Validation/Qualification Issues for Enabling Technologies for Drug Discovery

David M. Stresser, Ph.D.
BD Biosciences
Who is BD?

- **FORTUNE 500** company (#316)
- Locations in **more than 50 countries**
- Approximately **29,000 associates** worldwide
- Serves healthcare institutions, life science researchers, clinical laboratories and the general public
- Sells a broad range of medical supplies and services, devices, laboratory equipment and diagnostic products
- **BD Gentest**\(^{SM}\) Contract Research Services provides *in vitro* drug metabolism services
My goals today

• Bring a CRO industry perspective on assay validation
• Offer a view on challenges to successful validation
• Review a case study from our laboratory
• Recommendations and closing thoughts
What is Validation?

• Validation is demonstrating you can repeatedly do what you want to do.
  – That means knowing how your inputs relate to your outputs
  – It starts early in the process/product development and continues throughout commercial life of the product.

• Must be fit for purpose
  – Simple QC assays for a research use only product
  – Safety study assay that is correlated (or predictive) of a clinical outcome.
CRO Perspective

• Not significantly different than any other life science business

• We must meet expectations of:
  – Regulatory agencies
  – Customers (funding)
  – Auditors (agencies, customers, consultants)

• Our customer base
  – Mostly large and small pharma
  – They require “research grade” to GLP standard assays
  – Customer philosophies vary – as a service provider we need to achieve a validation standard representative of the highest standard among our target customer base
Challenges to Assay Validation

- It is resource intensive
- Requires multi-disciplinary expertise
  - Scientists
  - Statisticians
  - Quality Assurance
  - Project managers
- Relative to the science and other end goals, the process can be dull and tedious
- Communication - keeping all project team members aligned and stakeholders informed
General Strategy for Assay Validation

- **Prework**
  - Lock in on project goals
  - Input from various sources [Regulatory guidelines, “Voice of customer”, Direct study of customer environment (e.g. scientific literature, etc)]

- **Verification**
  - Conduct the needed experiments to become adequately familiar with the assay conduct, QCs, reproducibility and robustness
  - Adequate verification data sets make the validation exercise much easier from a quantitative and statistical perspective.
  - With robust data going in, validation should be a coronation.

- **Validation**
  - Demonstration and documentation that acceptance criteria can be met
  - Make recommendations for standard assay conditions
Validation Components

• Intra-assay precision (i.e. multiple replicates of the same conditions are tested in one assay)

• Inter-assay precision (i.e. repeat assays conducted by the same operator). If the repeat assays are conducted on separate days, this test may also be referred to as inter-day precision.

• Inter-operator precision (i.e. identical assays conducted by different operators either side by side or sequentially as applicable)
Validation Components

• Acceptance criteria
  – The validation protocol must specify acceptance criteria for all parameters to be validated.
  – Acceptance criteria are determined by the Study Director (with input from stakeholders if applicable).
  – Acceptance criteria may be based on a number of considerations, including, but not limited to
    • Historical in-house data
    • Results of verification experiments
    • Voice of customer
    • Industry standards
    • Competitive environment
    • Regulatory guidelines
    • Scientific literature
Validation Components

- The number of QC parameters and statistical precision will dictate failure rate. For example, 4 QC values at 95% equals a 20% failure rate
- Focus on the right number of QCs and the right level of statistical failures.
- The "flip side" of broad statistical criteria is the acceptable range may be excessively large
Validation Components

With data accumulation over time...

- The limits may evolve over time as more data accumulates
- Other driving factors for modifying limits includes changes in customer expectations, waste elimination targets, etc.

Actual mean and distribution
Validation Components

Long-Term Capability

- Is an upward/downward trend observed? Examine reasons and take corrective action if possible.
Assay “Qualification”, not validation

• Demonstrate reproducibility only
  – Typically interday
• Establish a positive and negative control
• “Research grade”
  – No formal protocols, reports
  – No formal involvement of QA
• For many customers, this meets their expectations
Structured process to validation

• Establish a process with check points to promote care and proper planning (e.g. peer review, QA review)

• Check points represent “control” points in the process
Case Study - Validation

- **Project Objective Statement:** Adapt cytochrome P450 inhibition assays to mass spectrometry analytical methodology and introduce preincubation to standard protocol.

- Cytochrome P450 inhibition is a required drug-drug interaction test for small molecule drug candidates

- Analytical method validation
  - 8 metabolites; 8 validation protocols

- Assay method validation
  - 8 assays; 16 Validation protocols for IC$_{50}$, $K_i$, time-dependent inhibition analysis

- 48 Validation protocols and Reports
Acceptance Criteria

- **Analytical**
  - FDA guidance document for analytical method validation (2001)
    - Selectivity
    - Standard Curve
    - Stability
      - Autosampler
      - Freeze/thaw
      - 4 weeks @ -20 ºC
    - Accuracy and Precision

- **Assays**
  - Incubation time(s) selected must fall within linear portion of the response
  - Protein concentration(s) selected must fall within linear portion of the response
  - Total metabolism must be less than 15%.
  - $K_M$ value must be within 5-fold of literature values reported by Obach and Walsky, Drug Metab. Dispos. 32: 647, 2004.
  - $IC_{50}$ values must be $< X \mu M$ and duplicate determinations within 5-fold
  - $K_i$ values must be within 5-fold of the $IC_{50}$ value and less than twice the $IC_{50}$ value. Duplicate determinations within 5-fold
Example Assay Development and reproducibility: CYP2C9/Diclofenac 4’-hydroxylase

- Resulting Data Set
  - Linearity of metabolite formation with incubation time and HLM protein concentration
  - $K_M$ determination
    - 3.5 µM, 3.9 µM
  - $IC_{50}$ and $K_i$ determination with sulfaphenazole
    - $IC_{50}$: 0.41 µM, 0.63 µM
    - $K_i$: 0.20 µM, 0.19 µM
## Assay Validation Results

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Criteria</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time dependence</td>
<td>Incubation time(s) selected fall within the linear portion of the assay</td>
<td><strong>Pass</strong> (5 min)</td>
</tr>
<tr>
<td>Protein Dependence</td>
<td>Protein concentration(s) selected fall within the linear portion of the assay</td>
<td><strong>Pass</strong> (0.02 mg/mL)</td>
</tr>
<tr>
<td>Total metabolism</td>
<td>Less than 15%. If assay sensitivity is a problem the study director will determine if up to 30% total metabolism is acceptable.</td>
<td><strong>Pass</strong> (7% at 0.25 µM midazolam, 0.04 mg/mL protein, 5 min)</td>
</tr>
<tr>
<td>$K_M$</td>
<td>Within 5-fold of literature values reported by Stresser et al; Drug Metab. Dispos. 32: 105-112, 2004 (3.0 µM) or as determined by Obach and Walsky, Drug Metab. Dispos. 32: 647-660, 2004 (2.3 µM).</td>
<td><strong>Pass</strong> (2.0 µM, 2.3 µM)</td>
</tr>
<tr>
<td>$IC_{50}$</td>
<td>Ketoconazole: &lt; 1 µM; Duplicate determinations within 5-fold</td>
<td><strong>Pass</strong> (0.013 µM, 0.019 µM)</td>
</tr>
<tr>
<td>$K_i$</td>
<td>Within 10-fold of the $IC_{50}$ value and less than twice the $IC_{50}$ value; Duplicate determinations within 5-fold</td>
<td><strong>Pass</strong> (0.0086 µM, 0.0092 µM)</td>
</tr>
<tr>
<td>Parameter</td>
<td>Criteria</td>
<td>Results</td>
</tr>
<tr>
<td>---------------</td>
<td>----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>----------------------------------------------</td>
</tr>
<tr>
<td>IC$_{50}$ shift</td>
<td>The shift in IC$<em>{50}$ for azamulin, verapamil, and diltiazem should be &gt; 2-fold at the 30 min preincubation time point; the shift in IC$</em>{50}$ for ketoconazole should be &lt; 2-fold</td>
<td>Pass</td>
</tr>
<tr>
<td></td>
<td>Ketoconazole (0.9, 1.0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Azamulin (76, 44)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Verapamil (62, 97)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Diltiazem (&gt;33, &gt;26)</td>
<td></td>
</tr>
<tr>
<td>$K_i$</td>
<td>Within 5-fold of the literature value reported in Obach et al (2006) for verapamil (1.8 µM) and diltiazem (4.5 µM). Within 10-fold of the mean value obtained during feasibility experiments for azamulin (0.17 µM). Duplicate determinations within 5-fold of each other.</td>
<td>Pass</td>
</tr>
<tr>
<td></td>
<td>Azamulin (0.10 µM, 0.23 µM)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Verapamil (1.6 µM, 2.4 µM)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Diltiazem (13 µM, 4.4 µM)</td>
<td></td>
</tr>
<tr>
<td>$k_{inact}$</td>
<td>Within 5-fold of the literature value reported in Obach et al (2006) for verapamil (0.043 min$^{-1}$) and diltiazem (0.012 min$^{-1}$). Within 10-fold of the mean value obtained during feasibility experiments for azamulin (0.50 min$^{-1}$). Duplicate determinations within 5-fold of each other.</td>
<td>Pass</td>
</tr>
<tr>
<td></td>
<td>Azamulin (0.54 min$^{-1}$, 0.82 min$^{-1}$)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Verapamil (0.023 min$^{-1}$, 0.022 min$^{-1}$)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Diltiazem (0.0024 min$^{-1}$, 0.0076 min$^{-1}$)</td>
<td></td>
</tr>
</tbody>
</table>
Outcome and observations

• All validations met their acceptance criteria
• Not always “smooth sailing”
• Amended protocols or protocol deviations
• Amendments and deviations should be avoided
  – Unexpected time and effort to discuss, resolve & document
  – Frustration to project teams
  – Represent obstacles to successful validation
Example deviations

1. Organic solvent used by analytical chemist was slightly different than that used by the assay biochemist (2.5% vs 0.3%). Eventually required amended protocol to demonstrate lack of an effect.

2. Protocol created unattainable mandate
   - “IC_{50} value will be reported” (was greater than highest concentration tested – no effect on conclusion)

3. Unanticipated results during validation experiment caused a change in substrate concentration

4. Analyst forgot a (non-critical) step

5. Instrument malfunction meant exceeding the stability time point specified in the protocol.
Tips to avoid deviations

• Ensure analysts understand the task and are aware of what could go wrong. Don’t assume.

• Incorporate specificity into the protocol to provide guidance, but adequate flexibility to avoid painting yourself into a corner

• Don’t skimp on time needed for verification
Closing thoughts

• It is unlikely you will have all the information and forethought needed to avoid deviations, amendments and other “issues”
  
  – Resolution can range from simple to down right “painful”. Input from key stakeholders, “voices of reason” and experienced individuals result in best outcomes.

• With cell-based assays, variability is larger than for biochemical endpoints described here. Long term drift is more of a concern.

• An ounce of planning is worth a pound of reactive effort
Thank you for your attention

• Acknowledgments:
  – Bill Doherty
  – Elke Perloff
  – Charles Crespi
  – Shangara Dehal