Review

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Quality specifications for peptide drugs: a regulatory-pharmaceutical approach

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Peptide drugs, as all types of pharmaceuticals, require adequate specifications (i.e. quality attributes, procedures and acceptance criteria) as part of their quality assurance to ensure the safety and efficacy of drug substances (i.e. active pharmaceutical ingredients) and drug products (i.e. finished pharmaceutical dosage forms). Compendial monographs are updated regularly to keep up with the most recent advances in peptide synthesis (e.g. reduced by-products) and analytical technology. Nevertheless, currently applied pharmacopoeial peptide specifications are barely harmonized yet (e.g. large differences between the *European Pharmacopoeia* and the *United States Pharmacopeia*), increasing the manufacturers' burden of performing analytical procedures in different ways, using different acceptance criteria. Additionally, the peptide monographs are not always consistent within a single pharmacopoeia. In this review, we highlight the main differences and similarities in compendial peptide specifications (including identification, purity and assay). Based on comparison, and together with additional information from peptide drug substance manufacturers and public evaluation reports on registration files of non-pharmacopoeial peptide drugs, a consistent monograph structure is proposed. Copyright © 2009 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: peptide drug substance; quality attributes; acceptance criteria; regulatory affairs; ICH guidelines; *Ph. Eur.* and USP pharmacopoeial monographs; related substances thresholds

Introduction

Peptides show great pharmaceutical potential as active drugs and diagnostics in several clinical areas such as endocrinology, urology, obstetrics, oncology, etc. and as functional excipients in drug delivery systems to overcome tissue and cellular membrane barriers. From a pharmaceutical point of view, peptides are situated somewhere between classical organic drug substances and high molecular weight biopharmaceuticals. Therefore, their development toward pharmaceutical compounds poses some unique challenges: the data-supported rationalization of quality specifications being one of the major issues in this process. Especially, the establishment of justified acceptance criteria forms a well-known complication in this respect, where toxicological and clinical aspects (e.g. qualification of impurities: establishment of biological safety levels) are to be combined with chemical-pharmaceutical considerations (e.g. process capability). Recently, practical guidelines on related impurities in synthetic peptides have been issued in the Ph. Eur. [1] as a supplement to the Q3A guideline of the ICH [2] based on the conclusions of the EDQM symposium 'New Impurities Control: Setting Specifications for Antibiotics and Synthetic Peptides' [3]. Although not intended for compounds still in the R&D phase, it should be noted that changes in the route of synthesis may result in an altered impurity profile, which is of importance for the interpretation of toxicological and clinical studies.

A classification of the different types of peptide and derivatives that are currently available or under investigation as therapeutic drugs is given in Table 1. For the purpose of this review, peptide drugs are defined as native or modified peptides with a molecular weight up to 6 kDa [4] (i.e. about 50 amino acids, including insulins).

An overview of the peptide drugs currently described in the Ph. Eur. [1] and/or USP [5] is given in Table 2. These 28 pharmacopoeial peptides are obtained from various origins: chemical synthesis ($n = 13$) [6], fermentation ($n = 7$), tissue extraction ($n = 7$) and rDNA technology ($n = 5$). In four cases (i.e. salmon calcitonin, human insulin, oxytocin USP and vasopressin USP), the pharmacopoeial peptides may be obtained from two different origins. Supplementary synthesis- and manufacturer-

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Abbreviations used: *AAA, amino acid analysis; AES, atomic emission spectrometry; API, active pharmaceutical ingredient, syn. drug substances; CEP, Certificate of Suitability to the Monographs of the European Pharmacopoeia; CFU, colony forming units; EDQM, European Directorate for the Quality of Medicines & Healthcare; EMEA, European Medicines Agency; EPAR, European Public Assessment Report; FDP, finished drug product, syn. finished pharmaceutical product (FPP); ICH, International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use; ICP, inductively coupled plasma; IR, infrared; IT, identification threshold; LAL, Limulus amoebocyte lysate; ODI, other detectable impurity; PDE, permitted daily exposure; Ph. Eur., European Pharmacopoeia; QC, quality control; QT, qualification threshold; R&D, research and development; RT, reporting threshold; TAMC, total aerobic microbial count; TLC, thin-layer chromatography; TYMC, total combined yeasts/moulds count; USP, United States Pharmacopeia; UV, ultraviolet.*

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Biography

Bart De Spiegeleer was born in 1959 in Aalst, Belgium. He studied pharmacy at Ghent University, where he also completed his Ph.D. on radiosynthesis, characterization and functionality of anticancer platinum complexes. He spent a research stage at the University of Manchester. He continued to work in the pharmaceutical industry as QA, RA and technical manager, first at Federa (Brussels) and then at Couvreur (Puurs). He started in 1994 his own pharmaceutical consulting company, Health Engineering and Assurance (HEA), heading a group specialized in chemical-pharmaceutical development encompassing also GMP/GLP and regulatory affairs. He was a board member of several start-up companies in the pharmaceutical field. In 2005, he became part-time professor at Ghent University. Since 2008, becoming full-time professor, he has been Head of Drug Quality and Registration (DruQuaR) laboratory at the Department of Pharmaceutical Analysis in Ghent University. The laboratory is recognized by the Health Authorities for chemical drug analysis, including ICH stability.

He is a member of the national pharmacopoeial commission, and gives lectures regularly for professional organizations about quality aspects of drug development and regulatory affairs. He is author or coauthor of over 100 publications, including several patents in collaboration with pharmaceutical companies. His current main scientific interests include functional quality aspects of peptide drugs; transmembrane (blood-brain barrier, skin-mucosa) properties of peptides and other compounds; chemical and physico-chemical characterization of modified peptides, proteins and other drugs; adsorption and surface behavior; pharmaceutical development of peptide APIs (active pharmaceutical ingredients) and FPPs (finished pharmaceutical products); GMP/GLP regulatory aspects of drugs; chemical and metabolic stability as well as bioactivity of peptides.

Christian Burvenich obtained his B.Sc. (biology) and degree in veterinary medicine at Ghent university. After graduation, he received an extensive training in experimental medicine in veterinary and human physiology labs. In 1983 he obtained his Ph.D.-degree in physiology and pathophysiology. In 1985 he was appointed as professor in physiology at the faculty of veterinary medicine of the same university. Since then, he has been studying the molecular basis of the variation of underlying mechanisms of inflammation and sepsis. Innate immunity is studied in several '*in vivo*' and '*in vitro*' models (at bone marrow, circulation and mammary gland level). In this area he published several peer-reviewed papers cited on SCI. Since 1990 he has been the promoter and co-promoter of several Ph.D. students in his university and in Utrecht (the Netherlands). He has spent sabbaticals at USDA-ARS (Beltsville MD, USA) in 1992, 1997 and 2001. His basic research on mammary inflammation was honored with national and international scientific degrees. In 2001 he was honored for his international

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(*Continued*) work by the American dairy science association. In 2003–2005 he was awarded with the Belgian Francqui chair at Liege University. C. Burvenich is a member of the editorial board of several reviews. He is regularly asked as a referee and keynote speaker. He has been the chairman of the 'mammary immunology section of a European project on mammary gland biology (COST 825) and was a member of COST 811 (apoptosis group). He has been a member of several academic evaluation commissions at universities in the United States, Canada, the Netherlands, France, Finland and Israel. He has been a member of several international and national Ph.D. commissions. Since 1998 he is the Chair of the international office at the faculty of veterinary medicine of Ghent University. He has been a member of the Flemish FWO commission for physiology (selection of projects and fellowships) in Belgium.

Christophe Van de Wiele is a senior professor in the Department of Nuclear Medicine, at the University Hospital in Ghent, Belgium. Dr Van de Wiele received his medical degree in 1991 from the University of Ghent, Belgium and his training in Nuclear Medicine at the University Hospital Ghent from 1991 to 1996. In 2001 he received his Ph.D. degree from the University of Ghent. From 2002 to 2003 he followed master courses in biotechnology. His primary clinical and research focus is nuclear oncology: basic science development and clinical evaluation of new tracers for prediction of early assessment of response to cancer therapy. He is a member of different scientific societies and commissions, the IRIST section editor of the Quarterly Journal of Nuclear Medicine, and the author or co-author of several manuscripts.

Mr Valentijn Vergote received his M.Sc. degrees in Chemistry and Pharmaceutical Sciences at Ghent University in 1994 and 1998, respectively. Since then, he worked in the quality control and regulatory affairs departments of several pharmaceutical companies. Currently he is a staff scientist in the DruQuaR lab of Ghent University, preparing his Ph.D. thesis on quality analytics of peptide drugs.

dependent specifications for drug substances can be found in CEPs (e.g. currently, $n = 6$ for leuprorelin) [7].

For a large number of marketed peptide drugs (e.g. ganirelix and ziconotide), no compendial monographs are currently available. However, some quality-related information on these products can be found in, e.g. EPARs published by the EMEA [8].

Drug substance and drug product mandatory quality attributes and acceptance criteria are generally categorized into three subdivisions: (i) identification, (ii) tests and (iii) assay. The tests given in the 'identification' section are not designed to give a full characterization of the peptide, but are intended to provide sufficient confirmation on the identity. The 'tests' section normally consists of limit tests on process- and drug-related organic impurities, inorganic contaminants and residual solvents, supplemented

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Table 1. Classification of drugs interacting with specific peptide receptors

#	Product type	Description
1	Native peptides	Endogenous peptides, either extracted from natural sources, prepared by chemical synthesis or using recombinant DNA technology.
2	Chemically modified peptides	Peptides closely matching the native peptide ligand.
2.1	Sequence modifications	Synthetic incorporation of amino acid analogs and isosteres, e.g. replacement with D-stereoisomer.
2.2	Functional group modifications	Chemical transformation on a side-chain or on the termini, e.g. *C*-terminal ethylamide, PEGylation.
2.3	Backbone modifications = pseudopeptides (syn. amide bond surrogates)	One or more amide bond replacements, e.g. ψ[CH_2NH] amide bond surrogate.
3	Peptidomimetics	Nonpeptide drugs.

by specific tests such as physicochemical properties and microbial purity. The 'assay' section generally includes a single, specific, stability-indicating method for the quantitation of the active moiety content. In many cases, the same procedure (e.g. LC) is used for both assay and determination of impurities.

The current regulatory quality status for peptide drug substances is presented here. Differences and similarities in guidelines and pharmacopoeias are highlighted, and their relevance discussed. Although peptide FDPs are not discussed, several API quality attributes are also applicable to the peptides in their marketed formulations. Of course, the quality of the FDPs is dependent on that of the API, with the impurity profile of the latter carried over into the formulation. Additional degradation compounds are to be expected due to interactions with the FDP excipients and the manufacturing/compounding process itself, resulting in higher acceptance levels for the impurities present.

Developmental Characterization

The quality specifications for batch release are to be regarded as being part of the overall pharmaceutical assurance strategy to ensure high product quality and consistency. Other aspects are extensive developmental characterization, product design, adherence to appropriate good manufacturing practices (aGMP) consistent with the developmental stage, manufacturing process validation, starting materials testing, in-process controls, API and FDP stability testing under different storage conditions, etc.

Developmental characterization, a mandatory part of the marketing authorization application, comprises the full battery of appropriate analytical procedures. These include elaborate structural identity proof, determination of physicochemical properties, biological activity, immunoreactive properties, purity and impurities, and allows the establishment of relevant specifications for routine testing of production lots with suitably, fully characterized reference standards being generated.

For full characterization of peptide drug substances, as required in development and for references, at least the following analytical procedures are to be considered [9,10]: elemental analysis, amino acid analysis, Edman sequencing, proteolytic mapping, tandem MS analysis, two-dimensional NMR, IR and UV/VIS spectral analysis, X-ray diffraction, differential scanning calorimetry, circular dichroism, optical rotation, analysis of enantiomeric purity of amino acids (e.g. hydrolysis in DCl/D_2O followed by derivatization and subsequent analysis using a chiral column [11]), LC techniques (reversed-phase, size exclusion, ion-exchange, affinity, etc), electrophoretic techniques (e.g. capillary electrophoresis and isoelectric focusing), microscopic techniques, analytical ultrafiltration, light scattering, biological assay methods (animal-based, cell culture-based and biochemical), etc. New analytical technologies and modifications to existing technologies are continuously being developed and should be utilized when appropriate. For method validation as well as for investigation of the peptide properties, developmental characterization includes also information obtained from relevant stress tests (e.g. light, heat, humidity, acid/base hydrolysis and oxidation).

The following sections discuss the routine API specifications, which are less elaborate than those used for drug candidates still in the R&D phase as discussed above. Emphasis will be placed on the pharmacopoeial approach, which serves as a basis for new peptides not yet described in an API compendium.

Characters

The pharmacopoeial information given under this heading is provided for those who use, prepare and dispense drugs solely to indicate descriptive (i.e. visual aspect & other organoleptic characteristics such as taste and odor) and solubility properties. Although these properties may indirectly assist in the preliminary API quality evaluation, they are not requirements in a strict sense.

In peptide monographs, a common description of appearance is 'white to almost white, hygroscopic powder'. Frequently mentioned solvents for stating solubility are water, ethanol and dilute solutions of acids and alkali hydroxides.

Production

Pharmacopoeial statements on particular aspects of the manufacturing process are mandatory requirements. However, this section is usually absent for classical chemical manufacturing processes. In most cases, the tests described cannot be easily verified on a sample of the final article by an independent analyst, and hence, testing needs to be carried out during production. The methods are normally not detailed as this is the responsibility of the manufacturer.

In peptide monographs, these are mostly related to APIs produced by methods based on rDNA technology with additional requirements for, e.g. biological activity using a bioassay, 'host-cell-derived proteins', 'host-cell or vector-derived DNA' and 'single

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Table 2. Pharmacopoeial peptides

#	Peptide	*European Pharmacopoeia*		*United States Pharmacopeia*	
		Monograph	Origin	Monograph	Origin
1	Bacitracin	01/2008 : 0465 (6.0)	Fermentation	32 (2009) pp. 1620	Fermentation
2	Bacitracin zinc	01/2008 : 0466 (6.0)	Fermentation	32 (2009) pp. 1623	Fermentation
3	Buserelin acetate	01/2008 : 1077 (6.3)	Chemical synthesis	–	–
4	Calcitonin acetate, salmon	01/2008 : 0471 (6.0)	Chemical synthesis rDNA technology	32 (2009) pp. 1747	Chemical synthesis rDNA technology
5	Colistimethate sodium	01/2008 : 0319 (6.0)	Semi-synthetic/fermentation	32 (2009) pp. 2021	Semi-synthetic/fermentation
6	Colistin sulphate	01/2008 : 0320 (6.0)	Fermentation	32 (2009) pp. 2022	Fermentation
7	Desmopressin acetate	07/2009 : 0712 (6.5)	Chemical synthesis	32 (2009) pp. 2075	Chemical synthesis
8	Felypressin acetate	01/2008 : 1634 (6.0)	Chemical synthesis	–	–
9	Glucagon, human	01/2008 : 1635 (6.0)	rDNA technology	–	–
10	Glucagon, porcine	–	–	32 (2009) pp. 2504	Pancreas extraction
11	Glucagon, bovine	–	–	32 (2009) pp. 2504	Pancreas extraction
12	Gonadorelin acetate	01/2008 : 0827 (6.0)	Chemical synthesis	32 (2009) pp. 2519	Chemical synthesis
13	Gonadorelin hydrochloride	–	–	32 (2009) pp. 2520	Chemical synthesis
14	Goserelin acetate	01/2008 : 1636 (6.0)	Chemical synthesis	–	–
15	Gramicidin	01/2008 : 0907 (6.0)	Fermentation	32 (2009) pp. 2528	Fermentation
16	Insulin aspart	01/2008 : 2084 (6.0)	rDNA technology	–	–
17	Insulin lispro	01/2008 : 2085 (6.0)	rDNA technology	32 (2009) pp. 2644	rDNA technology
18	Insulin, bovine	01/2008 : 1637 (6.0)	Pancreas extraction	32 (2009) pp. 2639	Pancreas extraction
19	Insulin, porcine	01/2008 : 1638 (6.0)	Pancreas extraction		
20	Insulin, human	01/2008 : 0838 (6.0)	Pig pancreas extraction + enzymatic modification rDNA technology	32 (2009) pp. 2642	Pig pancreas extraction + enzymatic modification rDNA technology
21	Leuprorelin acetate	01/2008 : 1442 (6.0)	Chemical synthesis	32 (2009) pp. 2761	Chemical synthesis
22	Oxytocin acetate	01/2008 : 0780 (6.0)	Chemical synthesis	32 (2009) pp. 3185	Chemical synthesis Pituitary extraction
23	Polymyxin B sulphate	01/2008 : 0203 (6.0)	Fermentation	32 (2009) pp. 3326	Fermentation
24	Protirelin acetate	01/2008 : 1144 (6.0)	Chemical synthesis	–	–
25	Somatostatin acetate	01/2008 : 0949 (6.0)	Chemical synthesis	–	–
26	Tetracosactide acetate	01/2009 : 0644 (6.3)	Chemical synthesis	–	–
27	Tyrothricin	01/2008 : 1662 (6.0)	Fermentation	32 (2009) pp. 3830	Fermentation
28	Vasopressin	–	–	32 (2009) pp. 3849	Chemical synthesis Pituitary extraction

chain precursor'. For human insulin produced by enzymatic modification of porcine insulin, additional evaluation of residual proteolytic activity is required.

Identification of Peptides

Guidelines

According to ICH guideline Q6A [2], identification testing in routine QC (giving an acceptable degree of assurance, and not intended as a full elucidation, cf. developmental characterization) should optimally be able to discriminate between compounds of closely related structure that are likely to be present. Generally, adequate specificity is obtained by applying a combination of different (mostly two) principles. In case of salts, identity confirmation should also include the counter-ions.

In case of peptide drug substance identification, the methods applied should at least be able to differentiate from (1) peptides with altered sequences or functional groups that may be formed due to a problem during the synthesis (e.g. amino acid deletion, truncation, remaining protective group, modification of disulfide bridge and perhaps even peptide sequences with two or more adjacent amino acids in the wrong order), (2) other peptides from the same therapeutic class and (3) other peptides available at the manufacturing site.

Current Pharmacopoeial Situation

Several techniques are currently in use for identity confirmation of pharmacopoeial peptide drug substances (Figure 1). For the vast majority of these compounds, this includes LC-UV analysis based on retention of intact and/or digested peptide by comparison with reference material. In the Ph. Eur., alternative tests mainly for use in pharmacies ('second identification') are described in six older peptide antibiotic monographs, all of them involving TLC. The retention time-based specificity is further increased by AAA, spectrometric techniques (e.g. IR, MS, NMR and UV), color reactions (e.g. biuret and ninhydrin) and biological tests (e.g. isolated organ assay). Only in some particular cases, the nonpeptide moiety of the active pharmaceutical ingredient is identified (e.g. sodium, sulphates and zinc for antibiotic peptides).

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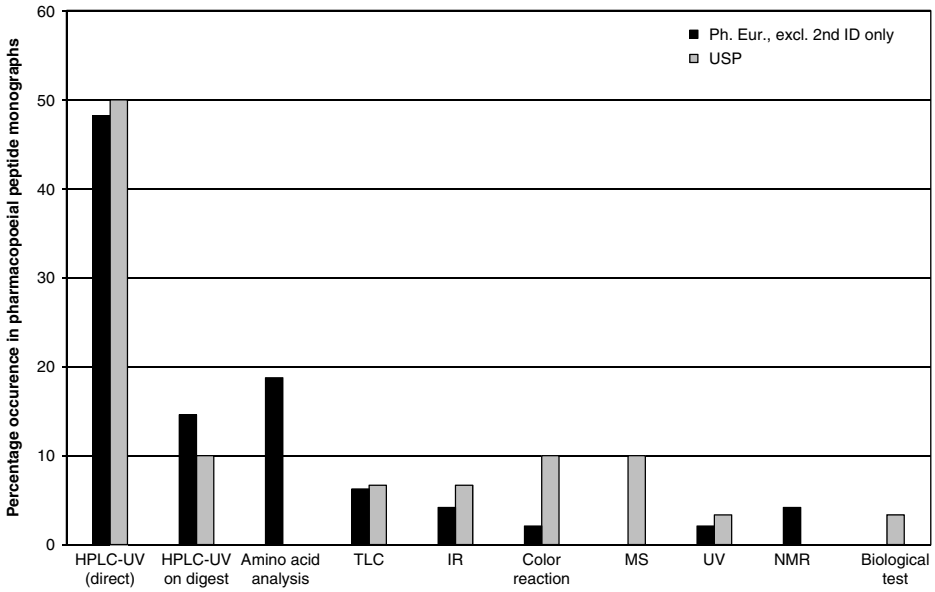

Figure 1. Techniques currently in use for pharmacopoeial peptide drug substance identity confirmation.

Pharmacopoeial Inconsistencies

With the exception of an almost universal LC-UV retention-time-based identification, there is a current lack of intra- and inter-pharmacopoeial harmonization in peptide monographs. The inconsistencies within the identification tests (i.e. next to the retention-time-based approach) are best demonstrated by the human gonadotropin-releasing hormone (gonadorelin) and its nonapeptide analogs, buserelin, leuprorelin (*syn.* leuprolide) and goserelin (Table 3). While AAA is not considered as an identification test by the USP, this analysis is described in the Ph. Eur. under this section for these peptides, except for gonadorelin where TLC is applied. In the latter case, AAA is included in the Ph. Eur. monograph under the 'tests' section, similar to the USP monographs on gonadorelin acetate and leuprolide. The spectroscopic techniques used for identification of these four strongly related peptides are ^{1}H NMR, ^{13}C NMR and IR for the Ph. Eur. with no intra-pharmacopoeial consistency, while IR and MS are being used by the USP. Next to the identification using LC-UV, the inter-pharmacopoeial harmonization is thus only observed for the IR spectrophotometry on leuprorelin.

Justification of Test Methods

Although none of the above tests are considered to be inadequate for the intended purpose, the adaptation of a single set of peptide identification tests throughout the pharmacopoeias is recommended to reduce the manufacturers' analytical burden, especially for the more expensive spectrometric techniques where NMR is mostly applied by Ph. Eur. and MS by USP.

NMR spectroscopy can provide unique information about peptide structure, dynamics, hydration and folding in the solution state [12–14]. Due to the structural complexity of most peptides, especially those larger than 15 amino acids, ^{1}H and ^{13}C NMR spectra are full of partially overlapping signals that can only be assigned by means of two-dimensional techniques. However, in the Ph. Eur., one-dimensional NMR is applied for identity confirmation based on profile comparison *versus* a reference NMR spectrum [15,16]. The information content of these analyses is considered to be similar to that of tandem MS experiments with typical fragment ions allowing the elucidation of the peptide sequence [17,18]. As opposed to NMR, suitable mass spectrometers and even LC-MS^{n} instrumentation have become routinely available for QC labs. Hence, it would seem logical to prefer identification of the primary peptide structure by MS over NMR, as supported by the USP. Moreover, LC-UV-MS^{n} would allow to obtain at the same time more detailed peptide impurity profiles, requires smaller amounts of substance to be examined and is also applicable to most finished peptide drug products.

AAA is not considered to be fully complementary to the sophisticated spectrometric techniques, but may be presented as an alternative. Biological tests using animal experiments (such as that described in the USP monograph on oxytocin) are no longer considered to be justified for routine QC of peptides, as physicochemical alternatives are available (cf. European Convention of the protection of animals used for experimental and other scientific purposes, 1986).

Counter-Ions

As both gonadorelin acetate and hydrochloride are available as active pharmaceutical ingredients, identification of the counter-ion would normally be required as per ICH Q6A. Furthermore, introduction of this additional test for all peptide API monographs would be justified as peptide trifluoroacetates are also frequently available as synthesis intermediate [19]. It should be mentioned that the acetate counter-ion also be identified by its chromatographic assay, color reactions and even NMR analysis allowing distinction between mono- and diacetate [3].

Tests

From the occurrence of the pharmacopoeial tests in peptide monographs (excluding specific tests based on intended use; see Figure 2), it was found that the USP presents a greater variety in tests than the more consistent Ph. Eur., although there is still much room for improvement.

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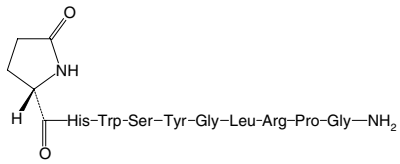
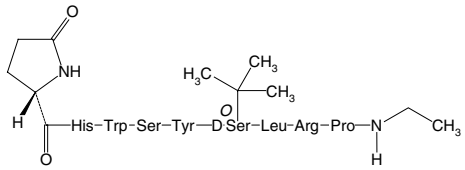
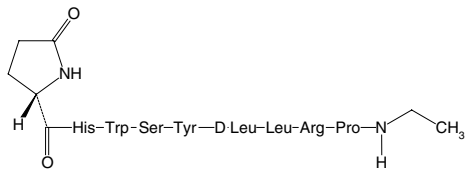
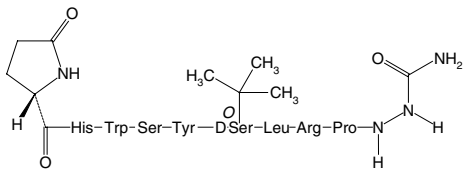
Table 3. Identification of gonadorelin and analogs

Peptide	Identification tests	
	European Pharmacopoeia 6.5	United StatesPharmacopeia 32
Gonadorelin [$C_{55}H_{75}N_{17}O_{13}$; M_r 1182.31] 	*Mono-acetate:* A. Liquid chromatography B. Thin-layer chromatography	*Di-acetate:* A. Mass spectrometry B. Liquid chromatography *Hydrochloride:* Liquid chromatography
Buserelin [$C_{60}H_{86}N_{16}O_{13}$; M_r 1239.44] 	A. Liquid chromatography B. ^{1}H NMR spectrometry C. Amino acid analysis	–
Leuprorelin [$C_{59}H_{84}N_{16}O_{12}$; M_r 1209.42] 	A. IR spectrophotometry B. Liquid chromatography C. Amino acid analysis	A. IR spectrophotometry B. Liquid chromatography
Goserelin [$C_{59}H_{84}N_{18}O_{14}$; M_r 1269.43] 	A. ^{13}C NMR spectrometry B. Liquid chromatography C. Amino acid analysis	–

– : No monograph available.

Related Peptide Impurities

In contrast to low molecular weight drugs, the synthesis of peptides consists of many steps coupling different amino acids to each other, resulting in a greater diversity of potential impurities [20–22] (Table 4). For the majority of peptide drug substances, the presence of related peptide impurities is checked by gradient reversed-phase LC with UV detection. Although most of the pharmacopoeial methods currently apply non-MS compatible mobile phases, it is expected that future monographs will prescribe more convenient mobile phase additives to allow also direct identification by LC-MS [3,46].

Since the release for public consultation of the Q3 guideline draft on impurities in new drug substances (excluding peptides) in March 1994, a rationale for the reporting and control of related peptide impurities was under discussion [3,47]. Recently, thresholds for reporting, identification and qualification of related substances in synthetic peptide drug substances were included in the Ph. Eur. (monograph 07/2009 : 2034 'Substances for pharmaceutical use'): 0.1%, 0.5% and 1.0%, respectively. Hence, all unspecified impurities, including ODIs as defined by the Ph. Eur. in a separate section listing potential impurities (designated by a letter of the alphabet), are expected to comply with these new thresholds: see Table 5. However, specific (lower) thresholds should be applied for impurities known to be unusually potent or to produce toxic or unexpected pharmacological effects. As even small peptides can be highly toxic [48], this risk should not be underestimated. Furthermore, it has been demonstrated that even small changes in a peptide sequence by one or two amino acids can convert a receptor agonist into an antagonist and vice versa [49,50].

All potential impurities observed during the developmental and stress stability studies are classified into specified and unspecified, depending on their 'real-life' presence and concentration levels. The consequence is that specified impurities will be targeted and individually be assessed and reported during QC analysis. The limits for the related impurities are related to the thresholds, with the qualification threshold as a safety-alert threshold. Of course, specified impurities in the pharmacopoeial monographs are qualified at the indicated limit, as the basis of a monograph is the existing product where the impurity profile is 'qualified by its use'.

In the Ph. Eur., a disregard limit (which is not necessarily identical to the reporting threshold) was commonly used for the calculation

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Table 4. Peptide impurities

Peptide impurity	Modification	Typical examples	References
Diastereomeric (racemized) peptides	Single L- to D-amino acid conversion	[2-D-histidine]leuprorelin (Ph. Eur. impurity B)	[23,24]
	Single D- to L-amino acid conversion	[6-L-leucine]leuprorelin (Ph. Eur. impurity C)	
	Double D/L- to L/D-amino acid conversion	[2-D-histidine,4-D-serine]leuprorelin (Ph. Eur. impurity F)	
Deamidation peptides	Asn to (iso-)Asp conversion	[5-L-aspartic acid]desmopressin (Ph. Eur. impurity A)	[20,25–27]
	Gln to Glu conversion	[4-L-glutamic acid]desmopressin (Ph. Eur. impurity B)	[20,26,28]
	C-terminal deamidation	[9-glycine]desmopressin (Ph. Eur. impurity C)	[29]
Deletion peptides	Single/double amino acid deletion	des-22-tyrosine-calcitonin (salmon) (Ph. Eur. impurity C)	[21,30,31]
Insertion peptides	Single amino acid insertion	endo-8a-L-proline-goserelin (Ph. Eur. impurity J)	[21,31]
	Double amino acid insertion	endo-8a,8b-di-L-proline-goserelin (Ph. Eur. impurity I)	
Truncated peptides	Missing amino acids at *N*-terminus	buserelin-(3–9)-peptide (Ph. Eur. impurity C)	[30]
	Missing amino acids at *C*-terminus	5-oxo-L-prolyl-L-histidine (protirelin Ph. Eur. impurity C)	[29]
Acetylated peptides	Serine *O*-acetylation	O^4-acetylgoserelin (Ph. Eur. imp. K)	[32]
	Threonine *O*-acetylation	[12-(O-acetyl-threonine)]somatostatin (R0-CEP 2005-245-Rev 00)	[33]
	Lysine *N*-acetylation	(N-ε -acetyl-lysine)somatostatin (R0-CEP 2005-245-Rev 00)	
	N-terminal acetylation	N^1-acetylfelypressin (Ph. Eur. impurity E)	
Oxidation peptides	Met oxidation to sulfoxide or sulfone	Tetracosactide sulphoxide (Ph. Eur. impurity A)	[28,34]
	Oxidation of other amino acids (e.g. Trp, Cys, His)	Trp-oxidized leuprolin	[21,34–37]
	Formation of trisulfide from disulfide	Salmon calcitonin trisulfide	[38]
β-Elimination peptides	Formation of dehydroalanine, thiocysteine, etc.	[4-dehydroalanine]leuprorelin (Ph. Eur. Impurity K)	[39]
Reduction peptides	Reduction of, e.g. Trp and disulfide	Trp-reduced leuprolin	[20,21]
Reaction by-products from incomplete deprotection	Acetylaminomethyl (Acm) derivatives	S^1,S^6-bis[(acetylamino)methyl]-(reduced felypressin) (Ph. Eur. impurity A)	[40]
	t-Bu, Fmoc, etc derivatives	*t*-Bu/Fmoc-leuprolin	[21,41]
Other reaction by-products	*C*-terminal dimethylamidation	$N^{1.9}$,$N^{1.9}$-dimethyldesmopressin (Ph. Eur. Impurity G)	[34,42]
	C-terminal diazane to hydrazide	[9-L-prolinohydrazide]goserelin (Ph. Eur. Impurity E)	
	Side-chain reactivity (e.g. arginine)	[8-[5-*N*-[imino(1*H*-pyrazol-1-yl)methyl]-L-ornithine]]leuprorelin (Ph. Eur. impurity J)	[31]
Cyclization	Intramolecular amide bond formation	Cyclo-(L-histidyl-L-prolyl-) (Protirelin Ph. Eur. impurity E)	[43]
Oligomers and/or aggregates	Covalent self-association, e.g. intermolecular disulfide exchange and di-Tyr cross-links	Bis(reduced felypressin) (1,6′),(1′,6)-bis(disulfide) (Ph. Eur. impurity C)	[28]
	Noncovalent self-association	Glucagon fibrils	[44,45]

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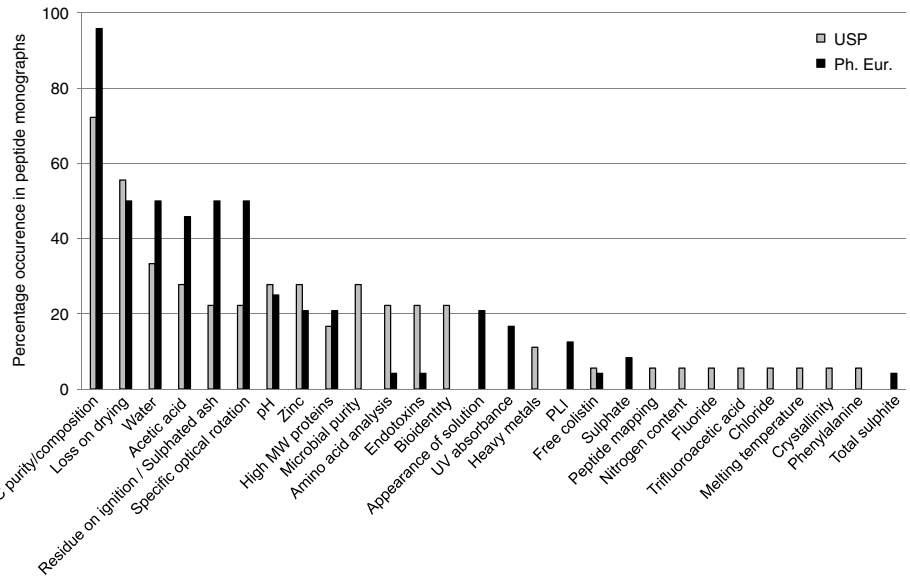

Figure 2. Occurrence of purity tests in pharmacopoeial peptide monographs (excluding those intended for use in the manufacture of specific dosage forms).

Table 5. Acceptance criteria[a] for related substances in synthetic peptide drug substances

		Individually listed and limited with a specific acceptance criterion[b] ?	
		Yes	No
Identified?	Yes	Specified identified	Unspecified identified
		AC : Specified limit	AC : Not more than 1.0% (QT), unless general AC[c] is lower[d]
		E.g. *Leuprorelin Ph. Eur. impurity C* (i.e. [*6-L -leucine*]*leuprorelin*)	E.g. *Leuprorelin Ph. Eur. impurity H* (i.e. [*7-D-leucine*]*leuprorelin given under 'Other detectable impurities'*)
	No	Specified unidentified	Unspecified unidentified
		AC : Specified limit	AC : Not more than 0.5% (IT), unless general AC[(3)] is lower
		E.g. *Tetracosactide Ph. Eur. impurity B* (*unidentified impurity at RRT = 0.95*)	*'Unidentified impurity at RRT = x.xx'*

[a] Four types of acceptance criteria (AC) are applicable: specified AC, general AC, identification threshold (IT) and qualification threshold (QT).
[b] Specified ACs: approved by the competent authorities (cf. pharmacopoeia, CEP and DMF).
[c] For substances already evaluated by the competent authorities, a general acceptance criterion on 'any other impurity' may be applicable.
[d] According to the Ph. Eur. definition of 'Other detectable impurities', these compounds are not known to be normally present above the identification threshold (IT). Consequently, when these are observed above the IT, it is common practice to include these related substances in specified impurities list.

of the sum of impurities. The applicability of this disregard limit next to the reporting threshold is currently not clear.

Concerning the Ph. Eur. synthetic peptide monographs, disregard limits and total sum of impurities are ranging from 0.03% to 0.1% and 1.5% to 12%, respectively. The largest limit for unspecified impurities was observed in the tetracosactide monograph: 2.5%, with 9% sum of impurities allowed other than the specified sulphoxide (limited at 3%).

As per ICH Q3A, related substances results are to be reported (e.g. on a certificate of analysis) as an impurity profile, which lists all individual specified impurities and all unspecified impurities above the stated reporting threshold with identification (if > IT) or appropriate descriptor (e.g. relative retention time), together with the applicable acceptance criteria and test outcome as values with one or two decimal places (for results ≥1.0% and <1.0%, respectively). Results for specified impurities below the reporting threshold should be given as 'not more than' followed by that threshold (which should be higher than the limit of detection). A supplementary chromatogram with impurities indicated may be given as well. Although intended for the registration applications of new drugs, it is recommended to apply the impurity profile reporting as described above also during the earlier research stages of peptide drug development [46].

High Molecular Weight Peptides and Proteins

High molecular weight peptide/protein impurities encountered in peptide APIs may arise from several aggregation or self-association mechanisms [51]. Peptide aggregates are generally classified into covalent and noncovalent interaction products, with the extent of their formation dependent on a wide variety of environmental

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factors. Covalent aggregates are typically formed from two or more monomers by disulfide bonds or oxidation of tyrosine to bityrosine, while hydrophobic and electrostatic interactions are involved in the formation of noncovalent aggregates. Because these impurities (i.e. dimers to polymers) can have serious toxicological, immunological or pharmacological consequences, adequate control of their presence in potentially susceptible peptide APIs is required. Quantification of these impurities is currently rarely included in the Ph. Eur. and USP monographs. Nevertheless, peptide aggregation/fibrillation were also reported in literature for salmon calcitonin and glucagon [52]. In the pharmacopoeias, these impurities are explicitly analyzed as a separate test on insulin drug substances involving size-exclusion chromatography. It should also be noted that in some cases, covalently bound oligomers can also be determined using the reversed-phase LC method for related substances [46]. This approach is rarely followed in the pharmacopoeias, e.g. felypressin Ph. Eur. impurities C and D (both limited as specified impurities at 0.5% each).

The pharmacopoeial acceptance limits on the sum of all impurities with a molecular mass higher than that of insulin monomer are harmonized and ranged from 0.25% (insulin lispro) to 1.0% (bovine, human and porcine). Although it is not fully clear whether the thresholds given in Ph. Eur. monograph 07/2009 : 2034 (i.e. RT, IT and QT) are applicable to these organic impurities as well, it seems logical to use the QT of 1.0% as an upper limit for these high molecular weight peptides and proteins, unless justified otherwise.

Amino Acid Analysis

Based on the new qualification threshold of 1.0%, AAA as purity test for related peptides (cf. gonadorelin acetate Ph. Eur./USP, calcitonin salmon USP and leuprolide acetate USP) is clearly considered to be obsolete for synthetic peptides as even the narrowest limits on individual amino acid content are equivalent to 5% [1].

Water and Acetic Acid

Except for oxytocin USP and vasopressin USP, residual water and acetic acid are consistently described in the pharmacopoeial synthetic peptide monographs using Karl Fischer coulometric titration and LC, respectively. For nonsynthetic peptides, the less selective 'loss on drying' is a commonly applied test. The tests for water and acetic acid are considered to be essential quality attributes as these factors may affect the stability of the peptide. Furthermore, as water and acetic acid are generally major constituents of peptide drug substances, their presence requires factorization for FDP production (i.e. compounding based on active moiety content) and has economical consequences as well.

Peptide monographs in the Ph. Eur. consistently give single-sided acceptance criteria for the tests 'water' and 'loss on drying' ranging from not more than 3% to not more than 14%, dependent on the hydrophilicity of the compound.

Acetic acid acceptance criteria in the two pharmacopoeias are normally given as a range (±2.0% to 8.0% absolute) with the target value for the fully protonated species being related to the number of positively charges in the peptide (Arg, Lys, *N*-terminus, etc) normalized for the molecular mass. Attention has to be paid to differences between pharmacopoeias, e.g. both the Ph. Eur. and the USP include a gonadorelin acetate monograph. However, Ph. Eur. specifies 4.0% to 7.5% of acetic acid (i.e. monoacetate), while 8% to 12.5% (i.e. diacetate) in USP.

Optical Rotation

The Ph. Eur. currently includes 12 peptide monographs containing specifications on specific optical rotation; the majority concerning synthetic peptides ($n = 9$). Only for two other synthetic peptides, oxytocin and calcitonin, no such test is included. The optical rotation is measured on solutions containing between 2 and 50 g/l of peptide with high equipment performance requirements for the lower concentrations (i.e. exceeding the Ph. Eur. specifications of 0.01° readability), especially for goserelin where the product acceptance criteria are given as a quite narrow range. However, contrasting the used high concentrations, it should be noted that ideally this measurement involves a strongly diluted solution due to concentration-dependent phenomena (e.g. dimerization) [53]. In all 12 Ph. Eur. monographs, the specific optical rotation specification is defined as an acceptance range around a target value: ±2° to 6° absolute, or ±3.7% to 11.9% *versus* target. No logical explanation (other than historic or economic) could be given for the rather large variability of the acceptance ranges. However, in most cases (e.g. acceptance limits broader than those for diastereomers-discriminating assay), this test is considered to be superfluous for the control of impurities in synthetic peptide drug substances: even if all theoretical diastereomeric impurities would be present at a concentration just below the reporting threshold, these are controlled by LC analysis for assay. Furthermore, the incorporation of D-amino acids in the peptide sequence decreases the sensitivity of the optical rotation test, making it even less suitable for its intended purpose. The usefulness of this test is currently under debate, and it seems that the optical rotation was incorporated in pharmacopoeial peptide monographs based on historic rather than scientific reasons [3].

Absorbance

The Ph. Eur. currently includes four peptide monographs containing specifications on UV absorbance. All four include the determination of a specific absorbance at approximately 278 nm, and concerned synthetic peptides containing tryptophan residues (i.e. a prerequisite for quantitative determinations at this wavelength). However, Ph. Eur. monograph on two similar synthetic peptides (i.e. goserelin and leuprorelin) lack this test, indicating that regulatory authorities do not always consider it to be necessary for adequate quality control testing. For buserelin, gonadorelin and tetracosactide, the acceptance limits are given as two-sided acceptance criteria, ranging from ±5.2% to 9.0% relative to the center value, which fits more or less the theoretical value [54,55]. Based on these broad acceptance criteria, this test is no longer considered to be adequate for the control of impurities in peptide drug substances.

Residual Solvents and Reagents

In addition to specified related substances, peptide CEPs frequently include supplementary quality attributes and acceptance criteria on residual solvents (e.g. isopropanol and acetonitrile) and reagents (e.g. trifluoroacetic acid and triethylamine) as required by the mandatory Ph. Eur. general monograph 07/2009 : 2034 'Substances for pharmaceutical use'. Although residual solvents are expected to comply with ICH guideline Q3C, PDE values for reagents are not always available to calculate the acceptance limits (e.g. trifluoroacetic acid), leaving the justification of the accepted levels for these residual compounds in the hands of the manufacturer. For trifluoroacetic acid, which is also a

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metabolite of isoflurane inhalation anesthetic, the limits applied were found to range from 0.1% to 1% (e.g. somatostatin: Bachem R0-CEP 2005-245-Rev 00 and Lipotec R0-CEP 2000-078-Rev 01, respectively). An explanation for these differences is that the acceptance criteria applied for specific impurities should also be based on capability considerations whenever qualification is not a concern. While residual solvents are normally determined using (static headspace) gas chromatography, trifluoroacetic acid is commonly determined using ion chromatography [56].

Microbiological Attributes

Although synthetic compounds are rarely contaminated with micro-organisms, it should be noted that these organisms clearly have the propensity to degrade/metabolize a peptide active constituent. If intended for use in the manufacture of nonsterile pharmaceutical preparations, TAMC and TYMC are expected to adhere respective acceptance criteria of 10^3 CFU/g and 10^2 CFU/g, as per harmonized ICH Q4B annex 4c guideline (i.e. Ph. Eur. monograph 01/2009:50104 and USP <1111>). However, more stringent acceptance criteria for nonsterile dosage forms may be applicable dependent on route of administration (e.g. nasal use: 10^2 CFU/g and 10^1 CFU/g, respectively; with additional tests for specified micro-organisms).

Where the label states that the peptide is sterile, it should meet the requirements for sterility testing (i.e. Ph. Eur. 01/2009:20601 and USP<71>) as one of the aspects of process sterility assurance.

If intended for use in the manufacture of parenteral preparations (i.e. sterile dosage forms intended for administration by injection, infusion or implantation) without a further appropriate procedure for the removal of bacterial endotoxins, endotoxins from Gram-negative bacteria should be quantified using a LAL test (i.e. Ph. Eur. 01/2008:20614 and USP<85>). Acceptance limits given in Ph. Eur. peptide drug substance monographs are ranging from 0.7 to 500 IU/mg (protirelin and desmopressin, respectively), and are based on the maximum recommended human dose of product per kg of body weight in a single hour period. In the USP, limits are sometimes expressed as IU per unit of biological activity. Only when justified and authorized, the LAL test can be replaced by the pharmacopoeial 'pyrogens' test (i.e. Ph. Eur. 01/2008:20608 and USP<151>) which involves i.v. injection in rabbits and subsequent body temperature recording.

Immunogenicity

Unlike proteins, the risk of evoking an immune reaction is quite low for peptides [3]. Although related peptide impurities, with the exception of truncated sequences, could in theory be more immunogenic as compared with their parent peptide, these are considered to be sufficiently safe at levels below the 1.0% qualification threshold. However, for peptides not obtained from chemical synthesis, the immunogenic potential of protein impurities should be carefully examined. In the Ph. Eur., an immunochemical test 'proinsulin-like immunoreactivity' (PLI) is described for insulins obtained from animal pancreas with and without enzymatic modification to assess the drug substance immunogenicity with an applied limit of 10 ppm, calculated on dried substance. Although the suitability of this test is well-documented [57], no such test is incorporated in the USP monographs. However, the relevance of these monographs is questionable as these older insulin types obtained from animal pancreas are already replaced by more modern versions being marketed.

Bioidentity

In the USP, a 'bioidentity' test is described in four peptide API monographs, while not present in the Ph. Eur. monographs. For USP salmon calcitonin, this test involves a quantification of cAMP produced within human mammary tumor cell line T-47D; and for the three USP monographs on insulins, this is a qualitative test with subcutaneous injection in rabbits with dextrose determined in the blood samples taken. This test is intended to assess the ability or capacity of a biotechnological/biological product to achieve a defined biological effect (cf. ICH guideline Q6B). However, as peptides are normally not considered to be as complex molecules as large proteins, this test is considered to be redundant provided that sufficient physicochemical characterization is established and a well-established manufacturing history is available. Nevertheless, the USP seems to take a different position, requiring the use of rabbits for compliance of insulin 20 years after this was abandoned by the Ph. Eur.

Inorganic Impurities

Tests such as 'sulphated ash'/'residue on ignition' (for determination of the inorganic impurities content in organic substances) and 'heavy metals' are not often observed in recent pharmacopoeial monographs on peptide drug substances.

The presence of heavy metals in peptides can arise from starting materials and reagents, leaching from pipes and equipment, etc, and as residual catalysts. From the latter category, palladium was very frequently mentioned as a supplementary test in the peptide CEPs. EMEA guideline EMEA/CHMP/SWP/4446/2000 on the specification limits for residues of metal catalysts or metal reagents gives PDEs for palladium (i.e. 100 and 10 μg/day for oral and parenteral use, respectively). Two options are considered for the calculation of the residual metal concentration limit as the PDE divided through the daily dose. The first one assumes a daily dose of 10 g, resulting in respectively 10 and 1 ppm of palladium when considering oral and parenteral use, respectively. Option 2 takes into account the actual daily dose of the API in a FDP, leading to much lower limits for peptides that are normally used at doses far below 10 g per day. The inclusion of a test for this Pd catalyst, classified by EMEA as metal with significant safety concern (i.e. class 1), was frequently observed in CEPs with stringent acceptance criteria ranging from 1 to 10 ppm (e.g. Bachem somatostatin R0-CEP 2005-245-Rev 00 and Bachem gonadorelin acetate R0-CEP 2005-022-Rev 00, respectively).

As inorganic impurities (such as metals salts) can be toxic and/or negatively influence the stability of the peptide compound, considering heavy metal binding properties of peptides, the incorporation of an adequate heavy metals test in the peptide drug substance specifications is recommended. Recent documents from the authorities suggest the implementation in the near future of ICP (both with AES and MS detection) for determining heavy metals, replacing the 'heavy metals' colorimetric tests with thioacetamide [58]. This technique offers a more sensitive, specific and reproducible way to determine heavy metal concentration while including all elements of pharmaceutical interest at high recoveries [59,60].

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Table 6. Monograph proposal for peptides obtained by chemical synthesis or using rDNA technology

#	Quality attribute	Typical method	Typical acceptance criteria
Characters			
1	Appearance	Visual aspect	White or almost white powder
2	Solubility	Visual aspect	Solubility in water, ethanol and dilute acetic acid
Identification			
3	Active moiety	RP-HPLC-UV	Retention time *versus* reference
4	Active moiety	(LC-)MS	(Tandem) mass spectrum *versus* reference
5	Counter-ion	Color reaction/HPLC-UV	Positive
General purity tests			
6	Related substances	RP-HPLC-UV	Individual, identified $\leq$1.0% Individual, unidentified $\leq$0.5% Total $\leq$5.0%
7	Counter-ion content	HPLC-UV	Acetic acid: target $\pm$5.0%
8	Water content	Karl-Fisher	$\leq$10.0%
Purity tests based on intended use			
9	Total aerobic microbial count	TAMC plate count	Nonsterile API: $\leq 10^2$ CFU/g
10	Total combined yeasts/moulds count	TYMC plate count	Nonsterile API: $\leq 10^1$ CFU/g
11	Absence of specified micro-organisms	Pathogens test (*S. aureus, P. aeruginosa*)	Nonsterile API, depending upon route of administration: absence in 1 g
12	Bacterial endotoxins	LAL test	API for parenteral use without appropriate removal: $\leq$10 IU/mg
13	Sterility	Sterility test	Sterile API: complies
Peptide-specific purity tests			
14	Higher molecular weight peptides and proteins	SEC/RP-HPLC-UV	Total $\leq$1.0%
Synthesis-specific purity tests			
15	Residual reagents	HPLC-UV (IEC)	Trifluoroacetic acid $\leq$1.0%
16	Residual solvents	(Headspace) GC	Isopropanol $\leq$0.5%
17	Residual catalysts	AAS/ICP-AES	Palladium $\leq$10 ppm
18	Immunogenicity	Immunochemical test	$\leq$10ppm
Assay			
19	Peptide content	RP-HPLC-UV	94.0% to 102.0%, calculated on anhydrous and counter-ion-free substance
20	Mass balance	Calculation	100% – water – counter-ion – sum of impurities

Other Tests

Other tests such as 'pH' and 'appearance of solution' are less frequently observed in recent pharmacopoeial monographs on peptide drug substances. These quality attributes might be useful in specific cases, and can be added to the specifications based on the developmental characterization results.

Assay

According to ICH guideline Q6A, a specific stability-indicating procedure should be included in the drug substance specifications to determine the content of the drug substance. There are two main approaches to determine the API content: (1) absolute assay as a functional group analysis and (2) relative assay against a reference standard. While the first approach is generally less selective and requires a standardized titrant in most cases, the latter assumes that the standard remains unchanged and that its original purity determination is valid in the conditions in which it will be used [61]. Generally speaking, absolute assay methods (such as titrations with potentiometric endpoint detection) are still more abundant in the Ph. Eur. when compared with the USP (where the breakthrough of LC-based assay methods was much faster) [62].

For many peptide APIs, it is possible to employ the same LC procedure for both assay and quantitation of related substances using appropriately characterized reference substances. In some particular cases, a microbiological assay of antibiotics is included in the pharmacopoeial peptide monographs, and also a biological test and an amino acid analysis are described in two separate USP monographs (glucagon and gonadorelin acetate, respectively) as peptide assay. Sophisticated high-precision absolute peptide assay methods, such as metal coded tagging in combination with LC-ICP-MS [63], are currently not described in either pharmacopoeia. All pharmacopoeial LC assays are performed using analytical (i.e. mostly 3.9 to 4.6 mm internal diameter) C_{18}

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reversed phase columns with UV detection at 210 to 220 nm, targeting the amide bond, except for Ph. Eur. monograph 01/2009 : 0644 'Tetracosactide' where 275 nm is prescribed as wavelength for quantification, targeting the aromatic amino acids.

Except for five monographs on peptide antibiotics, the acceptance criteria are expressed in Ph. Eur. 6.5 as % m/m (in most cases, based on the anhydrous and acetic acid-free substance). The peptide antibiotics described in the current pharmacopoeia are complex peptide mixtures obtained from fermentation. Each component (which is generally designated by an alphanumerical code) demonstrates its own specific antimicrobial activity. Hence, the active moiety content is expressed in units of biological activity.

In the USP, the peptide content of nonantibiotic peptide compounds is still frequently expressed in specific USP units (e.g. glucagon, insulin, oxytocin and vasopressin).

Combining both pharmacopoeias, excluding antibiotics and other monographs where only upper limits are given or limits in USP units, the mean lower and upper acceptance limit $\pm$ standard deviation are 93.9% $\pm$ 2.3% and 103.6% $\pm$ 1.3%, respectively. The frequent use of asymmetric specification intervals for these selective assays arises from taking into account the total sum of impurities (average = 2.5%). The upper acceptance limit for assay also demonstrates that amino acid analysis does not suit the requirements as the variability of this method is considered to be too high (up to 5% is acceptable for well-recovered amino acids according to Ph. Eur. monograph 01/2008 : 20256).

The application of complementary acceptance criteria on mass balance (i.e. 100% – total impurities, counter-ions, residual solvents and water, e.g. as part of the internal release specifications) may be advisable [64,65]. This is especially helpful in designing biomedical experiments or for production purposes, where the quantity of peptide active moiety is required.

Conclusion

A peptide-drug monograph should basically consist of appearance, solubility information (important for analytical/product development), identification by LC-UV supplemented by another test such as MS, related peptides by LC-UV (including higher molecular weight derivatives), residual solvents (water, acetic acid, others), residual reagents, inorganic impurities (such as catalysts), microbiological quality attributes and assay by LC-UV. Such a typical monograph is given in Table 6. Related substances are expected to adhere to thresholds of reporting (0.1%), identification (0.5%) and qualification (1.0%). Individual impurities should primarily focus on synthesis impurities such as diastereomeric and deamidated peptides. Total related impurities are generally below 5%.

Pharmacopoeias are legally binding reference works for the quality control of medicines and pharmaceutical substances. Hence, the monographs given therein are expected to contain frequently updated, scientifically justified test and acceptance criteria, reflecting the current state-of-the-art techniques and quality levels of products on the market. Nevertheless, the current relevance of some tests, as observed for peptide APIs (e.g. UV absorbance, optical rotation, amino acid analysis) in both Ph. Eur. and USP, can be questioned. Also, potential improvements were suggested (e.g. identification of counter ion, use of MS-compatible LC mobile phases). Based upon the outcome of developmental characterization, supplementary specifications (e.g. racemization of individual amino acids within the peptide sequence) might be included as well.

Furthermore, to diminish the workload in QC labs, full harmonization between the pharmacopoeias would be welcomed, with consistency between the different peptide monographs also being one of the objectives. Clear-cut decisions would be beneficial as to how peptides should be identified (i.e. MS or NMR), and whether or not to use bioassays for routine QC testing.

For R&D peptides, careful attention should be paid to sufficient characterization and quality control [46]. Ideally, these should adhere to similar thresholds for impurities as described above, applying one or more suitable purity tests (e.g. LC-MS with adapted mobile phase gradient).

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