LC-MS/MS assay transfer: a journey through the method cross-validation challenges

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Agenda

Case study: Small molecule LC-MS/MS method cross-validation between 3 bioanalytical sites

• Issues faced
• Troubleshooting performed
• Robustness tests
• Impact of analytical methods variability on the PK

References:
Xiaohui Xu et al., Fit-for-purpose bioanalytical cross-validation for LC–MS/MS assays in clinical studies, Bioanalysis (2013) 5(1), 83–90
Stephen White et al., The quest for assay robustness across the life cycle of a bioanalytical method, Bioanalysis (2015) 7(7), 815–824
Alexandra Georgiou et al., An inter-laboratory transfer of a multi-analyte assay between continents, Bioanalysis (2015) 7(7), 825–831
Per EMA and FDA guidelines during the course of a study, incurred and spiked samples should be analyzed and cross checked between the involved laboratories when clinical study samples are measured by 2 or more laboratories.
Results: Method cross comparison
-Spiked QC samples and clinical samples-

- **Spiked QC samples Acceptance criteria:** at least 2/3 of the data within ± 15% of the normalized difference. **Cross-check: OK**

- **Clinical study samples Acceptance criteria:** at least 2/3 of the data within ± 20% of the normalized difference. **Cross-check: Failed**
ISRs Methods reproducibility assessment -Clinical samples-

• ISR assessments demonstrate methods reproducibility both at Novartis and ESP1 sites
## What went wrong?

<table>
<thead>
<tr>
<th></th>
<th>Novartis Basel</th>
<th>BA-CRO ESP1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Validation</td>
<td>OK</td>
<td>OK</td>
</tr>
<tr>
<td>Long term stability</td>
<td>Fulfilled</td>
<td>Fulfilled</td>
</tr>
<tr>
<td>Stock solution stability</td>
<td>Fulfilled</td>
<td>Fulfilled</td>
</tr>
<tr>
<td>Selectivity, (N-Oxide)</td>
<td>Fulfilled</td>
<td>Fulfilled</td>
</tr>
<tr>
<td>Sample processing</td>
<td>Consistent between the two laboratories</td>
<td>Consistent between the two laboratories</td>
</tr>
<tr>
<td>Chromatography</td>
<td>Consistent between the two laboratories</td>
<td>Consistent between the two laboratories</td>
</tr>
<tr>
<td>Mass spectrometer</td>
<td>API 5000</td>
<td>API 6500</td>
</tr>
<tr>
<td>UPLC</td>
<td>Symbiosis</td>
<td>Shimadzu</td>
</tr>
<tr>
<td>Samples</td>
<td>Concentrations consistent between primary and back up samples</td>
<td></td>
</tr>
<tr>
<td>ISR</td>
<td>Passed acceptance criteria in both laboratories</td>
<td></td>
</tr>
</tbody>
</table>

We don’t know!
Method robustness assessment - sample extraction -

- The calculation of pharmacokinetic parameters for a drug assumes that the concentrations measured in clinical samples are reliable.

- The concentration measured in clinical samples were confirmed by a different sample extraction strategy.

**Diagram:**
- Liquid/Liquid extraction
- Protein Precipitation (Validated sample Extraction)
- LC-MS/MS

**Graph:**
- Concentration Bias of study samples
  - Protein precipitation vs Liquid/Liquid extraction
  - BA-CRO (ESP1)

**Legend:**
- Normalized difference (Bias %)
  - 0 5 10 15 20 25 30
  - -25.0 -20.0 -15.0 -10.0 -5.0 0 5 10 15 20 25 30
Summary of BA activities -First in man study-

Backup Samples Transfer

- NOV
  - June 2015: Study Outsourced
  - Aug 2016: Study Outsourced
- ESP1
  - June 2016: Study Outsourced
  - Feb 2017: Study Outsourced

Time
Concentration bias of re-measured samples at the ESP1

- 61% of the concentration measured are within acceptance criteria (±20% of normalized concentration bias).
**Impact of the concentration Bias on key PK parameters (NVS vs ESP1)**

<table>
<thead>
<tr>
<th>PK Parameters</th>
<th>Effects of the methods bias</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC</td>
<td>Not of clinical significance</td>
</tr>
<tr>
<td>T1/2</td>
<td>In some individuals and for some cohorts of clinical significance</td>
</tr>
<tr>
<td>Cmax</td>
<td>In some individuals and for some cohorts of clinical significance</td>
</tr>
<tr>
<td>Impact on the clinical strategy</td>
<td></td>
</tr>
<tr>
<td>Dose escalations</td>
<td>Not impacted</td>
</tr>
<tr>
<td>Recommended Phase II Dose</td>
<td>Possibly impacted</td>
</tr>
<tr>
<td>Food effect cohort samples measured between the two BA sites</td>
<td>PK results of difficult comparison</td>
</tr>
</tbody>
</table>

Graphs Courtesy of Yi (Gary) Gu, PKS Novartis

NIBR, PK Sciences

Business Use Only
Summary of BA activities
- First in man study -

Backup Samples Transfer

- June 2015
  Novartis Basel
- June 2016
  Study Outsourced
- August 2016
- February 2017
  Sample reanalysis started
- March 2017
  Biological sample import restriction at the ESP1 country
Results: Method cross comparison -Spiked QC samples and clinical samples-

**Spiked QC samples Acceptance criteria:** at least 2/3 of the data within ± 15% of the normalized difference. **Cross-check: OK**

**Clinical study samples Acceptance criteria:** at least 2/3 of the data within ± 20% of the normalized difference. **Cross-check: Failed**
Method robustness assessment - Selectivity assessment -

- The concentration measured in clinical samples were confirmed by a different chromatographic set up.
BA activities to support a first in man study

Backup Samples Transfer

- **June 2015**: Novartis Basel
- **June 2016**: Study Outsourced
- **August 2016**: Biological sample import restriction
- **February 2017**: Sample reanalysis started
- **March 2017**: Biological sample import restriction
- **November 2017**: Analysis of clinical study samples
Conclusions/lessons learned

- Method transfer between different laboratories is the “optimal” robustness test.
- Method robustness assessment should be designed early on during the drug development program.
- Study samples should be used for the method robustness assessment.
- The effect of methods bias should be assessed in the context of the PK objectives of a clinical program.
Thank you!