Drug development of highly potent therapeutic peptides – Regulated “microdose” Bioanalysis

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Introduction

- Drug development of agonists towards oxytocin/vasopressin receptors
- Highly potent and selective peptides
- Therapeutic “microdoses”
- Regulated “microdose” Bioanalysis support needed
What does it take?

- **Method Development**
  - Sophisticated and complex method needed
  - Method development time = at least 2 x “standard LC-MS/MS method”

<table>
<thead>
<tr>
<th></th>
<th>Sub-therapeutic microdoses</th>
<th>Therapeutic microdoses</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Method Validation</strong></td>
<td>Qualified method normally sufficient</td>
<td>Full validation required</td>
</tr>
<tr>
<td><strong>Sample Analysis</strong></td>
<td>“Fit for purpose” compliance</td>
<td>Full compliance required</td>
</tr>
</tbody>
</table>
Approach

- Sample preparation
  - Sample enrichment and thorough clean-up using ion exchange SPE + solvent evaporation is a prerequisite

- Chromatography
  - Multidimensional LC (different types of stationary/mobile phases) to achieve maximum selectivity

Lövgren et al., Journal of Pharmaceutical and Biomedical Analysis 53 (2010) 537-545
Chromatographic set-up

LC-pump 3 — Auto-sampler — Analytical column (C18) — LC-pump 4

Valve 1:
- 1: Waste
- 2: CN column

Valve 2:
- 1: Waste
- 2: Trap column (C4)

Valve 3:
- 1: Mass Spectrometer
- 2: LC-pump 1
- 3: LC-pump 2
- 4: LC-pump 2
- 5: LC-pump 1
- 6: Waste

Sciex API5000
Recent experience

- Three therapeutic peptides (all around 1000 Da)
  - Case 1: New Chemical Entity
  - Case 2: Life Cycle Management
  - Case 3: New Chemical Entity

- Stable label internal standards
Case 1 – background

- FIH design, SAD
- 6 hours i.v. infusion
Analytical background

- LLOQ = 5 pg/mL

- Validation data, inter-assay CV: 11% at LLOQ
- ISR, 90 % within acceptance criteria (20% from original)
PK outcome

- Mean PK profile, cohort 1 males (3.92 µg)
Sample throughput

Total cycle time: 13 minutes
Non-specific binding

- Approach used to support several nonclinical studies and FIH study, all of a sudden problem!

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Calibration Conc.</th>
<th>Response Per ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAL 1</td>
<td>0.126</td>
<td>0.016294</td>
</tr>
<tr>
<td>CAL 2</td>
<td>0.304</td>
<td>0.017849</td>
</tr>
<tr>
<td>CAL 3</td>
<td>0.734</td>
<td>0.031072</td>
</tr>
<tr>
<td>CAL 4</td>
<td>1.76</td>
<td>0.038598</td>
</tr>
<tr>
<td>CAL 5</td>
<td>4.26</td>
<td>0.101249</td>
</tr>
<tr>
<td>CAL 6</td>
<td>10.0</td>
<td>0.091589</td>
</tr>
<tr>
<td>CAL 7</td>
<td>25.6</td>
<td>0.096501</td>
</tr>
</tbody>
</table>

- Non-specific binding to polypropylene plates used for preparation of calibration samples
Matrix effects

- Normally not a problem, despite the large plasma volumes
- Matrix effects seen in PoC study (septic shock patients with heavy co-medications)

Mean IS response (CAL and QC)

50% of mean IS response
Case 2 – background

- Explorative PK study (LCM)
- Oral administration, 240 µg
- Low bioavailability, 0.25%
Analytical background

- LLOQ = 2 pg/mL

- Validation data, inter-assay CV: 10% at LLOQ
- ISR, 90 % within acceptance criteria (20% from original)
PK outcome

- Mean PK profile, 240 µg

- With 100 µg dose, LLOQ of 2 pg/mL make it possible to detect 80% of AUC.
Integration
Carry-over

- Carry over in 5 out of the first 10 runs
- Sample analysis halted
- Explorative study and samples analysed in profile order

↓

- Revised procedure for evaluating and mitigating carry over
Carry-over

- **Original criteria (blank samples)**
  - > 20% of CAL1 = Carry over!

- **Revised criteria**
  - <20% of CAL1 → no problem
  - <20% of CAL2 → raise LLQ to CAL2, reanalyse samples below CAL2
  - >20% of CAL2 but ≤100% of CAL1, look at impact on next sample
    - if area(n-1)/area(n) ≤ 30 → OK
    - If area(n-1)/area(n) ≥ 30 → reanalyse sample
  - >100% of CAL1 → run failed

- In total, no carry-over run failure (n=30) with this revised procedure
Case 3 – background

- FIH design, SAD part
- Intranasal
- Expected Bioavailability: 1-5%

Expected therapeutic dose
Analytical background

- LLOQ = 20 pg/mL

- Validation data, inter-assay CV: 9 % at LLOQ
Alternative method approach needed

- Less polar uncharged peptide → our standard approach could not be used
- RP SPE + 2-column chromatographic system → less selectivity → less robust
- S/N criteria indicated LLOQ at 5 pg/mL, but LLOQ set at 20 pg/mL to get robust method performance
PK outcome

- At two lowest dose groups, 5 and 15 µg → all results below LLOQ
- PK outcome, mean curve (300 µg)
Future challenge

- Develop a robust method that can give kinetic data at therapeutic doses, LLOQ at 1-5 pg/mL required

- Upcoming milk transfer study → need to develop and validate a method in breast milk at low pg/mL levels
Summary

- Development of agonist peptides targeting the oxytocin/vasopressin receptors requires therapeutic “microdoses”
- Support to these studies → Regulated “microdose” bioanalysis
- It is a bioanalytical challenge, both scientifically and operational, but it is feasible to develop and validate LC-MS/MS methods down at low pg/mL levels in a regulated bioanalysis environment, if required
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