Phase Appropriate Validation Design for Potency Assays – from IND Enabling Studies through Method Validation for Licensure

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Outline

• General overview of potency assay validation strategy
• Determination of validation acceptance criteria and sample size
• Case studies
Analytical Method Lifecycle

Method validation study

Confirm suitable method performance

Method design, development and understanding

- Method selection & optimization
- Define method performance target
- Develop method with desirable performance

Method performance verification

Routine monitoring of the method performance
Potency Method Validation Requirements

Required validation parameters for potency methods

- Accuracy, Precision, Linearity, Range, Specificity

Other relevant assessments

- Method robustness
  - Can be evaluated during assay development
- Stability indicating properties
  - Required for licensure studies (FDA 2015 guidance for industry: *Analytical Procedures and Methods Validation for Drugs and Biologics*)
  - Data for meaningful assessment may not be available for IND enabling studies
- System suitability criteria
  - Integral part of potency methods
  - Phase-appropriate approach
Validation Objective and Approach

Validation objective

Demonstrate suitable method performance for intended use

Elements for a meaningful validation approach

- Simple and efficient study design
- Meaningful acceptance criteria
- Appropriate data analysis
- Standardized approach
Potency Method Validation Study Design

Considerations

Efficient assessments of required validation parameters
- Combined study design can often be used to assess accuracy, precision, linearity and range simultaneously

Sufficient sample size
- Independent of mechanism of action

Replication strategy
- Validation experiment can be based on single replicate
- Reportable results based on multiple-replicates may be used during routine testing ($\sigma_{\text{reportable}} = \sigma_{\text{single}}/\sqrt{n}$, where $n$ is the number of replicates)

Co-validation
- Pros: Very common; data from multiple laboratories
- Cons: Logistics; Parameter estimation may be challenging
- Technology transfer in combination with validation in representative QC lab can have advantages
Example: Validation Study Design and Data

Cell-based bioassay, mid stage (Phase II)

<table>
<thead>
<tr>
<th>Nominal Potency</th>
<th>50%</th>
<th>75%</th>
<th>100%</th>
<th>125%</th>
<th>150%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Run</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>104</td>
<td>103</td>
<td>98</td>
<td>109</td>
<td>103</td>
</tr>
<tr>
<td>2</td>
<td>108</td>
<td>101</td>
<td>108</td>
<td>105</td>
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<td>3</td>
<td>100</td>
<td>93</td>
<td>98</td>
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<td>4</td>
<td>98</td>
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<td>5</td>
<td>94</td>
<td>96</td>
<td>101</td>
<td>102</td>
<td>95</td>
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<tr>
<td>6</td>
<td>104</td>
<td>96</td>
<td>102</td>
<td>95</td>
<td>105</td>
</tr>
</tbody>
</table>

Mean % Recovery  101  98  101  102  99

Overall SD (single replicates)  4.5

• Validation study is based on single replicates
• One dataset (total sample size N=30) covers accuracy, precision, linearity and range
• Replicates at each nominal potency level are from independent assay runs

Replication Strategy
The reportable value for routine sample testing is defined as average of n=3 independent replicates, the predicted SD for reportable results is $4.5/\sqrt{3} = 2.6$

High accuracy and precision result in a powerful assay (strong capability of differentiating true changes)
Example: Validation Outputs

Summary of assay accuracy, precision, linearity and range

<table>
<thead>
<tr>
<th>Nominal Potency (%)</th>
<th>50</th>
<th>75</th>
<th>100</th>
<th>125</th>
<th>150</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Accuracy</strong> (Mean % Recovery)</td>
<td>101</td>
<td>98</td>
<td>101</td>
<td>102</td>
<td>99</td>
</tr>
<tr>
<td><strong>Intermediate Precision</strong> (Overall SD of N=30 %Recovery Results)</td>
<td></td>
<td></td>
<td></td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td>Predicted SD of Reportable Results (Mean of 3 Replicates)</td>
<td></td>
<td></td>
<td></td>
<td>2.6</td>
<td></td>
</tr>
</tbody>
</table>

**Linearity**

Linear Regression Plot

\[ y = 1.00x - 0.20 \]

\[ R^2 = 1.00 \]

Validated Range: 50-150%
Validation Acceptance Criteria Considerations

“Not too narrow”
Suitable method should be expected to pass the validation criteria

The uncertainty of calculated results associated with sample size should always be taken into consideration

“Not too wide”
Passing the validation criteria should provide sufficient confidence that the method is suitable for intended purpose

Scientifically / statistically meaningful validation acceptance criteria
Strategy for Setting Meaningful Validation Acceptance Criteria

1. **Anticipated specification limits**
2. **Define assay performance target**
3. **Develop assay with desirable performance (suitable for intended purpose).**
   - Initial proposal of replication strategy

- **Define validation design based on assay performance knowledge**
  - (“not too narrow”)

- **Define validation criteria based on expected specification**
  - (“not too wide”)

**Final validation acceptance criteria and study sample size**
Step 1: Setting Assay Performance Target

**Step 1**

**Statistical Assessment**
Define assay performance that will ensure acceptable method capability

- **Anticipated specification limits** → **Define assay performance target** → **Define validation design based on assay performance knowledge** (“not too narrow”) → **Define validation criteria based on expected specification** (“not too wide”) → **Final validation acceptance criteria and study sample size** → **Develop assay with desirable performance (suitable for intended purpose). Initial proposal of replication strategy**

Required additional inputs for method capability:
- Expected overall product mean potency
- $\sigma_{\text{process}}$, variability due to manufacturing process.

(OOS calculation uses the total variability from process and method)

$$\sigma_{\text{total}} = \sqrt{\sigma^2_{\text{process}} + \sigma^2_{\text{assay}}} = \sqrt{\sigma^2_{\text{process}} + \sigma^2_{\text{single replicate}}/n} $$
Example Contour Plot: Assay Performance Criteria (Specification: 50-150%)

- **X-axis:** Assay accuracy (Recovery: 50-150%)
- **Y-axis:** Assay precision (reportable results) (SD: 0-200)

**Contour lines:** Specified OOS rate

**Area below the contour line:** assay performance with expected OOS rate less than the specified value

Assumption for this plot:
Product mean potency=100%, variability from manufacturing process is negligible
Example: Contour Plot Details (Specification Limit: 50-150%)

X-axis: Assay accuracy (Recovery: 70-130%)
Y-axis: Assay precision (reportable results) (SD: 0-20)

Contour lines:
Specified OOS rate

Area below the contour line:
assay performance with expected OOS rate less than the specified value

Assumption for this plot:
Product mean potency=100%, variability from manufacturing process is negligible
Example Contour Plot: Assay Performance Criteria (Specification: 70-130%)

X-axis: Assay accuracy (Recovery: 70-130%)
Y-axis: Assay precision (reportable results) (SD: 0-20)

Contour lines:
Specified OOS rate

Area below the contour line:
assay performance with expected OOS rate less than the specified value

Assumption for this plot:
Product mean potency=100%, variability from manufacturing process is negligible
Example Contour Plot: Assay Performance Criteria  
(Specification: 80-120%)

X-axis: Assay accuracy  
(Recovery: 70-130%)  
Y-axis: Assay precision  
(reportable results)  
(SD: 0-20)

Contour lines:  
Specified OOS rate

Area below the contour line:  
assay performance with  
expected OOS rate less than  
the specified value

Assumption for this plot:  
Product mean potency=100%, variability from manufacturing process is negligible
### Numerical Examples – OOS Rate vs. Assay Performance

<table>
<thead>
<tr>
<th>Assay performance</th>
<th>Corresponding SD for single replicates (Reportable results based on 3-replicates)</th>
<th>Upper bound of OOS rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50-150%</td>
<td>60-140%</td>
</tr>
<tr>
<td>Accuracy (Recovery)</td>
<td>Precision (SD for reportable)</td>
<td>≤ 1.7</td>
</tr>
<tr>
<td>98-102%</td>
<td>≤ 1.7</td>
<td>≤ 1.7</td>
</tr>
<tr>
<td>98-102%</td>
<td>≤ 2.9</td>
<td>≤ 2.9</td>
</tr>
<tr>
<td>95-105%</td>
<td>≤ 1.7</td>
<td>≤ 1.7</td>
</tr>
<tr>
<td>95-105%</td>
<td>≤ 2.9</td>
<td>≤ 2.9</td>
</tr>
<tr>
<td>95-105%</td>
<td>≤ 4.6</td>
<td>≤ 4.6</td>
</tr>
<tr>
<td>93-107%</td>
<td>≤ 1.7</td>
<td>≤ 1.7</td>
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<tr>
<td>93-107%</td>
<td>≤ 2.9</td>
<td>≤ 2.9</td>
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</tr>
<tr>
<td>90-110%</td>
<td>≤ 1.7</td>
<td>≤ 1.7</td>
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<tr>
<td>90-110%</td>
<td>≤ 2.9</td>
<td>≤ 2.9</td>
</tr>
<tr>
<td>90-110%</td>
<td>≤ 4.6</td>
<td>≤ 4.6</td>
</tr>
<tr>
<td>90-110%</td>
<td>≤ 5.8</td>
<td>≤ 5.8</td>
</tr>
</tbody>
</table>
Step 1 Summary: Setting Assay Performance Target

- There is a direct correlation between acceptance criteria for specification vs. assay performance requirements.
- The required assay performance can be defined based on acceptable method capability resulting in acceptable OOS rate during routine testing (e.g., ≤ 1%).
- Desirable assay precision may be achieved using reportable value based on multiple replicates.
Step 2: Develop Assay with Desirable Performance

1. **Optimize assay parameters.**
2. **Define proper data analysis model and system suitability criteria.**
3. **Propose replication strategy.**

- Anticipated specification limits
- Define assay performance target
- Develop assay with desirable performance (suitable for intended purpose).
  - Initial proposal of replication strategy
- Define validation design based on assay performance knowledge ("not too narrow")
- Define validation criteria based on expected specification ("not too wide")
- Final validation acceptance criteria and study sample size
Step 3: Define Validation Design Based on Assay Performance Knowledge ("not too narrow")

**Step 3**

**Statistical Assessment**

Calculate probability of passing given validation acceptance criteria with expected assay performance

- Anticipated specification limits
- Define assay performance target
- Develop assay with desirable performance (suitable for intended purpose). Initial proposal of replication strategy

Define validation design based on assay performance knowledge ("not too narrow")

Define validation criteria based on expected specification ("not too wide")

Final validation acceptance criteria and study sample size
### Example: Probability of Passing Validation Criteria with Given Assay Performance and Validation Study Design

<table>
<thead>
<tr>
<th>Expected performance (single replicate)</th>
<th>Validation study design (based on single replicates)</th>
<th>Probability of passing validation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accuracy (mean recovery)</td>
<td>Precision (SD)</td>
<td>Accuracy criteria (mean recovery per level)</td>
</tr>
<tr>
<td>93%</td>
<td>13</td>
<td>90-110% ≤ 15</td>
</tr>
<tr>
<td>85-115%</td>
<td>15</td>
<td>30</td>
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<tr>
<td>85-115%</td>
<td>20</td>
<td>30</td>
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<td>85-115%</td>
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<tr>
<td>80-120%</td>
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<td>80-120%</td>
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<td>75-125%</td>
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<tr>
<td>70-130%</td>
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<td>60</td>
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<td>70-130%</td>
<td>25</td>
<td>30</td>
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</tbody>
</table>

The validation study design with acceptable passing probability (green boxes) will be considered for next step.
Example: Impact of Validation Sample Size N (Validation Criteria: Accuracy 80-120%, precision ≤ 20)

Area below the line: assay performance that will pass the validation criteria with ≥ 95% probability with specified validation sample size N

X-axis: assay accuracy (80-120% recovery)
Y-axis: assay precision SD (0-20)
Step 3 Summary: Define Validation Design Based on Assay Performance Knowledge (“not too narrow”)

- Probability of passing validation acceptance criteria can be calculated based on expected assay performance and validation sample size $N$
- Select validation study design based on acceptable probability of passing the validation with expected performance (e.g., $\geq 95\%$)
- Increasing the validation sample size $N$ may help achieving the acceptable probability of passing
Step 4: Define Validation Criteria Based on Expected Specification (“not too wide”)

Anticipated specification limits → Define assay performance target → Develop assay with desirable performance (suitable for intended purpose).

Initial proposal of replication strategy

Define validation design based on assay performance knowledge (“not too narrow”)

Define validation criteria based on expected specification (“not too wide”)

Final validation acceptance criteria and study sample size

Step 4

Statistical Assessment
Calculate “worst case” probability of failing anticipated specification

Ensure suitability for intended purpose
# Example: Probability of Not Passing Anticipated Specification at Limits

<table>
<thead>
<tr>
<th>Expected performance (single replicate)</th>
<th>Validation study design (based on single replicates)</th>
<th>Probability of passing validation</th>
<th>Anticipated specification limits</th>
<th>Worst case probability of not passing specification at limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accuracy (mean recovery)</td>
<td>Precision (SD)</td>
<td>Accuracy criteria (mean recovery per level)</td>
<td>Precision criteria (SD)</td>
<td>N</td>
</tr>
<tr>
<td>93%</td>
<td>13</td>
<td>90-110%</td>
<td>≤ 15</td>
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<td></td>
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<td>80-120%</td>
<td>≤ 15</td>
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<td>75-125%</td>
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<td>75-125%</td>
<td>≤ 20</td>
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<td></td>
<td>70-130%</td>
<td>≤ 25</td>
<td>30</td>
</tr>
</tbody>
</table>

Assumptions for this example:
1. Product mean potency=100%, variability from manufacturing process is negligible.
2. Reportable value is the mean of 3 replicates.
Step 4 Summary: Define Validation Criteria Based on Expected Specification (“not too wide”)

- Probability of not passing anticipated specification at limits will inform selection of final validation acceptance criteria
- In case desirable validation acceptance criteria (“not too narrow” and “not too wide”) can not be achieved, consider further assay development and / or re-assessment of replication strategy.
Determine meaningful validation acceptance criteria for an early-phase study

<table>
<thead>
<tr>
<th>Anticipated specification limits</th>
<th>50-150%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall product mean potency</td>
<td>100%</td>
</tr>
<tr>
<td>Potency variability component (SD) due to manufacturing process</td>
<td>$\sigma_{\text{process}} = 3$</td>
</tr>
<tr>
<td>Acceptable OOS rate during routine testing</td>
<td>$\leq 1%$</td>
</tr>
<tr>
<td>Acceptable probability of passing validation acceptance criteria</td>
<td>$\geq 95%$</td>
</tr>
</tbody>
</table>
Step 1: Setting Assay Performance Target

OOS rate within specification 50–150%

Set assay performance target based on OOS assessment using overall SD:

$$\sigma_{total} = \sqrt{\sigma_{process}^2 + \sigma_{assay}^2}$$

Note: Tighter assay performance requirement compared to the case when $\sigma_{process}$ is negligible
Step 2: Develop Assay with Desirable Performance

Actual performance of the developed assay (single replicate)

Accuracy: 108%

Precision: SD=12

Proposed replication strategy for reportable value:

Mean of 3 replicates

SD for reportable values = $12/\sqrt{3} = 7$

Overall SD (process + method): $\sqrt{3^2 + 7^2} = 7.5$

Estimated OOS rate: 0.0%
### Step 3: Define Validation Design Based on Assay Performance Knowledge (“not too too narrow”)

<table>
<thead>
<tr>
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Step 4: Define Validation Criteria Based on Expected Specification (“not too wide”)

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<th>Expected performance (single replicate)</th>
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</tr>
</tbody>
</table>
Summary of Case Study

• Define assay performance target based on OOS assessment using anticipated specification and process knowledge

• Based on assay performance knowledge, identify selections of validation criteria that will allow the assay to pass (“not too narrow”)

• Based on specification, define final validation criteria that ensure suitable performance (“not too wide”)

• The final validation criteria are “not too narrow” and “not too wide”.
Summary of Presentation

A scientific/statistically-driven approach has been defined to provide a practical solution for determining meaningful validation acceptance criteria for potency methods

• Define assay performance target based on OOS assessment
• Develop assay with desirable performance. Gain good knowledge of assay performance.
• Based on assay performance knowledge, identify validation criteria that are “not too narrow”
• Based on specification, define final validation criteria that ensure suitable performance (“not too wide”)

The concept can be applied across methodologies beyond potency assays
Acknowledgement

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Jeff Glenn
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Weiguo Cai

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