UNIVERSITEIT GENT

FACULTY OF **PHARMACEUTICAL SCIENCES**

Stability indicating method development for low level calcitonin

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1. Peptide drugs

Peptide drugs overview:

- Native peptides
- Chemically modified peptides
- Peptidomimetics

Examples:
Somatostatin, enfuvirtide, insulin,…

Protein and peptide drug market:
2007: $ 47.4 billion
2010: $ 55.7 billion

2. Peptide drug challenges

Anatomic compartment	Degradation		Anatomic compartment	Degradation
Stomach	• Acid hydrolysis • Gastric enzymes		Liver	• Liver enzymes
Duodenum	• Pancreatic enzymes		Blood	• Proteases • Other enzymes
Intestinal brush	• Exo/endopeptidase • Adsorption barriers		Kidney	• Excretion

3. Improving peptide biopharmaceutical characteristics

A. Modification of peptide structure

e.g. cyclization

B. Linking to other molecules

e.g. PEGylation

C. Formulation

e.g. sustained release formulation

4. Peptide delivery routes

A. Invasive administration: current mainstream route

Injection: SC, IM, IV,…

B. Non-invasive administration: new developments

1. Oral *e.g.* cyclosporin
2. Nasal *e.g.* salmon calcitonin
3. Pulmonal *e.g.* insulin

…

5. Calcitonin

A. Structure

Linear, 32 amino acid residues

B. Therapeutic use

1. Postmenopausal osteoporosis
2. Paget's disease

C. Origin

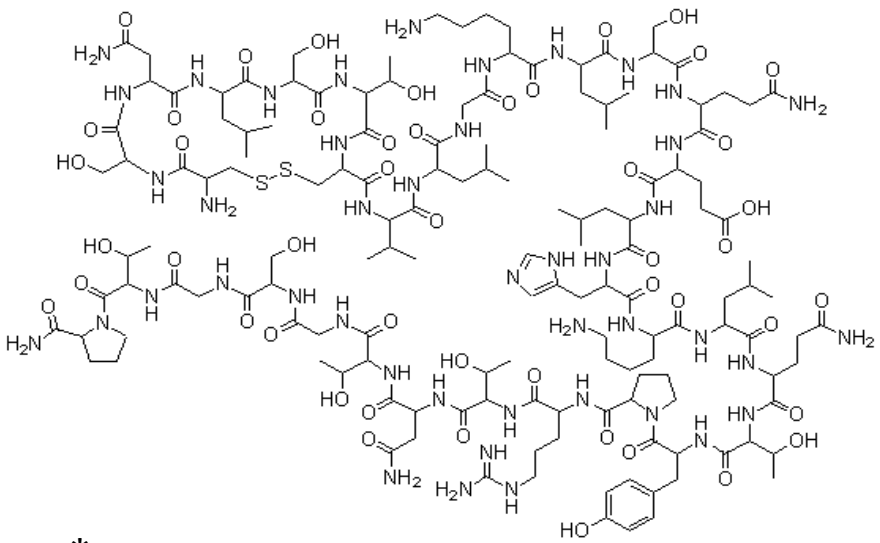
1. Salmon*
2. Human
3. Eel
4. Porcine

D. QSAR requirements

1. Cys^{1}-Cys^{7} disulphide bridge
2. AA residues 1-8
3. C-terminal prolinamide moiety

E. Current formulations

1. Solution for injection *e.g.* Miacalcic®
2. Nasal spray *e.g.* Fortical®



* H-Cys-Ser-Asn-Leu-Ser-Thr-Cys-Val-Leu-Gly-Lys-Leu-Ser-Gln-Glu-Leu-His-Lys-Leu-Gln-Thr-Tyr-Pro-Arg-Thr-Asn-Thr-Gly-Ser-Gly-Thr-Pro-NH_2

6. Low level salmon calcitonin formulation

Newly developed salmon calcitonin (sCT) formulation:

sCT: 400 ppm in
polymeric matrix (containing carbomer)

↓

63 µm particles in a nasal aerosol

↓

25 mg/powder puff ~ 10 µg sCT ~ 60 IU

Objective:
- Assay method development
- Stability evaluation

⟺

Challenges:
- low levels of sCT
- sCT analytical stabilitiy

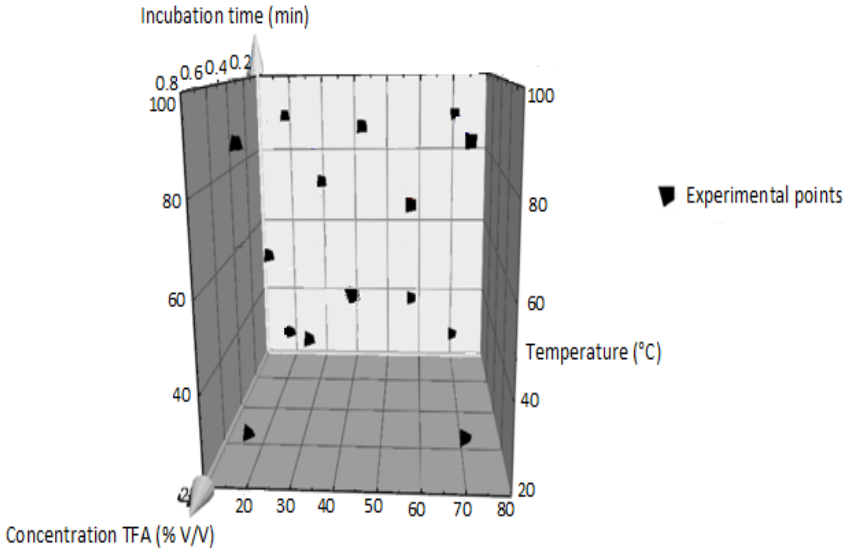
7. sCT chromatographic profiling

Parameter	HPLC-UV (assay)			LC-MS (identification)		
Column (+ guard column)	Everest C_{18} (300 Å), 250 × 4.6 mm, 5µm					
Column temperature	40°C			30°C		
Mobile phase	A: 0.1% V/V TFA in H_2O B: 0.085% V/V TFA in ACN			A: 0.1% V/V FA in H_2O B: 0.1% V/V FA in ACN		
Gradient program	Time (min) 0 20	%A 73 63	%B 27 37	Time (min) 0 60	%A 80 65	%B 20 35
Flow rate	1 ml/min					
Injection volume	100 µl					
Detection	DAD UV @ 195 nm			ESI - ion trap MS^1 / data dependent MS^2		

8. Sample extraction – Onion design

Onion design:

- Quadratic model fitting with PLS
- Limited experiments (n=15)
- Experimental space (3 variables)



Extraction variables:

- TFA concentration (0.1-0.75% V/V)
- Incubation temperature (20-70°C)
- Incubation time (30-90 min)

Result (*i.a.* contour plots)

For optimal recovery of 95% sCT calculated:
0.55% V/V TFA, 55°C incubation temperature and 45 min incubation time

Experimental confirmation: 93.9 (± 1.4)% sCT recovery (n=3)

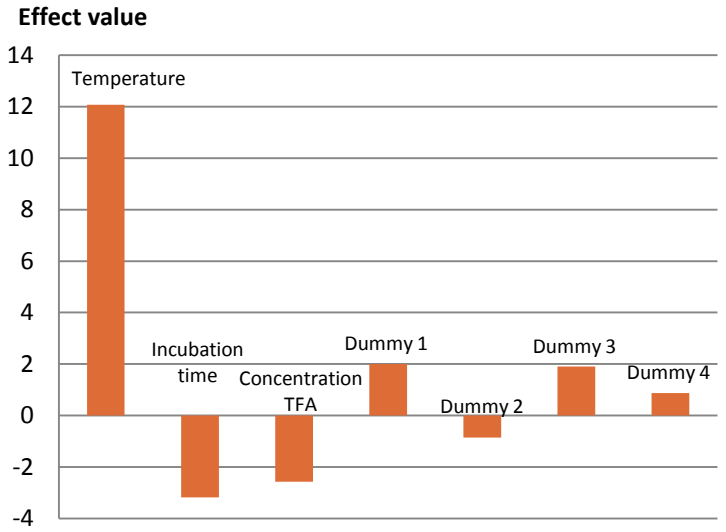
8. Sample extraction – Placket Burman design

Placket Burman design:

Onion model robustness evaluation

Extraction variables:

- TFA concentration (0.45-0.55-0.65% V/V)
- Incubation temperature (50-55-60 °C)
- Incubation time (40-45-50 min)



Results:

- Temperature: positive significant effect ($P < 0.05$) → (± 2°C) range
- Maximal recovery: 0.45% V/V TFA, 55°C (± 2°C) and 45 min
  99.6% sCT recovery (no significant analytical degradation)

9. Formulation stability results

Design

✓Time interval: 6 weeks

✓Conditions:

- Room temperature (20°C)
- In refrigerator (5°C)
- In freezer (-35°C)

Time point	Storage condition	Assay (% vs. T_0)
$T_{0\ wks}$	n.a.	100
$T_{6\ wks}$	-35°C	93.9
	5°C	83.9
	20°C	69.8

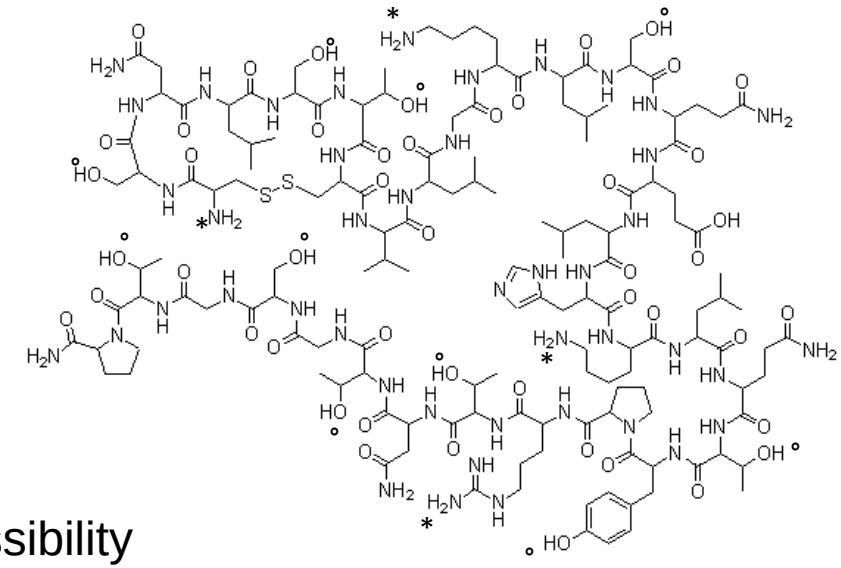
Result:

- Temperature dependent decrease in sCT assay
- No equivalent increase of degradation

10. Conclusion

Hypothesis:

- Chemical interaction between sCT and carbomer (chemabsorption)
- Amide (*) and ester (°) formation

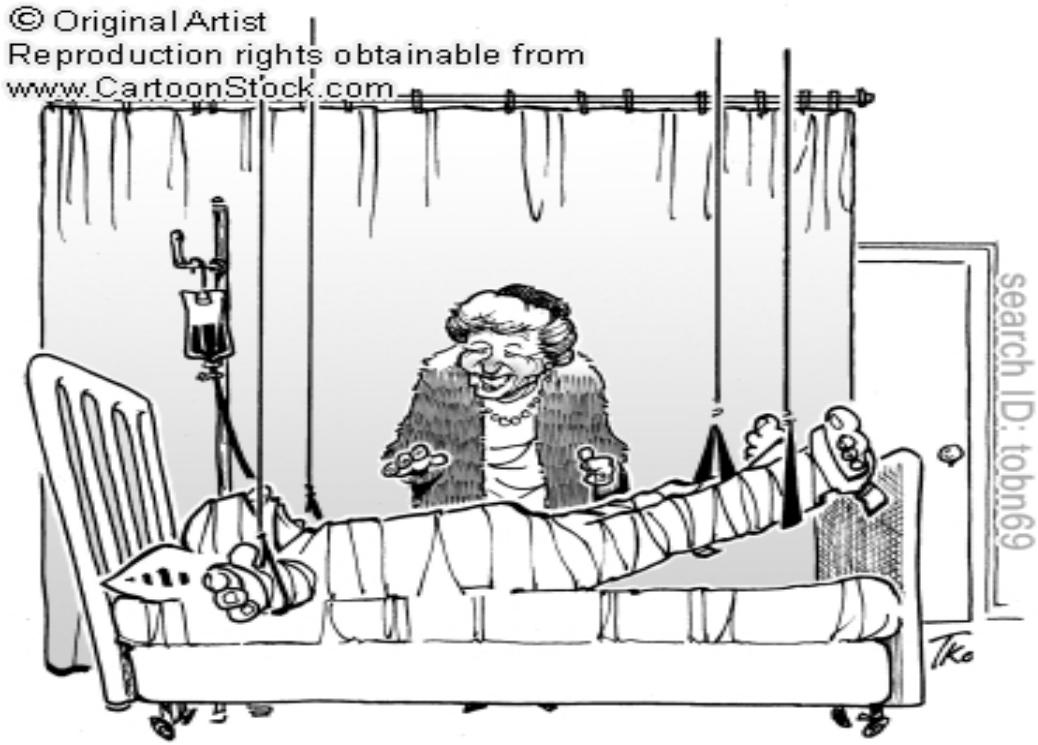


Validation:

- IR: sCT concentration insufficient
- SERS: in progress
- EW-CRDS: envisioned

→ New peptide-polymer linkage possibility

Thank you for your attention!!!



"No, no - don't get up. I'll show myself out."