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MEDICINE ON THE BODY'S OWN TERMS

Drug development of highly potent therapeutic peptides – Regulated "microdose" Bioanalysis

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Presented at EBF Open Meeting "Less is More" Barcelona, 16-18 November 2011

Introduction

- Drug development of agonists towards oxytocin/vasopressin receptors

- Highly potent and selective peptides

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- Therapeutic “microdoses”

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- 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"

	Sub-therapeutic microdoses	**Therapeutic microdoses**
Method Validation	Qualified method normally sufficient	Full validation required
Sample Analysis	"Fit for purpose" compliance	Full compliance required

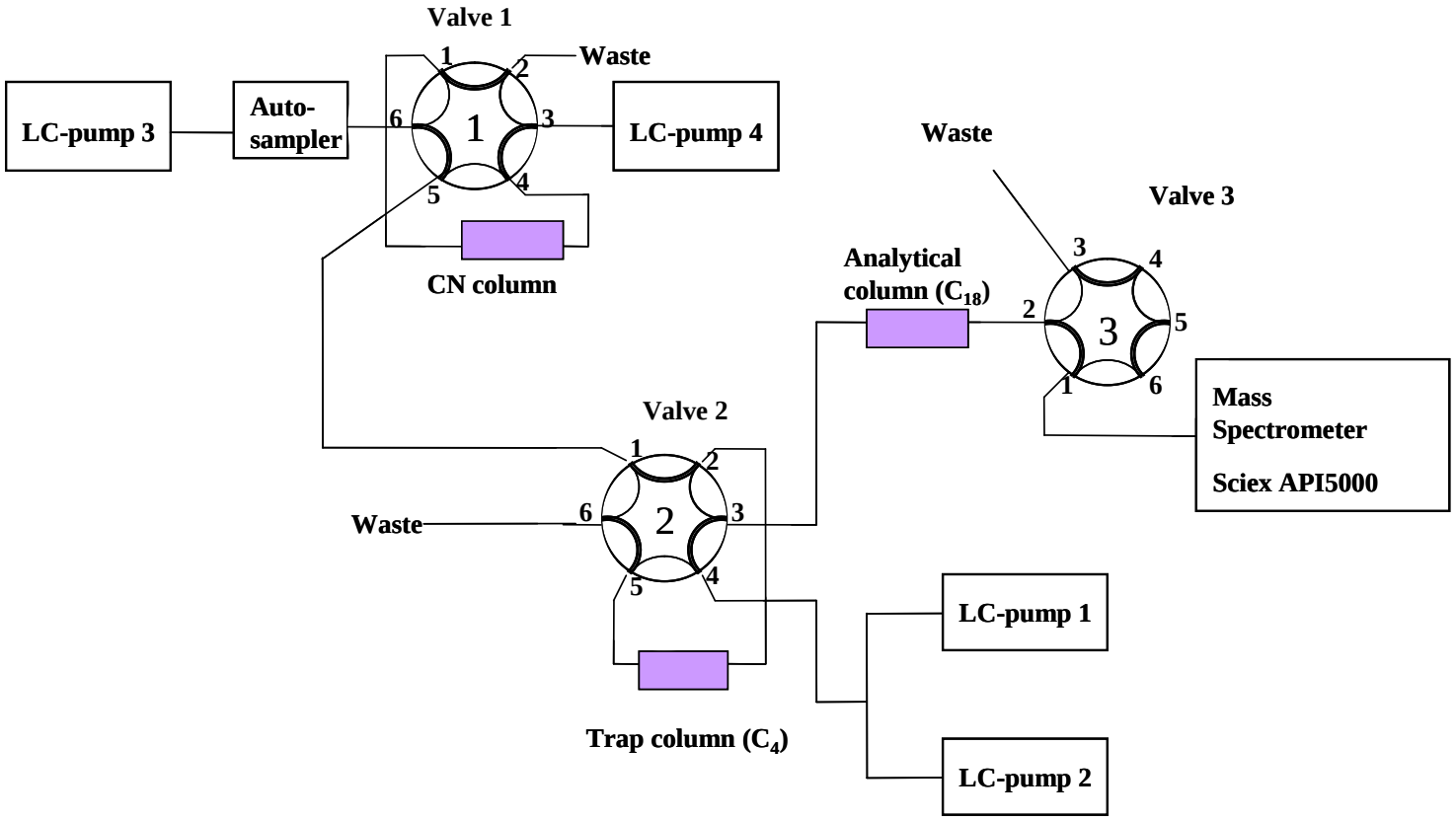
Approach

- 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



Recent experience

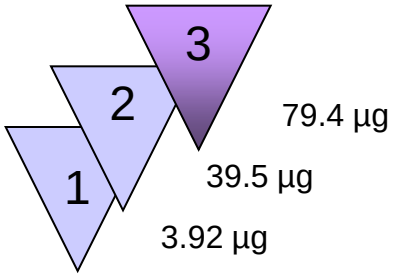
- Three therapeutic peptides (all around 1000 Da)
  - Case 1: New Chemical Entity
  - Case 2: Life Cycle Management
  - Case 3: New Chemical Entity

- Stable label internal standards

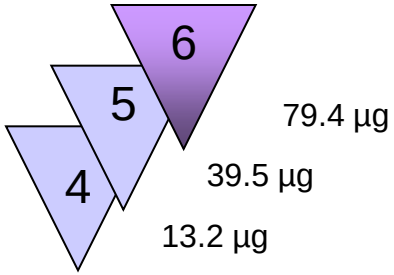
Case 1 – background

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

Males

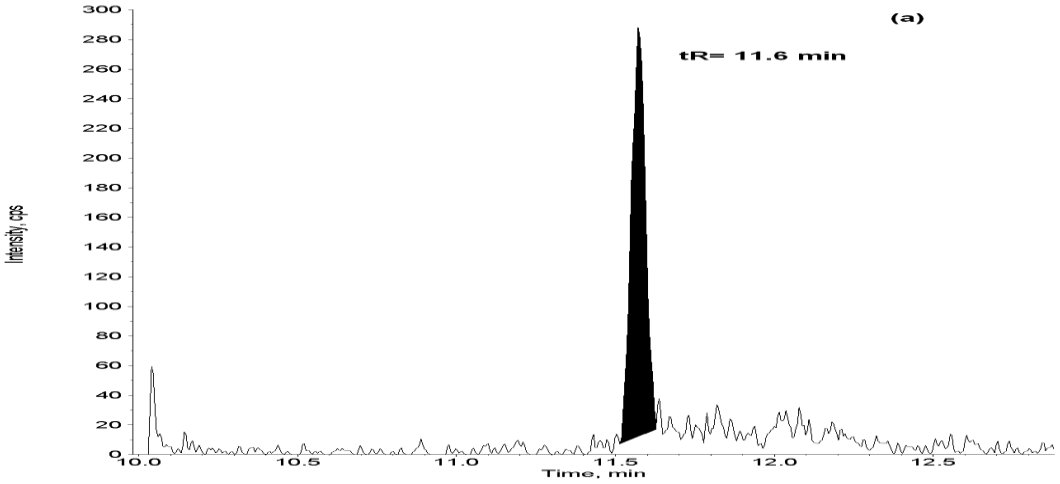


Females



Analytical background

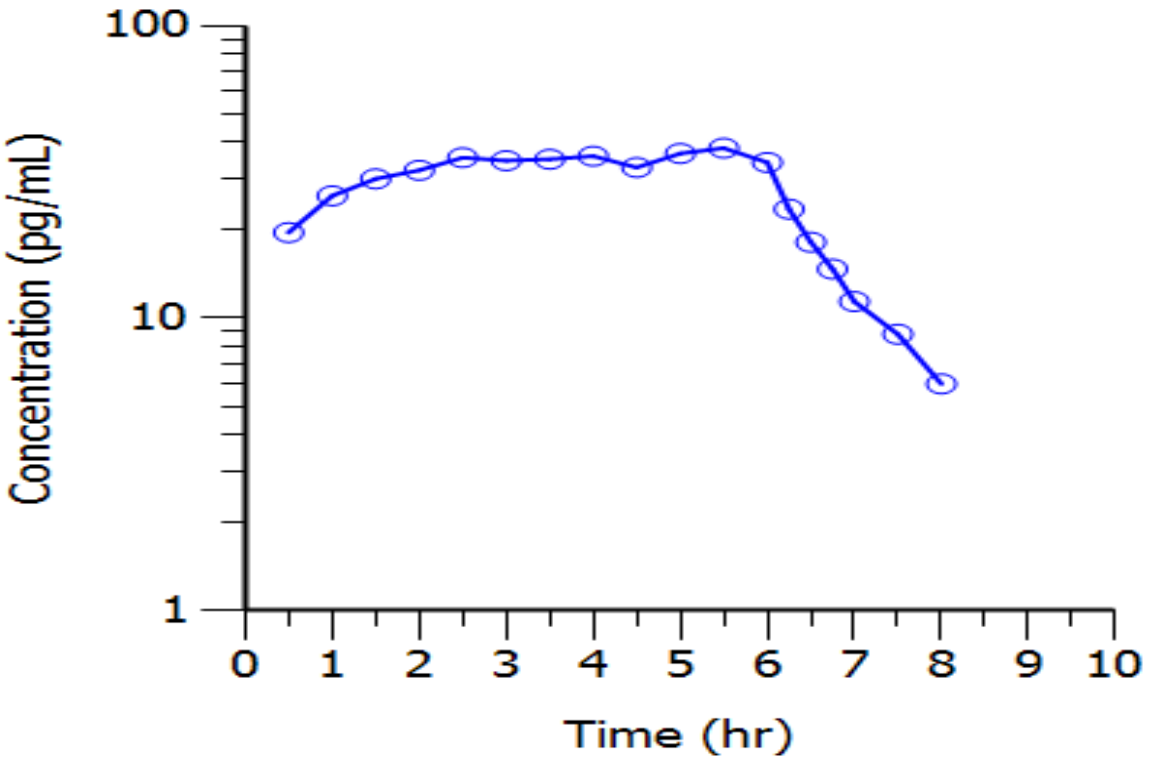
- LLOQ = 5 pg/mL



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

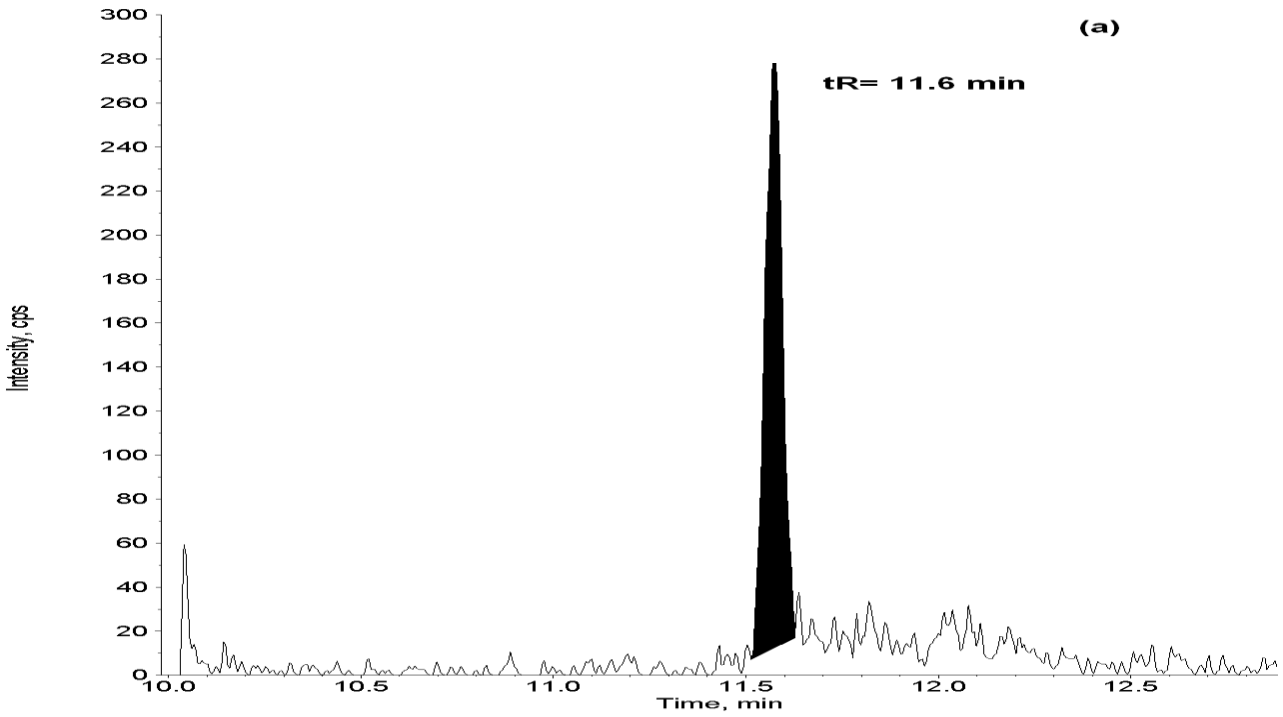
PK outcome

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



Sample throughput

Total cycle time: 13 minutes



Non-specific binding

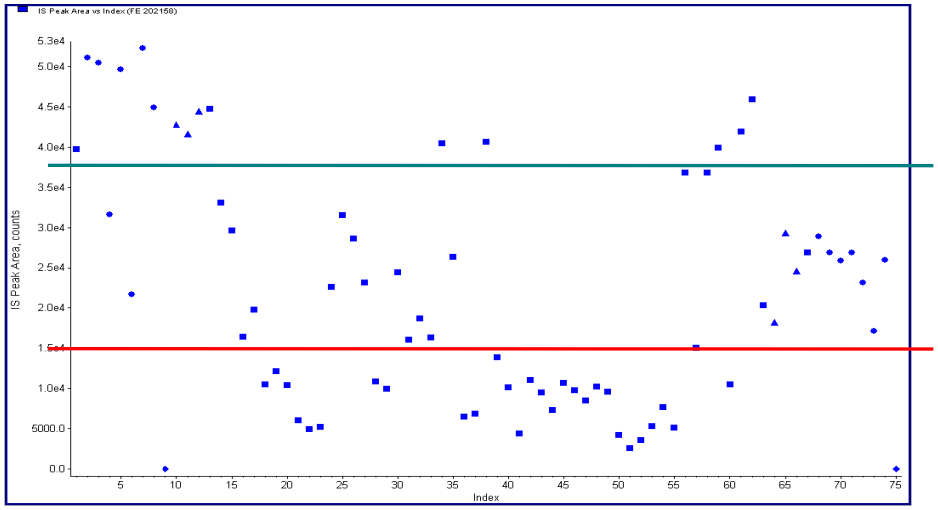
- Approach used to support several nonclinical studies and FIH study, all of a sudden problem!

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

- 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-medication)



Mean IS response (CAL and QC)

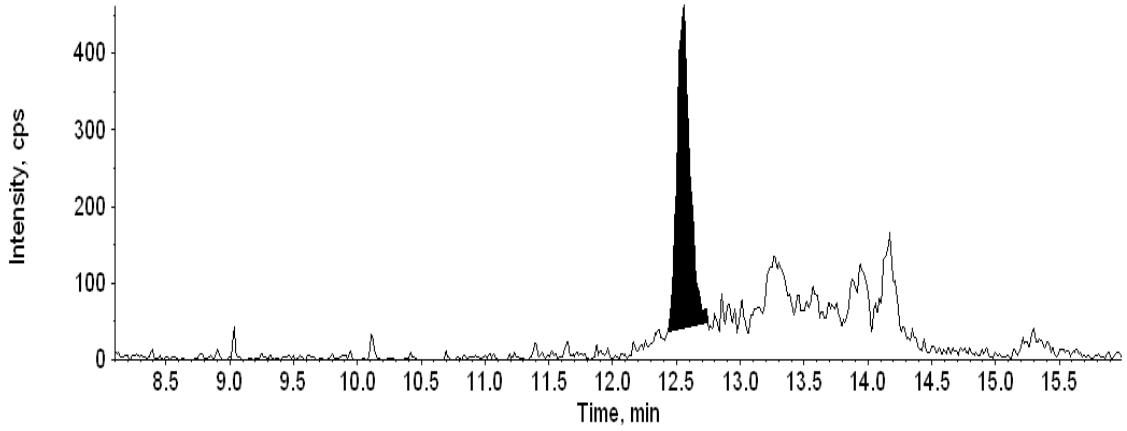
50% of mean IS response

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)

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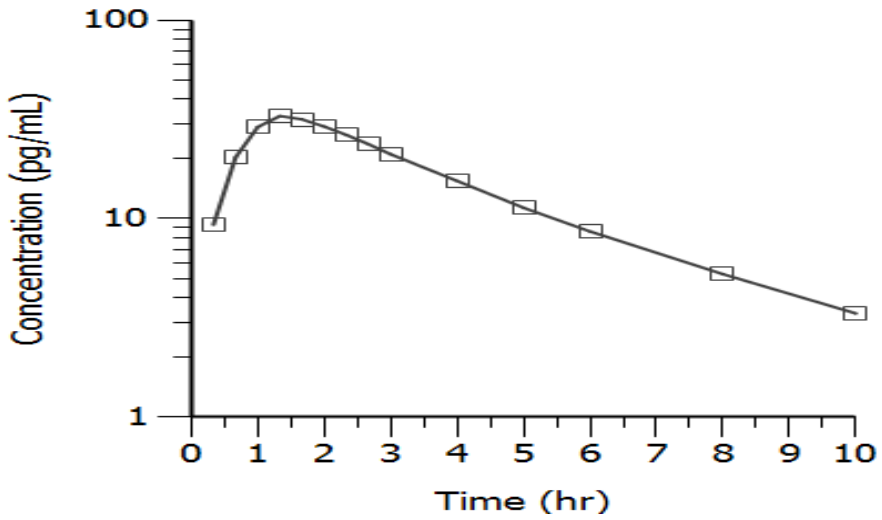
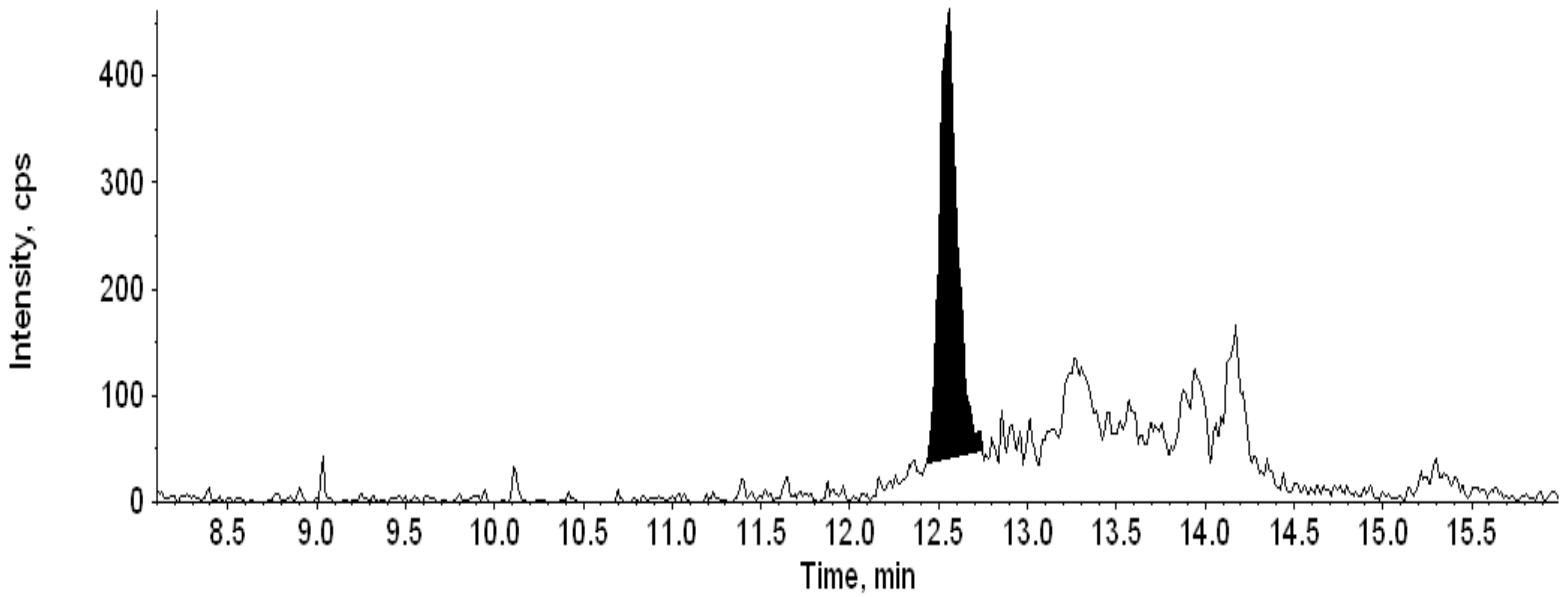
PK outcome

- 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

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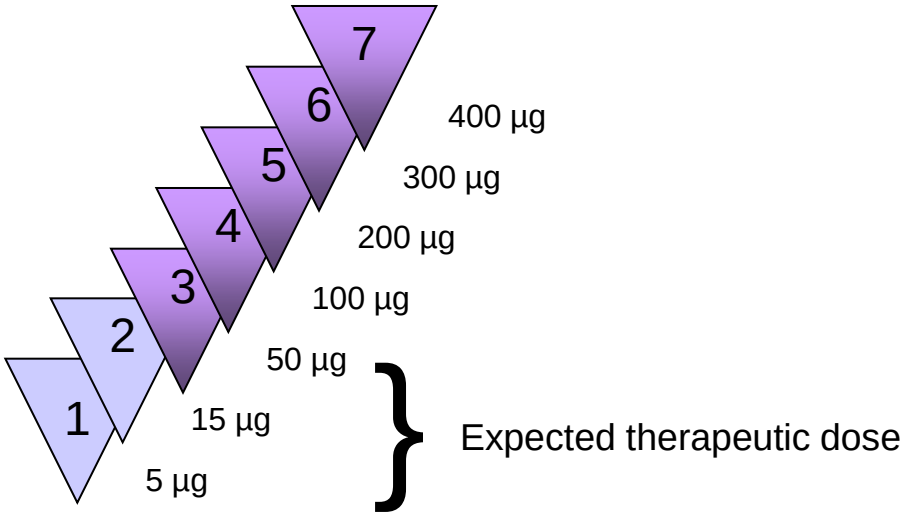
- Revised procedure for evaluating and mitigating carry over

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) $\leq$ 30 → OK
    - If area(n-1)/area(n) $\geq$ 30 → reanalyse sample
  - >100% of CAL1 → run failed
- In total, no carry-over run failure (n=30) with this revised procedure

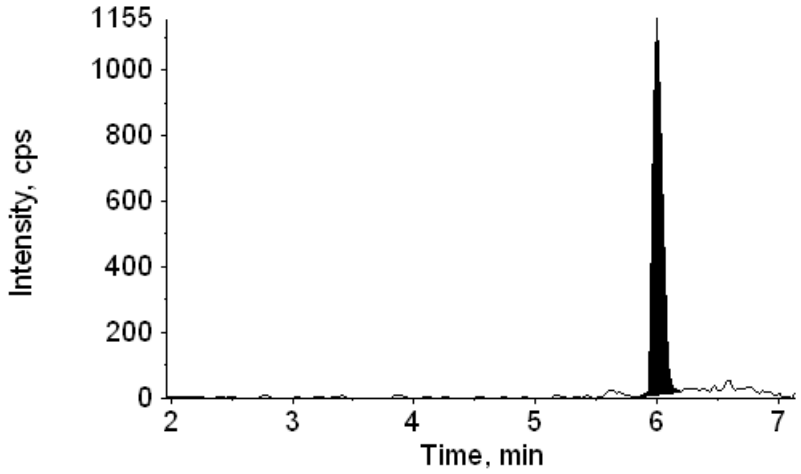
Case 3 – background

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



Analytical background

- LLOQ = 20 pg/mL



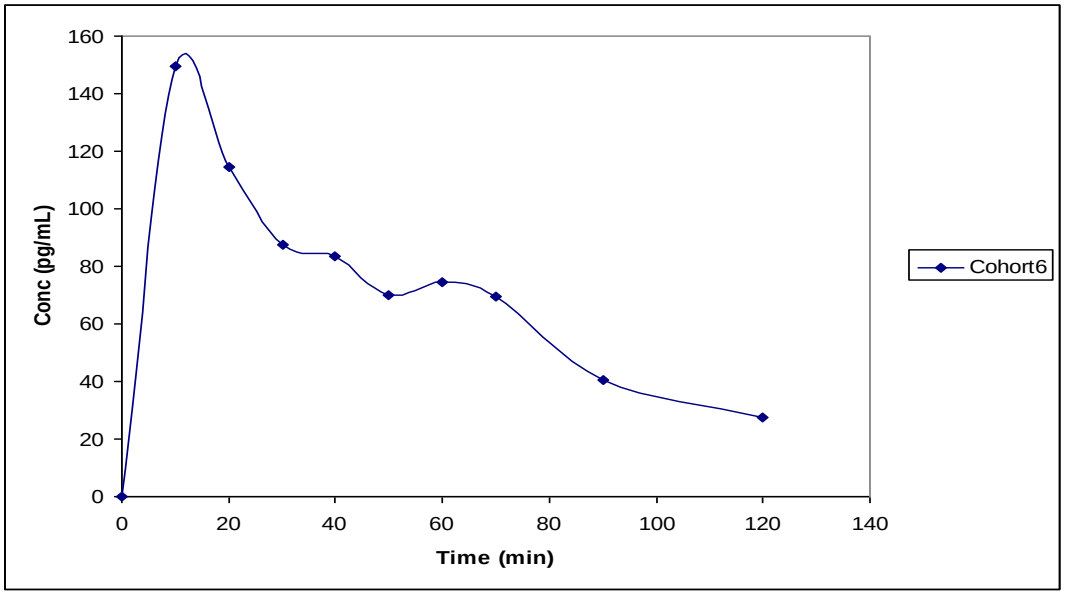
- Validation data, inter-assay CV: 9 **%** at LLOQ

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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Acknowledgements

- **Bioanalysis**
  - Alf Carlshaf
  - Anders Sonesson
  - Anna-Karin Wendel
  - Carina Ekström
  - Carina Johansson
  - Sara Johansson
  - Ulf Lövgren

- **PK guys**
  - Niclas Petri
  - Rikard Sandström