already stated above in the same section).	Delete sentence "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."	Not accepted	The listed SPPS critical steps are meant as example and are not intended to be prescriptive.
PolyPeptide Group	Specific comment	270	272	Add a sentence that clarify that coupling reactions can be controlled by well defined PARs and NORs for process paramenters e.g. time and temperature, instead of Kaiser test.	Insert on row 272: "Alternatively, process control may be achieved by using well established PAR and NOR for process parameters."	Comment noted	The text already provides information on it
Bachem AG	Specific comment	270	272	With ref. to: "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." It is true that colour tests are widely used to monitor the absence and/or presence of free amino groups during SPPS. However, recent investigations demonstrated that the colour tests are becoming less and less important. Furthermore, only mentioning the Kaiser test would already exclude a very common amino acid, i.e., proline. We therefore recommend to either omit this paragraph entirely or to reduce it to a more general text to avoid propagation of outdated information. For automated large-scale processes, they are completely omitted.	Commonly, the monitoring of SPPS reactions can be performed with colorimetric tests, e.g., based on the reaction of ninhydrin with primary amines.	Accepted	Relevant amendments have been made
EFPIA	Specific comment	271	271	Comment: Narrow scope - Kaiser test Proposed change: Include other tests and reference to PAT techniques like refractive index measurement.		Accepted	Relevant amendments have been made
United States Pharmacopeial	Specific comment	272	272	Due to the KCN reagent for the Kaiser test, it would be better to include the use of alternative colorimetric methods (Chloranil test and/or TNBS test).	We suggest adding "Other equivalent alternative tests are Chloranil test and TNBS test.".	Accepted	Relevant amendments have been made
Aspen Oss B.V.	Specific comment	272	272	Add monitoring during LPPS	Add sentence: During LPPS, coupling and deprotection steps may be monitored by more quantitative chromatographic techniques.	Comment noted	No amendment is deemed necessary
AstraZeneca	Specific comment	273	273	Single pool purity acceptance criteria are not required. The acceptance criteria should be applied on the pooled fractions only.		Comment noted	The relevant text has been amendment to provide further clarity

EFPIA	Specific comment	273	275	Comment: Narrow scope. Proposed change: Extend to include continuous chromatography approaches with automated side fraction recycling.		Accepted	Relevant amendments have been made
Bachem AG	Specific comment	273	275	With ref. to: "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." The paragraph is not taking into account automated fractioning systems collecting adequate main fractions based, e.g., on conductivity or UV measurements.	During peptide purification by preparative chromatography, fractions are usually combined into a pool of fractions. Main fractions may also be collected by automated fractioning systems, e.g., based on conductivity or UV measurements. The pooling strategy should be defined and appropriate acceptance criteria for purity should be stated, as applicable.	Accepted	The relevant text has been amendment to provide further clarity
EFPIA	Specific comment	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.		Comment noted	The relevant text has been amendment to provide further clarity
United States Pharmacopeial	Specific comment	274	276	The main pool originating from the last purification run is the critical point for the quality definition of the processed peptide, so it should be the only one impacted by the specification setting.	We suggest removing "individual fractions and".	Comment noted	The relevant text has been amendment to provide further clarity
PolyPeptide Group	Specific comment	274	275	It is stated that "..acceptance criteria for the purity of individual fractions and the main pool should be stated". The pooling strategy varies between different processes. It may be relevant to have acceptance criteria only on individual fractions, only on the pool, or possibly both. Now, this sentence requires acceptance criteria on both the individaul fractions and the pool. Please add and/or as indicated in the proposed text to the right.	"The pooling strategy should be defined and acceptance criteria for the purity of individual fractions and/or the main pool should be stated."	Comment noted	The relevant text has been amendment to provide further clarity
NMEU-Nuclear Medicine Europe	Specific comment	274	276	Criteria for individual impurities are not always necessary in early-phase development. More important in such cases is understanding of criticality of specific impurities.	... defined and acceptance criteria for the purity of individual fractions should only be stated based on the criticality of each individual impurity fractions and the main pool should be stated.	Comment noted	The relevant text has been amendment to provide further clarity
EFPIA	Specific comment	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):		Comment noted	N/A
Bachem AG	Specific comment	275	276	With ref. to: "These criteria for purity usually include overall purity and criteria for individual impurities." The pooling strategy should be defined with appropriate acceptance criteria (e.g. purity) on defined process steps (e.g. main pool)	These criteria for purity usually include overall purity and criteria for individual impurities, if applicable.	Comment noted	N/A
Bachem AG	Specific comment	276	278	With ref. to: "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." To be in line with terminology used in line 188	In case re-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.	Accepted	Relevant amendments have been made

PolyPeptide Group	Specific comment	278	279	It is redundant to include the criteria for discarding fractions in the regulatory application. The important criteria to include in the regulatory application is the criteria for accepting fractions/pool.	Delete sentence "It should be stated which fractions are discarded."	Not accepted	No amendment is deemed necessary.
Bachem AG	Specific comment	278	279	With ref. to: “It should be stated which fractions are discarded.” It is understood that fractions outside of potential requirements for side fractions are discarded by default. However, it should always be possible to discard also fractions inside of purity criteria for side fractions, e.g., for commercial reasons. In the former case, information of discarding is self-evident and would be redundant. In the latter case it is irrelevant for a quality description in 3.2.S. It is therefore not considered meaningful to include this information in the dossier.	(text 278-279 deleted, no text)	Not accepted	No amendment is deemed necessary.
EFPIA	Specific comment	279	279	Suggest replacing “filtration and lyophilisation” with "filtration and drying." in order to reflect that other drying methods can also be relevant.		Accepted	Relevant amendments have been made
United States Pharmacopeial	Specific comment	280	281	Intermediate could be used without testing before the next stage if a re-test period is defined based on stability data generated.	We suggest adding “if no stability data are available” at the end of the sentence.	Not accepted	Not within the scope of the guideline
PolyPeptide Group	Specific comment	280	288	Strictly, by the definition of an intermediate in ICH Q7, the SPPS process contains many intermediates (formed during the stepwise SPPS process). These "peptide-on-resin" intermediates are not possible to test before next step in the process. In the final product of SPPS, the peptide-resin, the peptide is chemically bound to the resin and testing is not possible without chemical modification of the intermediate (i.e. cleavage). That is, it is not possible to test intermediates in the SPPS process step. It is, thus, proposed to delete the sentence that all intermediates should be tested. Regarding the sentence "Only intermediates complying with specifications may be pooled"; it is a fundamental GMP requirement that only intermediates that comply are pooled and, thus, this statement is not needed here. A somewhat reworded text is proposed.	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-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. For the crude peptide after cleavage and deprotection, adequate and justified in-process control and/or specifications should be presented. Intermediates during purification are normally controlled in-process as described above. The methods used for in-process control and/or intermediate testing should be described and confirmation of validation provided where applicable.	Accepted	The text has been amended for clarity
Almac Sciences	Specific comment	280	282	An on-resin peptide is also an intermediate, but cannot be analysed unless the peptide is cleaved from the resin. What do the EMA think is acceptable in terms of specification and testing for an on-resin peptide, or indeed is anything formal required?		Comment noted	The text has been amended for clarity
Steve McIntyre/ Almac	Specific comment	280	288	Crude peptides are classified as intermediates and should be tested against a specification. What about the peptide on-resin on completion of the synthesis. We do test this by performing a trial cleave of the on-resin peptide and performing LC-MS to confirm the desired peptide is present and also obtain an estimate of purity - is this approach acceptable	On-resin peptides should be tested via a trial cleave and LCMS to confirm the correct peptide has formed	Comment noted	The text has been amended for clarity
Bachem AG	Specific comment	281	282	With ref. to: “The methods used for in-process control and/or intermediate testing should be described, and confirmation of validation provided where applicable.” To be more precise, “critical” is to be added.	The methods used for critical in-process control and/or intermediate testing should be described, and confirmation of validation provided where applicable.”	Not accepted	No amendment is deemed necessary

EFPIA	Specific comment	285	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".		Not accepted	No amendment is deemed necessary
Bachem AG	Specific comment	285	286	With ref. to: "For all intermediates, justified specifications should be presented. Only intermediates complying with specifications may be pooled." It is acknowledged that intermediate (sub)lots need to comply with adequate criteria. However, IPC criteria are not synonymous with "specifications". Therefore, an adjustment of wording is recommended.	For all intermediates, justified criteria should be presented. Only intermediates complying with these criteria may be pooled.	Not accepted	No amendment is deemed necessary
Aspen Oss B.V.	Specific comment	286	286	We propose to add the following paragraph, which is relevant for both SPPS and LPPS:	Since in peptide chemistry the origin of impurities can be easily derived by anyone skilled in the art to either the quality of the amino acid derivatives or specific steps in the synthesis and since generally no substantial purification from product-related impurities takes place at the intermediate stages, it is not deemed necessary to set specifications for specific impurities in the intermediate stages (for on-resin intermediates this is not even possible), provided that the purging of the relevant impurities is monitored in the final chromatographic purification and controlled by the specifications on the API. For intermediates it is, consequently, preferred to define critical process parameters and associated operating ranges instead, where applicable. It should be demonstrated that the ensuing critical impurities are appropriately controlled at the API level. Critical impurities are those impurities at the (crude) API level, which show low purging in the final chromatographic purification step(s) and/or observed levels close to the limits set for these specific impurities in the final API.	Comment noted	No amendment is deemed necessary.
EFPIA	Specific comment	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.		Comment noted	The text has been amended for clarity
NMEU-Nuclear Medicine Europe	Specific comment	287	288	Crude peptide after cleavage and deprotection may not always be an intermediate and be released. In some cases, an in-process control may be more adequate.	For the crude peptide after cleavage and deprotection adequate and justified IPCs or specifications should be presented depending on the criticality of the step and the form in which the crude peptide is available.	Comment noted	The text has been amended for clarity
Bachem AG	Specific comment	287	288	With ref. to: "For the crude peptide after cleavage and deprotection adequate and justified specifications should be presented." This sentence is considered superfluous as it is covered by the preceding sentence. In addition, please see comment on line 285.	(text 287-288 deleted, no text)	Accepted	The sentence has been deleted.
Aspen Oss B.V.	Specific comment	289	290	We do not deem this sentence of added value, if our previous comments (line 286) are implemented.	Delete sentence.	Not accepted	No amendment is deemed necessary.

Granzer Regulatory Consulting & Services GmbH	Specific comment	292	292	The guidance stipulated in ICH Q7 (Section 12.5) should be cited due to the complex nature of the synthetic manufacturing process.		Comment noted	N/A
NMEU-Nuclear Medicine Europe	Specific comment	292	292	Guideline should clarify at which stage a process validation of peptide precursors for radiopharmaceuticals is expected		Comment noted	Please refer to the Scope of the guideline
BCN Peptides	Specific comment	298	304	The information to be included in this section as per M4Q guideline is not focused on the justification of how the manufacturing conditions are defined. It is focused on significant changes made to the manufacturing process and/or manufacturing site of the drug substance used in producing nonclinical, clinical, scale-up, pilot, and, if available, production scale batches. ” Following the same approach, for products that are existing Drug substances, the information that should be included in this section is the description of the changes performed to the manufacturing process once the maufacturing process is validated.. It is noted that such kind of information on the process development has been never requested during DMF assessment, and is considered within the know how of the company and the development procedures applied by each company. In summary is it not considered relevant for the Quality of the product to explain how each manufacturing conditions is defined.	reagents). Information on the starting material designation, the identification of the critical material attributes and the justification on the control strategy applied along the manufacturing process should be included in this section. Additionally, the changes applied to the manufacturing process after process validation should be described.	Not accepted	It is not deemed necessary to amend this section, as the highlighted aspects are already covered by the guideline on the Chemistry of the Active substances
Aspen Oss B.V.	Specific comment	298	298	If appropriate in-process controls are prescribed, reaction times may generally be skipped. Only critical reaction times need to be included (e.g., if side reactions occur at unacceptable levels in the course of time).	change ‘reaction time’ to ‘critical reaction times’.	Not accepted	Sufficient level of flexibility is ensured
Medicines for Europe	Specific comment	307	308	Not all peptides (depending on the size and nature of amino acid residues) possess the higher order structure (secondary, tertiary, and/or quaternary, aggregates). If the absence of higher order structure is clearly evident from literature data (very small peptide size, literature data for exactly the same compound) and/or the absence is confirmed with analytical data gathered during development, the additional evaluation during characterization should not be required / necessary.	The structure of the peptide should be confirmed by analytical data, this includes the primary, secondary, tertiary, and quaternary structure where relevant. If the absence of higher order structure of peptide is evident from the literature data and the absence is additionally confirmed with the data gathered during development, the additional evaluation is not required during characterization.	Accepted	Relevant amendments have been made
ProPharma	Specific comment	307	308	Please clarify, if correct, that the higher order structures only need to be provided as part of S.3.1 and not as part of S.1.2.		Not accepted	No reference to specific guideline text
EFPIA	Specific comment	308	308	Use for secondary, tertiary and quaternary structure, general and simple terminology such as high-order structure. Lines 308, 339, 646 and 717		Accepted	Relevant amendments have been made
Bachem AG	Specific comment	313	314	With ref. to: Amino acid analysis as described in the Ph. Eur. general chapter 2.2.56 usually complements the characterisation of synthetic peptides. General chapters of the Ph. Eur. are not to be part of a regulatory guideline since they are already described in the Pharmacopeia. Furthermore, the word "usually" should be avoided in this case.	Amino acid analysis may complement the characterisation of synthetic peptides.	Not accepted	Sufficient level of flexibility is provided
EFPIA	Specific comment	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.		Accepted	Relevant amendments have been made
United States Pharmacopeial	Specific comment	315	315	Several studies have demonstrated that the Elemental analysis can yield inaccurate results for small molecules (An International Study Evaluating Elemental Analysis (ACS Cent. Sci. 2022, 8, 855−863)). The application of this analysis in peptide could also give misleading results. Therefore, elemental analysis is not recommended for structure confirmation for peptides, due to the complexity of the structure, the usual high moisture and/or volatile counter-ions content.		Accepted	Relevant amendments have been made

Medicines for Europe	Specific comment	315	315	In our opinion elemental analysis does not unambiguously confirm the peptide structure, since there are usually peptide-related impurities, water, residual / counter ions, and residual solvents also present in the peptide. Other techniques (i.e., different mass techniques), are much more appropriate and reliable for structure confirmation. Therefore, we suggest that the line 315 is omitted (if it is a requirement).	Line 315 should be omitted.	Accepted	Relevant amendments have been made
Medicines for Europe	Specific comment	316	329	While we understand the importance of NMR spectrometry in determining the structure of peptides, we believe that for generic peptides, the requirement "One- and two-dimensional techniques should be used to assign the structure by means of 1H, 13C and 15N NMR data" should not be necessary. It might be a reasonable requirement for synthetic peptides in reference medicinal products, however, we propose that a comparison of fingerprints, as mentioned in Ph. Eur. 2.2.64., should be sufficient to demonstrate the similarity of generic peptide to the synthetic peptide in reference product during sameness studies. In the context of generic peptide manufacturing, the goal is to demonstrate that the generic product is the same as the reference product. The use of different well-established mass techniques (i.e., peptide mapping, MS/MS sequencing) for primary structure determination and circular dichroism spectroscopical methods for higher order structure determination can provide a robust and efficient means of demonstrating this sameness.	NMR spectrometry is described in Ph. Eur. general chapter 2.2.64, 'Peptide Identification by Nuclear Magnetic Resonance Spectrometry.' However, the scope described in that general chapter is qualitative and consists of comparing the NMR spectrum of a test sample with that of a reference sample acquired under identical conditions. Furthermore, the scope is restricted to one-dimensional NMR spectrometry. NMR experiments are recommended to be part of the characterisation studies. One- and two-dimensional techniques should be used to assign the structure by means of 1H, 13C and 15N NMR data where relevant, unless other methods such as mass spectrometry provide conclusive evidence of the chemical structure.	Partly Accepted	Relevant amendments have been made
Regeneron Pharmaceuticals, Inc.	Specific comment	316	322	The guideline should address additional NMR considerations a sponsor may have given the potentially different challenges faced when characterizing synthetic peptides. Two considerations would be (1) the use of alternative nuclei for NMR and (2) the use of semi-quantitative statistical spectral similarity or visual overlay as appropriate. The guideline should note that other nuclei such as Fluorine (F) and Phosphorous (P) are sensitive to NMR. Using NMR for these nuclei may allow innovators to understand better the overall structure of an analyte when a peptide incorporates modified or synthetic amino acids. Therefore, the guideline should allow for sponsors to use NMR alternatives as appropriate when evaluating critical quality attributes (CQAs). In addition, the guideline discusses the limitations of Ph. Eur. 2.2.64 as there are events where the sponsor is unable to obtain assignments for the synthetic peptide construct due to construct size or sequence degeneracy. In such cases, semi-quantitative statistical spectral similarity approaches may be applied to NMR data to demonstrate consistency in peptide quality (ECHOS, Principal Component Analysis). This added clarity to the guideline would help communicate how a sponsor could demonstrate comparability with atomic resolution even when discrete assignments may not be obtainable.	Revise Line 322 to add: "For peptides that contain other NMR visible nuclei (such as F or P), appropriate NMR experiments may be used to verify consistency in nucleus incorporation or other related quality attributes. In addition, for large systems, or for systems where discrete assignments are not obtainable, spectral similarity approaches with data and justification may be used to establish primary, secondary, and tertiary structural comparability ."	Not accepted	Sufficient level of flexibility is provided
Bachem AG	Specific comment	316	329	With ref. to: "NMR spectrometry is described in Ph. Eur. general chapter 2.2.64, 'Peptide Identification by Nuclear Magnetic Resonance Spectrometry.' However, the scope described in that general chapter is qualitative and consists of comparing the NMR spectrum of a test sample with that of a reference sample acquired under identical conditions. Furthermore, the scope is restricted to one-dimensional NMR spectrometry. NMR experiments are recommended to be part of the characterisation studies. One- and two-dimensional techniques should be used to assign the structure by means of 1H, 13C and 15N NMR data where relevant. NMR can be used for • Determination of the number and types of proton nuclei • Determination of the peptide sequence • Identification of amino acids • Assignment of carbon atoms • Assignment of nitrogen atoms • Secondary and tertiary structure elucidation" General chapters of the Ph. Eur. are not to be part of a regulatory guideline since they are already described in the Pharmacopeia. For technical reasons, 15N data is not accessible in the native sequence and marking with 15N is necessary to obtain response, and hence should not be indicated in this section. One- and two-dimensional techniques may be, not should be used, since analysis of peptides exceeding 10-15 residues can be challenging, and structure analysis by NMR may be difficult and inaccurate (EP: Technical Guide for the elaboration of monographs on synthetic peptides and recombinant DNA proteins (2018)). Information with respect to the expected data like MS spectroscopy should be provided. Examples should be deleted like MS is referred in lines 308-312.	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 1H and 13C NMR or other heteronucleii data where relevant. Representative spectra and relevant interpretation should be included.	Accepted	Relevant amendments have been made
EFPIA	Specific comment	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.		Accepted	Relevant amendments have been made
EFPIA	Specific comment	330	334	Comment: narrow scope to chiral gas chromatography. Proposed change: allow alternatives chromatographic techniques.		Accepted	Relevant amendments have been made

United States Pharmacopeial	Specific comment	330	336	Chiral gas chromatography is not the only technique to identify and quantify enantiomers. USP chapter <1503> Quality Attributes of Synthetic Peptide Drug Substances mentions non-routine determination of stereoisomeric purity is possible using chiral AAA. Apart from it, spiking with synthesized diastereomeric analogs and testing by HPLC is a reliable technique. The technique does not involve a degradation step (hydrolysis) and avoids the risk of epimerization.		Accepted	Relevant amendments have been made
BCN Peptides	Specific comment	330	337	The company experience working with chiral gas chromatography allowed to conclude that this technique is not accurate and most of times yield overestimated results that are not confirmed by HPLC (analyzing a reference Standard of the corresponding enantiomer to identify the elution time of the impurity). It is known that authorities often request such test, but in most cases the results obtained are not reliable. This lack of reliability is attributed to the fact that the peptide sample needs to be hydrolized and submitted to further derivatization of the resulting amino acids. This technique could be used to sreen which impurities are potentially to be present, and further investigate them to confirm its presence or not, but should not be considered as a definitive technique..	Chiral gas chromatography (GC) is often used to identify and quantify the enantiomers of the different amino acids after acid hydrolysis. As the hydrolysis is known to induce some level of racemisation, it is carried out in deuterated hydrochloric acid, yielding deuterated amino acids if the racemisation takes place at this stage; deuterated and non-deuterated amino acid residues are detected separately by a mass spectrometric detector placed in tandem with the chiral GC system. However it is noted that the results obtained are often overestimated, and are not accurate. Other more reliable techniques, such as HPLC analysis (using reference standards of the most potential epimer peptide impurities) is recommended. Enantiomeric purity can be controlled by several means during the manufacture of a synthetic peptide. However, it should be justified that it is sufficient to perform the test on enantiomeric purity as a characterisation test and that no routine release control is required.	Accepted	Relevant amendments have been made
Bachem AG	Specific comment	330	334	With ref. to: "Chiral gas chromatography (GC) is often used to identify and quantify the enantiomers of the different amino acids after acid hydrolysis. As the hydrolysis is known to induce some level of racemisation, it is carried out in deuterated hydrochloric acid, yielding deuterated amino acids if the racemisation takes place at this stage; deuterated and non-deuterated amino acid residues are detected separately by a mass spectrometric detector placed in tandem with the chiral GC system." "GC" is not the only techniques applied, so a rephrasing is needed. Likewise other forms of hydrolyses can be used, so we suggest a more general wording. A detailed description of a particular analytical procedure including hydrolysis, measuring technique and evaluation should not be part of a guideline since other analytical techniques may be applied as well. We therefore recommend deleting this section.	Chiral chromatography (e.g. GC) is often used to identify and quantify the enantiomers of the different amino acids usually after suitable hydrolysis.	Accepted	Relevant amendments have been made
Granzer Regulatory Consulting & Services GmbH	Specific comment	330	337	It should be clarified which data are expected for confirming the drug substance has no propensity toward racemisation (refer also to 827-828), i.e., forced degradation and data from accelerated stability studies during development.		Comment noted	Relevant amendments have been made
Regeneron Pharmaceuticals, Inc.	Specific comment	330	337	The guideline should allow for sponsors to obtain supporting data from test methods other than chiral gas chromatography (GC) for the establishment of enantiomer identities and quantities. Furthermore, the guideline should allow for a risk-based justification where GC may be performed as a characterization test, but not required as a release test. The guideline lists chiral GC as the method "often used" to identify and quantify the enantiomers of the different amino acids. However, chiral GC may not be an informative method depending on the substrate nor is chiral GC broadly available in testing labs. Revising the text to explicitly allow for other scientifically valid approaches would provide a benefit to industry and the agency, allowing scientific flexibility while developing robust control strategies for chiral purity.	Revise Lines 335-337: However, it should be justified by means of a risk analysis, that it is sufficient to perform the chiral GC test or an appropriate alternative analytical method to determine enantiomeric purity as a characterization test and that no release control is required.	Accepted	Relevant amendments have been made
piCHEM Forschungs- und Entwicklungs GmbH	Specific comment	334	337	"It should be justified that it is sufficient to perform the Enantiomeric Purity test as characterization only and that no routine release control is required." Enantiomeric Purity is a critical quality attribute that needs to controlled. This possibility should be better elaborated and explaned via examples.		Accepted	Relevant amendments have been made

Aspen Oss B.V.	Specific comment	336	336	add information on justification	….it should be justified e.g. by prior general knowledge on the mechanisms of epimerization during peptide manufacturing processes that …..	Accepted	Relevant amendments have been made
PolyPeptide Group	Specific comment	338	339	UV and IR spectroscopy have limited analytical values for peptide. The techniques does not provide information on secondary structure.	Delete sentence "Additional information on the secondary structure can be gathered from these techniques."	Not accepted	Amendments have been made to allow sufficient level of flexibility
Bachem AG	Specific comment	338	339	With ref. to: "Ultraviolet (UV) and infrared (IR) spectroscopy are part of the standard characterisation programme. Additional information on the secondary structure can be gathered from these techniques." UV and IR spectroscopy can only be optional; it is no standard in case of peptides. The description is misleading since "normal" UV and IR techniques would not provide the expected information, especially on the secondary structure. We therefore recommend rephrasing the section.	Special Ultraviolet (UV) and infrared (IR) spectroscopy techniques may be part of the characterisation programme. Additional information on the secondary structure may be gathered from these techniques.	Accepted	Relevant amendments have been made
PolyPeptide Group	Specific comment	340	347	Higher order structure depends on the solution in which the analysis is run. That is, analysis performed on the drug substance itself in a standard matrix (e.g. water, standard buffer) may not involve the same supramolecular forces as in the formulation. Consequently, the higher order structure may differ. It is proposed that the guideline try to bring more clarity into what characteristics and studies that are relevant for the drug substance and what characteristics that are more relevant to study in the context of drug product formulation.		Comment noted	The applicant should cover these aspects in development studies
Bachem AG	Specific comment	340	347	With ref. to: "Circular dichroism (CD) spectroscopy can be used to determine the absorption, e.g. in the presence of chromophores such as tryptophan, tyrosine, phenylalanine, disulfide bonds and peptide bonds. It measures differences in absorbance between left and right circularly polarized light and hence asymmetric properties of the chromophores. Changes in the structure and hence aromatic environments result in different CD spectra. Near-UV CD spectroscopy determines the tertiary structure due to asymmetric environments of tryptophan, tyrosine, phenylalanine and disulfide. Far UV CD spectroscopy determines the secondary structure due to asymmetric environments of the peptide." A detailed description of the interaction of certain chromophores with polarized light and the evaluation / interpretation should not be part of a guideline. The paragraph needs to be rephrased, shortened and re-focused.	Circular dichroism (CD) spectroscopy can be used to provide information on secondary and tertiary structure elements by the absorption of polarized light.	Accepted	Relevant amendments have been made
EFPIA	Specific comment	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"		Not accepted	N/A
EFPIA	Specific comment	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.		Comment noted	The wording has been amended.
EFPIA	Specific comment	348	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.		Comment noted	The wording has been amended.
Medicines for Europe	Specific comment	348	349	On smaller peptides with no secondary structure the main influence on bioactivity is the primary structure of peptide. Therefore, we propose that biological assay testing during characterization can be omitted on smaller peptides with no secondary structure when appropriate characterisation of primary structure by physicochemical testing can be done and the absence of higher order structure is proven.	Usually, no biological assay is required for the routine release of synthetic peptides. Nevertheless, biological assays can serve as additional tools for the characterisation of synthetic peptides. When the drug substance is a small peptide and the biological activity is dictated only by primary structure, biological assay testing can be omitted during routine testing as well as during characterization. In such cases, the primary structure, physicochemical properties, and the absence of secondary structure must be thoroughly evaluated.	Partially accepted	The wording has been amended.

NMEU-Nuclear Medicine Europe	Specific comment	348	348	To avoid misinterpretation suggest sentence is proposed	No biological assay is required for the routine release of synthetic peptides.	Partially accepted	The wording has been amended.
AstraZeneca	Specific comment	348	348	biological activity is described as a tool for characterisation of synthetic peptides.	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.	Not accepted	The wording has been amended.
ProPharma	Specific comment	348	349	"Usually" seems a bit vague. Could you please provide more specific examples of when biological assays need to be used for routine control purposes? Please refer also to row 493 (S.4.2) and row 645 (Chapter 5) of this form below.		Partially accepted	The wording has been amended.
Bachem AG	Specific comment	348	349	With ref. to: "Usually, no biological assay is required for the routine release of synthetic peptides. Nevertheless, biological assays can serve as additional tools for the characterisation of synthetic peptides." For peptides with higher-order structures, it is generally necessary to confirm its structural fit for intended purpose. This can be done either by biological activity measurement (quantitative, semi-quantitative or qualitative) or by physico-chemical methods.	Usually, no biological assay is required for the routine release of synthetic peptides. Nevertheless, biological assays may serve as additional tools for the characterization of synthetic peptides in cases where the higher order structure cannot be sufficiently characterized by physicochemical tests.	Partially accepted	The wording has been amended.
BCN Peptides	Specific comment	349	349	Biological assays have not been required by authorities for the characterization of APIs. So adding this sentence will provoque a request that has been not an issue up to date. If reference to biological assays is mantained we suggest to include an explanation of the cases where the biological assay may be appropriate.	Usually, no biological assay is required for the routine release of synthetic Peptides, nor for its characterization.	Not accepted	The wording has been amended.
United States Pharmacopeial	Specific comment	350	350	What is considered longer peptides? Need clarification.		Partially accepted	Relevant amendments have been made
Viatris	Specific comment	350	351	The draft guideline states that peptide mapping may be applicable for longer peptides and to consider Ph. Eur. General chapter 2.2.55. Synthetic peptide sequences are generally known and providing the data for peptide sequencing negates the requirement for peptide mapping. However, we request that agency define what is meant by "longer peptides" to address confusion for when to complete peptide mapping.		Partially accepted	Relevant amendments have been made
BCN Peptides	Specific comment	350	351	Definition of longer peptides should be included. Otherwise it may arise questions in cases where it is not relevant. Peptide mapping is aplicable to proteins according to the Ph. Eur monograph 2.2.55.	Peptide mapping may be applicable for longer peptides (e,g: size close to proteins). Ph. Eur. General chapter 2.2.55, 'PeptideMapping', may be considered if needed.	Partially accepted	Relevant amendments have been made
PolyPeptide Group	Specific comment	350	351	Clarify that peptide mapping is redundant when MS/MS data is available.	Peptide mapping can be applicable for longer peptides, where the MS/MS sequencing data may be difficult.	Accepted	Relevant amendments have been made
EFPIA	Specific comment	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.		Partially accepted	Relevant amendments have been made
NMEU-Nuclear Medicine Europe	Specific comment	350	351	Definition of longer peptide should be provided. Previously, the wording ‚complex peptide' has been used in this document. Are these terms equivalent? See comment related to page 3 footnote and comment related to lines 222-224		Partially accepted	Relevant amendments have been made
BCN Peptides	Specific comment	352	353	The formation of aggregates is dependent on many factors includding the API solubility and therefore the solvents used to solubilize the sample may have a big impact on the aggragates formation. This information is relevant for the Drug product and depends on the conditions used for the manufacture of the Drug product. So it is proposed to not include this information in this section, and include it un the impurities section in case it is relevant.	Deletion of the lines	Not accepted	Sufficient level of flexibility is provided

PolyPeptide Group	Specific comment	352	353	Aggregation and fibrillation properties of the drug substance are highly dependent on solution matrix. Such studies should, primarily, be done by the formulator in relevant conditions. It is questionable if a general characterization of the drug substance of such properties has much value. It is proposed that it's clarified in the guideline that aggregation and fibrillation studies shall be performed, primarily, by the formulator and, thus, described in the drug product part of the registration application. Furthermore, it would be useful for Industry if agency could provide more information/insight on aggregation study like potential causes of aggregation formation during manufacturing and storage of active substance and drug product. Different types of aggregates to be studied (covalent, non-covalent, fibrils etc.) and the analytical tools respectively. Also, a discussion and recommendations on control strategy and acceptance criteria would be valuable.	For some peptides, it may be relevant to perform characterization by Thioflavin T (ThT) dye assays and intrinsic tryptophan fluorescence to investigate whether the peptide can form fibrillary aggregates.	Partially accepted	Relevant amendments have been made
Bachem AG	Specific comment	352	353	With ref. to: "Peptides can also be characterized by Thioflavin T (ThT) dye assays and intrinsic tryptophan fluorescence to investigate whether a peptide can form fibrillary aggregates." Description is too specific since ThT is only one out of multiple techniques. An adaptation of the formulation is recommended (see lines 668-670). For very small peptides aggregation is not expected. "if relevant" therefore needs to be added.	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.	Accepted	Relevant amendments have been made
EFPIA	Specific comment	352	352	Comment: Other methods besides Thioflavin T assay test should be considered to investigate aggregation, e.g. fibrillary aggregates.		Accepted	Relevant amendments have been made
Bachem AG	Specific comment	354	354	With ref. to: "Where relevant disulfide bridge confirmation should be part of the characterisation studies." Disulfide bridges need to be characterized. Confirmation may not be possible. A rephrasing is proposed.	Where relevant disulfide bridge characterisation should be part of the studies.	Accepted	Relevant amendments have been made
Bachem AG	Specific comment	355	359	With ref. to: "Evidence of chemical structure ... should be addressed on a case by case basis." The information is partly redundant and partly in contradiction with the text of lines 306-354. Furthermore, there is the risk that some Authorities interpret the table as a list of tests expected to be used, and not as suggestion of tests which may be used.	Delete lines 355-359, including the table	Not accepted	N/A
EFPIA	Specific comment	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.		Not accepted	Relevant amendments have been made to clarify the EU requirements
United States Pharmacopeial	Specific comment	357	357	Table should include other potential techniques for enantiomerical purity testing.		Accepted	Relevant amendments have been made
BCN Peptides	Specific comment	357	357	NMR is commonly used for sequence characterization. For chiral analysis , see the rationale of lines 330-337. Biological activity & Seconday, tertiary and quaternary structures apply to Drug product since it is dependent on the solvent where the formulation is prepared. Drug substance manufacturers not necessary know this conditons. So this information should be part of the Drug product dossier.	Addition of NMR (bidimensional H-NMR and C-NMR) to the AAA sequence confirmation. Enantiomeric purity: replace Chiral GC-MS by HPLC. Deletion of the raws for secondary, tertiary, quaternary structures and biological characterisation.	Partially accepted	Relevant amendments have been made
Granzer Regulatory Consulting & Services GmbH	Specific comment	357	357	The text should clarify that one or more of the analytical techniques can be employed. As per USP '<1503 Quality Attributes of Synthetic Peptide Drug Substances, Table 2', it would we useful to provide comments/useful notes to accompany the respective analytical techniques e.g., why such methods are useful under certain circumstances.		Comment noted	The comment is noted. No amendment has been proposed.
Regeneron Pharmaceuticals, Inc.	Specific comment	357	357	The table of example techniques provides sponsors a sampling of potential assays to use for various structural interrogation, however this table does not include all assays previously addressed in the text of section 4.3.1, such as NMR. The exclusion of NMR in the current table may presume preference to only those assays included. Addition of NMR and other discussed methods to the table would reduce the implication of preference by simply summarizing those techniques previously addressed within the text.	Line 357: Modify the example table to associate all previously discussed techniques, including NMR, to the structural aspects of a peptide, providing a summary table.	Accepted	The table has been amended.
EFPIA	Specific comment	357	357	Example table-> Comment: Inclusion of additional analytical techniques (e.g. enantiomeric purity after partial or complete enzymatic digestion).		Accepted	Relevant amendments have been made

EFPIA	Specific comment	358	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.		Accepted	Relevant amendments have been made
PolyPeptide Group	Specific comment	358	359	A clarification, or example, is needed for when studies of tertiary structure are relevant. See also comment above on lines 352-353.		Accepted	Clarification has been included in the text
AstraZeneca	Specific comment	358	358	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?	change wording of 'quaternary structure / association state' or remove CG-MALS as exemplar technique.	Accepted	Relevant amendments have been made
EFPIA	Specific comment	360	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?		Accepted	Relevant amendments have been made
AstraZeneca	Specific comment	360	360	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?		Accepted	Relevant amendments have been made
NMEU-Nuclear Medicine Europe	Specific comment	360	362	Diagnostic radiopharmaceuticals are not meant to have pharmacological activity, as their use is restricted to a microdosing approach. Therefore, this should not apply to peptides used in diagnostic radiopharmaceuticals. (refer to comment for line 81-83)	….should be discussed (for veterinary products see Investigation of Chiral Active Substances 3CC29a, 362EMEA/CVMP/128/95) and for radiopharmaceuticals it shall not be included.	Comment noted	N/A
Aspen Oss B.V.	Specific comment	360	362	This sentence is related to drug product, not drug substance. It is not clear to us why this sentence is included.	Delete sentence.	Accepted	Relevant amendments have been made
Bachem AG	Specific comment	360	362	With ref. to:"'The relevance of the eventual and possible isomers regarding biological/pharmacological activity should be discussed (for veterinary products see Investigation of Chiral Active Substances 3CC29a, EMEA/CVMP/128/95)." If the peptide consists of different diastereomers, discussion should be part of the characterization study. If diastereomers are not wished, they are part of the impurity profile and not of the characterization study. Discussion on their presence/absence should be part of the justification of specification (section 3.2.S.4.5). The relevance of possible isomers regarding biological/pharmacological activity should not be part of the characterization study.	Delete lines 360-362	Accepted	Relevant amendments have been made
Medicines for Europe	Specific comment	363	367	The morphology examination is usually performed using different microscopic techniques. PXRD and DSC are techniques used for solid state characterization including crystal and amorphous state.	Physicochemical characterisation of the drug substance could include solubility and hygroscopicity studies, determination of the isoelectric point (pI) and thermogravimetric studies. The morphology may be examined by light microscopy while amorphous state may be examined by powder X-ray diffraction (PXRD) and differential scanning calorimetry (DSC).	Accepted	Relevant amendments have been made
BCN Peptides	Specific comment	364	367	The pI is usually calculated theoretically. Up to date its experimental determination has been never considered relevant for the poduct characterization. It is known that lyophilised Peptides are hygroscopic without the need to perform hygroscopicity studies. This kind of studies have been never requested during DMF assessment. If hygroscopicity studies are requested: clarification on the conditions of the study should be indicated. The scanning calorimetry (DSC) does not provide relevant information for Peptides.	Physicochemical characterisation of the drug substance could include solubility and Optical rotation . The morphology may be examined by powder X-ray diffraction (PXRD), or light microscopy.	Accepted	Relevant amendments have been made

Bachem AG	Specific comment	364	367	With ref. to: "Physicochemical characterisation of the drug substance could include solubility and hygroscopicity studies, determination of the isoelectric point (pI) and thermogravimetric studies. The morphology may be examined by powder X-ray diffraction (PXRD), differential scanning calorimetry (DSC) and light microscopy" In most cases, peptides are in an amorphous form. We recommend rephrasing the text to reflect the most relevant techniques for amorphous powders, obtained by lyophilisation. Isoelectric point to be eliminated (see comment on lines 136-137): The determination of the isoelectric point bears uncertainties and may be affected with significant systematic errors, therefore it should not be reflected as general property. Further, isoelectric point is of more importance for proteins than for peptides, especially for their electrophoretic separation. Nevertheless, the isoelectric point influences membrane permeability and thus the absorption of peptides which may be of relevance for designing the composition of certain drug product formulations. Such an information, however, is not relevant for a drug substance.	Physicochemical characterisation of the drug substance could include, e.g. solubility, hygroscopicity or morphology studies. The morphology may be examined by powder X-ray diffraction (PXRD) or other techniques, as relevant.	Accepted	Relevant amendments have been made
PolyPeptide Group	Specific comment	365	365	Clarify that pI can be determined by theoretical calculation	Physicochemical characterisation of the drug substance could include solubility and hygroscopicity studies, determination or calculation of the isoelectric point (pI) and thermogravimetric studies	Accepted	Relevant amendments have been made
piCHEM Forschungs- und Entwicklungs GmbH	Specific comment	365	365	"The morphology may be examined…." Peptides are in most cases amorphous. Contradiction with previous statement in section 4.1.3 (lines 141-142).		Accepted	Relevant amendments have been made
NMEU-Nuclear Medicine Europe	Specific comment	365	367	Morphology characterization is not applicable for radiopharmaceuticals due to the microdosing character of the formulation. Therefore for clarification purposes, add sentence proposed	Add sentence 'Morphology characterization is not applicable for peptides used in radiopharmaceuticals '	Not accepted	No amendment is deemed necessary.
EFPIA	Specific comment	368	368	**Comment:** It should be clarified that identification of impurities is performed at a later stage.		Accepted	Please refer to the scope of the guideline and to section 7) Requirements for Clinical trial applications (human products only)
NMEU-Nuclear Medicine Europe	Specific comment	368	368	In this section it should be clarified that identification of impurities is performed at a later stage. And it must be clarified at which stage this is expected	Add following sentence at line 368: A list of specified impurities shall not be considered necessary before analytical method validation (or equivalent, before Phase III readiness)	Accepted	Please refer to the scope of the guideline and to section 7) Requirements for Clinical trial applications (human products only)
Granzer Regulatory Consulting & Services GmbH	Specific comment	368	447	No reference with regards to the control, the risk considerations and the requirements for nitrosamine impurities that are applicable to synthetic peptide active substances has been discussed and should be included.		Accepted	Relevant amendments have been made (please refer to section 5) Medicinal product Consideration)
Aspen Oss B.V.	Specific comment	382	383	add examples.	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.	Accepted	Relevant amendments have been made
Aspen Oss B.V.	Specific comment	384	384	This is also applicable for LPPS.	..impurities may be incorporated in the sequence during the assembly of the peptide by SPPS and LPPS.	Accepted	Relevant amendments have been made
EUCOPE	Specific comment	388	388	Replace "narrow" with conservative". Narrow is subjective while conservative can be justified by product and process knowledge.		Not accepted	Proposal not agreed as the term "conservative" is not a recognised term for acceptance criteria
EUCOPE	Specific comment	388	388	Acceptance criteria of impurities should be assessed and supported based on knowledge, available data, mfg. capability e.g. purging, clearance, and their impact of downstream product quality, safety. A "narrow" specification which does not provide added quality assurance to the end product is not considered appropriate.		Accepted	Relevant amendments have been made

Granzer Regulatory Consulting & Services GmbH	Specific comment	388	388	It is stated that: 'Narrow acceptance criteria for those impurities should be set for each starting material used in the manufacture of the peptide.'	'Suitably justified acceptance criteria for those impurities should be set for each starting material used in the manufacture of the peptide.'	Accepted	Relevant amendments have been made
AstraZeneca	Specific comment	388	388	Remove 'Narrow acceptance criteria' as terminology not aligned to developing a science & knowledge holistic control strategy.	Consider 'Appropriate and justified criteria for these impurities should be set for each starting material used in the manufacture of the peptide to ensure API of appropriate quality.'	Accepted	Relevant amendments have been made
EFPIA	Specific comment	388	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)…".		Not accepted	Justified acceptance criteria should be provided also for processes different than SPPS
NMEU-Nuclear Medicine Europe	Specific comment	388	389	Acceptance criteria to be determined based on carry over and final peptide control strategy		Accepted	Relevant amendments have been made
Regeneron Pharmaceuticals, Inc.	Specific comment	388	389	The guideline states that narrow impurity acceptance criteria for starting materials used in peptide manufacturing should be set. Without properly qualifying this recommendation of narrow acceptance criteria, differences in definitions between sponsors and the agency may unnecessarily hinder product development. To avoid this, the agency should state that acceptance criteria for starting materials should be as narrow as reasonably justified. By updating this text, the guideline would be taking into account that what is considered narrow acceptance criteria may differ based on the product phase, manufacturing process, and analytical variability associated with monitoring these substances. This clarification will benefit industry and the EMA by clearly stating the criteria proposed be established as supported by the available scientific evidence.	Revise Line 388-389 to read: Appropriately justified acceptance criteria for those impurities should be set for each starting material used in the manufacture of the peptide.	Accepted	Relevant amendments have been made
Aspen Oss B.V.	Specific comment	388	389	add more detailed information	Narrow acceptance criteria by appropriate analytical techniques for those impurities as well as for any other individual impurity should be set for each starting material used in the manufacture of the peptide.	Accepted	Relevant amendments have been made
Bachem AG	Specific comment	388	389	With ref. to: "Narrow acceptance criteria for those impurities should be set for each starting material used in the manufacture of the peptide." Narrowing is one possible, not the only one. We therefore recommend using "suitable" as a more generic wording.	Suitable acceptance criteria for those impurities should be set for each starting material used in the manufacture of the peptide.	Accepted	Relevant amendments have been made
Aspen Oss B.V.	Specific comment	392	392	add more detailed information	…during SPPS, LPPS, fragment condensation, cleavage and other synthetic steps.	Accepted	Relevant amendments have been made
EFPIA	Specific comment	395	395	Lines: 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		Accepted	Relevant amendments have been made
Aspen Oss B.V.	Specific comment	397	397	add more detailed information	However, SPPS, LPPS and fragment condensation conditions…	Accepted	Relevant amendments have been made
Aspen Oss B.V.	Specific comment	398	398	Add additional example	(e.g. Fmoc and Z)	Accepted	Relevant amendments have been made

Aspen Oss B.V.	Specific comment	402	402	add more detailed information	Deletion sequences are peptides with one or several amino acids missing either by incomplete coupling or deprotection reactions.	Accepted	Relevant amendments have been made
Aspen Oss B.V.	Specific comment	404	404	add more detailed information	…acetylation to cap unreacted coupling sites during SPPS.	Accepted	Relevant amendments have been made
Aspen Oss B.V.	Specific comment	405	405	Add additional information for LPPS.	Add sentence: 'During LPPS, the formation of deletion sequences can be generally better controlled, since completion of coupling and deprotection reactions can be quantitatively monitored by chromatographic techniques.'	Comment noted	No amendment is deemed necessary.
Aspen Oss B.V.	Specific comment	408	408	Add more accurate description	…….result of the presence of free amino acid in the coupled protected amino acid, of premature...'.	Accepted	Relevant amendments have been made
EFPIA	Specific comment	417	430	Comment: It is suggested to complete the list of potential degradation pathways by including cyclic imide formation (aspartamide).		Accepted	Relevant amendments have been made
United States Pharmacopeial	Specific comment	420	420	Isomerization could also occur during the process and during storage, especially in basic conditions. It should be included as a bullet point.		Accepted	Relevant amendments have been made
EpiVax	Specific comment	428	429	We agree that aggregation of synthetic peptides may be related to safety issues, particularly in the case of immunogenicity. How do you propose sponsors evaluate the immunogenicity risk associated with aggregation- innate immune cell assays, PBMC assays?	Please specify how you would like sponsors to investigate immunogenic risk (e.g. acceptable methods)	Not accepted	The referred text has been deleted. However, please be informed that the topic may be covered by future work of the Agency.
BCN Peptides	Specific comment	428	429	Aggregation should be investigated in the drug product not in the drug substance, since it may depend on the conditions used for the formulation. Additionally, since aggregation is not alway occurring we consider important to add the clarification that this parameter is not always relevant.	Aggregation may occur for synthetic peptides and could potentially be related to safety issues, including immunogenicity and should therefore be investigated, when relevant.	Not accepted	Aggregation may occur for synthetic peptides and should be investigated.
PolyPeptide Group	Specific comment	428	429	It is agreed that aggregation/multimer formation is a potential safety concern and should be investigated. Covalently bound multimers that may form during manufacture and/or in stability and should be studied as part of the impurity investigation of the drug substance and if relevant, at release and in stability testing. Non-covalently bound aggregates are dependent on the solution matrix and should be studies by the formulator. Reference is made to lines 668-670 where this is already addressed. It is proposed that the guideline explains and defines the different forms of multimer/aggregates that may form and outline when and by whom such forms shall be studied. See also comment above on lines 352-353.		Comment noted	No amendments have been made since too specific.
Aspen Oss B.V.	Specific comment	428	429	Aggregation is primarily an issue for drug product. It is not clear to us why this sentence is included.	Delete the sentence about aggregation.	Partly accepted	Relevant amendments have been made
Bachem AG	Specific comment	428	429	With ref. to: "Aggregation may occur for synthetic peptides and could potentially be related to safety issues, including immunogenicity and should therefore be investigated." For very small peptides aggregation is not expected and thus a more general wording is needed, as proposed.	Aggregation may occur for synthetic peptides and could potentially be related to safety issues, including immunogenicity and should therefore be discussed, if applicable.	Not accepted	The referred text has been deleted.
EUCOPE	Specific comment	430	430	Possible routes of degradation should be discussed in 32S32 together with potential related substances discussions, instead of 32S71.		Accepted	Relevant amendments have been made

EFPIA	Specific comment	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 …		Not accepted	No amendment is deemed necessary.
EFPIA	Specific comment	432	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.).		Partly accepted	Relevant amendments have been made
EUCOPE	Specific comment	432	436	Please provide justification of including method capability, and copies of chromatograms in 32S32 instead of in 32S42. Please clarify the need to report actual impurities detected in the batch samples in 32S32 instead of in 32S44.		Not accepted	If impurities are characterised by methods not used in routine testing, it is mostly appropriate to provide information in S.3.2. No amendment to the guideline text is considered necessary.
Bachem AG	Specific comment	432	434	With ref. to: "Highly specific analytical methods (with appropriate limits of detection (LOD) and limits of quantitation (LOQ) used to detect each of the likely impurities considered above, or other related impurities, the exact identities of which may be unknown, should be described." We suggest rephrasing this section to align the wording with ICH Q2 (R2).	Highly specific analytical methods with appropriate lower range limit used to detect each of the likely impurities considered above, or other related impurities, the exact identities of which may be unknown, should be described.	Accepted	Relevant amendments have been made
NMEU-Nuclear Medicine Europe	Specific comment	432	436	Peptides used for radiopharmaceuticals should be exempted from this, as for the Ph. Eur 2902 applies. (refer to comment for line 81-83) Ph. Eur. 2902 has less strict identification thresholds than Ph. Eur. 2034. (and no qualification thresholds). This has an effect on the analytical method development and validation. This exception for radiopharmaceuticals and related products should be clarified here.	Add sentence: Peptides used in radiopharmaceuticals (including in cold kits) are exempted.	Not accepted	For radiopharmaceuticals, reference to the relevant Guideline (i.e., EMEA/CHMP/QWP/306970/2007) on Radiopharmaceuticals is made. For synthetic peptides used in radiopharmaceuticals or precursors, the synthetic peptides guideline applies only regarding synthesis and starting materials.
Granzer Regulatory Consulting & Services GmbH	Specific comment	437	447	Cleaved protection groups may further react with side chains of sensitive amino acids and thus result in formation of peptide-related by-products (see addressed under `related substances formed during cleavage´ in lines 411-416) or may also result in break-down products (without further reacting with amino acids). Lines 446-447 state that ´… peptide-related impurities are not within the scope of ICH M7 …´. It should be clarified whether peptide-related side products containing protection groups need not be included in the ICH M7 impurity assessment, and whether consideration of direct break-down products of cleaved protection groups which have not further reacted with amino acids (and as such do not represent peptide-related but process-related impurities, but are currently not mentioned in subsection ´Process-related impurities´) are expected to be considered in the ICH M7 assessment.		Comment noted	No amedment is deemed necessary since too specific.
ProPharma	Specific comment	438	439	Please clarify that non-peptide (small molecule) impurity limits are recommended to follow ICH Q3A(R2), Impurities in New Drug Substances guideline.	If not genotoxic, non-peptide (small molecule) impurity limits are recommended to follow ICH Q3A(R2), Impurities in New Drug Substances guideline.	Accepted	Relevant amendments have been made
ProPharma	Specific comment	441	442	Please consider to add the clarification that based on risk assessment outcome, the residual solvent included in the release specification may be limited to solvent(s) used in the final steps of the manufacturing process.	If justified, based on risk assessment outcome, the residual solvent(s) release test may be limited to solvent(s) used in the final steps of the manufacturing process.	Not accepted	Not specific for peptides
Aspen Oss B.V.	Specific comment	441	445	These lines are also applicable for LPPS, not only SPPS.	Add LPPS.	Not accepted	No amendment is deemed necessary.
AstraZeneca	Specific comment	443	444	Genotoxic should be replaced with mutagenic in line with M7 guidance.		Accepted	Relevant amendments have been made
EFPIA	Specific comment	451	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)….'		Accepted	Relevant amendments have been made

EFPIA	Specific comment	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 (while non-stability indicating parameters may be omitted from these studies)."		Not accepted	No amendment is deemed necessary, as covered by more general guidelines.
PolyPeptide Group	Specific comment	451	452	AAA should not be listed as separate analysis, it is one of the potential identification tests as already explained on rows 490-493.	Typical specification tests included as an attribute in the specification are as follows (non-exhaustive list). For the identification of the peptide, at least two orthogonal methods is recommended to be used.	Accepted	Relevant amendments have been made
AstraZeneca	Specific comment	451	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)….'	Accepted	Relevant amendments have been made
ProPharma	Specific comment	451	467	Please consider to include: Residual fluoride, required if hydrofluoric acid (HF) is used during the manufacturing process (e.g., due to tert-butyloxycarbonyl protecting group or tert-butoxycarbonyl protecting group [Boc]-chemistry).	Residual fluoride content.	Accepted	Relevant amendments have been made
BCN Peptides	Specific comment	454	455	Amino acid analysis is considered an identification test. Typically two identification tests are required.	Identification by two suitable tests: (Eg, Amino acid analysis, MS, HPLC, etc)	Comment noted	N/A
Bachem AG	Specific comment	454	454	Examples of identification test should be given	identification test (e.g. RRT, MS, MS/MS)	Not accepted	No amedment is deemed necessary. Examples of identification tests are already provided under section 4.4.2. Analytical procedures
EFPIA	Specific comment	455	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.		Accepted	Relevant amendments have been made
Bachem AG	Specific comment	455	455	Amino acid analysis is considered a characterization test, not a release test and should therefore be deleted from the list. If it is part of a release specification, it is example of an identification test and should not be listed separately.	No text (text of 455 deleted)	Accepted	Relevant amendments have been made
EFPIA	Specific comment	457	458	Replace the specific instrument type 'HPLC' with broader technique 'LC'.		Accepted	Relevant amendments have been made
United States Pharmacopeial	Specific comment	457	457	There could be more techniques for aggregation content other than SEC-HPLC.	We suggest changing to "Aggregates / oligomers by appropriated technique (if relevant)".	Accepted	Relevant amendments have been made
Bachem AG	Specific comment	457	457	The term "high molecular weight impurities" is commonly used and includes all possible types of high molecular weight variants including aggregates and oligomers as well as other product related high molecular weight impurities.	High molecular weight impurities by SEC-HPLC (if relevant)	Accepted	Relevant amendments have been made
AstraZeneca	Specific comment	457	458	Replace the specific instrument type 'HPLC' with broader technique 'LC'.		Accepted	Relevant amendments have been made

United States Pharmacopeial	Specific comment	458	458	Elemental analysis is not reliable for these types of products and should be deleted.		Not accepted	No amendment deemed necessary.
BCN Peptides	Specific comment	458	458	It is important to diferentiate the Net Peptide content (value needed for the manufacture of the drug product) from the assay. It often causes missunderstanding on the value relevant for the manufacture of the drug product.	Net peptide content; e.g. by HPLC or elemental analysis;	Not accepted	No amendment deemed necessary.
Granzer Regulatory Consulting & Services GmbH	Specific comment	458	458	More detailed considerations should be provided on strategy to determine drug substance ´assay/ content´, refer to the below considerations on lines 552-556. Relevant information should be added either under section 4.4.1 or 4.4.5, accordingly.		Not accepted	No duplication of information is deemed necessary.
ProPharma	Specific comment	458	458	Please consider to add more alternative tests: AAA, nitrogen analysis by Kjeldahl, qNMR. The determination of peptide content by elemental analyses can be affected by the following two properties of the APIs: - The counterion constitutes up to x% of the peptide weight. - The peptides can be very hygroscopic of nature.	Assay/content; e.g., by HPLC, elemental analysis, AAA, nitrogen analysis by Kjeldahl, or qNMR.	Accepted	Relevant amendments have been made
Bachem AG	Specific comment	459	459	The current phrasing is misleading and needs to be modified	counter-ion content, e.g. acetic acid, if relevant	Accepted	Relevant amendments have been made
EFPIA	Specific comment	459	459	Lines: 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		Accepted	Relevant amendments have been made
EFPIA	Specific comment	460	460	Recommend removing “TFA content” – it is dependent on the process and reagents used. Suggest replacing with “residual solvents / reagents / acids / bases (when relevant)”.		Accepted	Relevant amendments have been made
NMEU-Nuclear Medicine Europe	Specific comment	460	460	Clarification about TFA limits should be provided either here or elsewhere in this peptide guideline, as TFA is a common solvent in peptide chemistry, has not been classified according to ICH Q3C (R8), and current used limits as usually justified with ‘common practice’		Not accepted	No amendment deemed necessary. No defined limit is currently available, and an ALARP (as low as reasonably possible) approach should be favoured.
Bachem AG	Specific comment	460	460	The current phrasing is misleading. Other residual ions than TFA might be present and thus need to be determined. “Residual” needs to be without any bracket as it should be defined that trace amounts are meant	residual ion content, e.g. TFA	Accepted	Relevant amendments have been made
United States Pharmacopeial	Specific comment	461	461	pH degree is a very uncommon specification for peptide drug substances. pH of the solution is tested for drug products and it depends on the concentration and the solution used. It is not relevant to the peptide manufacture.		Accepted	Relevant amendments have been made
Medicines for Europe	Specific comment	461	461	Peptides as drug substances are mostly lyophilized powders. While we agree that a pH is an important parameter during manufacturing of peptides as drug substances, as well as during manufacturing od peptide drug products, we think it is not a relevant drug substance specification parameter for solid peptides. It should, however, be evaluated during characterization. Therefore, we suggest that this line is either omitted or reworded as proposed.	pH of solution (not relevant for peptides in solid state)	Accepted	Relevant amendments have been made
BCN Peptides	Specific comment	461	461	pH of solution is not includded in any of the Ph. Eur or USP peptide monographs. It is not a parameter considered to be relevant for the quality of the product. It is noted that it has been never requested by authorities during the ASMF assessed.	Deletion of the line	Accepted	Relevant amendments have been made
PolyPeptide Group	Specific comment	461	461	pH of solution should be included in the specification only if there is a specific rationale. It is proposed to remove pH as a typical test on the specification.	Delete "pH of solution"	Accepted	Relevant amendments have been made
EDQM	Specific comment	461	461	pH of solution is not a parameter included in the Ph. Eur. synthetic peptides monographs since it depends on the concentration and the solution used and is more relevant for a medicinal product.	It is proposed to list pH of solution only as a general property and not a specification test.	Accepted	Relevant amendments have been made

piCHEM Forschungs- und Entwicklungs GmbH	Specific comment	461	461	The pH of the solution is irrelevant as a specification. pH depends on the counter ions. As for many peptides no stoichiometric ratio is present, the specification of the pH value does not make sense. The determination of the pH might be described either in the general properties or in characterisation section.		Accepted	Relevant amendments have been made
Bachem AG	Specific comment	461	461	pH of solution is a characterization test, not a release test and should therefore be deleted from the list	No text (text of 461 deleted)	Accepted	Relevant amendments have been made
EFPIA	Specific comment	463	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.		Accepted	Relevant amendments have been made
United States Pharmacopeial	Specific comment	463	463	Mass balance is usually not a release test.		Accepted	Relevant amendments have been made
BCN Peptides	Specific comment	463	463	The assay calculated by HPLC against reference standard as anhydrous and counterion free) is indicative of the mass balance.	mass balance /Assay	Comment noted	N/A
PolyPeptide Group	Specific comment	463	463	The value of the test for mass balance is limited. The components of the drug substance (i.e. peptide, impurities, water, counter ion) are controlled by individual specification criteria. It is proposed to remove Mass balance as a typical test on the specification.	Delete "mass balance"	Accepted	Relevant amendments have been made
EUCOPE	Specific comment	463	463	Please clarify the need for the "mass balance" requirement in 32S41.		Accepted	Mass balance has been deleted
piCHEM Forschungs- und Entwicklungs GmbH	Specific comment	463	463	Recommended specification for mass balance for synthetic peptides is put in question. Requirements should be elaborated in the guideline. Water content and counter ion content, as well as their test accuracy have a significant impact on the mass balance, especially when testing small quantities and/or hygroscopic peptides. Therefore, our opinion is that mass balance calculation does not give additional information about the drug substance. In addition, for peptides presented in units below 1 mg/container, the determination of this parameter is not possible at all.		Accepted	Relevant amendments have been made
Bachem AG	Specific comment	463	463	To avoid any confusion with the notion of “mass balance” used to assess the stability indicating property of a method, we recommend replacing this term by “corrected assay”. The definition and condition of use of corrected assay, aligned with USP <1503>, also needs to be given.	• corrected assay (corrected for water content and counter ion content, as applicable)** ** In case an appropriate qualified reference standard is not available, correct assay is replaced by the sum of peptide content, water and counter ion	Comment noted	Mass balance has been deleted
ProPharma	Specific comment	465	465	Elemental impurities testing may be required based on the risk assessment outcome and in case of intentionally used metal catalyst.	Elemental impurities based on risk assessment outcome (e.g. in case of use of metal catalyst).	Not accepted	It is considered unnecessary to specify
Viatris	Specific comment	467	467	When the draft guidance lists “microbiological purity” for typical specification tests to be included, which test does agency recommend (i.e., is agency requesting microbiological content or microbiological assay)?		Not accepted	No amendment is deemed necessary.
Bachem AG	Specific comment	467	467	The precise terms of the Ph. Eur. should be used for the microbiological tests	Microbiological quality	Not accepted	Reference is made to the terminology used in the Guideline on the Chemistry of Active Substance
Bachem AG	Specific comment	468	470	With ref. to: “The acceptance criteria laid down in the drug substance specification are identical with the limits that apply for stability studies” This is the other way round. The specifications of reference are the release ones. They are valid all along the retest period.	The limits that apply for stability studies are identical with the acceptance criteria laid down in the drug substance specification	Not accepted	No amendment is deemed necessary.

EDQM	Specific comment	470	474	Ph. Eur. monograph "Substances for pharmaceutical use (2034)" excludes chemical precursors for radiopharmaceutical preparations. The definition of monograph 2034 states "This monograph does not apply to chemical precursors for radiopharmaceutical preparations which are the subject of a separate monograph (Chemical precursors for radiopharmaceutical preparations (2902)." In monograph (2902), chemical precursors are defined as non-radioactive substances obtained by chemical synthesis for combination with a radionuclide. For info: in the monograph there is no distinction between non-radioactive substances used in radiopharmaceutical preparations and non-radioactive substances used in kit preparations.	It is proposed that at the end of the text in line 474 the following is added: "In case of peptides to be used in the preparation of radiopharmaceuticals, the general monograph "Chemical precursors for radiopharmaceutical preparations (2902)" applies, peptide-related impurities should be reported above 0.2% and identified above 2.0 per cent."	Not accepted	Please refer to the Guideline on Radiopharmaceuticals. The synthetic peptides guideline applies to peptides used in radiopharmaceuticals only regarding synthesis and starting materials.
NMEU-Nuclear Medicine Europe	Specific comment	472	473	We understand that in the case of synthetic peptides used in radiopharmaceuticals, the EP 2034 does not apply. The EP 2902 is used as reference (cross ref to lines 82-84). different thresholds are mentioned in Ph. Eur. 2092.	Add this sentence: For peptides used for radiopharamceuticals (including kits for radiopharmaceutiacals), EP 2902 is used as reference.	Comment noted	This guideline does not apply with respect to the requirements for peptide-related impurities to be applied for peptides used in radiopharmaceutical preparations. Please refer to the Guideline on Radiopharmaceuticals.
Kazumasa Yoshikawa, Ph.D.	Specific comment	472	474	DRAFT says "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%." Ph. Eur. general monograph 'Substances for Pharmaceutical Use (2034)' describes those thresholds as "of organic impurities in peptides obtained by chemical synthesis". "Organic impurities in peptides" have a wider scope than mere "peptide-related impurities" mentioned in this DRAFT because organic impurities may not always be peptide-related. For example, dicyclohexylcarbodiimide (DCC) used in the chemical synthesis is an organic impurity but not peptide-related. I would like you to adapt the DRAFT sentence so as to be consistent with the Ph. Eur. general monograph.	peptide-related impurities ==> organic impurities	Not accepted	No amendment is deemed necessary.
Bachem AG	Specific comment	476	477	With ref.: "For synthetic peptides, usually acetate is used as counter ion, however, other counter-ions are also possible (e.g. trifluoroacetic acid or TFA)" Acetate is less and less the most common counterion. Trifluoroacetic and TFA are the same. This sentence does not bring any additional information and we therefore suggest deleting it.	No text (text of 476-477 deleted)	Partially accepted	Duplication of reference to TFA is noted. The text has been amended accordingly.
United States Pharmacopeial	Specific comment	477	477	TFA is trifluoroacetic acid.	We suggest replacing TFA with sodium.	Partially accepted	Duplication of reference to TFA is noted. The text has been amended accordingly.
NMEU-Nuclear Medicine Europe	Specific comment	477	477	Clarification about TFA limits should be provided either here or elsewhere in this peptide guideline, as TFA is a common solvent in peptide chemistry, has not been classified according to ICH Q3C (R8), and current used limits as usually justified with 'common practice'		Comment noted.	Following a review of available data on TFA, no limit is currently available, and an ALARP (as low as reasonably possible) approach should be favoured.
Bachem AG	Specific comment	477	478	With ref.: "The type of counter ion should be justified" Justification of counterion is part of development and not of release specs. We propose to replace "justified" by "defined".	The type of counter ion should be defined.	Accepted	Relevant amendments have been made
piCHEM Forschungs- und Entwicklungs GmbH	Specific comment	477	479	"the amount of counter ions should be controlled in the drug substance specification with an upper and a lower limit." In section 4.1.3, lines 143-144 the guideline mentions: "The counter ion needs to be indicated, if relevant, and whether it is present in a stochiometric or non-stochiometric ratio." These two scenarios should be better explained in the guideline. A lower counter ion limit might not applicable, especially when the counter ion is present in the drug substance in a non-stochiometric ratio. Compare also USP <1503> which reads: "Most peptide drug substances contain a counter ion, commonly acetate or chloride. The counter ion acceptance criterion is specific to each drug substance and is usually set based on batch data history." In the USP guideline, there is no requirement for a lower limit of the counter ion. We recommend the requirements in the guidelines to remain harmonized worldwide.		Accepted	Relevant amendments have been made
BCN Peptides	Specific comment	478	478	The lower limit is not always relevant for the quality of the product.	The amount of counter ions should be controlled in the drug substance specification at least with an upper limit.	Accepted	Relevant amendments have been made
EDQM	Specific comment	479	479	In certain cases when the content of a counterion is low, defining only the upper limit may be sufficient. This is also reflected in the Ph. Eur. monographs on synthetic peptides (e.g. Protirelin (1144)).	It is proposed to allow only one limit if justified, for example as follows: "… and the amount of counter ions should be controlled in the drug substance specification with an upper and lower limit, unless otherwise justified".	Accepted	Relevant amendments have been made

AstraZeneca	Specific comment	479	479	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		Comment noted.	N/A
ProPharma	Specific comment	481	481	No test for assay has been discussed under the Analytical Development chapter. Please consider to add: A test for Assay.	A test for Assay (e.g., based on a chromatographic method usually the same as for related substances) using a reference standard should be considered.	Accepted	A section for assay has been included.
Granzer Regulatory Consulting & Services GmbH	Specific comment	481	488	Reference to ICH Q2(R2) and ICH Q14 should be included. It should also be indicated as to where this information on method development should be included in the dossier.		Partly accepted	Relevant amendments have been made
Bachem AG	Specific comment	486	488	With ref.: "The development of analytical procedures to control the quality of peptides, specifically the identity and the peptide-related impurities, could be a challenge due to the complexity of the structure of these molecules and the risk of co-eluting impurities" This sentence does not contribute to the analytical procedure section. We recommend deleting it	No text (text of 486-488 deleted)	Not accepted	No amendment is deemed necessary.
Bachem AG	Specific comment	491	493	With ref: "…use of at least two orthogonal methods is recommended. Identification by mass, relative retention time (RRT), LC-MS, LC-MS/MS, peptide mapping, bioactivity, amino acid analysis or NMR are considered appropriate" We recommend to re-focus on the most prominent examples. Furthermore, there is the risk that some Authorities interpret the examples as a list of tests expected to be used, and not as suggestion of tests which may be used.	…use of at least two orthogonal methods is recommended (e.g. Identification by mass, relative retention time (RRT), MS/MS)	Not accepted	No amendment is deemed necessary.
ProPharma	Specific comment	492	493	Please consider to add examples of more alternative identification tests, for instance: • enantiomeric purity (preferably Chiral chromatography compared to optical rotation), • N-Terminal sequence analysis (Edman), • higher order structures (CD, NMR and FTIR).		Not accepted	No amendment is deemed necessary.
ProPharma	Specific comment	493	493	Please clarify that bioactivity is no longer a routine identification test for most peptides; but may be required for large peptides or those with complex sequences. As indicated in: - ICH Q6B guidelines (e.g., scope), synthetic (poly)peptides do fall under chemical substances. - EDQM technical guide: Synthetic peptides may be considered to differ from products of recombinant DNA technology in two structural aspects: • they are usually small, typically below 5000 Da; • they may have chemical structures that do not occur naturally in proteins or peptides. As a consequence, they can generally be sufficiently characterised by a battery of physico-chemical tests.		Partly accepted	No amendment is deemed necessary.
EFPIA	Specific comment	495	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.		Comment noted	No amendment is deemed necessary.
EDQM	Specific comment	498	500	In relation to the comment on lines 470-474, two different cases are to be distinguished.	It is proposed that the statement in lines 498-500 reads: "At least, the analytical methods used for the control of purity should be suitable to fulfil the requirement for the Ph. Eur. reporting threshold of 0.1% for synthetic peptides (0.2% for synthetic peptides used in the preparation of radiopharmaceuticals).	Comment noted	This guideline does not apply with respect to the requirements for peptide-related impurities to be applied for peptides used in radiopharmaceutical preparations. Please refer to the Guideline on Radiopharmaceuticals.
NMEU-Nuclear Medicine Europe	Specific comment	499	499	Refer to comment for lines 472-473		Accepted	Relevant amendments have been made

EFPIA	Specific comment	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.		Not accepted	No clear proposal to amend the text has been received and no amendment is deemed necessary.
Bachem AG	Specific comment	501	502	With ref.: "If one analytical method for detection and quantification of all the peptide-related impurities is not appropriate to separate all peaks, additional independent method(s) may be needed" More accurate wording is recommended	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"	Accepted	Relevant amendments have been made
ProPharma	Specific comment	501	503	Please consider to add: Care should be given to the quantification of co-eluting impurities – it can't be assumed that each impurity has the same response factor as the API. The following variables should be taken into account: different response factors, different ionization efficiencies due to different structural features, and ion suppression by the API.	Care should be given to the quantification of co-eluting impurities and the following variables should be taken into account: different response factors, different ionization efficiencies due to different structural features, and ion suppression by the API.	Not accepted	No amendment is deemed necessary since not specific to synthetic peptides.
EFPIA	Specific comment	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."		Partially accepted	The comment is acknowledged. The text has been revised to clarify that when co-eluting impurities are observed as one peak the qualification threshold of 1.0% applies unless otherwise justfified.
AstraZeneca	Specific comment	502	544	Line 502 Highlights to separate 'all peaks'. Line 544 Indicates grouping of peaks not recommended unless justified and based on demonstrated analytic effort.	It is not always feasible to separate all peaks and it is not clear what is the threshold for effort required to justify not resolving these?	Partially accepted	Same as above.
EFPIA	Specific comment	505	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.		Comment noted.	Sufficient level of flexibility is provided. No additional amendment is considered necessary.
BCN Peptides	Specific comment	505	505	Diastereomers do not need chiral methods since they are detectable by reverse phase chromatography.	Deletion of the line	Not accepted	Deletion of the sentence is not accepted, however the sentence was amended.
ProPharma	Specific comment	505	505	Please consider to add that Enantiomeric purity should preferably be performed using a Chiral chromatography method compared to optical rotation*. * The appropriateness of optical rotation as a "chiral" purity test is very doubtful (J. Pept. Sci. 2009; 15: 697–710), it is therefore expected that diastereoisomeric compounds are separated and detected by for instance an UPLC-UV method (especially if not properly controlled during the synthesis).		Not accepted	Not relevant as optical rotation is not mentioned in the text.
Bachem AG	Specific comment	505	505	With ref.: "Control of diastereomers may require the development of specific chiral methods." Diastereoisomers are usually determined by non-chiral methods (e.g. ordinary reverse-phase methods). These methods are often -specifically developed to address control of specific diastereomers.	Control of diastereomers may require the development of specific methods.	Accepted	The proposed amendment has been included.
ProPharma	Specific comment	507	509	Please clarify that the detailed description of the Identification test is expected to include for the system type and its characteristics e.g. resolution, including SSTs.		Not accepted	The level of details requested is not considered necessary for a guideline on synthetic peptides.
Bachem AG	Specific comment	507	509	With ref.: "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." This sentence does not fit with the header in line 506 (Changes of analytical methods during development) nor in line 481 (Analytical development), and we recommend deleting it or moving it to between line 480 and 481.	No text (text in rows 486-488 deleted)	Accepted	It was agreed to move the sentence before Analytical development heading

EFPIA	Specific comment	513	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.		Comment noted	The comment is noted, however the paragraph has been deleted since no additional requirement than for other chemical active substances is foreseen.
BCN Peptides	Specific comment	513	514	Analytical methods used in initial phases of the development are often improved at later stages. So the validation is relevant once the method is implemented according to the Quality control applied in the DMF Registration.	The analytical procedures used for the control of the drug substance, should be fully validated	Comment noted	Same as above.
PolyPeptide Group	Specific comment	513	513	Analytical procedures used during development of the peptide are normally not fully validated, refer to EMA guideline EMA/CHMP/QVP/545525/2017rev2 och ICH Q14.	The analytical procedures used for the control of the drug substance, should be fully validated.	Comment noted	N/A
piCHEM Forschungs- und Entwicklungs GmbH	Specific comment	513	514	"The analytical procedures… including the analytical procedures used during the development of the peptide should be fully validated." Validation of analytical methods during the development phase of the product is not an ICH requirement. During development, the methods as well as the specification limits are subject to changes (please compare also ICHQ7, chapter 19 which reads: "19.80 While analytical methods performed to evaluate a batch of API for clinical trials may not yet be validated, they should be scientifically sound.)"		Comment noted	N/A
Bachem AG	Specific comment	513	514	With ref.: "The analytical procedures used for the control of the drug substance, including the analytical procedures used during the development of the peptide, should be fully validated." Analytical methods used during development may still be under development as well and may not yet be fully validated according to ICH. The retrospective statement on "during development of the peptide" should be removed.	The analytical procedures used for the control of the drug substance should be fully validated.	Comment noted	N/A
EUCOPE	Specific comment	514	514	Replace "fully validated" with "validated in a phase appropriate manner". This is because the knowledge of the analytical procedure commensurate with development stage, and the parameters evaluated in the validation exercise differs at different stages of development.		Comment noted	N/A
BCN Peptides	Specific comment	522	522	Typically CoA of 3 batches are acceptable. Along the life of the dossier múltiple batches may be referred (for exemple 3 validation batches +additional revalidation batches + bathes used for determination of impurities, or fate and purge of reagents, or additional stability data, etc). So the request to include all batches in this section is considered excessive.	This section should summarize the batch analysis data for representative peptide batches .	Comment noted.	The relevant text has been deleted since the previously mentioned requirements are not different from those as stated in the guideline on the Chemistry of Active Substances.
Granzer Regulatory Consulting & Services GmbH	Specific comment	522	523	The requirement to provide all batch analysis data (including early development batches) in the section S.4.4 is considered not to be in line with eCTD.	This section should summarize the batch analysis data for all the peptide batches that support the proposed commercial process and specification presented in the section S.4.1. Further batch results (e.g. applicable for nonclinical program, earlier development) which may also not have been obtained based on the final commercial methods & specifications should commonly be provided in S.2.6 / S.4.5 / S.7 (and not necessarily for all but only for selected, relevant tests).	Comment noted	The relevant text has been deleted since the previously mentioned requirements are not different from those as stated in the guideline on the Chemistry of Active Substances.
piCHEM Forschungs- und Entwicklungs GmbH	Specific comment	522	530	There is a clear guideline on chemistry of active substances (EMA/454576/2016) which should be referred to and no separate regulation which might be interpreted differently should be introduced. Development batches should be discussed in the development section of a dossier. Only representative batches which were produced according to the process described in section 2.2 should be listed in section 4.4. Batches produced in development should be presented in section 2.6.		Comment noted	The relevant text has been deleted since the previously mentioned requirements are not different from those as stated in the guideline on the Chemistry of Active Substances.
Bachem AG	Specific comment	528	530	With ref: "Usually, early development batches are tested using a slightly different specification. The differences in the results obtained in the batches used in earlier development and pilot/commercial batches should be explained and justified" This section appears to be referring to clinical trial applications. We therefore recommend moving these 2 sentences to between lines 824 and 825.	No text (text in rows 486-488 deleted, to be moved to between rows 824 and 825)	Comment noted	As per above, the relevant text has been deleted.

Bachem AG	Specific comment	530	531	With ref.: "The improvement in the analytical methods during development of the peptide could lead to the observation of new impurities in pilot/commercial batches." No "new" impurities appear because an improvement of the analytical method, either by better peak separation or improved sensitivity. We recommend changing the wording	The improvement in the analytical methods during development of the peptide could lead to newly observed impurities in pilot/commercial batches.	Accepted	The relevant text has been amended.
BCN Peptides	Specific comment	531	534	The information on which batches are used for clínica/preclinical batches is owned by the Drug product MAH, and not by the Drug substance manufacturer. So this information should be part of the drug product dossier and not part of the Drug substance dossier.	Deletion of the lines	Not accepted	Clarification has been included in the text.
BCN Peptides	Specific comment	536	537	The information on which batches are used for clinical/preclinical batches is owned by the Drug product MAH, and not by the Drug substance manufacturer. So this information should be part of the drug product dossier and not part of the Drug substance dossier.	The proposed specification should be supported by representative batch data combined with an adequate understanding of the manufacturing process of the peptide	Not accepted	Clarification has been included in the text.
AstraZeneca	Specific comment	536	536	Lines 536 and 541-3 Over emphasis on using batch data to justify specification acceptance criteria.	EFPIA paper: https://www.vaccineseurope.eu/wp-content/uploads/2023/06/efpia-ve-position-paper-on-ich-q6-specifications_5june2023.pdf	Comment noted	General requirements for setting specification are provided in the guideline. The applicant can justify any different approach.
Medicines for Europe	Specific comment	538	541	The lines state that impurities above Ph. Eur. qualification threshold should be qualified, however they do not state which qualification studies are required. We propose that ICH Q3A/B qualification strategy (systemic toxicological evaluation) is applied.	In case that the limit for identified or unidentified impurities is above the prescribed Ph. Eur. qualification threshold, qualification of these impurities is expected based on the principles of ICH Q3A/B guidelines.	Not accepted	No amendment is need as already covered by relevant guidance.
EDQM	Specific comment	538	539	In relation to the comment on lines 470-474, two different cases are to be distinguished.	It is proposed that the statement in lines 538-539 reads: "The limits applied for peptide-related impurities should be based on the general monographs of the European Pharmacopoeia "Substances for pharmaceutical use (2034). In case of peptides used for the preparation of a radiopharmaceutical preparations, the limits applied for peptide-related impurities should be based on the general monographs of the European Pharmacopoeia "Chemical precursors for radiopharmaceutical preparations (2902)."	Comment noted.	With respect to the limits for peptide-related impurities to be applied for peptides used in radiopharmaceutical preparations, please refer to the Guideline on Radiopharmaceuticals.
NMEU-Nuclear Medicine Europe	Specific comment	539	539	Refer to comment for lines 472-473		Comment noted	As per above comment, please refer to the Guideline on Radiopharmaceuticals.
EFPIA	Specific comment	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.		Not accepted	Ph. Eur. provides the limits for peptide-related impurities, which should be applied. For impurities above these limits, qualification studies are needed.
ProPharma	Specific comment	541	543	Please consider to change wording into: The acceptance criteria are best justified based on available synthesis performance/process capability, batch and stability data. Any widening of assay limits during storage should be justified by a corresponding increase in qualified degradation products.		Not accepted	No amendment is deemed necessary.

EFPIA	Specific comment	552	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.	Nevertheless, the acceptance criteria for the pep	Comment noted	No amendment is deemed necessary.
PolyPeptide Group	Specific comment	552	556	For peptides, there is often confusion around the terms assay, content, counter-ion free, anhydrous substance and mass balance. It is proposed that definitions are clarified in this guideline and that terminology is stringent. In this guideline section, the term "counter-ion free, anhydrous substance" is used which probably means the same thing as mass balance on line 463. On line 458 the terms assay / content are used. It is proposed that the term assay is used and that it is defined as the net peptide content, i.e. the relative amount of peptide in the drug substance.	The assay and how it is calculated should be clearly defined. The assay is typically expressed as the net peptide content corrected for total impurities with a lower specification acceptance criterion only, e..g. Assay [peptide name] ≥ 85%.	Not accepted	The mentioned text has been re-worded and provides sufficient level of clarity.
Bachem AG	Specific comment	552	553	With ref: "The assay and how it is calculated should be clearly defined. The assay limits are typically expressed in terms of the counter-ion free, anhydrous substance, unless otherwise justified." Assay limits other than "counter-ion free, anhydrous" can be applicable without the need of further justification, e.g., in case of tests based on elemental analysis. To avoid misunderstandings, the text should be slightly modified.	The assay and how it is calculated should be clearly defined. Limits of assays determined by HPLC are typically expressed in terms of the counter-ion free, anhydrous substance, unless otherwise justified.	Accepted	Relevant amendments have been made
Granzer Regulatory Consulting & Services GmbH	Specific comment	552	556	The current wording and proposed calculation of assay limits suggests that the assay value shall be expressed referring to the anhydrous, counter-ion free and purity-corrected substance. However, here, as well as in section 4.4.1 or 4.4.2, there is no guidance provided on differentiation between possible assay and content determination strategies (line 458 states only the example of ´assay/content; e.g. by HPLC or elemental analysis; see also comment above). As per USP <1503 Quality Attributes of Synthetic Peptide Drug Substances>, section ´Assay and Peptide Content', it would we useful to indicate which analytical principles may be applied for assay and for content assessment. It would also be helpful to provide guidance whether an assay determination which is based on an absolute content determination method in combination with water content, counter-ion content and chromatographic purity test(s) rather than an assay method by quantitative HPLC may be acceptable.		Comment noted	Relevant amendments have been made
United States Pharmacopeia l	Specific comment	554	554	Explanation of "permitted assay reproducibility" is needed. For example, permitted assay reproducibility should be set according to the results of the assay method validation.		Comment noted	Such level of details is not considered needed as not specific to synthetic peptides
EFPIA	Specific comment	557	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".		Comment noted	The relevant text has been revised.
BCN Peptides	Specific comment	557	557	The biological assay is only requested in peptide monographs where the product is obtained by recombinant technology, but it is not requested for synthetic Peptides. So, if reference to biological assay is kept in the guideline it is requested to clarify which are the cases where the biological assay would be recommended. It is noted that biological assay has been never requested (by competent authorities) to be included in specifications for the synthetic Peptides manufactured by the company.	Delete this line	Comment noted	The relevant text has been revised.
PolyPeptide Group	Specific comment	557	557	It is stated on line 348 "Usually, no biological assay is required for the routine release of synthetic peptides". On line 557 it is then stated that "The absence of a biological assay should be justified" which seems to contradict the earlier statement. Also it is unclear how such a justification could be expressed other than in very general words.	Delete sentence "The absence of a biological assay should be justified."	Accepted	The sentence has been deleted and the text revised.
BicycleTx Limited	Specific comment	557	557	The proposed edit is in line with ICH Q6B—Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products, which applies to proteins, allows bioassays to be replaced by physicochemical tests when a well-established manufacturing history exists and when "sufficient physicochemical information about the drug, including higher-order structure, can be thoroughly established by such physicochemical methods, and relevant correlation to biologic activity demonstrated". Synthetic peptides are not subject to the inherent variability of biological active substances and can be expected to be routinely fully characterised by physicochemical methods.	Delete: The absence of a biological assay should be justified. Add a sentence: Biological assays are not typically required for synthetic peptides but may be required if structure cannot be thoroughly established by physicochemical methods.	Partially accepted	The sentence has been deleted and the text revised.

Granzer Regulatory Consulting & Services GmbH	Specific comment	557	557	The considerations provided on the drug product under lines 662-667 are also relevant for the decision & justification whether a potency test is deemed necessary or may be deleted for drug substance testing. It is therefore recommended to add corresponding wording (or a cross-reference to these Drug Product passages).		Comment noted	The relevant text has been revised.
Bachem AG	Specific comment	557	557	With ref: "The absence of a biological assay should be justified." We see a discrepancy with "no biological assay is required for the routine release of synthetic peptides (348-349)". It is indicated in the draft guideline that the test is usually not needed, so we do not see the reason for justifying its absence. We therefore recommend deleting this sentence.	No text (text of 557 deleted)	Accepted	The relevant text has been revised.
EFPIA	Specific comment	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.		Accepted	The relevant text has been revised.
Bachem AG	Specific comment	561	562	With ref: "The origin of the reference standards should be briefly indicated (e.g. batch synthesised according to the commercial process)." The reference standard does not need to be synthesised according to the commercial process. It should be appropriately prepared, identified, tested for the intended use. Indicating even with e.g. something else would go beyond any International regulatory and GMP requirement and should therefore be deleted.	The origin of the reference standards should be briefly indicated	Accepted	Relevant amendments have been made
PolyPeptide Group	Specific comment	571	573	The section state suggest that desiccant or inert atmosphere should be used for hygroscopic powders. PolyPeptide's normal storage condition, as proven effective for peptides manufactured for many years, is under air without desiccant. It is suggested that the wording of the section is changed to reflect the different options.	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.	Accepted	Relevant amendments have been made
Bachem AG	Specific comment	571	573	With ref.: The container closure system should be suitable, considering the substance properties, storage conditions and use: e.g. for hygroscopic powders, appropriate desiccant should be included. Alternatively, storage under inert atmosphere could be considered. No example is required in this section. The fact that the container closure system should be suitable considering the substance properties, storage conditions and use gives enough directions.	The container closure system should be suitable, considering the substance properties, storage conditions and use.	Accepted	Relevant amendments have been made
Aspen Oss B.V.	Specific comment	572	572	The use of a desiccant is an example for appropriate measures to take for storage of hygroscopic powders.	….for hygroscopic powders, appropriate measure should be taken, e.g. include desiccant.	Accepted	Relevant amendments have been made
AstraZeneca	Specific comment	572	572	Use of desiccants and packaging under inert atmosphere in packaging of peptide APIs are not common, suggest these are removed		Accepted	Relevant amendments have been made
EFPIA	Specific comment	575	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.		Not accepted	It is considered that the topic is currently still premature to be included in this guideline that is specific for synthetic peptides.
EUCOPE	Specific comment	581	581	Editorial: replace "freeze" with "frozen".		Accepted	Relevant amendments have been made

Bachem AG	Specific comment	581	584	With ref.: "…but the use of higher temperatures/humidities are also expected not only to address short term excursions, but to obtain a comprehensive overview of the degradation pathways of the drug substance; these data might be especially important for the development of the drug product." The thermo-degradation is evaluated as part of the forced degradation study, as mentioned in lines 587-588. We therefore suggest deleting this sentence from the long-term and accelerated conditions section	No text (text of 581-584 deleted)	Partially accepted	The relevant text has been amended.
EFPIA	Specific comment	585	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.		Comment noted	The comment is noted, however reference to forced degradation studies could be part of analytical procedures or stability studies.
BCN Peptides	Specific comment	585	586	Degradation pathway is usually includded in the impurities section	The potential degradation pathways of the peptide should be discussed (in this section or in section 3.2.S.3.2) taking into account the amino acids composition and sequence: e.g. oxidation of Cys and Met residues, deamidation, hydrolysis, β- Asp-containing sequences .	Accepted	Please refer to the impurity section and revised text regarding possible routes of degradation
Bachem AG	Specific comment	585	587	With ref.: "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." To avoid scattered information on potential and actual impurities in a peptide drug substance, it is recommended to allow for the possibility to refer to section 3.2.S.3.2 here.	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. If discussed in 3.2.S.3.2 instead, a reference to the relevant part of the impurity discussion is acceptable.	Accepted	Please refer to the impurity section and revised text regarding possible routes of degradation
Aspen Oss B.V.	Specific comment	586	586	Add examples	…e.g. oxidation of Cys and Met residues, deamidation, acetylation, disulfide exchange, hydrolysis, β-Asp-containing sequences.	Accepted	The list of pathways contributing to the formation of degradation products in section 4.3.2. has been amended accordingly.
EFPIA	Specific comment	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.		Comment noted	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.
EpiVax	Specific comment	590	591	We agree that aggregation of synthetic peptides may be related to safety issues, particularly in the case of immunogenicity. How do you propose sponsors evaluate the immunogenicity risk associated with aggregation- innate immune cell assays, PBMC assays?	Please clarify how sponsors should investigate the immunogenic risk associated with peptide aggregates	Comment noted	The referred text is no longer relevant due to deletion.
PolyPeptide Group	Specific comment	590	591	Please refer to the comment on line 428-429.		Comment noted	No amendments have been made since too specific.
BicycleTx Limited	Specific comment	590	591	Synthetic peptides active substances are typically lyophilised so cannot evaluate aggregation on stability. It may also be more appropriate to evaluate aggregation on the finished product.	Add: Evaluation of aggregation may not be relevant for synthetic peptides where the final active substance is a lyophilised powder or where aggregation is investigated as part of the drug product stability.	Not accepted	No amendment is deemed necessary
AstraZeneca	Specific comment	590	590	what is expected by the term "aggregation" on stability – oligomers, particles, HMWs?		Comment noted	The text has been amended for clarity
Bachem AG	Specific comment	590	591	With ref.: "Aggregation may also occur for synthetic peptides and could potentially be related to safety issues, including immunogenicity and should therefore be investigated during stability." To avoid the misunderstanding that aggregation should also be investigated during stability for peptides that are not prone to aggregation, the paragraph should be slightly amended.	Aggregation may also occur for synthetic peptides and could potentially be related to safety issues, including immunogenicity. For peptides where a tendency to aggregate is known or suspected, it should therefore be investigated during stability.	Not accepted	Aggregation should be investigated irrespective of prior knowledge.

EFPIA	Specific comment	599	602	This statement is not specific to synthetic peptides. Recommend considering replacing with a reference to existing guideline.		Accepted	Relevant amendments have been made
EFPIA	Specific comment	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.		Not accepted	No amendment is deemed necessary.
BCN Peptides	Specific comment	603	644	Conjugated products are out of the scope of this guideline.	Deletion of all this section	Not accepted	N/A
NMEU-Nuclear Medicine Europe	Specific comment	603	644	It is not clear whether conjugation with a metal-free chelator to form the precursor for radiolabeling is in scope of this chapter. -NMEU expresses concerns regarding to definition of API Starting Material, and it would apply in this case	Add following sentence to line 607: Peptide conjugates that include organic modifications up to 30% of the total peptide mass will be considered peptides according to the definition of this guideline.	Not accepted	No amendment is deemed necessary.
EUCOPE	Specific comment	604	644	It is not clear if peptide conjugated oligonucleotide products would fall under this guidance or the proposed Oligonucleotide draft guidance (not yet released).		Not accepted	Currently experience is too limited to include specific text.
Bachem AG	Specific comment	604	612	With ref.: "Conjugation has emerged as a popular mechanism to alter or enhance the properties of peptide drug candidates. Conjugation to poly(ethylene glycol) (PEG), lipids and proteins has been used as a half-life extension strategy. Conjugation can also be used to deliver a cytotoxic payload or imaging agent to specific cell types targeted by the peptide. However, there is added complexity with respect to the characterisation and control of these conjugates. The control of the unconjugated peptide which is usually classified as an intermediate is essential. Adequate specifications and control methods should be established for these intermediates. In cases where no intermediate is isolated these approaches should be justified and an adequate control strategy should be developed (see also 4.2.3)." Everything indicated in this section is only applicable for late-stage conjugation, and thus needs to be mentioned	Add between 607 and 608: Conjugation has emerged as a popular mechanism to alter or enhance the properties of peptide drug candidates. Conjugation to poly(ethylene glycol) (PEG), lipids and proteins has been used as a half-life extension strategy. Conjugation can also be used to deliver a cytotoxic payload or imaging agent to specific cell types targeted by the peptide. Further considerations need to be made in case of the late-stage conjugation. In such cases, there is added complexity with respect to the characterisation and control of these conjugates. The control of the unconjugated peptide which is usually classified as an intermediate is essential. Adequate specifications and control methods should be established for these intermediates. In cases where no intermediate is isolated these approaches should be justified and an adequate control strategy should be developed (see also 4.2.3).	Not accepted	No amendment is deemed necessary.
Medicines for Europe	Specific comment	608	609	The guideline currently considers all conjugates, including those with PEG moieties, lipids, or other proteins, as equally complex, however, this oversimplifies the diverse nature of these conjugates. The characterization and control requirements for different types of conjugates may vary. PEGylated peptides often require additional detailed structural characterization due to PEG heterogeneity, site of PEG addition, and number of attached PEG moieties. Similar control and characterization requirements can also be applicable to peptide-protein conjugates. Since in both types of conjugates the main part of the molecule is normally the carrier/linker (i.e., PEG moiety, carrier protein), the analytics of peptide part is more difficult and the control of both peptide and carrier part is essential. On the other hand, lipid-conjugated peptides possess much less complexity, since the peptides are usually conjugated to fatty acids or cholesterol, which all possess discrete, exactly defined structure with the main/biggest part of the molecule still being the peptide. In our opinion the analytics of this type of conjugates do not differ from the analytics of peptides and the same methods and level of control are sufficient. Additionally, some PEG moieties are also monodisperse pure compounds with a precise, discrete molecular weight (normally of less than 1 kDa), which when conjugated to a peptide possesses much lower level of complexity than PEGylated peptides with PEG part exceeding 10 kDa. Therefore, we propose that the requirements for smaller conjugates with exactly defined structure, molecular weight, and number of atoms (e.g., conjugation with exactly defined lipid part or conjugation with exactly defined PEG part) should not be as strict / extensive as the requirements for larger conjugates, where peptides are conjugated to non-homogeneous molecules or where several attachment sites are possible.	However, there is added complexity with respect to the characterisation and control of these conjugates, which is not applicable for conjugates with analytically confirmed discrete / exactly defined chemical structure.	Accepted	Relevant amendments have been made

Bachem AG	Specific comment	615	616	With ref: "Purging of process-related impurities from the conjugation process should be investigated." Purging is not the only option for getting the process-related impurities under control. We suggest using a more general phrasing. The sentence should be adapted accordingly.	Process-related impurities from the conjugation process should be considered as part of the control strategy of the drug substance.	Accepted	Relevant amendments have been made
Bachem AG	Specific comment	618	619	With ref.: "Di-PEGylation or multi-PEGylation (or other conjugation moieties) may also occur and should be adequately controlled." A more general wording is recommended	High molecular weight impurities (or other conjugation moieties) may also occur and should be adequately controlled.	Not accepted	No amendment is deemed necessary.
EFPIA	Specific comment	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).		Not accepted	No amendment is deemed necessary.
PolyPeptide Group	Specific comment	620	626	The section includes the reference to ICH Q11 for the definition of starting materials. This statement is sufficient and it is not necessary to state that GMP shall be applied from the defined starting material and it is not necessary to take the example of an activated ester not being suitable as a starting material (ICH Q11 should be applied).	Delete sentences: It has to be assured that all steps of the intermediate synthesis starting from the defined starting material are performed under good manufacturing practice (GMP). Consequently, e.g. the activation of the suitable PEG starting material is considered a part of the manufacturing process and an activated PEG derivative (e.g. in the form of an N-hydroxysuccinimide (NHS) ester) may not be suitable as starting material and is considered to be an intermediate itself.	Not accepted	No amendment is deemed necessary.
EFPIA	Specific comment	623	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.		Not accepted	No amendment is deemed necessary.
Aspen Oss B.V.	Specific comment	624	626	Is this not allowed as a non-GMP starting material, just like the protected amino acid derivatives, if the activated derivative is commercially available, defined by appropriate specifications, supported by information, in the form of flowcharts, indicating the synthetic process(es) including details of reagents, solvents and catalysts used, and a criticality assessment of which starting material impurities may have an impact on the impurity profile of the peptide conjugate?		Not accepted	No amendment is deemed necessary.
Granzer Regulatory Consulting & Services GmbH	Specific comment	635	638	With regards to the documentation expected on the peptide and the conjugation moiety (e.g. cytotoxic payload) from each manufacturer, it should be clarified whether there is an option to refer to confidential part information provided by the corresponding manufacturer via the ASMF procedure, knowing that both moieties (i.e. peptide and conjugation moiety) may also be developed and registered as "stand alone" drug substances in drug product formulations. (This should be considered in the EU ASMF guideline, which is currently under revision).		Not accepted	Please refer to Section 4.2.4. of the Guideline on Chemistry of Active Substances for options to submit data for either the conjugation moeity or the peptide itself. Note that ASMF and CEP procedures are only applicable to active substances. If the conjugation moiety or the non-conjugated peptide are not active substances full documentation on the manufacture and control of the conjugation moiety as well as the peptide is required in the dossier.
EFPIA	Specific comment	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.		Partially accepted	Relevant amendments have been made

EFPIA	Specific comment	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.		Accepted	Relevant amendments have been made
AstraZeneca	Specific comment	639	640	This is a conservative approach. A risk-based approach for determining comparability of suppliers of new conjugate API SM is proposed.		Accepted	Relevant amendments have been made
EFPIA	Specific comment	643	644	Comment: Use of abbreviation should be aligned (SmPC vs SPC), see also line 712		Accepted	Relevant amendments have been made
piCHEM Forschungs- und Entwicklungs GmbH	Specific comment	645	717	The sections 5 and 6 should be reconsidered as they do not deal with the development and manufacture of synthetic peptides. The discussed topics should be covered by other guidelines		Not accepted	There has been significant interest in Sections 5 and 6 by stakeholders.
NMEU-Nuclear Medicine Europe	Specific comment	649	653	NMEU considers that thresholds of Ph. Eur 2902 should be applicable for cold kits for radiolabeling. For all other Drug Products with peptides, thresholds of ICH Q3B should apply, and not Ph. Eur. 2034	Add sentence to line 653: Cold kits for radiopharmaceutical preparation (which are also considered Drug Products) shall be released according to thresholds specified in EP2902.	Not accepted	No amendment is deemed necessary.
Granzer Regulatory Consulting & Services GmbH	Specific comment	649	653	Lines: 649-653 and 696-700 Considering that ICH Q3B excludes synthetic peptides from its scope, and accordingly, reference here in this guideline is made to impurity thresholds of Ph. Eur. ´Substances for Pharmaceutical Use´ it should be clarified whether the impurity controls and purity/impurity specification should focus on Drug Product-relevant impurities, i.e., (potential) degradation & reaction products of the Drug Substance following the spirit of ICH Q3B, or should consider all types of peptide-specific impurities. Further, it is stated in the Drug Substance section lines 446-447 that ´Synthetic peptides themselves and peptide-related impurities are not within the scope of ICH M7 …"). It should therefore be confirmed whether it will be acceptable to omit peptide-related impurities / degradation products applicable for the Drug Product from ICH M7 assessment.		Not accepted	No amendment proposed nor deemed necessary.
Medicines for Europe	Specific comment	659	661	Based on PQRI threshold, it is understood that leachables below the PQRI threshold of 5 mcg/day are not expected to raise any concerns for sensitization or potential interactions to produce sensitization. Hence, leachables below the PQRI threshold can be excluded from the possibility of producing any immunogenic reactions through interactions with peptides.	Potential interactions of the peptide with the excipients present in the formulation and leachables **above the PQRI threshold of 5 µg/day (sensitization threshold)** that could result from manufacturing materials and packaging materials such as stoppers should be evaluated during pharmaceutical development.	Not accepted	Not agreed: PQRI thresholds not relevant. Please refer to ICH Q3E guideline developments.
NMEU-Nuclear Medicine Europe	Specific comment	659	659	The word ‚leachables' needs to be defined		Not accepted	No amendment is deemed necessary, as covered by referenced ICH guidelines.
EFPIA	Specific comment	662	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.		Comment noted	No further amendment is deemed necessary. Please refer to amendments to sections 4.3.1 and 4.4.5 of the guideline.

Medicines for Europe	Specific comment	662	663	The sentence uses the term potency assay, which according to ICH Q6B can be either a biological assay or a physicochemical assay. The latter is even the case for larger proteins such as human growth hormone and insulin, where the physicochemical content assay is required for release testing according to European Pharmacopoeia. However, as written, we assume the author meant "biological assay" only, which would be inconsistent with line 348, where the guideline states that "Usually, no biological assay is required for the routine release of synthetic peptides". We propose alternative wording for clarity and to avoid misinterpretation of the guideline.	Proposal to delete: 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. Proposal to add: Usually, no biological assay is required for the routine release of synthetic peptides, independently whether the peptide forms a stable secondary or tertiary structure. However, applicants are encouraged to give more details on the possible (absence of) 3-D (secondary) structure, e.g. based on NMR and FTIR, as well as computation investigations.	Comment noted	No further amendment is deemed necessary. Please refer to amendments to sections 4.3.1 and 4.4.5 of the guideline.
AstraZeneca	Specific comment	662	662	Inclusion of bioassay in the section feels absolute? What would a package of work look like to show no potency assay is required?	add text to clarify that confidence in the activity of the sequence and its relation structure would mean no potency testing required.	Comment noted	As above.
ProPharma	Specific comment	662	667	Please confirm if the following is correctly understood: As peptides are normally not considered to be as complex molecules as large proteins, the in vivo bioassay/potency test is considered to be redundant provided that sufficient physicochemical characterization (of structural aspects of the molecule known to affect the biological activity in vivo) is established and presented in 3.2.S.3.1 and a well-established manufacturing history (presented in 3.2.S.2.6/3.2.P.2.3) is available.		Comment noted	Please refer to amendments to sections 4.3.1 and 4.4.5 of the guideline.
EpiVax	Specific comment	668	670	Sponsors are asked to discuss the immunogenicity risk associated with aggregation after formulation development and under stress conditions. When being asked to discuss the risk, are sponsors expected to evaluate such immunogenic risk in in vitro assays such as innate immune cell assays or in a T cell assay?	It is recommended that the agency clarify if there is a need to perform in vitro assays, and if so which ones are requested to investigate the immunogenic risk associated with aggregates. In vitro studies are necessary to support any such claims of non-risk and a simple discussion cannot accurately address this concern.	Comment noted.	Further discussions related to immunogenicity may be covered by future Agency work in this area.
EFPIA	Specific comment	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?		Comment noted	Please refer to the Guideline on the sterilisation of the medicinal product, active substance, excipient and primary container.
United States Pharmacopeial	Specific comment	673	673	Reference to the new Annex 1 of the GMP published in August 2022 should be included.		Not accepted	Please refer to the Guideline on the sterilisation of the medicinal product, active substance, excipient and primary container, where reference to GMP Annex 1 is made.
EFPIA	Specific comment	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."		Not accepted	Please refer to the Guideline on the sterilisation of the medicinal product, active substance, excipient and primary container.
United States Pharmacopeial	Specific comment	675	675	Terminal sterilization is the most recommended method for peptide sterilization. We recommend using wording consistent with Annex 1, section 8.34.		Not accepted	The present wording is considered in line with the Guideline on the sterilisation of the medicinal product, active substance, excipient and primary container

NMEU-Nuclear Medicine Europe	Specific comment	675	678	NMEU considers that, especially for cold kit for radiopharmaceutical preparation, sterile filtration (including the use of pre-sterilized container closure systems and aseptic processing), eventually followed by lyophilization, is sufficient, as several of the excipients typically used in said kits would degrade during a thermal sterilization process or a sterilization process with high gamma-radiation. In certain cases, as described in the bullet points below, the use of aseptic processing may be accepted, even if the formulation itself can be terminally sterilised. The approach should be clearly documented, explained and scientifically justified. Such cases could be justified by: Enabling as long a shelf-life as possible for radiopharmaceutical medicinal products with a shelf-life of less than one week. Cross-Reference to: "Guideline on the sterilisation of the medicinal product, active substance, excipient and primary container"	Modify sentence in 677-679 to: A combination of sterile filtration, pre-sterilised container closure system and aseptic processing is acceptable if the applicant demonstrates suitability during process validation. Add sentence: For radiopharmaceuticals, conditions for the choice of sterilization methods described in guideline EMA/CHMP/CVMP/QWP/850374/2015 shall apply.	Not accepted	No amendment is deemed necessary, as reference to the Guideline on the sterilisation of the medicinal product, active substance, excipient and primary container is considered sufficient.
EFPIA	Specific comment	677	683	**Comment:** A clear definition of "significant" and "moderate degradation", together with some examples would be helpful.		Not accepted	No definition is deemed necessary in this guideline, as the Guideline on the sterilisation of the medicinal product, active substance, excipient and primary container sufficiently covers such concept.
United States Pharmacopeial	Specific comment	677	683	Clarification of the level of acceptable degradation is needed for the sentence: "In case of moderate degradation, exceeding the qualification threshold is not a valid argument in itself to reject terminal sterilization". Except steam sterilization, other processes like gamma irradiation can be used. Do you consider adding them?		Comment noted	No amendment is deemed necessary, as reference to the Guideline on the sterilisation of the medicinal product, active substance, excipient and primary container is considered sufficient.
BCN Peptides	Specific comment	677	690	This conditions are too strict and do not agree with existing guidelines. Reference to current guidelines should applied instead of adding stricter conditions. Guideline on the Sterilisation of the Medicinal Product, Active Substance, Excipient and Primary Container' states:" In case of medicinal products containing highly sensitive active substances, (e.g. proteins or other heat labile biological substance), where it is well known that terminal sterilisation is not possible, a justification based on a scientific rationale is generally acceptable and further justification of the choice of aseptic processing discussed later in section 4.3 may not be needed." This guideline also states that: "Terminal sterilisation should not be ruled out purely on the basis of an increase in degradation products above the qualification thresholds in ICH Q3A/VICHGL10 (active substances), ICH Q3B/ VICH GL11 (finished products) or the impurity limits in ICH M7 for products in the scope of that guideline without additional justification. If impurities are either metabolites or are generated at levels already qualified, then terminal sterilisation is still considered feasible. However, if the degradation products are not qualified at the level at which they occur, then sterile filtration and aseptic processing may be selected. For medicinal products for human use impurities which occur above the identification threshold should be specified in the finished product specification." Difference between generics and new Drug products should be includded, since for generics, no clinical studies apply. So for generic products, asseptic filtration should be accepted based on the original product assessment. On the other hand, let me mention that usually, manufacturers of sterile API peptides do not have a standard packaging. This means that they dispense the amount of product once the manufacturing is finished. The rest of the product is kept as bulk. These last operations are performed as mentioned in the chapter into aseptic conditions.	A combination of sterile filtration, pre-sterilised container closure system and aseptic processing is acceptable when allowed by Guideline on the Sterilisation of the Medicinal Product, Active Substance, Excipient and Primary Container For existing Drug products, reference to the original Drug assessment report can be made to justify the use of a sterile filtration.	Not accepted	The wording is considered to be in line with the Guideline on the sterilisation of the medicinal product, active substance, excipient and primary container. No stricter requirements are applied.
BioNTech SE	Specific comment	677	679	The Agency is highlighting that the combination of sterile filtration, pre-sterilization of container closure system and aseptic processing is acceptable only in case of significant degradation with a terminal steam sterilization under least stressful conditions. We suggest specifying the criteria for defining the significance of the degradation based on the following considerations: - The combination of sterile filtration, pre-sterilization of container closure system and aseptic processing is acceptable if the terminal steam sterilization under least stressful conditions results in progressive degradation of the active substance after terminal steam sterilization of the Drug Product (DP) by forming new impurities or increasing impurity level, thus impacting the shelf life of the DP. - The combination of sterile filtration, pre-sterilization of container closure system and aseptic processing is acceptable if the terminal steam sterilization under least stressful conditions results in degradation of the active substance leading to an increase of the impurity level in the DP by more than 5% compared to the Drug substance total impurity level.	A combination of sterile filtration, pre-sterilised container closure system and aseptic processing is only acceptable if the applicant demonstrates by data that the use of a terminal steam sterilisation process under the least stressful conditions (F0 ≥ 8 minutes) causes significant degradation. The term "significant degradation" in this context includes but is not limited to (i) progressive degradation of the active substance after terminal steam sterilization of the Drug Product (DP) by forming new impurities or increasing impurity level, thus impacting the shelf life of the DP and (ii) degradation of the active substance leading to an increase of the impurity level in the DP by more than 5% compared to the Drug substance total impurity level	Not accepted	No amendment is deemed necessary, as reference to the Guideline on the sterilisation of the medicinal product, active substance, excipient and primary container is considered sufficient to cover this concept.

BicycleTx Limited	Specific comment	677	679	Steam sterilisation is only applicable for aqueous drug products, need also to reflect that some finshed products can be powders (eg if lyophilised) and a different decision tree will apply in accordance with the EMA Guideline on the sterilisation of the medicinal product, active substance, excipient and primary container.	Remove 'Steam' and 'F0 >= 8 minutes' so that the sentence reads: A combination of sterile filtration, pre-sterilised container closure system and aseptic processing is only acceptable if the applicant demonstrates by data that the use of a terminal sterilisation process under the least stressful conditions causes significant degradation.	Accepted	Proposed deletions have been made.
AstraZeneca	Specific comment	684	684	What does moderate degradation towards heat stress mean? Could EMA be more specific please?		Comment noted	Deletion of the term "moderate" has been made. Please refer to the Guideline on the sterilisation of the medicinal product, active substance, excipient and primary container for further details.
EFPIA	Specific comment	685	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.		Comment noted	The relevant text has been amendment to clarify that feasibility of terminal sterilisation should be considered from early development.
AstraZeneca	Specific comment	685	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.		Comment noted	The relevant text has been amendment to clarify that feasibility of terminal sterilisation should be considered from early development.
EFPIA	Specific comment	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).		Comment noted	Feasibility of terminal sterilisation should be considered/investigated from early development.
EFPIA	Specific comment	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.		Comment noted	No mention to overages is present in the text and no amendment is deemed necessary.
EpiVax	Specific comment	688	690	It is unclear when an immunogenicity risk assessment is "relevant" after sterilization. Is the sponsor expected to provide post-sterilization microbiology data to ensure no contamination? Should the sponsor provide data from innate immune response and or T cell studies alongside microbial testing data? Assuming contaminating pathogens are filtered out or destroyed during sterilization, sponsors should be expected to determine if Toll-like receptor agonists and other pathogen associated molecular patterns (refered to as IIRIMS by the US FDA) are present and capable of eliciting an innate immune response. Such a response could induce an unwanted T cell response that could lead to the activation of anti-drug B-cells and therefore antidrug antibodies.	The agency should specify that residual microbial components can stimulate innate immunity that could turn on adaptive immunity leading to an ADA response. The agency should further clarify what types of immunology risk assessment studies should be performed to support that sterilization is effective both in neutralizing microbial contaminants and eliminating innate immune stimulating microbial components	Not accepted	No amendment is deemed necessary. The interpretation of the referenced text is incorrect, as the text referred to degradation products and other physicochemical properties of the product.
EpiVax	Specific comment	696	700	It is unclear why the agency is setting these thresholds and the determination of requested information was determined for each of the impurity concentrations. Additionally we are curious why the request deviates from other regulatory agencies like the US FDA ANDA guidance where qualification and justification is required for impurities above 0.1% with the limit not exceeding 0.5% for a generic peptide drug. Does the agency have data regarding the threshold necessary for potential immunogenicity that can be cited?	Requesting the agency to provide supporting data or qualifying information to justify what is requested for impurities at 0.1, 0.5 and 1.0%	Not accepted	No clear proposal for amendment has been made.

Medicines for Europe	Specific comment	696	700	The term “qualification limit” for impurities as per the ICHQ3A/B guidance relates to a limit that is devoid of any type of toxicity, i.e., covering all toxicological endpoints. It also includes sensitization. Considering that peptide related impurities are not expected to be as immunogenic and would be more similar to small molecules, qualification limit of 1.0% would be appropriate to control peptide related impurities. This is in line with ICHQ3B qualification limit for products with the maximum daily dose below 10 mg/day, as majority of the peptide products have a MDD of less than 10 mg/day and also the dosing regimen for majority of the products does not include daily dosing.	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% for toxicity and immunogenicity endpoint. The qualification threshold of 1.0% would also apply to aggregates /oligomers.	Not accepted	The current wording is considered sufficient and no further amendment is deemed necessary.
EUCOPE	Specific comment	696	700	The proposed ID and tox qualification limits described for peptide related impurities (peptide-related impurities should be reported above 0.1%, identified above 0.5%, and qualified above 1.0%) are too restrictive for conjugated peptides. For conjugated peptides, the limits should be on the order of 1.0% and 1.5% for identification and qualification as described in the white paper, Impurities in Oligonucleotide Drug Substances and Drug Products (see – Capaldi et al. Impurities in Oligonucleotide Drug Substances and Drug Products.Nucleic Acid Therapeutics.Dec 2017.309-322 – http://doi.org/10.1089/nat.2017.0691)		Not accepted	The referenced paper is related to oligonucleotides and not to synthetic peptides. Compendial requirements (Ph.Eur. 2034) are applicable to synthetic peptides products.
NMEU-Nuclear Medicine Europe	Specific comment	696	697	Refer to comment in lines 649-653		Not accepted	As above.
United States Pharmacopeial	Specific comment	698	700	It is suggested to revise the last sentence of the paragraph to “If aggregation/ oligomerization occurs during finished product manufacture and/or storage, aggregates/ oligomers should be adequately characterized in the manufacturer dossier.”.		Accepted	The proposed amendment has been included.
Granzer Regulatory Consulting & Services GmbH	Specific comment	707	712	In-line with the comments under lines 552-556 on assay value definition of the Drug Substance, it should be confirmed whether the label claim of Drug Product shall be expressed referring to the counter-ion free and purity-corrected substance, hereby indicating also the salt form counter-ion) used to present the Drug Substance in the Drug Product in the Drug Product label.		Accepted	The strength of the finished product should be defined with respect to the mass of peptide base (not including salt or counter-ion). This clarification has been included in the section.
PolyPeptide Group	Specific comment	718	718	Many of the suggested comparability studies must be made product to product in order to provide relevant information. It is suggested that it is made clear in the introduction for this chapter that the comparability studies with the reference product shall be done using the synthetic peptide in formulation or the "naked" peptide, or both, as relevant.		Not accepted	No amendment is deemed necessary
EpiVax	Specific comment	726	728	It is specified here that a biosimilar is highly similar to another approved biologic medicine, in terms of several factors including immunogenicity. What requirements do you have on biosimilar sponsors for comparative evaluation of immunogenicity. How is a sponsor to prove similarity?	The agency is requested to clarify how immunogenic risk similarity is to be evaluated and reported	Comment noted	Further discussions related to immunogenicity may be covered by future Agency work in this area.
EpiVax	Specific comment	732	735	"Typically, structural heterogeneity and post-translational modifications are not relevant for these molecules". In the absence of citations or rationale, this is a completely erronious statement and is quite misguided. Such modifications can all impact safety profiles of these products. The agency provides no justification to support this statement	The agency is asked to cite relevant sources or provide rational for the statments made in this paragraph	Comment noted	The sentence has been amended to improve clarity
EFPIA	Specific comment	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).		Not accepted	Guidance on the legal basis falls outside the scope of this guideline
EpiVax	Specific comment	738	742	"Should be considered" is non-committal. Please clarify what is requested of product sponsors. For immunogenicity, what type of assessment do you require- in silico epitope prediction? Innate immune cell assays? T cell assays? Comparative measurement of ADA response?	The agency is requested to specify what assays and measurements of "biosimilarity" are required by the agency.	Not accepted	Further discussions related to immunogenicity may be covered by future Agency work in this area.

Medicines for Europe	Specific comment	738	742	Currently the Chapter 6 only applies to the synthetic peptide development programmes using biological medicinal product as a reference product. It should be noted that at the moment there are also several reference products on the European market present, where the peptide is fully synthetic, and are therefore not addressed by this guideline. However, equal principles as mentioned for synthetic drug product vs recombinant reference product are going to be applicable also for synthetic vs synthetic product, since during comparability testing sameness with regards to physicochemical properties, primary structure, higher order structures, aggregation profile, and biological activity will have to be shown, normally with the same techniques as mentioned in this guideline and chapter 6. We would, therefore, propose that peptides with synthetically prepared reference products are also included.	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)). Where relevant, the same principles are also applicable to synthetic peptide development programmes using a reference product of synthetic or semi-synthetic origin.	Not accepted	The section regards synthetic peptides developed with reference to a biological product. Therefore, the proposal is not considered aligned with the scope of this section.
Bachem AG	Specific comment	743	744	Ref. : "Analytical comparability testing, comprising physicochemical (structural) and biological (functional) assays and conventional analytical testing, forms the basis of the demonstration of comparability" We find that the biological (functional) assay being part of the basis for comparability is too strict, especially compared to the requirements for a synthetic reference. We would recommend rephrasing this section and focusing the biological (functional) assays to large and complex peptides.	Analytical comparability testing, comprising physicochemical (structural) and conventional analytical testing, forms the basis of the demonstration of comparability. Comparability of biological (functional) assays may be considered, as relevant, e.g. for large and complex peptides.	Not accepted	No amendment is needed.
Medicines for Europe	Specific comment	745	745	The text refers to the section 4.1.3 Characterisation 3.2.S.3. This is probably a typo, since in this guideline the chapter 4.3. refers to Characterisation 3.2.S.3. We propose to correct that typo.	(see section 4.3 Characterisation 3.2.S.3)	Accepted	The reference has been corrected.
Medicines for Europe	Specific comment	745	747	In chapter 6 analytical comparability testing advice is given. However, there is no guidance on whether the comparability exercise is a one-time study or should it be done at different time points prior expiry on test drug product and reference medicinal product. We suggest that the text includes the diction that the drug product should be tested on or near release and at the end of the proposed shelf life. Additionally, the reference product should be tested at different time points prior to expiry, after aging under conditions consistent with the labelled storage conditions.	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 side-by-side comparability studies. The drug 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.	Accepted	The proposed amendment has been included.
Medicines for Europe	Specific comment	747	748	To align closer with EMA guidelines on biological medicinal products [1] and CMDh guidelines on reference product for bioequivalence studies [2], and support global development programs of synthetic peptides, the draft guideline should not eliminate the ability for applicants to use non-EEA authorised comparator products to demonstrate comparability between the reference medicinal product and the synthetic peptide. Lines 747-748 should be revised to clarify that the reference medicinal product should be a medicinal product authorised in the EEA, but allow a non-EEA authorised comparator to be used in comparability studies if justified. It will be the applicant's responsibility to establish an acceptable bridge between the non-EEA authorised comparator and the EEA-authorised medicinal product. Furthermore, the guideline mentions that reference product sourced from European market and drug product batches from commercial process should be used for side-by-side comparability study. It is, however, lacking the clarification on the number of lots that should be used for this study. We propose that at least 3 batches of drug product and reference medicinal product be used for comparability study (see the comment on lines 800-803). Especially in the case of orphan drug status of reference medicinal product, it is sometimes difficult / impossible to obtain several different batches of reference product for the side-by-side comparability study (with valid shelf life at the time of performing this study). To bypass this obstacle, we propose the use of frozen batches of reference medicinal product to perform the comparability study (age of sample at the time of freezing will be used as the age of sample at the time of testing) – their use will additionally be justified by stability data and no impact of freeze/thawing will be shown during development. This is a commonly used approach for biological products (biosimilars). References: [1] Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: quality issues (revision 1) EMA/CHMP/BWP/247713/2012 (May 2014). [2] Q&A – Generic Applications CMDh/272/2009/Rev.6 (March 2020).	It is important to note that the synthetic version be compared to a reference medicinal product, which has been granted a marketing authorisation the European Economic Area (EEA). The usage of frozen reference product is allowed if adequate stability data are provided.	Not accepted	The rationale for the proposal is not agreed.

Viatris	Specific comment	747	748	To align closer with EMA guidelines on biological medicinal products and CMDh guidelines on reference product for bioequivalence studies, and support global development programs of synthetic peptides, the draft guideline should not eliminate the ability for applicants to use non-EEA authorised comparator products to demonstrate comparability between the reference medicinal product and the synthetic peptide. Lines 747-748 should be revised to clarify that the reference medicinal product should be a medicinal product authorised in the EEA, but allow a non-EEA authorised comparator to be used in comparability studies if justified. It will be the applicant's responsibility to establish an acceptable bridge between the non-EEA authorised comparator and the EEA-authorised medicinal product. See EMA's Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: quality issues (revision 1) EMA/CHMP/BWP/247713/2012 (May 2014). Also see CMDh's Q&A – Generic Applications CMDh/272/2009/Rev.6 (March 2020).	It is important to note that the chemically synthesised version be compared to a reference medicinal product, which has been granted a marketing authorisation the European Economic Area (EEA). Remove: reference product used in the comparability studies should be sourced from the European market.	Not accepted	It is out of the remit of this guideline to grant additional flexibility concerning the source of the reference product. The current statement is correct as referring to the requirements for generics and hybrid applications, therefore the proposal for amendment is not accepted.
EpiVax	Specific comment	749	753	It is unclear what differences between synthetic and recombinant peptides should be evaluated. In several places in this draft guidance, you specify that immunogenicity is not likely to be impacted unless aggregation is detected (which is a false assumption). In alignment with US FDA guidelines, sponsors should perform comparative immunogenicity risk assessments between recombinant and synthetic peptides, evaluate the immunogenic potential of synthetic peptide impurities and also evaluate peptide drug products for their ability to induce innate immunity	Please specify which parameters sponsors are expected to evaluate in order to demonstrate comparability and safety.	Not accepted	Further discussions related to immunogenicity may be covered by future Agency work in this area.
United States Pharmacopeia l	Specific comment	754	755	Amino acid analysis can be used for the primary structure analysis, while IEF and western blotting are not good techniques for the primary structure analysis. FTIR, fluorescence and DSC are also not commonly used for higher-order structure.		Accepted	The relevant text has been amended.
United States Pharmacopeia l	Specific comment	758	761	Thioflavin T is a sensitive technique for highly ordered protein amyloid (e.g in Alzheimer disease) and not for smaller systems like peptide aggregates and fibrils.	For this reason, it is proposed to remove the phrase related to Thioflavin T Assay.	Partially accepted	The wording has been amended.
AstraZeneca	Specific comment	758	765	Consider distinguishing between covalent and non-covalent impurities and provide guidance on how to address these types.		Comment noted.	N/A
Medicines for Europe	Specific comment	761	763	Not all peptides (depending on the size and nature of amino acid residues) are prone to aggregation. If the absence of aggregates is clearly evident from literature data (very small peptide size, literature data for exactly the same compound) and/or the absence of aggregates is confirmed with analytical data gathered during development, the additional evaluation of fibrillary aggregates should not be required. We propose that "if relevant" phrase is used before the lines for cases such as the one described above. This way there will be no discrepancy with the Line 668.	If relevant, aggregation propensity should also be investigated by suitable techniques detecting fibrillary aggregates such as Thioflavin T (ThT) assay.	Accepted	Relevant amendments have been made
Bachem AG	Specific comment	761	763	Ref: "Aggregation propensity should also be investigated by suitable techniques detecting fibrillary aggregates such as Thioflavin T (ThT) assay." The aggregation propensity should not be focused on fibrillation and the associated analytical technique of ThT assay. We therefore recommend rephrasing this part.	Aggregation propensity should also be investigated by suitable techniques.	Partially accepted	The wording has been amended.
EpiVax	Specific comment	763	765	Peptide impurities, even with single amino acid changes have the potential to promote an unwanted immune response to a synthetic peptide drug. New impurities found in the synthetic product should be evaluated for T cell epitopes using a combination of in silico and in vitro assays to determine if peptide impurities contain new epitopes that can change HLA binding properties and T cell responses that could spread to the API impacting the safety profile of the product.	Please specify how sponsors are expected to determine and qualify the immunogenic risk of new synthetic peptide impurities.	Not accepted	Further discussions related to immunogenicity may be covered by future Agency work in this area.
Medicines for Europe	Specific comment	763	765	The guideline says that impurities in the synthetic peptide not present in the biological reference product are qualified and do not raise concerns regarding immunogenicity. We proposed that it is additionally clarified that only impurities exceeding qualification limit of 1.0% should be qualified.	When differences in the impurity profiles are observed it should be demonstrated that the impurities in the synthetic peptide not present in the biological reference product are qualified above 1.0% and do not raise concerns regarding immunogenicity.	Accepted	Relevant amendments have been made

Viatris	Specific comment	763	765	The draft guideline states that when "differences in the impurity profiles are observed" between a synthetic peptide and its reference product applicants should demonstrate that impurities in the synthetic peptide "are qualified and do not raise concerns regarding immunogenicity." The guideline continues to describe methods to assess immunogenicity that are not considered useful, which we agree. However, there is no guidance on the recommended methods to assess immunogenicity risk when differences in impurity profiles are observed. We request EMA amend the draft guideline to include guidance on what methods are considered suitable to evaluate immunogenicity risks when there is a difference in (including novel) impurity profiles.		Comment noted.	Further discussions related to immunogenicity may be covered by future Agency work in this area.
BCN Peptides	Specific comment	763	778	The wording is confusing. We have added modifications to clarify some points.	When differences in the aggregation impurity profiles are observed it should be demonstrated that the aggregation impurities in the synthetic peptide not present in the biological reference product are qualified and do not raise concerns regarding immunogenicity. Apart from to the aggregates, regarding the assessment of impurity related immunogenicity, experience has shown that immunogenicity of peptides is of lesser concern than that of proteins due to their size. Furthermore, changes or modifications (e.g. deamidations) of a small number of amino acids are not noticeably immunogenic. If the total amount of peptide-related impurities does not exceed the respective amount of peptide-related impurities of the originator product, this is not considered as a concern even if a given peptide-related impurity is not present in the originator preparation. In case a novel type of impurity occurs, i.e. differing from the drug substance in more than a few amino acid modifications, this novel impurity should be reduced as far as possible since reliable prediction of immunogenicity is not feasible. For these new impurities, 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.	Not accepted	Current wording is considered correct and clear.
EpiVax	Specific comment	766	774	Reliable prediction of immunogenicity (of a novel peptide impurity compared to the API) is not only reliable but accurate. Manufacturers applying for approval of their peptide drug products with the US FDA employ a combination of in silico and in vitro methods to determine the risk potential. New impurities that result from synthetic manufacturing processes can absolutly differ from products manufactured in recombinant expression systems. The focus should not be on the overall abundance of the impurities in the product but rather on the qualification of new impurities on an individual basis to determine their risk potential relative to the API peptide. SIngle amino acid changes can in fact be noticably immunogenic and in silico analysis can predict the risk which can be further qualified in vitro T cell and HLA binding assays. This can be especially true if the innate immune system is triggered due to several factors such as contamination or aggregation, providing the proper conditions for a T cell and subsequent ADA response.	The agency should provide specific references or rational to support these claims and if none are available, this paragraph should be removed because it is both false and misguided.	Not accepted	Further discussions related to immunogenicity may be covered by future Agency work in this area.
Viatris	Specific comment	766	767	The draft guideline states that when assessing impurity related to immunogenicity, "experience has shown that immunogenicity of peptides is of lesser concern that that of proteins due to their size" and "changes or modifications (e.g., deamidations) of a small number of amino acids are not noticeably immunogenic." We agree with this sentiment and request that agency further clarifies in guidance when an immunogenicity study is not required (e.g., include chain length of amino acid change that would require a study) to avoid agency requests for unnecessary studies.		Comment noted	Further discussions related to immunogenicity may be covered by future Agency work in this area.
EFPIA	Specific comment	769	771	This is not agreed. New impurities not present in the reference product need to be justified.		Accepted	The sentence has been deleted.

Medicines for Europe	Specific comment	769	771	We think that lines 769–771 (»If the total amount of peptide-related impurities does not exceed the respective amount of peptide-related impurities of the originator product, this is not considered as a concern even if a given peptide-related impurity is not present in the originator preparation.«) are not in line with lines 763–765 (»When differences in the impurity profiles are observed it should be demonstrated that the impurities in the synthetic peptide not present in the biological reference product are qualified and do not raise concerns regarding immunogenicity.«), since they could be wrongly understood as: impurities, present in synthetic peptide but not in biological reference product, should be qualified above 1.0%, unless the total amount of impurities in the synthetic peptide does not exceed the respective amount of peptide-related impurities present in originator product. If understanding is correct, we suggest that lines 769–771 should be omitted.	We propose lines 769–771 to be omitted.	Accepted	The sentence has been deleted.
AstraZeneca	Specific comment	769	771	New impurities not present in the reference product need to be justified.		Accepted	The sentence has been deleted.
United States Pharmacopeial	Specific comment	772	772	The definition of "differing from the drug substance in more than a few amino acid modifications" should be clarified (putting relevant examples) or removed for the immunogenic risk evaluation.		Accepted	The sentence has been removed
EFPIA	Specific comment	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.		Accepted	Proposed deletions have been made.
United States Pharmacopeial	Specific comment	775	777	In-silico prediction of immunogenicity, e.g. based on predicted binding T-cell receptors (TCR), or in-vitro tests of T-cell activation, has been considered helpful by other regulatory agencies since the regulatory T cells have the potential to help develop and maintain tolerance, and therefore control the T-cell independent immune responses (e.g. heparin-induced thrombocytopenia (HIT)).	It is suggested to revise to "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 not be sufficient since also T-cell independent immune responses are described (e.g. heparin-induced thrombocytopenia (HIT))." Delete "Mainly intended for vaccine 777...".	Accepted	Proposed deletions have been made.
EpiVax	Specific comment	775	778	This statement is false and misleading. In silico T cell epitope prediction tools, of which there are many options, predict the binding of a T cell epitope to HLA, not the interaction between the peptide epitope and the T cell receptor. Furthermore, while in silico T cell epitope algorithms may have been developed for development they have been widely used in the evaluation of peptide impurities in synthetic peptide drugs and to evaluate the risk of HCPs in recombinant biosimilar products. In silico assessment should be used in conjunction with in vitro T cell assays and innate immune response assays to develop a global assessment of immunogenic risk potential as a part of any drug development plan. We agree that T-cell-independent immune responses have been an issue, but innate responses can induce an unwanted T cell response given the proper circumstances. Saying that T cell response evaluations are not useful because T cell independant responses are described is dangerous and and extremely misguided.	This paragraph should be removed from the finalized guidance and it contains several false and misleading claims. If these claims are supported by relevant literature, the agency is asked to cite sources and qualify the claims made here.	Accepted	Further discussions related to immunogenicity may be covered by future Agency work in this area.
Medicines for Europe	Specific comment	781	782	The guideline mentions several times that peptide-related impurities should be reported above 0.1%, identified above 0.5%, and qualified above 1.0%. The meaning of the lines 781–782 is somehow ambiguous, therefore we propose that it is clarified in more details that peptide-related impurities at the levels 0.1–0.5% should be reported and quantified, and the levels of each peptide-related impurity should be compared to their corresponding levels in RLD.	This monograph allows an identification threshold of 0.5%. However, for comparability purposes peptide-related impurities at levels 0.1-0.5% should be reported and quantified, and the levels of each peptide-related impurity compared to their corresponding levels in reference product. A limit of quantification (LOQ) of 0.1% for HPLC purity testing is required.	Accepted	Relevant amendments have been made

Viatris	Specific comment	784	785	When discussing synthetic peptide development programs using a biological medicinal product as a European Reference Medicinal Product, the draft guideline states that comparative forced degradation studies are also recommended. However, forced degradation is not necessary when the routes of degradation and the suitability of the analytical procedures can be determined through other methods.	Remove: Comparative forced degradation studies are also recommended and the suitability of the analytical purity methods to fully characterize the impurity profiles of both products should be demonstrated.	Partially accepted	Clarification has been included to the text
Bachem AG	Specific comment	784	785	Ref "Comparative forced degradation studies are also recommended and the suitability of the analytical purity methods to fully characterize the impurity profiles of both products should be demonstrated." We understand and support the fact that the impurity profile of both products needs to be compared. However, we do not understand the relationship with the forced degradation. The interchangeability of both products takes place under normal use conditions and not under stress conditions. Therefore, we strongly recommend deleting this section.	No text (text of 784-785 deleted)	Partially accepted	Clarification has been included to the text
Bachem AG	Specific comment	790	792	Ref: "Functional assays (e.g. cell based assays using appropriate cell lines) should be developed and used in the comparability studies. It depends on the mechanism of action which additional functional assays may be needed to demonstrate similarity (e.g. binding kinetics)." Functional assays may serve as additional tools for the characterization of synthetic peptides in cases where the higher order structure cannot be sufficiently characterized by physicochemical tests. In these cases, comparison between the synthetic peptide and the reference needs to be done. In the other cases, we do not see the rationale in the CMC section of a dossier, for performing functional assays. If suspicion of biological non-comparability exists, relevant tests should be performed as clinical or non-clinical tests, on the drug product (Module 4 or 5)	Functional assays (e.g. cell based assays using appropriate cell lines) may be developed and used in the comparability studies. It depends on the mechanism of action which additional functional assays may be needed to demonstrate similarity (e.g. binding kinetics).	Not accepted	No amendment is deemed necessary.
Bachem AG	Specific comment	793	795	Ref "The absence of a biological assay in the release specifications for drug substance and drug product should be appropriately justified, e.g. by commercial-scale batch biological assay data and, in addition, by appropriate characterisation of higher-order structure by physicochemical testing" We would like to focus the justification of the absence of a biological assay on projects where such an assay was established during development since other cases are covered in line 557.	When used during the development, the absence of a biological assay in the release specifications for drug substance and drug product should be justified, e.g. by relevant scale batch biological assay data and, in addition, by appropriate characterisation of higher-order structure by physicochemical testing	Not accepted	Biological assays should be used during development.
Medicines for Europe	Specific comment	800	803	The guideline references the 'Reflection paper on statistical methodology for the comparative assessment of quality attributes in drug development' - EMA/CHMP/138502/2017). However, this reflection paper provides theoretical, partly conflicting considerations on the determination of the number of batches in biosimilar development, but no concrete guidance how to determine an adequate number of batches. Therefore, we propose to delete this reference, and provide pragmatic guidance on the number of batches. A minimum of 3 batches should be included in the comparability exercise.	Batches preferably from the commercial process should be used for the side-by-side analyses. The number of batches used in the comparability studies should be at least 3.	Not accepted	The number of batches should be justified by the applicant.
EFPIA	Specific comment	806	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."		Accepted	The proposal has been implemented.
EFPIA	Specific comment	807	808	Recommend adding reference to "Guideline on the requirements to the chemical and pharmaceutical quality documentation concerning investigational medicinal products in clinical trials".		Accepted	The introduction to the section has been amended to clarify that this section is complementary to the Guideline on the requirements to the chemical and pharmaceutical quality documentation concerning investigational medicinal products in clinical trials for those quality aspects specifically related to synthetic peptides.
NMEU-Nuclear Medicine Europe	Specific comment	807	808	The document does not provide clarity which requirement is valid in which phase. This lack of definition is found throughout the document. It should be clarified throughout the document which requirement is expected at which state of the development process. A statement like "….applies for registration phase and commercial phases, and differences may be applied during clinical development…." may be worthwhile		Accepted	Clarification regarding the clinical trial phases has been included.
Granzer Regulatory Consulting & Services	Specific comment	807	837	See general comment section.		Not accepted	The scope of the entire guideline is for MAA. Guidance on IMPD for synthetic peptides is specifically provided within section 7.

piCHEM Forschungs- und Entwicklungs	Specific comment	807	837	Minimum requirements during clinical development should be elaborated in more detail		Partially accepted	Further clarification regarding CT phases has been included.
EUCOPE	Specific comment	809	812	"requirements for peptides intended to be used in the course of clinical studies are evolving depending on the stage of development… the main focus should be on the safety of the synthetic peptide, especially in the early stages of development." Additional guidance for earlier Phase (Phase 1 and 2) versus Phase 3 studies should be considered and incorporated into this section of the guidance document as it was incorporated in the EMA's Guideline on the Requirements to the Chemical and Pharmaceutical Quality Documentation Concerning Investigational Medicinal Products in Clinical Trials.		Partially accepted	Further clarification regarding CT phases has been included.
EFPIA	Specific comment	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.		Comment noted.	For clarity, the sentence refers to starting materials' requirements.
BicycleTx Limited	Specific comment	816	819	By setting the expectation that amino acids are the RSMs from early in development, this may result in excessive detail in the IMPD and undue burden on the applicant to maintain this information through product development. ICH Q11 requirements are only applicable to commercial products.	Add: At early stages of development, amino acids may not be required to be designated as regulatory starting materials, instead compounds later in the drug substance process may be selected as RSMs. However, appropriate RSMs in accordance with this guideline and ICH Q11 expectations must be designated by the time of MAA.	Comment noted	The wording has been revised to allow more flexibility.
EFPIA	Specific comment	817	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)…"		Accepted	The relevant text has been amended.
BCN Peptides	Specific comment	819	819	Clarification on what means later development.	expected for later development in drug product registration.	Accepted	The sentence has been amended accordingly.
EFPIA	Specific comment	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."		Accepted	The sentence has been deleted following addition of an introduction to the section to clarify that section 7 is complementary to the Guideline on the requirements to the chemical and pharmaceutical quality documentation concerning investigational medicinal products in clinical trials.
BCN Peptides	Specific comment	825	828	Aggregation should be investigated in the drug product not in the drug substance, since it may depend on the conditions used for the drug product manufacture.	The novel peptide should be fully characterised in terms of primary structure; The propensity toward racemisation should also be investigated.	Not accepted	The mentioned text does not refer to the active substance only, but to the finished product as well.
Bachem AG	Specific comment	825	827	Ref: "The novel peptide should be fully characterised in terms of primary structure; particular attention should be paid to its potential for aggregation from as early as possible in the development in order to avoid problems during formulation of the drug product." As indicated in the Note for Guidance on IMP (EMA/CHMP/QWP/545525/2017 Rev. 2), the level of information to be provided should consider, among others, the state of development/clinical phase as well as the duration of the clinical phase. The aggregation propensity should be considered at all stages of development to avoid issues when the drug product is to be produced and is subject to patient exposure. The extent of the characterization should then depend on the phase of the clinical trial and should not be "full", for a phase I study. "As early as possible in the development" is subject to misinterpretation by the Authorities, which would tend to request a superfluous full characterization for a clinical study of a short duration and limited patient exposure.	The novel peptide should be characterised in terms of primary structure; attention should also be paid to its potential for aggregation propensity in case indication of relevance for the drug product was observed.	Partially accepted	The text has been amended to remove "as early as possible in the development.
EFPIA	Specific comment	827	828	Comment: It should be clarified that this information is not required for inclusion in the IMPD until later development.		Not accepted	The aggregation propensity should be considered at all stages of development.

Bachem AG	Specific comment	827	828	Ref "The propensity toward racemisation should also be investigated." The propensity toward racemisation is a "tendency" and should only be investigated if there is reason to suspect its occurrence.	The propensity toward racemisation may also be investigated.	Accepted	Clarification has been added to the sentence.
EFPIA	Specific comment	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.		Not accepted	No amendment is deemed necessary.
BCN Peptides	Specific comment	829	830	Clarification on the wording	Impurities in the peptide active substances should be identified in the course of development. Peptide- related impurities above the threshold of 1.0%, should be identified, and further qualified by the preclinical studies performed on the drug substance containing these impurities.	Not accepted	The proposed amendement is not agreed since it does not improve clarity.
EDQM	Specific comment	829	830	In relation to the comment on lines 470-474, two different cases are to be distinguished.	It is proposed that the statement in lines 829-830 reads: "Impurities in the peptide active substances should be identified in the course of development. Peptide-related impurities above the threshold of 1.0% (2.0% in case of peptides used in the preparation of radiopharmaceuticals) should be identified and qualified in preclinical studies."	Not accepted	Please refer to the Guideline on radiopharmaceuticals. The synthetic peptides guideline applies to peptides used in radiopharmaceuticals only regarding synthesis and starting materials.
BicycleTx Limited	Specific comment	829	829	Structural identification of impurities not likely to be feasible early in development.	Peptide-related impurities above the threshold of 1.0%, should be identified (at a minimum by RRT) and qualified in preclinical studies.	Not accepted	It is not agreed that identification by RRT suffice.
Bachem AG	Specific comment	829	830	Ref "Peptide-related impurities above the threshold of 1.0%, should be identified and qualified in preclinical studies." The full identification of each impurity is not always performed in early clinical phases. For clinical studies it is important to capture new impurities which might occur during process development and are not covered by the non-clinical or toxicological studies. In early development phase, it is sufficient to capture these impurities by their relative retention time. We therefore recommend rephrasing this sentence	New peptide-related impurities above the threshold of 1.0% and not present in the batches subject to non-clinical/toxicity studies, should be identified at least by RRT and qualified in preclinical studies.	Not accepted	It is not agreed that identification by RRT suffice.
NMEU-Nuclear Medicine Europe	Specific comment	829	834	At early development stage individual acceptance criteria for specified impurities will not be available. It should be clarified, from which stage on this is needed. Reference to Ph. Eur. 2902 for peptides used for radiopharmaceuticals should be included.	Add sentence: Specified impurities shall be included for analytical method validation, the latest at the stage of a phase III clinical trial	Not accepted	Impurities are potentially a safety concern and should therefore be controlled to acceptable levels from an early stage.
Aspen Oss B.V.	Specific comment	830	830	Qualification of related impurities can also be performed in clinical studies.	Change 'preclinical studies' to '(pre)clinical studies)'.	Not accepted	Current wording is considered correct.
AstraZeneca	Specific comment	830	830	The 1.0 % qualification threshold from Ph. Eur. for marketed products is a good limit to include. However, applying the same limit to clinical supplies seems unreasonable considering the low number of individuals exposed and the shorter duration of exposure under medical supervision.		Not accepted	No amendment is deemed necessary.
AstraZeneca	Specific comment	831	831	Peptide related impurities above the threshold of 1.0%, should be identified and qualified in preclinical studies"	Clarify this is appropriate for clinical phases. Confirm identity limit is increased for clinical phase versus 'substances for pharmaceutical use, 2034.'	Comment noted	It is confirmed. No amendment to the guideline text is deemed necessary.

Bachem AG	Specific comment	831	834	Ref. "Orthogonal/complementary analytical procedures should be employed also in the early development stages in order to minimise the risk of co-elution of impurities and to adequately characterise the impurity profile of the synthetic peptide; if it can be shown in the course of development that one analytical procedure is sufficient to control all impurities, the other(s) could be omitted" The control of related impurities can be conducted by means of orthogonal analytical procedures or orthogonal detection techniques. Both options should be covered by this guidance. The control of related impurities should be in focus during relevant development steps not focusing on early stages. The impurities in focus of this section should be given as peptide related impurities.	Orthogonal/complementary detection techniques (e.g. mass spectroscopy) and/or analytical procedures should be employed in relevant development stages in order to minimise the risk of undetected co-elution of peptide related 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 the peptide related impurities, the other(s) could be omitted.	Not accepted	The statement that orthogonal detection techniques should be employed at relevant development stages is not agreed since it implies that they can be used at later stages. Co-elution of impurities should be avoided as early as possible in development.