Stability indicating method development for low level calcitonin

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## Outline

1. Peptide drugs
2. Peptide drug challenges
3. Improving peptide biopharmaceutical characteristics
4. Peptide delivery routes
5. Calcitonin
6. Low level salmon calcitonin formulation
7. sCT chromatographic profiling
8. Sample extraction
9. Formulation stability results
10. Conclusion
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

<table>
<thead>
<tr>
<th>Anatomic compartment</th>
<th>Degradation</th>
<th>Anatomic compartment</th>
<th>Degradation</th>
</tr>
</thead>
</table>
| 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. Pulmonary 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. Cys1-Cys7 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®
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 stability
## 7. sCT chromatographic profiling

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HPLC-UV (assay)</th>
<th>LC-MS (identification)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column (+ guard column)</td>
<td>Everest C$_{18}$ (300 Å), 250 × 4.6 mm, 5µm</td>
<td></td>
</tr>
<tr>
<td>Column temperature</td>
<td>40°C</td>
<td>30°C</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>A: 0.1% V/V TFA in H$_2$O</td>
<td>A: 0.1% V/V FA in H$_2$O</td>
</tr>
<tr>
<td></td>
<td>B: 0.085% V/V TFA in ACN</td>
<td>B: 0.1% V/V FA in ACN</td>
</tr>
<tr>
<td>Gradient program</td>
<td>Time (min) 0</td>
<td>%A 80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%B 20</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>60</td>
<td></td>
</tr>
<tr>
<td></td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>Flow rate</td>
<td>1 ml/min</td>
<td></td>
</tr>
<tr>
<td>Injection volume</td>
<td>100 µl</td>
<td>ESI - ion trap</td>
</tr>
<tr>
<td>Detection</td>
<td>DAD UV @ 195 nm</td>
<td>MS$^1$ / data dependent MS$^2$</td>
</tr>
</tbody>
</table>
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)

Result:
• Temperature dependent decrease in sCT assay
• No equivalent increase of degradation

<table>
<thead>
<tr>
<th>Time point</th>
<th>Storage condition</th>
<th>Assay (% vs. T₀)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₀ wks</td>
<td>n.a.</td>
<td>100</td>
</tr>
<tr>
<td>T₆ wks</td>
<td>-35°C</td>
<td>93.9</td>
</tr>
<tr>
<td></td>
<td>5°C</td>
<td>83.9</td>
</tr>
<tr>
<td></td>
<td>20°C</td>
<td>69.8</td>
</tr>
</tbody>
</table>
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!!!