Design of Experiments for Analytical Method Development and Validation

Thomas A. Little Ph.D.
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President Thomas A. Little Consulting
12401 N Wildflower Lane
Highland, UT 84003
1-925-285-1847
drlittle@dr-tom.com

Design of experiments (DOE) is a well proven characterization approach within product and process development and a key aspect of Quality by Design. Recently more attention has been
placed on applying DOE to analytical methods. DOE for analytical methods has three major applications: 1) method development for new methods or those that need improvement, 2) method validation and 3) quantitation of the influence of analytical methods on product and process acceptance and out-of-specification rates. Method development seeks to understand where critical process parameters are in the analytical method and to minimize their influence on accuracy and precision. DOE for method validation seeks to validate the analytical method for a range of concentrations so that changes in formulation or concentration will not require additional validation as they are changes within a characterized design space. Once methods have been developed, qualified and validated the impact they have on out-of-specification rates and process capability needs to be quantified and evaluated to determine their fitness for use.

A systematic approach for using DOE for analytical method development and validation is discussed in this paper and was written in line with the International Conference of Harmonization (ICH) Q2(R1), Q8(R2) and Q9 guidelines.

A quantitative understanding of the factors that influence resolution, linearity, precision and accuracy, is integral to applying DOE to method development.

Text book approaches to DOE generally suggest a sequential approach to DOE: 1) screening studies, 2) characterization studies and 3) optimization of the method or process. This approach applied to analytical methods is often not practical as 10-20 methods are often used for drug substance and drug product evaluation and the amount of time and materials needed to follow the three step, screen, characterize and optimize would consume unreasonable amounts of resources. The sequence generally recommended by the author for method development is 1) understanding the purpose of the study, 2) perform risk assessments to screen out factors that may or may not have an influence on the analytical method (screening variables by logic and an examination of their scientific potential for influence) and 3) characterization studies to quantify and minimize their influence on precision, accuracy and linearity.

Assays and measurement systems must be viewed as a process. The measurement process is made up of methods, standards, software, materials, chemistry, reagents, analysts, sample preparation methods, environmental conditions and instrumentation/equipment. Quality risk management and statistical data analysis techniques should be used to examine the process of measurement and identify factors that may influence precision, accuracy, linearity, signal to noise, limits of detection and quantification and/or any other assay attributes to achieve optimal assay results.

### Measurement Process

<table>
<thead>
<tr>
<th>Method</th>
<th>Software</th>
<th>Material</th>
<th>People</th>
<th>Quality Measurements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Prep</td>
<td>Environment</td>
<td>Instrument</td>
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</tbody>
</table>

**Figure 1. Measurement Process Elements**

**DOE for Method Development**

Design of experiments can be applied to many aspects of method development; however, the following will provide the typical steps for designing and analyzing experiments for analytical methods.

1. Define the purpose (repeatability, intermediate precision, accuracy, LOD/LOQ linearity, resolution, etc.)
2. Define the range of concentrations the method will be used to measure and the solution matrix it will be measured in.
3. Develop/define the reference standards for bias and accuracy studies.
4. Define the steps in the method and any associated documentation.
5. Determine the responses that are aligned to the purpose of the study.
6. Complete a risk assessment of all materials, equipment, analysts and method components aligned to the purpose of the study and the key responses that will be quantified.
7. Design the experimental matrix and sampling plan.
8. Identify the error control plan and run the study.
9. Analyze the study and determine settings and processing conditions that improve method precision and minimize bias errors. Document the design space of the method and associated limits of key factors.
10. Run confirmation tests to confirm settings improve precision, linearity and bias. Evaluate the impact of the method on product acceptance rates and process capability.

1. **Identify the Purpose of the Method Experiment**
   Make sure the purpose of the analytical method experiment is clear (repeatability, intermediate precision, linearity, resolution, etc.) The structure of the study, the sampling plan, and ranges used in the study all depend on the purpose of the study. Designing a study for accuracy determination is very different from a study that is designed to explore and improve precision. Accuracy, for example does not require sample replicates to estimate the mean change in the response, precision; however, requires replicates and duplicates to evaluation variation in the sample preparation and in other aspects of the method. The purpose of the study should drive the study design.

2. **Define the Range of Concentrations to be Evaluated**
   Define the range of concentrations the used to measure and the solution matrix. Ranges of the concentration will generate the characterized design space so they should be selected carefully as it will put restrictions on how the method may be used in the future. Normally five concentrations should be evaluated per ICH Q2R1.
3. **Define all Reference Standards Used in the Study**
   Develop/define the reference standards for bias and accuracy studies. Without a well characterized reference standard bias/accuracy cannot be determined for the method. Care should be made in selecting, storing and using reference materials. Stability of the reference is a key consideration and accounting for degradation when replacing standards is critical.

4. **Identify all Steps in the Analytical Method**
   Layout the flow or sequence used in the analytical method. Define the steps in the method (SOPs, procedures or work instructions), all chemistries, reagents, plates and materials used in the method and all instruments/sensors and equipment. Identify any steps in the process, materials, analyst techniques or equipment that may influence bias or precision.

5. **Determine the Responses**
   Determine the responses that are aligned to the purpose of the study. Raw data and statistical measures such as bias, intermediate precision, signal to noise ratio and CV are all responses and should be considered as independent results from the method. Make sure the data table is set up to collect both the raw data and then the statistics can be easily generated from the raw data and there is a direct link from the statistics to the data.

6. **Perform a Risk Assessment**
   A risk assessment of the analytical method is used to identify areas/steps in the method that may influence precision, accuracy, linearity, selectivity, signal to noise etc.
Specifically the risk question is “Where do we need characterization and development for this assay?” Complete a risk assessment of all materials, equipment, analysts and method components aligned to the purpose of the study and the key responses. The outcome of the risk assessment a small set (3-8) of risk ranked factors that may influence the reportable result of the assay. There are many kinds of factors, so factor identification and how to treat the factor in the analysis is critical to designing valid experiments. There are controllable factors: continuous, discrete numeric, categorical, and mixture. There are uncontrollable factors: covariate and uncontrolled. In addition there are factors used in error control: blocking and constants.

<table>
<thead>
<tr>
<th>CQA/Parameter</th>
<th>CQA/assay name (release)</th>
<th>USL</th>
<th>Target</th>
<th>LSL</th>
<th>Assay/Test name for Characterization Only</th>
</tr>
</thead>
<tbody>
<tr>
<td>Safety</td>
<td></td>
<td></td>
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<tr>
<td>Identity</td>
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<td>Purity/impurity</td>
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<td>Stability</td>
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<tr>
<td>Yield</td>
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<table>
<thead>
<tr>
<th>Analytical Method Process Step and or Process Changes</th>
<th>Risk Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unit Operation Number</td>
<td>Unit Operation Name</td>
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<tr>
<td>-------------------------------------------------------</td>
<td>---------------</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
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<td>2</td>
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<td>3</td>
<td>4</td>
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<td>4</td>
<td>5</td>
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</tbody>
</table>

Figure 3. Analytical Method Risk Assessment Example

7. Design the Experimental Matrix and Sampling Plan
For small studies using two or three factors a full factorial type design may be appropriate. When the number of factors rises above three a D-optimal type custom DOE design should be used to more efficiently explore the design space and determine factors that impact the method. There are many good software programs today that help the user define statistically valid experiments and can be customized to meet the user’s needs.

The experimental matrix is one consideration and the sampling plan is another. Replicates and duplicates are essential to quantification of factor influence on precision. Replicates are complete repeats of the method including repeats of the sample preparation, duplicates are single sample preparations but with multiple measurements or injections using the final chemistry and instrumentation. Replicates provide total method variation and duplicates provide instrument, plate and chemistry precision independent of sample preparation errors. If the experiment is designed properly many of the requirements for method validation (figure 4, Method Validation) can be directly met from the outcomes of the method DOE.
### Method Validation Quick Reference Guide

<table>
<thead>
<tr>
<th><strong>Assay Characterization</strong></th>
<th><strong>Specificity</strong></th>
<th><strong>Linearity</strong></th>
<th><strong>Range</strong></th>
<th><strong>Accuracy</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Understanding of the factors that influence the mean and standard deviation/CV of the assay.</td>
<td>To provide an exact result which allows an accurate statement on the content or potency of the analyte in a sample.</td>
<td>The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.</td>
<td>The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.</td>
<td>The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.</td>
</tr>
</tbody>
</table>

### Typical Factors
- Excipients, Concentrations, Assay Methods (# Dilutions)
- Sample prep method, controlled impurities or sample matrix
- 3-5 concentrations are typical with 3 min. concentration
- Well characterized standards with known potency etc.

### Recommended Data and Analysis Procedure
- For the establishment of linearity, a minimum of 5 concentrations is recommended. Other approaches should be justified. ICH Topic Q 2 (R1) Part II. Examination of residuals will indicate where the linear range has been established.
- Minimum of 9 determinations over a minimum of 3 concentration levels covering the specified range (e.g. 3 concentrations and 3 replicates each of the total analytical procedure).
- ICH Topic Q 2 (R1) Part II. 10+ determinations is even better for accuracy.

### Tip
- QRM, Process Mapping and FR Matrix to identify key factors in the analytical method
- Assay or analytical method designed to detect the specific drug attribute
- Linear fit, Adj R-squared, equation (slope/intercept) and residuals plots
- Make sure concentrations exceed drug application ranges and refer to linearity study for range
- Measure mean shift from reference standard

### JMP Platform
- DOE, Full Factorial, Custom Designs
- Fit Model and or Fit Y by X
- Fit Y by X or Fit Model, Residuals
- Fit Y by X
- Fit Y by X, Distribution and Graph Builder

Figure 4. Method Validation Quick Reference Guide

#### 8. Identify the Error Control Plan

Make sure to measure and record uncontrolled factors during the study, analyst name, equipment ID, out time, hold times, ambient temperature, temperature at the beginning and end of an operation, transfer times, pH, incubation time etc. may all hold valuable information on factors that impact the method. What factors will be restricted or held constant during the study? Do we need to block for batch, lot, sample prep or instruments that may have an influence on the reportable result.

#### 9. Analyze the DOE and Determine the Settings and Design Space

Use a good multiple regression/ANCOVA software package that allows the DOE factors and any uncontrolled variables to be correctly evaluated. Analyze the study and determine settings and processing conditions that improve method precision and minimize bias errors. When using statistics from the method (CV, mean, standard deviation) rather than raw data make sure and weight the analysis by the number of replicates or duplicates in order to assure statistical tests and confidence intervals are meaningful. Determine the design space and allowable ranges for all key factors that influence the method.
10. Verify the Modal and Determine the Impact of the Method on Specifications and Capability

Run confirmation tests to confirm settings improve precision, linearity and bias. Evaluate the impact of the method on product acceptance rates and process capability.

Using an accuracy-to-precision (ATP) model it is possible to visualize the relationship of precision and accuracy on product acceptance rates. The ATP model shows how changes in precision and accuracy impact product acceptance rates and the assay error design space relative to product acceptance specification limits.
The attention paid to method development, validation and control will greatly improve the quality of drug development, patient safety and predictable, consistent outcomes.

Summary

Design of experiment is a powerful and underutilized development tool for method characterization and method validation. Analytical professionals need to be comfortable using it to characterize and optimize the analytical method. If used properly and during development DOE will provide significant improvements in precision and a reduction in bias errors. It will further, help to avoid costly and time consuming validation studies as concentration are modified in formulations and dosing schemes are changed for drug product and drug substance.

References:

ICH Q2(R1) Validation of Analytical Procedures: Text and Methodology, 2005
ICH, Q8(R2) Pharmaceutical Development, 2009
ICH Q9 Quality Risk Management, 2006
Little, T.A. Engineering Statistics and Data Analysis-BPM, 2014