Parallelism – Feedback from the AAPS/EBF/JBF sister meetings

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on behalf of the EBF

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10 – A New Journey Begins
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Pre-Study Validation: Dilutional Linearity

- Dilutional linearity experiments are performed to demonstrate that high concentrations of the analyte of interest can be accurately measured by diluting into the assay’s quantitative range and multiplying the measured concentration by the dilution factor.
  - Particularly relevant to LBA assays where samples of high concentration may require significant dilution to achieve the working range of the assay
  - Hook effect is typically assessed in the same experiment by including samples spiked with very high concentrations of analyte which are tested without dilution beyond MRD

In-Study Validation: Parallelism

- The concept of parallelism is similar to dilutional linearity except that parallelism assesses incurred study samples.
- Incurred samples (pooled or individual) are tested at multiple dilutions that are expected to yield concentrations that fall above the assay ULOQ (to evaluate prozone or hook effect) as well as within the assay range.

Concept of Parallelism

Imagine a sample containing 4’000 ng/mL analyte

Final concentration should be independent of the sample dilution…!
Problem Statement

- Regulatory guidances provide differing levels of detail and requirements for assessment of parallelism
# Bioanalytical Guidelines

<table>
<thead>
<tr>
<th>EMA, 2011</th>
<th>FDA, 2013 draft</th>
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<tbody>
<tr>
<td><strong>7.1.1.10. Parallelism</strong>&lt;br&gt; If study samples are available, parallelism between the calibration standard curve and serially diluted study samples should be assessed to detect possible matrix effect or differing affinities for metabolites. A high concentration study sample (preferably close to Cmax) should be diluted to at least three concentrations with blank matrix. The precision between samples in a dilution series should not exceed 30%</td>
<td><strong>B. Bioanalytical Method Development and Validation / 1. Selectivity / b. Matrix Effects</strong>&lt;br&gt; Matrix effects should be evaluated. For example: The calibration curve in biological fluids should be compared with calibrators in buffer to detect matrix effects using at least ten sources of blank matrix. Parallelism of diluted study samples should be evaluated with diluted standards to detect matrix effects.</td>
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</tbody>
</table>
Questions and Answers (Q&A) for the Guideline on Bioanalytical Method (Ligand Binding Assay) Validation in Pharmaceutical Development

Q17. Is it not necessary to evaluate parallelism?
A17. Parallelism is defined as an established parallel relationship between a dose-response curve from a study sample dilution series and a curve from a calibration standard series, with no difference among back-calculated concentrations for multiple dilutions of a study sample. As of the issuance of this guideline, domestic and international knowledge has neither accumulated nor discussion yet matured regarding cases in which parallelism was not established, causes for failing to establish parallelism, and the extent of impact the failure might have on pharmaceutical development. Therefore, evaluation of parallelism is not necessarily required for all analytical methods. However, if parallelism is an intrinsic issue for an LBA-based bioanalytical method and is likely to cause a problem based on the nature of the analyte or method or data accumulated in the course of pharmaceutical development, scientifically valid evaluation and assessment of the impact on measured concentrations should be considered to the extent possible.
EBF Survey

- Survey of EBF member companies performed in May 2017 in lead up to Focus Workshop
  - Questions on ‘dilutional linearity’ and ‘parallelism for PK assays’
- 24 companies replied
  - 15 Pharma, 9 CRO
Q1: What do you perform in your lab?

- 24 responders

- LBA: 19
- Protein LCMS: 1
- Both: 4
Q2: Do you perform parallelism regularly for PK methods?

- 24 responders
Q3: How do you perform parallelism?

- 20 responders (18 routine, 2 non-routine)
Q4: Number of individual sera?

- 18 responders

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Q5: How many dilutions do you perform?

- 18 responders
Q6: When do you perform parallelism?

- 18 responders
Q7: How do you evaluate the acceptance of parallelism?

- 20 responders (18 routine, 2 non-routine)
Summary of the Survey

- Approx. ¾ responders perform parallelism assessment routinely

- Common approach for parallelism
  - to assess 3-10 individual samples
  - 3-5 dilutions in assay range
  - To apply %CV acceptance criteria
    - To a lesser extend evaluate %RE trends

- In line with GBC L2 white paper
Recommendation:

- If a parallelism assessment is deemed necessary, incurred samples should be tested at multiple dilutions across the quantitative range of the assay
  - Individual samples recommended
  - Pooled sample may be justified in some cases (e.g. low sample volume)
- Samples that fall above the assay ULOQ should be included (to evaluate hook effect), as well as samples within the assay range.
- Trends that may have meaningful impact on the study data should be evaluated.
Harmonization of the Guidances

Not Included as Recommendation:
- Precision (%CV) of the cumulative back-calculated concentrations for all in-range samples should be ≤30%.
  - Precision criteria should be used with caution, as precision of a dilution series can be misleading.
Evaluation of Trends

<table>
<thead>
<tr>
<th>Dilution Factor (Fd)</th>
<th>Mean Conc. (ng/mL)</th>
<th>Mean Conc. x Fd (ng/mL)</th>
<th>Precision of Series (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>279.6</td>
<td>279.6</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>159.5</td>
<td>319.0</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>98.1</td>
<td>392.2</td>
<td>20.5</td>
</tr>
<tr>
<td>24</td>
<td>48.4</td>
<td>387.4</td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>29.9</td>
<td>477.7</td>
<td></td>
</tr>
</tbody>
</table>

CV <30%

BUT…

Parallelism of Incurred Sample
Relative Error versus 1/3 Dilution Result

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Follow-up to the Survey

➢ Gather further data on incidence of parallelism failure
  – Type(s) of analyte, reason for failure
    o EBF Finger-on-the-Pulse (FotP) performed
Parallelism FotP

Q: How many parallelism assessments (approx.) have you performed in the past 2 years?

- 21 responders
- Total: approx. 215 parallelism assessments
- Note: Clinical and Preclinical assays
Q: What type of molecules have you assessed?
- Antibodies
- Antibody fragments
- Peptides
- Enzymes
- Fusion proteins
- ADC
- Therapeutic proteins
  - Hormones
  - Chemokines

Q: How many parallelism assessments have failed?
- 5
Parallelism Failures

Q: What type of molecules failed?
- Antibody (2)
- Peptide (1)
- Therapeutic protein (1)
- Not disclosed (1)

Q: Why did parallelism fail?
- ADA interference (2)
- Assay not reached equilibrium (1)
- Metabolism compound (1)
- Cause not identified (1)

Note: Instances of soluble target interference causing failure of some dilutions

Failures: 3 of 215 = 1.4%
Parallelism Failures

Q: What were the consequences of failure?
   – Assay redeveloped to address parallelism, and samples reanalysed (4)
   – Samples were analysed in multiple dilutions and reported as semi-quantitative (1)
Parallelism Recommendation

- **Recommendation:** The need to perform a parallelism assessment should not be mandated, but be driven by the characteristics of the drug, its binding partners and the assay reagents’ specificity. Scientific rationale should exist that explains why the assessment is warranted.
Acknowledgments

- EBF community for survey and FotP feedback
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- GBC L2 Harmonization Team
- EBF TT-35
- EBF TT-61
References


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