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UNIVERSITEIT GENT

FACULTY OF PHARMACEUTICAL SCIENCES

Development of quality specifications for peptide drugs

Bart De Spiegeleer[1,*], Valentijn Vergote[1] and Christian Burvenich[2]

[1] Drug Quality & Registration (DruQuaR) group, Faculty of Pharmaceutical Sciences, Ghent University, Harelbekestraat 72, B-9000 Ghent, Belgium.
[2] Department of Physiology and Biometrics, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, B-9820 Merelbeke, Belgium.
***Corresponding author: bart.despiegeleer@ugent.be (O.Ref.: 2008-276a; 11th Naples Workshop on Bioactive Peptides)**

INTRODUCTION

Peptides show great pharmaceutical potential as active drugs in different therapeutic areas like allergy, anti-infection, oncology, obesity, etc and as functional excipients in drug delivery systems to overcome tissue and cellular membrane barriers. The development of a peptide toward a pharmaceutical compound poses however unique challenges: the rational development of its quality specifications is one of the major issues in this process. We present here the current regulatory quality status for peptide drugs. Differences and similarities in guidelines and pharmacopoeial differences will be highlighted, leading to a proposal of a consistent basic monograph.

RESULTS

Table I: Pharmacopoeial peptides & related impurities acceptance criteria (incl. high molecular weight peptides)

#	Name	# AA	Pharmacopoeia [1]		Origin	Ph. Eur. acceptance criteria on related substances (%)				USP acceptance criteria on related substances (%)			
			Ph. Eur.	USP		Specified	Unspecified	Total	DL	Specified	Unspecified	Total	DL
1	Bacitracin	11	0465	1483	*B. licheniformis* or *B.subtilis*	6.0	20.0 [4]	-	0.5		No [2]		
2	Buserelin	9	1077	-	Synthetic	3 [6]	3	5	0.1		NA [3]		
3	Calcitonin (salmon)	32	0471	1595	Synthetic or rDNA	3.0 / 0.6 / 0.2	-	5.0	0.1	-	3.0	5.0	0.1
4	Colistin sulphate	10	0320	1847	*B.polymyxa* var. colistinus	-	4.0	23.0	× [5]				
5	Desmopressin	9	0712	1897	Synthetic	-	0.5	1.5	0.05	-	0.5	1.5	-
6	Felypressin	9	1634	-	Synthetic	0.5	0.1	3.0	0.05		NA [3]		
7	Glucagon	29	1635	2277	rDNA (Ph.Eur., human) Pork/ox pancreas (USP)	0.5 [6]	-	2.5	-	-	2.5	10.0	-
8	Gonadorelin acetate	10	0827	2291	Synthetic	-	2	5	0.05	-	1	2	0.05
9	Goserelin	9	1636	-	Synthetic	1.0 / 0.5	0.5	2.5	0.05		NA [3]		
10	Gramicidin	15	0907	2300	*Brevibacillus brevis* Dubos	2.0 / 1.0	2.0 / 1.0	-	× [5]		No [2]		
11a	Insulin aspart	51	2084	-	rDNA	1.0 / 2.0 & 0.5 [6]	1.5 [4]	-	-		NA [3]		
11b	Insulin, bovine	51	1637	2403	Ox (bovine) pancreas	3.0 & 1.0 [6]	3.0 [4]	-	-	1.0 / 10.0 & 1.0 [6]	5.0 [4]	-	-
11c	Insulin, human	51	0838	2405	Pork pancreas or rDNA	2.0 / 1.0 & 1.0 [6]	2.0 [4]	-	-	2.0 & 1.0 [6]	2.0 [4]	-	-
11d	Insulin lispro	51	2085	2408	rDNA	1.0 & 0.25 [6]	0.5 & 2.0 [4]	-	-	1.0 & 0.25 [6]	0.50 & 2.00 [4]	-	-
11e	Insulin, porcine	51	1638	2403	Pork pancreas	2.0 & 1.0 [6]	2.0 [4]	-	-	1.0 / 10.0 & 1.0 [6]	5.0 [4]	-	-
12	Leuprorelin (leuprolide)	9	1442	2510	Synthetic	1 / 0.5	0.5	2.5	0.1	1.0 / 0.5	0.5	2.5	-
13	Oxytocin	9	0780	2897	Synthetic	-	1.5	5	0.1	× [7]	-	5	-
14	Polymyxin B sulphate	10	0203	3023	*Paenibacillus polymyxa*	-	3.0	17.0	× [5]		No [2]		
15	Protirelin	3	1144	-	Synthetic	2	2	3	0.05		NA [3]		
16	Somatostatin	14	0949	-	Synthetic	-	1	2	0.03		NA [3]		
17	Tetracosactide	24	0644	-	Synthetic	4	5.0 / 2.5	-	-		NA [3]		
18	Tyrothricin	10 & 15	1662	3487	*Brevibacillus brevis* Dubos		No [2]				No [2]		
19	Vasopressin	9	-	3502	Synthetic or pork/ox pituitary		NA [3]			× [7]	-	5	-

-: absent; DL: disregard limit; [1] Ph. Eur.: 01/2008 monograph number; USP 31: page; [2] No: no related substances test described in monograph; [3] Not applicable: peptide is not described in pharmacopoeia; [4] Acceptance limit on sum of unspecified impurities; [5] No quantitatively defined disregard limit, but "diluted reference standard" operationally defined; [6] Acceptance limit on sum of two or more specified impurities; [7] Acceptance limit not defined in %, but as USP units.

Different origins of pharmacopoeial peptides:
- Biological: cells (n = 5/25) and tissues (n = 4/25)
- Biotechnological (rDNA) (n = 5/25)
- Synthetic (n = 11/25)

Non-pharmacopoeial peptides (*e.g.* ganirelix, cetrorelix, depreotide)

ICH guidelines:
- Explicit mentioned: Q5C, Q5E, Q6A/B
- Explicit excluded: Q3A/B
- Open/all types: Q1, Q2, Q3C, Q5A, Q7, Q8, Q9, Q10

Ph. Eur. Monograph 2034 + related 5.10 (concept of ODIs in decision tree):
- Currently: API peptides explicitly excluded from related substances section
- Proposal: RT = 0.1%, IT = 0.5%, QT = 1.0%

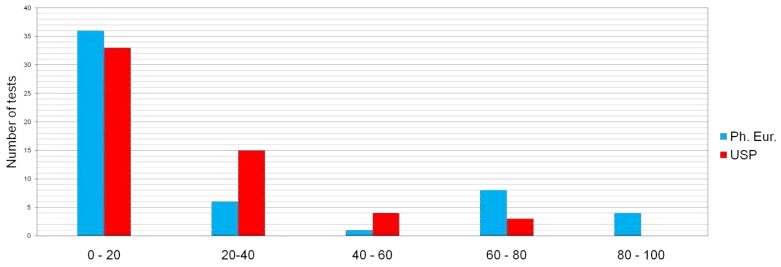

Fig. 1: % occurrence in peptide monographs of 55 pharmacopoeial tests

USP greater variety in tests than more consistent Ph. Eur.: room for improvement

Table II: Pharmacopoeial peptide test method & occurrence in monographs

Quality attribute	Method	No. of times included			Quality attribute	Method	No. of times included			Quality attribute	Method	No. of times included		
		Ph. Eur. only	USP only	Both			Ph. Eur. only	USP only	Both			Ph. Eur. only	USP only	Both
Description					**Tests (continued)**					**Tests (continued)**				
Appearance	Visual	10	0	8	Colour of solution	Visual	3	0	0	Pyrogens	Biological	0	0	1
Solubility H2O	Visual	9	0	8	Optical rotation	Polarimetry	8	0	3	Sterility	Microbiological	0	3	1
Solubility solvents	Visual	8	0	7	Specific absorbance	UV spectrophotometry	4	0	0	PLI	Immunochemical	1	0	0
Identification					Absorbance ratio	UV spectrophotometry	1	0	0	Zinc	AAS	0	1	1
	HPLC	8	0	9	Amino acid profile	AA analysis	1	2	1	Heavy metals	Chemical	0	3	0
	AA analysis	8	0	0	Related substances	HPLC				Bioidentitty	Biological	0	2	0
	TLC	6	0	1	Specified impurities		9	2	2	Residual solvents	GC	0	1	0
	HPLC peptide mapping	2	0	1	Unspecified impurities		9	1	4	Microbial purity	Microbiological	0	4	0
	2D planar peptide mapping	1	0	0	Sum of impurities		8	1	6	Nitrogen content	Kjeldahl	0	1	0
	MS	0	3	0	Related substances	TLC				Crystalinity	Optical microscopy	0	1	0
	Colour reaction	0	1	2	Unspecified impurities		1	0	0	Melting temperature	Melting in capillary	0	1	0
	UV spectrophotometry	0	0	1	Peptide content	HPLC	1	0	0	Phenylalanine	UV spectrophotometry	0	1	0
	IR	1	0	1	Acetic acid	HPLC or GC	7	0	4	Fluoride	Ion selective electrode	0	1	0
	Zinc (chemical)	0	0	2	Water	Karl Fischer	7	0	5	Trifluoroacetic acid	HPLC	0	1	0
	Sulphate (chemical)	1	0	0	Bacterial endotoxins	LAL	9	0	4	Particulate matter	Visual	0	1	0
	^{1}H NMR	2	0	0	Acetic acid + water	Calculation	1	0	0	**Assay**				
	Biological test	1	1	0	Composition	HPLC	3	0	0		HPLC	9	1	5
Tests					Loss on drying	Drying	0	0	6		Microbiological	0	2	3
pH of solution	Potentiometric	1	1	3	Sulphated ash	Ignition	3	1	4		Amino acid analysis	0	1	0
Clarity of solution	Visual	4	1	0	Sulphate	Titration	2	0	0		Biological	1	1	0

CONCLUSIONS

A peptide-drug monograph should basically consist of appearance, solubility information (important for analytical/product development, *e.g.* adsorption), identification by HPLC-UV, related impurities by HPLC-UV, residual solvents (water, acetic acid, others), sulphated ash, microbial purity and assay by HPLC-UV. 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 deamidation, epimers, oxidation and HMWP. Total related impurities are generally below 5%. Depending on the peptide-origin (synthetic, rDNA, cell- or tissue-based), this basic specification-set is to be supplemented with appropriate unrelated impurities (e.g. DNA, proteins, metals, specific organic solvents).