



Synthetic Peptides: Monograph specifications

EDQM Symposium: New impurities control, Strasbourg 22 September 2006

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Centre for Biological medicines and Medical technology



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- **Peptides** are the family of short molecules formed from the linking, in a defined order, of various α -amino acids.
- Proteins are **polypeptide** molecules. The distinction is that peptides are short and proteins are long.

Peptide or Protein:

- peptide chain short enough to be made synthetically

or:

- under 50 amino acids = peptide, above 50 amino acids = protein

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Peptides in Ph.Eur. 5.5

Monograph	Size(#AA)	
protirelin	3	thyrotropin RH
desmopressin	9	vasopressin/ADH mimic
felypressin	9	vasopressin/ADH mimic
oxytocin	9	
goserelin	9	GnRH analogue
leuprorelin	9	GnRH analogue
buserelin	9	GnRH analogue
gonadorelin	10	GnRH1
somatostatin	14	
tetracosactide	24	ACTH analogue
glucagon	29	
calcitonin salmon	32	

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Tests addressing 'purity' of peptides in PhEur monographs

- Related impurities: RP-HPLC 11 / 11
TLC 1 / 11
- Enantiomeric purity: Rotation 9 / 11
- Solvents 0 / 11
- Inorganic salts: Sulphated ash 1 / 11
- Appearance Solution S 2 / 11
- Endotoxins 10 / 11

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Limits related impurities in Ph.Eur. 5.5 monographs

Monograph	Total RP-HPLC impurities	
protirelin	3 %	specified
desmopressin	1.5 %	
felypressin	3 %	specified
oxytocin	5 %	
goserelin	2.5 %	specified
leuprorelin	2.5 %	specified
buserelin	5 %	specified
gonadorelin	5 %	
somatostatin	2 %	
tetracosactide	---	
calcitonin salmon	5 %	specified

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Related Impurities synthetic peptides

Many structural variants possible:

- Stereoisomers (epimers)
- Failures in sequence (lack or repetitive)
- Cleavage peptide bounds
- Coupling reagents/protective groups

- Deamidation (asn, gln)
- Oxidation (met)
- Aggregation

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General pharmacopoeial requirements on reporting, identification and qualification of impurities

Table 2034-1. – Reporting, identification and qualification of organic impurities in active substances

Use	Maximum daily dose	Reporting threshold	Identification threshold	Qualification threshold
Human use or human and veterinary use	≤ 2 g/day	> 0.05 per cent	> 0.10 per cent or a daily intake of > 1.0 mg (whichever is the lower)	> 0.15 per cent or a daily intake of > 1.0 mg (whichever is the lower)
Human use or human and veterinary use	> 2 g/day	> 0.03 per cent	> 0.05 per cent	> 0.05 per cent
Veterinary use only	Not applicable	> 0.1 per cent	> 0.2 per cent	> 0.5 per cent

- PhEur: Substances for pharmaceutical use (= ICH Q3A)
- These requirements on related substances do not apply to peptides.
- Thresholds do not apply, but the general concepts of reporting, identification and qualification are equally valid (chapter 5.10)

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Draft : Technical Guide for the elaboration of monographs for Synthetic Peptides and Recombinant DNA proteins

Related peptides

- Typically, monographs for synthetic peptides contain a reverse-phase LC test for related peptides. Such tests are validated for specified impurities known to be potential contaminants, and where possible, are transparent with respect to these impurities.
- Where appropriate, specified impurities reference substances are provided.
- Where necessary, specified impurities may have to be separately quantified in independent methods.
- Where a monograph depends on a single purity test, then the capacity of the method to measure all relevant impurities should be demonstrated.

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Impurities in PhEur monographs

Specified:

- protirelin
- felypressin
- buserelin
- goserelin
- leuprorelin
- calcitonin (salmon)

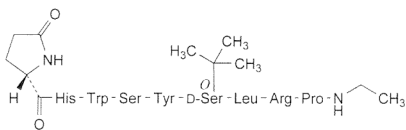
Not specified:

- desmopressin
- oxytocin
- *gonadorelin*
- *somatostatin*
- *tetracosactide*

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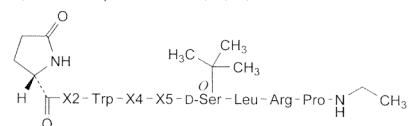
Buserelin monograph: 5 specified impurities

BUSERELIN



IMPURITIES

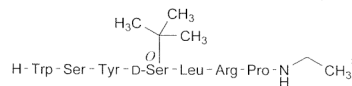
Specified impurities: A, B, C, D, E.



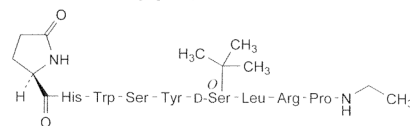
A. X2 = D-His, X4 = L-Ser, X5 = L-Tyr: [2-D-histidine]buserelin,

B. X2 = L-His, X4 = D-Ser, X5 = L-Tyr: [4-D-serine]buserelin,

D. X2 = L-His, X4 = L-Ser, X5 = D-Tyr: [5-D-tyrosine]buserelin,



C. buserelin-(3-9)-peptide,



E. [1-(5-oxo-D-proline)]buserelin.

riym

Specified impurities in PhEur monographs

	size	Total spec.	Epimer	Des-amido	-AA	+AA	S-S bridge	acetyl	other
protirelin	3aa	5	2	1	1				1
felypressin	9aa	6		2			3	1	
buserelin	9aa	5	4		1				
goserelin	9aa	12	7		1	2		1	1
leuprorelin	9aa	11	8					1	2
calcitonin [rDNA]	32aa	4 [+3]	1		1		[2]	2 [+1]	

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Things to do:

- Update monographs
 - Desmopressin
 - Oxytocin
- New monographs:
 - somatostatin analogues (octreotide)
 - nafarelin
- Introduce new technology (is RP-HPLC enough?)
- Establish guidance for related impurities

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Thanks for your attention !



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Toxicological aspects of peptide-related impurities

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Organon
The Netherlands

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Biological qualification of impurities

ICH Q3A/B (*peptides are exempted!*):

- *Genotoxicity* (in vitro mutagenicity and clastogenicity)
- *General toxicity* (e.g. \geq 2-week rat toxicity)
- *Other relevant toxicity endpoints* based on structure or pharmacological considerations

“Would a similar battery of qualification tests be applicable to peptides?”

“Would similar qualification thresholds (e.g. 1 mg as absolute threshold) be toxicologically acceptable for peptides?”



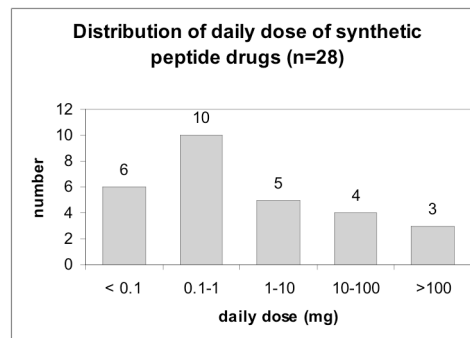
Characteristics of peptide drugs

- High biological activity
- High target specificity
- Unique 3-D structure important for biological effect
- Routes: i.v., s.c., i.m. and occasionally: inhalatory, intranasally, orally, intraocular, intra-articular
- Limited distribution (little accumulation, extracellular targeting)
- Rapid clearance (low biological stability, low $t_{1/2}$ (min))
- “Low toxicity” \Rightarrow low chemical toxicity
- Less immunogenic compared to monoclonals and other NBEs

\Rightarrow All of these characteristics potentially impact on the toxicity assessment of peptide-related impurities



Toxicity is ruled by *Dose (duration)*, not %



- On average peptides are dosed low (high potency!), but exceptions exist
- Same peptide: different indication, different route, different dose
- Dosing duration: single dose (e.g. GnRH antagonists) to chronic (e.g. calcitonins)



Types of peptide-related impurities

- Normal (A-B-C-D-E-F)
- Deletion sequences (A-B-C-D-F)
 - Insertion sequences (A-B-C-D-E-E-F)
 - Truncated sequences (A-B-C-D-E)
 - Diastereomers (racemization) (A-B-C-D-dE-F)
 - Modification of functional groups (deamidation, oxidation) (A-B-C-D-Ê-F)
 - Modification of disulfide bridges (disulfide exchange)
 - Oligomers, aggregates (for drug product)
 - *Genotoxic impurities*



Genotoxicity assessment (1)

Recent Guidance in theory also applicable for peptides:

- EMEA Guidance on the limits on genotoxic impurities, June 2006
- PhRMA Genotoxicity Task Force White Paper on Establishment of Allowable Concentrations of Genotoxic Impurities in Drug Substance and Product, 2005

3-step approach:

1. Classify *identified and predicted impurities* with regard to genotoxicity based on Structure Activity Relationship
2. Establish qualification strategy based upon impurity classification
3. Establish (phase-dependent) acceptance criteria for individual impurities and apply in specification setting

⇒ critical assessment of synthesis and manufacturing process, Q: “*What is the chance of forwarding toxic reagents, solvents or genotoxic impurities to the drug substance?*”



Genotoxicity assessment (2) critical assessment of the synthesis

Assess:

- Nature of synthesis conditions/type of chemistry (synthetic, possibly chemical modification)
 - Nature and quality of starting materials, solvents, resins, reagents, catalysts, protection + deprotection agents,
 - Effect of purification steps
 - Result of in-process controls
- ⇒ For peptides: remote risk for (high levels of) genotoxic impurities (Threshold of Toxicological Concern of 1.5 µg/day ^b)

^b higher levels might be advocated for short term use, for some classes of potent carcinogens lower thresholds apply



General toxicity assessment

Limitations of general toxicity tests for qualification:

- ⇒ toxicity studies mostly done with batches containing a limited % of impurity, at most comparison of batches
- ⇒ pharmacological effects of parent overrule and mask potential toxicity of peptide-related impurities.

On the other hand, large overdose does allow detection of serious toxicity

For peptides:

“*other relevant toxicity endpoints*” based on structure or pharmacological considerations are probably most relevant:

- ⇒ Altered pharmacological activity?
- ⇒ Immunogenicity?



Altered pharmacological activity

For many products extensive (quantitative) Structure-Activity-Relationship data is (publically) available as “lead optimization” data:

- Deletion sequences, insertion sequences, truncated sequences, diastereomers, disulfide exchange modification, (oligomers, aggregates) ⇒ mostly, loss of activity
- Terminal D-amino acids and acetylation ⇒ increased half-life, could occasionally result in ↑ activity in vivo, however chance for this type of modification is low

“Are in vitro or in vivo bioassays sensitive enough to reveal activity differences?”



Immunogenicity (1)

classical immunogenicity – breaking immuno tolerance

- Peptides < 8-10 aa: *not immunogenic*
- Linear epitopes are only weakly immunogenic
- With increasing length, the chance of exposing an immunogenic epitope increases importance of 3-D structure
- Many peptides are natural analogs or modified within the limits not to elicit immune response
- “Threshold dose for immune response”: for proteins µg range, for peptides 10-100 µg **BUT IN A CONTEXT!!!**
- peptides require conjugation/adhesion to surface and/or co-administration of adjuvant to elicit immune response
- Level and incidence of immune response correlates with route with generally s.c.>i.m.>inhaled>i.v.>p.o. and frequency of dosing



Immunogenicity (2)

In theory:

- truncation sequences ⇒ little impact on immunogenicity
- deletion and insertion sequences, diastereomers, functional group modification, disulfide exchange ⇒ possibly altered folding ⇒ increased chance of immunogenicity

However, in practice:

- The peptides are dosed low, the peptide-related impurities dosed even lower and immunizing context is absent!



In summary

For peptide related impurities:

- Risk of genotoxicity generally acceptable based on reagents, manufacturing, purification, ...
- Risk of chemical toxicity low, clearance high
- Pharmacological activity if present, mostly lower
- Risk of immunogenicity is low

⇒ Toxicity/immunogenicity is *ruled by dose, duration and route, not %*

⇒ This calls for a highly differentiated set of thresholds

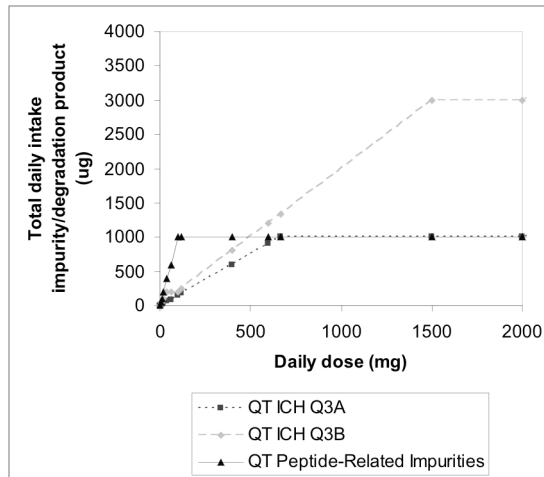
⇒ impractical

⇒ Process chemical and analytical challenges are high



One could consider ...

Qualification threshold for peptide related impurities:
e.g. 1% or 1 mg total daily intake, whichever is *lower*,
unless suspected toxicity calls for a lower level



Acknowledgements

Chris Rietveld
Harrie Joosten
Ernö Krajnc

Thank you for your attention!



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Quality, Experience And Flexibility
in GMP Bulk Peptides



**BCN Peptides is entirely focused on the GMP
manufacture of Bioactive Peptides by Solid Phase
Synthesis**

- Portfolio of Generic Peptides
- Custom Synthesis

Towards the identification of peptide impurities and interpretation of the monographs

Sergi Pavon
Quality Control Manager

Sílvia Arrastia
Regulatory Affairs Manager

3

Impurities coming from the route of Synthesis:

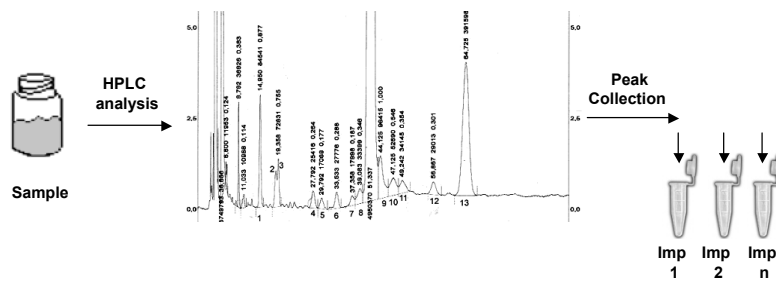
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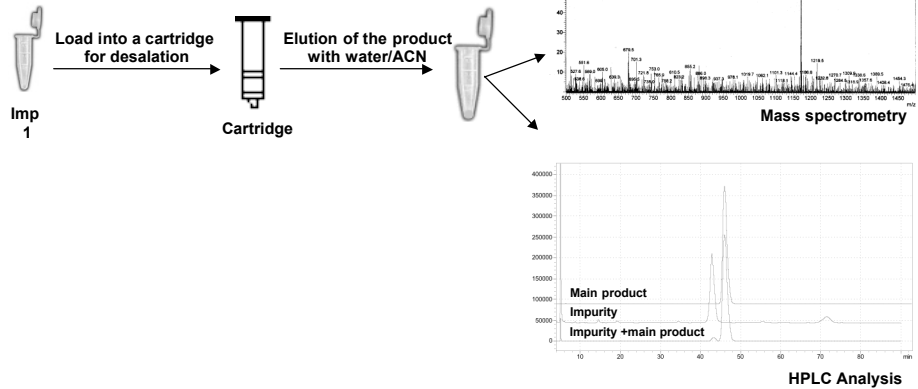
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- Hydrolysis
- Deamidation
- Oxidation
- Epimerization
- Acetylation
- ...

4

- Many potential impurities to be synthesized.
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- The identification of the impurities is performed using samples which are “rich” in impurities:
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7

→ Many Impurities present elute very close to the main peak:

- optimization of the conditions to allow separation of the peaks

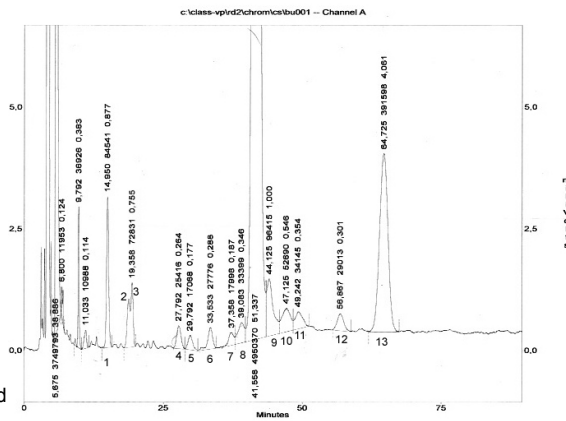
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coelute

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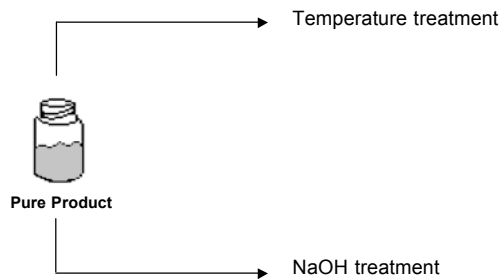
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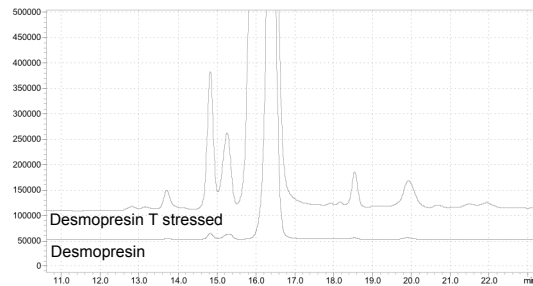
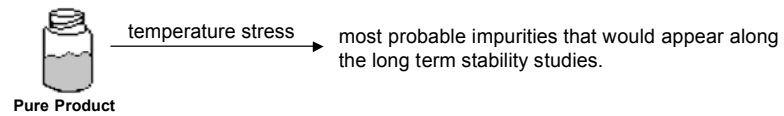
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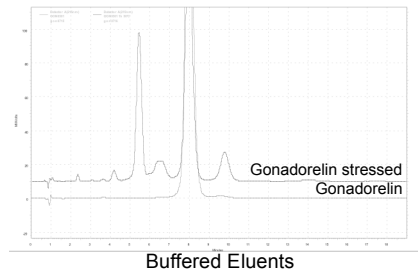
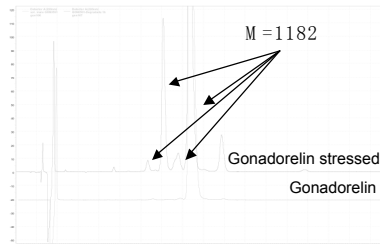
Treatments used to obtain samples rich in impurities to make easier the identification.



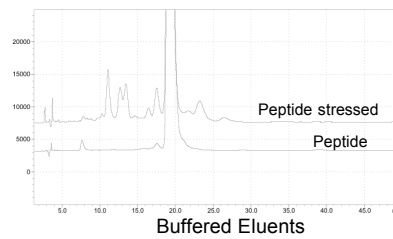
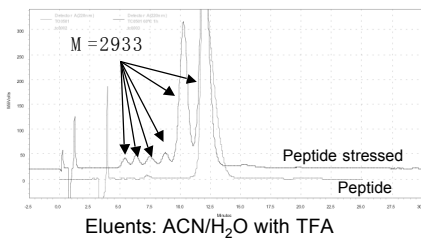
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Different profiles → second method

Related Peptides method Description:

➤ **Quantification:** → *Use of the Normalisation Procedure*

External Standard method → possible errors in the results

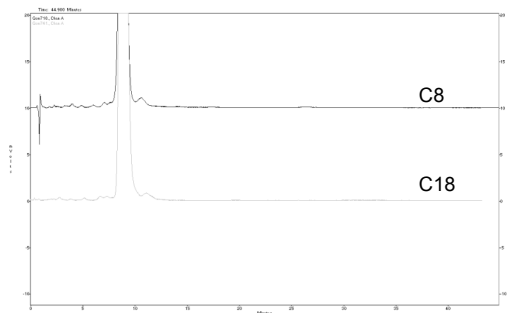
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➤ **Stationary Phase** → generic description such as reverse phase.

Stationary Phase

Different kind of reverse phase columns can lead identical results

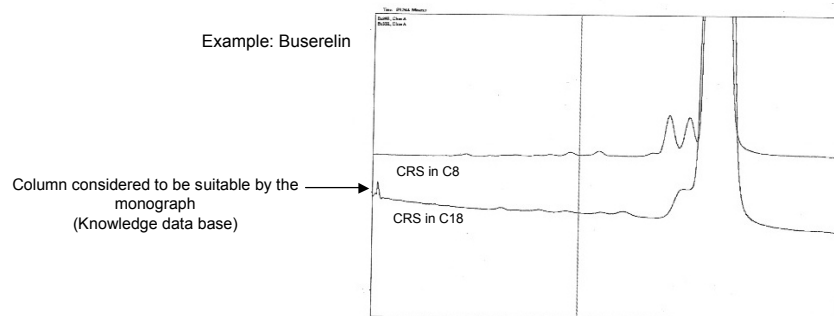


→ Description: Reverse Phase+System suitability

Stationary Phase

The separation does not depend only on the length of the aliphatic chain but also on other interactions.

Example: Buserelin



Proposal: CRS Standards + purity Chromatogram

Proposal for related peptide limits

General Monograph:

- Reporting Threshold (Disregard limit) >0.1%
- Identification Threshold > 0.5% (Tentative Identification)
- Qualification Threshold > 1.0%

Specific Monograph:

- A monograph can not cover all the impurities present in the products obtained by different synthetic strategies → **Unspecified impurities limit.**

- Homogenize the peptide monographs.
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Introduction *Related Peptide Impurities*

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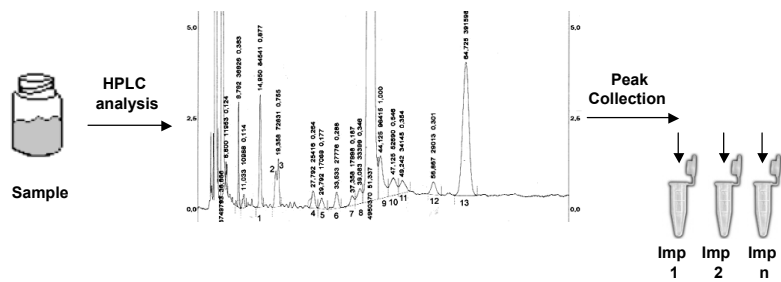
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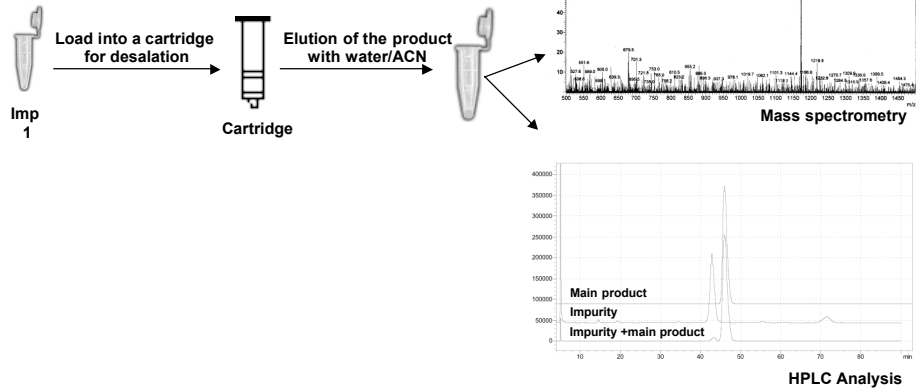
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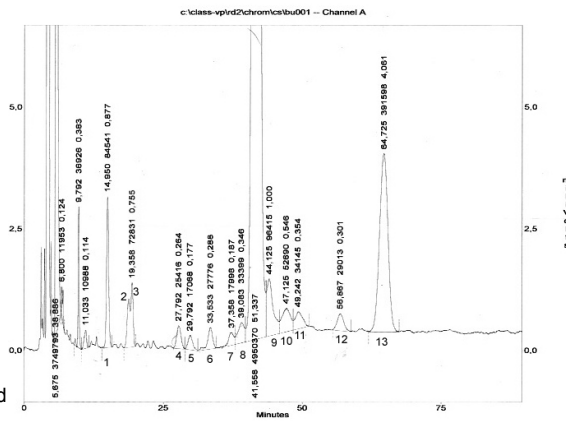
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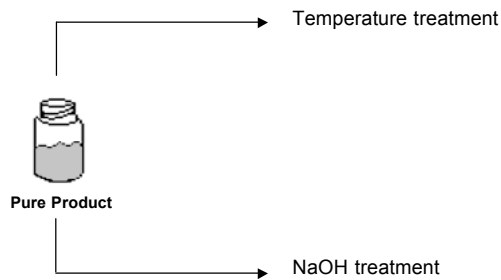
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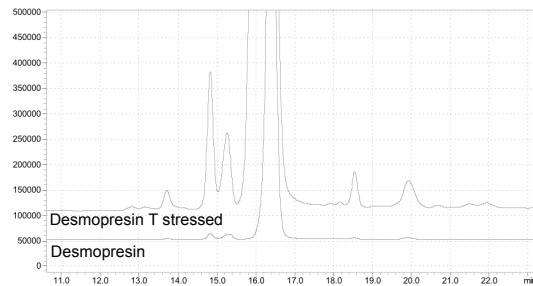
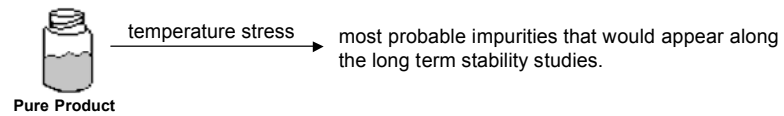
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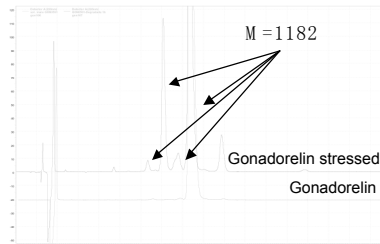
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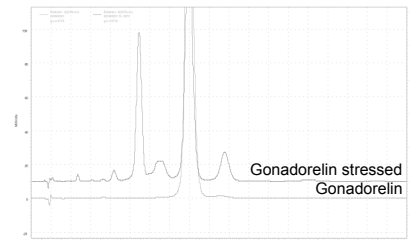
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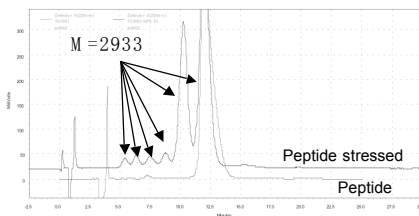


Eluents: ACN/H₂O with TFA

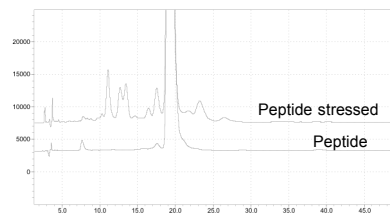


Buffered Eluents

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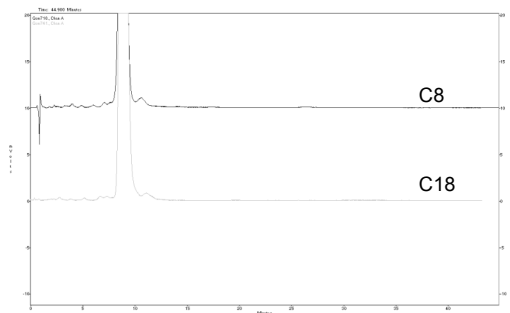
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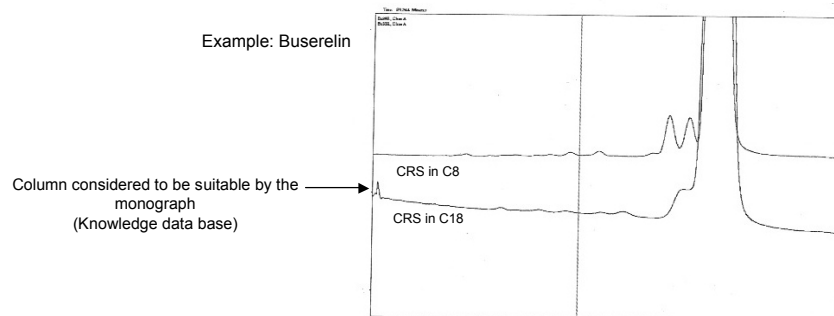


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Diosynth's Approach to Control Impurities in Peptide Manufacturing; A Proposal to Set Specifications for Synthetic Peptides

Ivo F. Eggen / Bernard M.M. van Genugten

EDQM meeting, 21-22 September 2006



Diosynth

Outline

- **Production of synthetic peptides**
- **Specs of starting amino acid derivatives**
- **Impurities in peptides**
 - What types of impurities?
 - How are they controlled?
- **Purification**
- **Efficiency**
- **Proposal for specifications**



Diosynth

Production of Synthetic Peptides

- **Assembly of the protected peptide sequence**
 - Solid-Phase Peptide Synthesis (SPPS)
 - Classical Solution-Phase Synthesis (CSPS)
 - Diosynth Rapid Solution Synthesis of Peptides (DioRaSSP)
- **Acidolysis: removal of protecting groups**
- **Other modifications like disulfide bond formation and fragment couplings**
- **Purification and desalting by preparative HPLC**
- **Lyophilization**



Diosynth

Specs of Starting Amino Acid Derivatives (1)

- **Typical specifications:**
 - assay $\geq 95.0 \%$
 - purity (HPLC) $\geq 98.5 \text{ a/a}\%$
 - optical antipode content $\leq 0.5 \%$
 - free amino acid content (TLC) $\leq 0.3 \%$
 - TSE-free statement



Diosynth

Specs of Starting Amino Acid Derivatives (2)

- **TSE-free statement:**
 - **origin/source of the starting material: synthetic/chemical or vegetal/plant or phytogetic/mineral (non-human, non-animal)**
 - **TSE risk of the starting material: no TSE risk**
 - **TSE risk of the manufacturing process:**
 - no materials used from human or animal source
 - no materials used with a TSE risk



Diosynth

Impurities in Peptides

- **Deletion sequences**
- **Insertion sequences**
- **Truncated sequences**
- **Diastereomers**
- **Modifications of functional groups**
- **Modifications of disulfide bridges**



Diosynth

Deletion Sequences

- Sequences lacking one or more residues
- Originating from incomplete coupling and/or deprotection steps
- Controlled by in-process analyses with narrow specifications (HPLC or TLC)
- Identified in API by LC/MS(/MS)



Diosynth

Insertion Sequences & Truncated Sequences



Diosynth

Insertion Sequences

- Sequences containing one or more “double” residues
- Originating from:
 - presence of free amino acid in starting protected amino acid derivatives ⇒ controlled by specs on s.m.
 - incomplete removal of excess carboxylic compound
- Identified in API by LC/MS(/MS)



Diosynth

Truncated Sequences

- N-Terminally truncated sequences may be generated when capping is part of the synthesis protocol ⇒ SPPS
- C-Terminally truncated sequences may be generated when quenching is part of the synthesis protocol ⇒ CSPA
- Identified in API by LC/MS



Diosynth

Insertion & Truncated Sequences

- **Controlled by DioRaSSP protocol:**
 - quenching with an anion-forming quencher
 - suitable extraction protocols
 - depending on the actual carboxylic compound



Diosynth

Quenching of Z-Amino Acid Derivatives

- **Extractibility tested for 20 common derivatives: His, Gly, Ala, Pro, Val, Ser(Bu^t), Ile, Leu, Thr(Bu^t), Asp(OBu^t), Glu(OBu^t), Phe, Trp, Orn(Boc), Lys(Boc), Tyr(Bu^t), Arg(Pbf), Asn(Trt), Gln(Trt), Trp(Boc)**
- **Category 1: quenching with DMAPA, washing upon hydrogenation not necessary**
- **Category 2: quenching with DMAPA, washing upon hydrogenation necessary**
- **Category 3: quenching with DMAPA, single-salt washing upon quenching or quenching with H-βAla-OBzl, double-salt washing upon quenching**
- **Category 4: quenching with H-βAla-OBzl**



Diosynth

Diastereomers



Diosynth

Diastereomers

- Sequences containing one or more residues in the undesired chiral form
- Originating from:
 - presence of optical antipode in starting protected amino acid derivatives
 - racemization of amino acid derivatives during stepwise coupling
 - racemization of C-terminal amino acid ester under basic conditions
 - racemization of C-terminal amino acid derivative during fragment coupling
- Identified in API using reference standards and/or chiral analysis method(s) (not LC/MS(/MS))



Diosynth

Starting Materials

- Presence of optical antipode in starting protected amino acid derivatives
- Controlled by narrow specification on starting amino acid derivatives
- Typically $\leq 0.5\%$ of optical antipode
- Analysis of all starting amino acid derivatives performed by chiral analysis method



Diosynth

Modifications of Functional Groups

- Some residues have sensitive side chains, which may be modified under specific conditions, e.g. Asn, Asp, Gln, Glu, Met, Ser, Trp; very problematic: Asn conversion to Asu, Asp, iso-Asp and racemization
- Extensive literature and internal data available
- Monitoring by LC/MS(/MS)
- Optimization of process conditions / identification of critical process parameters
- Reduction of impurities during purification process
- However, modification may also occur during storage of API \Rightarrow extensive stability testing



Diosynth

Modifications of Disulfide Bridges



Diosynth

Modifications of Disulfide Bridges (1)

- **Generally desired: cyclic monomer (intramolecular disulfide bridge)**
- **Impurities: (reduced) linear monomer, (parallel and anti-parallel) dimers, polymers**
- **Identified in API using reference standards and/or by LC/MS(/MS)**
- **Intramolecular cyclization promoted by dilution**



Diosynth

Modifications of Disulfide Bridges (2)

- Optimization of process conditions / identification of critical process parameters
- Reduction of impurities during purification process
- However, modification may also occur during storage of API ⇒ extensive stability testing



Diosynth

Purification

- Purification is based on:
 - initial screening procedure
 - knowledge on reduction factors for specific impurities built up during product development
 - meticulous in-process analysis
 - impurity profiling



Diosynth

Efficiency



Diosynth

Synthesis Efficiency

• Assumptions:

- reduction factor comprises loss of peptide material and loss of purity
- two chemical conversions per residue: coupling & deprotection
- mean efficiency per conversion: 93-95-97-99%
- linear synthesis

Number of residues	5	10	15	20	25	30
99% mean efficiency	90	82	74	67	61	55
97% mean efficiency	74	54	40	30	22	16
95% mean efficiency	60	36	21	13	8	5
93% mean efficiency	48	23	11	5	3	1



Diosynth

Purification Efficiency

• **Assumptions:**

- 85% recovery for 10-mer of 95% purity
- 10% loss going from 10 to 20 residues
- 20% loss going from 20 to 30 residues
- higher % loss for every % of additional purity

Number of residues	10	20	30	% Loss
95% desired purity	85	77	62	n.a.
96% desired purity	81	73	58	5
97% desired purity	73	66	53	10
98% desired purity	62	56	45	15
99% desired purity	49	44	35	20

AKZO NOBEL Diosynth

Proposal for Specifications

What about Synthetic Peptides in the ICH Q Guidelines?

- Q3A(R1) and Q3B(R2): ...The following types of drug substances are not covered in this guideline: biological/biotechnological, peptides, oligonucleotides,...
- Q6A: ...This guideline may be applicable to synthetic and semi-synthetic antibiotics and synthetic peptides of low molecular weight ...
- Q6B: ...This document does not cover antibiotics, synthetic peptides and polypeptides ...



Diosynth

What about Peptides in Ph.Eur.

- In Ph.Eur.'s General Monograph 2034 '*Substances for Pharmaceutical Use*': Peptides are excluded from the general concept as given in this chapter.
- In 5.10 '*Control of Impurities in Substances for Pharmaceutical Use*': Although the thresholds stated in the general monograph 2034 do not apply, the general concepts of reporting, identification (wherever possible) and qualification of impurities are equally valid for these classes'.



Diosynth

What's in Current Peptide Monographs? (1)

- **Oxytocin:** No specified impurities; Total impurities $\leq 5.0\%$, Individual $\leq 1.5\%$; Disregard limit: 0.1%.
- **Gonadorelin Acetate:** No specified impurities; Total impurities $\leq 5\%$, Individual $\leq 2.0\%$; Disregard limit: 0.05%.
- **Leuprorelin:** Specified impurities A through D; Impurity D: $\leq 1.0\%$, impurities A, B, C $\leq 0.5\%$; Any other impurity $\leq 0.5\%$; Total impurities $\leq 2.5\%$; Disregard limit 0.1%
- **Calcitonin:** $\leq 3.0\%$ for specified impurities A, B, C, D; Other unidentified, specified impurities may occur that co-elute with impurities A, B, C and D. Total impurities $\leq 5.0\%$; Disregard limit: 0.1%

What's in Current Peptide Monographs? (2)

- *In the Transparency Statement :*
- **Oxytocin:** No impurities.
- **Gonadorelin Acetate:** No impurities.
- **Leuprorelin:** total 11 impurities (4 spec. and 7 'other detect.'): 10 of them are the D-isomer instead of the L-isomer and one impurity is the acetylated serine.
- **Calcitonin:** 4 specified impurities, but also several unidentified impurities accepted, up to 3.0% individual. Two are modifications of the functional groups (acetylated), one is a diastereomer and one is a deletion sequence impurity.

General Considerations in Setting Specs

- Toxicology and safety data of peptides API's.
- Low daily doses for peptides: typically <10 mg / day.
- Analytical techniques evolve much faster than synthesis and purification techniques.
- Technical limitations regarding analytics; mainly selectivity, resulting in composite peaks.
- Technical/economical aspects regarding synthesis and purification.



Diosynth

Proposal for Synthetic Peptides

• In General Chapter (2034):

- Reporting threshold > 0.1%
- Identification threshold > 0.5%
- Qualification threshold > 1.0%

• Specifications in the Monograph:

- Disregard limit 0.1%
- Sum of all impurity peaks : $\leq 3.0\%$ (pept. of 2-15 AA)
- Sum of all impurity peaks : $\leq 5.0\%$ (pept. of > 15 AA)
- Specified peaks (all peaks with a limit > identification threshold) with their limits and RRTs
- "Any unspecified peak $\leq 0.5\%$ "

• In Transparency Statement (*List of Impurities*):

- information about all specified peaks e.g. peak with RRT 0.95: a single peak of impurity ... (name) ... ; or:
a composite peak, consisting of ... and ... an unidentified.



Diosynth

Why Difference in Total Impurities?

- Larger peptides will contain more different impurities.
- Also the purification of such peptides will be more difficult.

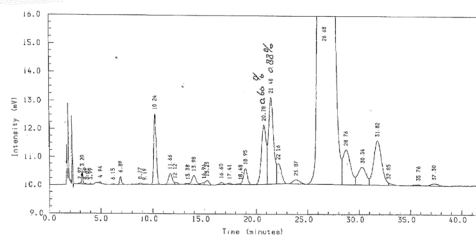
Why Impurity Peaks instead of Impurity Names?

- Often impurities co-elute, resulting in composite peaks. Occurs mostly at or around the main peak. Therefore, define peaks with RRT or RRT window rather than name(s) of impurities.

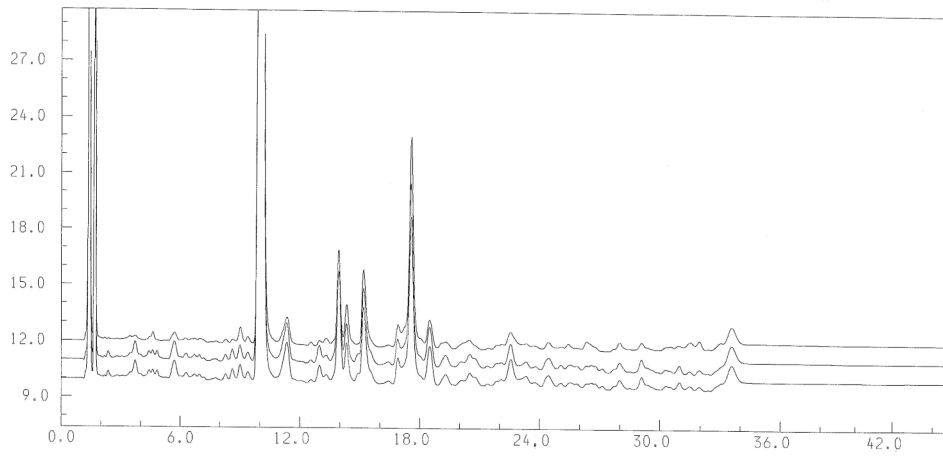
Co-elution of peaks - Example

Same HPLC Instrument
Same Brand of Column
(Supelcosil LC-18-DB)

but DIFFERENT Serial Numbers

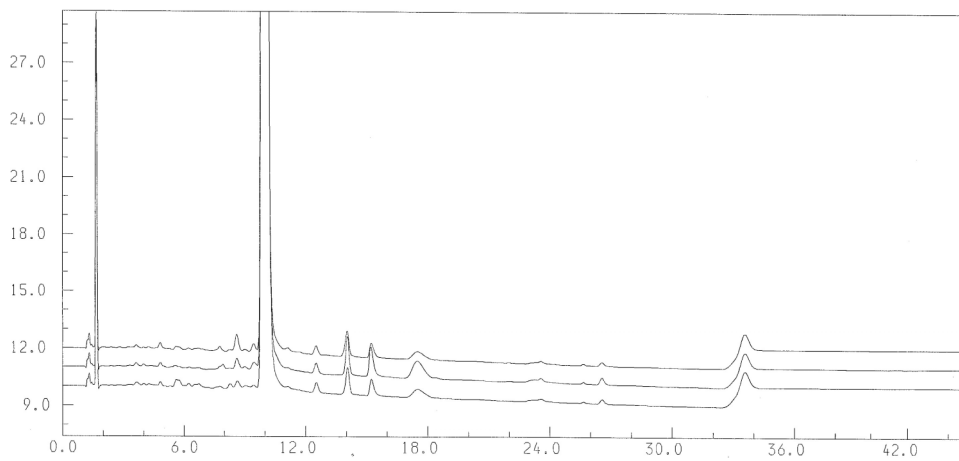


Chromatogram of Peptide before Purification



Diosynth

Chromatogram of Peptide after Purification



Diosynth

Qualification threshold at 1.0%.

- ICH Q3A(R1): up to 1.0 mg (=1000 µg) per day no concern. The level of 0.15% based on technical feasibility, not related to safety.
- For Peptides:
 - Low daily dose ;
 - Technically it is not possible to decrease all indiv. peaks to < 1.0%;
 - No concern about the toxicity/biological effects for peptide impurities, so:
acceptable to set the limit for qualification at 1.0%.
- Proposal in line with proposal as presented on TIDES® 2005 by Dr. Blair Fraser, FDA-CDER.



Diosynth

Level of Identification at 0.5% (1)

- Methods used for identification:
 - *LC/MS(/MS) on the purified peptide;*
 - Synthesis of the proposed structure;
 - Spiking experiments.
 - *First isolation from the (purified) peptide*
 - TOF-MS; MS/MS;
 - Amino acid analysis;
 - Determination of enantiomeric purity;
 - NMR.



Diosynth

Level of Identification at 0.5% (2)

- With LC/MS/MS: If MM=main component: possibly diastereomer, but on which amino acid? (often peptide impurities present at or around 0.5% are stereoisomers).
- Identification is often very difficult and time consuming.
 - Often peaks close to main peak consist of more than one impurity.
- Proposal in line with proposal as presented on TIDES® 2005 by Dr. Blair Fraser, FDA-CDER.



Diosynth

Summary (1)

- Peptides itself, but also peptide impurities have a low toxicity.
- Daily dose usually low: consequently the daily intake of an individual impurity is generally < 10 mg. Compared to the accepted 1.0 mg (ICH Q3A(R1)) there is no safety risk.



Diosynth

Summary (2)

- Peptides (and intermediates) do not crystallize; Consequently no intermediate purifications.
- Accumulation of impurities throughout route.
- Main impurities are diastereomers.
- Identification of peptide impurities may be very complicated.

Based on this all, we feel that the proposal as given earlier should be acceptable for defining the purity of peptides.



Diosynth

• In General Chapter (2034):

- Reporting threshold > 0.1%
- Identification threshold > 0.5%
- Qualification threshold > 1.0%

• Specifications in the Monograph:

- Disregard limit 0.1%
- Sum of all impurity peaks : $\leq 3.0\%$ (pept. of 2-15 AA)
- Sum of all impurity peaks : $\leq 5.0\%$ (pept. of > 15 AA)
- Specified peaks (all peaks with a limit > identification threshold) with their limits and RRTs
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• In Transparency Statement (*List of Impurities*):

- information about all specified peaks e.g. peak with RRT 0.95: a single peak of impurity ... (name) ... ; or:
a composite peak, consisting of ... and ... an unidentified.



Diosynth