Microdosing and Cold LC-MS/MS: Bioanalysis and It’s Evolving Role in Strategic Drug Development

Richard W. Abbott
Biosciences Group,
Shire Pharmaceuticals,
Basingstoke, UK
Synopsis

Pandora’s Cluster of Galaxies – where less is certainly more!

• Introduction
  • AMS vs LC-MS/MS
  • Sensitivity Improvements in Instrumentation Over Time
  • Changing Role of Bioanalysis in Pharmaceutical R&D

• Shire Case Example
  • Strategic Role of Bioanalysis
  • Assay Validation Strategy
  • Microdosing Study Application
  • Study Outcome

• Conclusions
• Acknowledgements
Introduction to Microdosing

Less is More: The Human Microdosing Concept; R.C. Garner; *DDT*; 10(7), 449, 2005

Advantages of the Microdosing Approach:

- Only gram quantities of drug are required for safety testing
  - Only 100 µg or 1/100 of the pharmacological dose is administered to the subjects, depending which is less
- A minimal toxicology package is required
  - Extended (14 day) single dose study in a single species
- Enables rapid progression into man
- Cost of microdosing approach is a fraction of FTIH approach
- Enables early decision between several drug candidates with similar pharmacological profiles
### Comparison of AMS vs LC-MS/MS

<table>
<thead>
<tr>
<th>AMS</th>
<th>Cold LC-MS/MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>- No widespread availability</td>
<td>- Already available routinely</td>
</tr>
<tr>
<td>- $^{14}$C material required</td>
<td>- No requirement for $^{14}$C-label</td>
</tr>
<tr>
<td>- $^{14}$C synthesis requires time</td>
<td>- Enables rapid decision making</td>
</tr>
<tr>
<td>- Exquisite sensitivity, fg/mL and ag/mL LLOQ’s attainable</td>
<td>- Can support studies where low pg/mL LLOQ’s required</td>
</tr>
<tr>
<td>- Can provide PK information on parent molecule and metabolite</td>
<td>- Can provide PK information on parent molecule</td>
</tr>
<tr>
<td>- Metabolism information, as well as absolute bioavailability and clearance</td>
<td>- No information on metabolism of drug</td>
</tr>
</tbody>
</table>

Lappin, G; Wagner, CC; Langer, O; van de Merbel, N; *Bioanalysis*, 1(2), 357, 2009
Ings, RMJ; *Bioanalysis*, 1(7), 1293, 2009
Evolution of Instrumentation Sensitivity

White Light : Multi Coloured

Less is More

Increasing Sensitivity

Time

UV/ GC/ RIA/ HPLC/ ELISA/ X-MS

Instrumentation

µg/mL ng/mL pg/mL

Sample Preparation
- SLE
- µ SPE
- Column Switching (2D-LC)

Chromatography
- Ultra high pressure LC pumps
- Decreasing particle size, 5 µm → 1.7 µm
- Increasing N and R_s
- Reduce column id

Mass Spectrometry
- Quadrupole MS refinements
e.g. API4000 to API5500, x10 fold sensitivity gain

In Combination

Sub ng/mL LLOQ

Increasing Trend

Single digit pg/mL LLOQ or lower

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Traditional Role of Bioanalysis in Pharmaceutical R&D

**Routine**

- Routine Assay Validation
- Routine Study Sample Analysis
- Study Scheduling
- Enabling Project Progression
- Dependable Study Support and Regulatory Submission Support

‘Back-room’ guys

[Only noticed if an issue arises!]
Strategic

- Enabling fresh thinking regarding sample collection and handling (e.g. DBS)
- Enabling new project strategy (e.g. microdosing)

Improvements in instrumentation sensitivity have made this possible

Exciting Times!
Recent paper on applicability of LC-MS/MS to microdosing studies


**Objective**
Investigation of the sensitivity of the LC-MS/MS approach as a tool in microdose clinical trials

**Methods**
31 of the 47 top selling drugs with a wide range of physicochemical properties were spiked in human plasma, extracted and analysed by LC-MS/MS
(Exclusions: High mol weight drugs, combination products, drugs requiring derivatisation)

**Results**
LLOQ’s varied from 0.08 to 50 pg/mL – These were < 1/8 of assumed $C_{\text{max}}$ at microdose for all drugs except losartan, indicating wide applicability for the generation of full PK profiles following microdosing

**Conclusions**
LC-MS/MS should be widely applicable for the support of clinical microdosing studies where generation of PK profiles of the parent molecule is the key endpoint.
Shire Case Example

• SSP-1252, Prodrug of Guanfacine (treatment for ADHD)

• Guanfacine

![Chemical Structure]

• Objective: Move project into man as rapidly as possible
  » Verify prodrug absorption and metabolism to guanfacine

• Development Team Goal:
  • Facilitate objective through a microdosing approach
PK Modelling and Bioanalytical Feasibility Study

Initial Plan

(1) Collaboration between Biosciences group and Clinical Pharmacology group to model projected Cmax for SSP-1252 and guanfacine following microdosing
   (1) Projected Cmax : 60 pg/mL
   (2) Calculated LLOQ required, based on $4 \times t_{1/2}$ : 4-5 pg/mL

(2) Bioanalytical feasibility study to check out that LLOQ of 5 pg/mL for each analyte was possible
   • As SSP-1252 was a prodrug, sample instability was also an issue, sample collection and handling was an additional complication
Shaping Clinical Development Strategy

• Based on the encouraging results from the bioanalytical feasibility study:
  
  • Clinical Development Strategy set to include microdosing
  • A first for Shire
  • A first for the bioanalytical group
  • A first experience where bioanalysis had been central in enabling the clinical development strategy

• NB Shire is a virtual company
• So this was achieved using an outsourced approach
Assay Validation Strategy

- Extent of validation required?
- Likely to use the assay only once at the extreme sensitivity required
- Regulatory guidance – not for bioanalysis in support of microdosing studies
- No previous discussion within EBF or AAPS regarding commonly accepted approach

- We did not feel comfortable taking anything other than the Full validation approach

- So we adopted this strategy and moved forward with full validation packages for both SSP-1252 and guanfacine
Method Summary

- **Matrix**
  - 220 µL acidified (10% formic acid) K_2 EDTA/NaF human plasma

- **Internal Standard**
  - Analogue, Guanabenz

- **Sample Preparation**
  - LLE with MTBE coupled with PP (MeCN)

- **HPLC**
  - HyperClone BDS C_{18} 130A, 5 µm, 150 x 2 mm with gradient elution

- **Mass Spectrometer**
  - Sciex API-5500
  - Turbo IonSpray, positive ion mode

- **Calibration**
  - Separate lines for guanfacine and SSP-1252, 5 to 2500 pg/mL
Example Chromatograms

- SSP1252 LLOQ
- Guanfacine LLOQ
- Double blank
- Double blank
## Headline Assay Validation Summary

<table>
<thead>
<tr>
<th></th>
<th>Intra-Day Data</th>
<th>Inter-Day Data</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LLOQ</td>
<td>Other QCs</td>
</tr>
<tr>
<td>1024 (%cv)</td>
<td>9.5 to 14.5</td>
<td>1.3 to 10.5</td>
</tr>
<tr>
<td>1024 (% bias)</td>
<td>-8.6 to 8.2</td>
<td>-12.5 to -2.7</td>
</tr>
<tr>
<td>1252 (%cv)</td>
<td>6.0 to 9.2</td>
<td>1.4 to 14.3</td>
</tr>
<tr>
<td>1252 (% bias)</td>
<td>-0.6 to 12.0</td>
<td>-11.6 to 5.2</td>
</tr>
</tbody>
</table>

### Stability

- At least 4h bench top acidified plasma stability (ice/water bath)
- At least 3 F/T cycles
- At least 31 days long term frozen acidified plasma stability (-70°C)
- At least 72 h extract reproducibility (4°C)
Clinical Microdosing Study Details

Primary Objective
Determination of the relative oral bioavailability of guanfacine from SSP-1252R compared to guanfacine HCl after a dose of 100 µg in healthy male volunteers

Study Design

<table>
<thead>
<tr>
<th>Period 1</th>
<th>7 day wash out</th>
<th>Period 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sequence 1</td>
<td></td>
<td>Sequence 2</td>
</tr>
<tr>
<td>SSP-1252R (6 subjects)</td>
<td>Guanfacine</td>
<td>SSP-1252R (6 subjects)</td>
</tr>
</tbody>
</table>

Sample Collection
- Blood samples (3 mL) collected into Vacutainers (2 mg NaF / 1.75 mg K₂EDTA)
- Place in crushed ice bath immediately and centrifuge within 30 minutes at 4°C
- Aliquot 0.6 mL plasma into tube containing 60 µL 10% formic acid, mix & freeze (-70°C)

NB – Training provided in pipetting by Bioanalytical CRO staff to clinical site staff

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Summary of Microdose Clinical PK Study Output

• Guanfacine concentrations measured to 24h (1252R administration) and 48h (guanfacine HCl administration)

• SSP-1252 rapidly absorbed and eliminated with measurable concentrations for 1h after administration

- Taking into account the different molar doses, relative F of guanfacine from SSP-1252R after oral dose of 100 µg was 28.3%
Bioanalytical Study Performance Details

- All 5 bioanalytical batches were acceptable
- QC data demonstrate acceptability of assay performance

<table>
<thead>
<tr>
<th></th>
<th>Low 15 pg/mL</th>
<th>Mid 200 pg/mL</th>
<th>High 2,000 pg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1024 (% cv)</td>
<td>10.3%</td>
<td>5.8%</td>
<td>4.1%</td>
</tr>
<tr>
<td>1024 (% bias)</td>
<td>-3.9%</td>
<td>-0.6%</td>
<td>4.3%</td>
</tr>
<tr>
<td>1252 (% cv)</td>
<td>9.7%</td>
<td>4.1%</td>
<td>10.2%</td>
</tr>
<tr>
<td>1252 (% bias)</td>
<td>0.8%</td>
<td>6.2%</td>
<td>4.4%</td>
</tr>
</tbody>
</table>

- 8 days from final sample receipt to availability of preliminary QC’d data
- Rapid sample analysis enabled rapid study data review and decisions
Conclusions

- The improvement in LC-MS/MS sensitivity is enabling the technique to be used as a tool in microdosing studies.

- The introduction of low pg/mL assay sensitivity is allowing bioanalysis to play a strategic role in drug development.

- Recent literature suggests the use of cold LC-MS/MS in microdosing may be widely applicable across therapeutic areas.

- Shire studies over the past year have verified these findings (4 cold microdosing studies completed).

- Wider discussion across industry should enable assay validation for microdosing studies to be appropriately tailored.
Acknowledgements

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• You, for your attention