QbD Approach to Assay Development and Method Validation

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Fundamental to all aspects of drug development and manufacturing are the analytical methods. Analytical methods require development, validation and controls just as any other product and process development activities. Measurement of API, key characteristics of the drug substance/product and impurities are essential for characterization and control of the drug.

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

A QbD approach to process development is often described and discussed in both guidance documents and the literature but do the same concepts and principles apply to method development? QbD is basically a systematic approach to development and includes requirements, risk assessments, characterization, design space, edge of failure and controls.

Historically insufficient attention has been paid to assay development, how it impacts the product, on-going release testing and product control. Simple coefficient of variation (CV) calculations for assay precision is a necessary measure of assay goodness during early development, but an insufficient measure and may be misleading as CV has no relationship to product acceptance and release testing limits. A QbD approach to method development will help to close those gaps.

Assays and measurement systems must be viewed as a process. The measurement process is made up of procedures, software, materials, chemistry, critical reagents, analysts, sample preparation, 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 performance.

Phase Appropriate Development and Validation

There is always a concern with method development of doing too much too soon. Phase appropriate method development is a consideration. Figure 1 provides some suggestions as to phase appropriate activities: 1) defined method, 2) qualified method, 3) validated method, and 4) method controls and technology transfer.

Method Development	Defined Method
Documented Method	x
Plates/Materials	x
Reagents, supplier ID	x
Instruments/Software	x
Representative Samples/Matrix	x
Standards/Controls	x
S/N, Concentration Range	x
Validity Criteria	x
Systems Suitability	x
Stability Indicating?	Y/N
Fitness for Use	x

Pre-Clinical Phase I *	Qualified Method
Robustness	x
Stability of the Method	x
Software and/or Scripts	x
Instrument Validation	x
Linearity	x
Bias	x
Repeatability	x

Phase I Phase II *	Validated Method
Specificity	x
Linearity	x
Range	x
Repeatability	x
Bias/Accuracy	x
ATP Model	x
LOB/LOD/LOQ	x
Intermediate Precision	x

Phase III + Tech Transfer & Control	
Tech Transfer**	x
Equivalence Testing**	x
DS/DP maturity	x
Monitoring and Controls	x

* Maturity of product and changes to product formulation need to be evaluated at each phase of qualification and validation.
** Tech Transfer and Equivalence Testing may occure post method development after the method has been qualified.

Figure 1. Phase Appropriate Method Development and Validation

Based on ICH guidance and the author's experience the following ten steps are recommended for analytical development and method validation:

1. Identify the purpose of the analytical method (characterization/release) and all critical quality attributes (CQAs).

2. Select the appropriate analytical method aligned with CQAs and development objectives. Make sure the method is fit for use.
3. Identify the process steps associated with the method
4. Determine all specification limits associated with release testing
5. Define systems suitability, assay controls (positive and negative) and validity criteria
6. Perform a risk assessment as to where assay development is needed and what may influence robustness and stability
7. Characterize the method (accuracy/bias/linearity etc.)
    a. System design (right technology, right instruments, right chemistry)
    b. Parameter design (set points)
    c. Tolerance design (allowable variation)
    d. Determine the design space and edge of failure
8. Complete method validation tests
    a. Train all analysts on the method
    b. Define the method validation requirements
    c. Make sure representative materials are used for the evaluation
    d. Conduct all method validation tests using defined protocols
    e. Achieve acceptable results for method validation of all analytical methods
    f. Determine if the analytical method is fit for use and ready to transfer
9. Define the control strategy for each method (closed loop and monitoring)
10. Determine the impact of the analytical method on process variation, validation and product acceptance rates.

These steps are covered in detail below.

1. Identify the Purpose

Make sure the purpose of the analytical method is clear. Will it be used for release testing and or for product/process characterization only? What are the target product profile parameters (ICH Q8(R2)) and CQAs the analytical method is associated with? Are there any CQAs that have no clearly defined measurement method? What impurities need to be measured and what is the risk of not measuring them. Is the assay correlated with other analytical methods? How orthogonal is each assay to other assays used to evaluate the product. How does the assay minimize or influence risk during drug development and manufacturing? Is the assay for characterization of drug substance/product or for release?

Target Product Profile				Product Design Requirements and Critical Quality Attributes						
Criteria	Minimum Product Profile	Target Product Profile	Optimal Product Profile	Attribute No.	Product Quality Attribute Name	CQA Purpose	Test or Measurement Defintion	Attribute Target	Attribute Upper Limit	Attribute Lower Limit
INDICATION				1						
				2						
				3						
				4						
				5						
ADMINISTRATION				1						
				2						
				3						
				4						
				5						
DOSING and DURATION of ADMINISTRATION				1						
				2						
				3						
				4						
				5						
POSSIBLE SIDE EFFECTS				1						
				2						
				3						
				4						
				5						

Figure 2 QTPP, CQAs and Associated Analytical Methods

2. Select the Analytical Method

There are many analytical methods. Make sure the method selected has appropriate specificity and has high validity. Valid analytical methods measure the condition of interest. Valid assays are fit for use. It is possible to have good precision with poor

measurement validity. For example it is possible to measure the quantity of a protein without knowing how active the protein is. Measures of activity and measures of quantity need to be correctly considered and balanced against other objective measures of the product.

3. Identify all Steps in the Analytical Method

Lay out the flow or sequence used in the analytical method. Using Visio or some other process mapping software lay out and visualize the sequence and flow used in performing the assay. This will be used for development, documentation, risk assessment and training. Make sure all steps are listed and detailed as to the flow and use of plates, materials and chemistry. Identify steps in the process that may influence bias or precision.

4. Determine Product Specification Limits

For those analytical methods that will be used for release testing, what are the specification limits that will be used to control the release of the product? Limits may be set from historical data, industry standards, based on statistical k sigma limits and/or tolerance intervals and or based on a transfer function. Limits need to reflect the risk to the patient, CQA assurance and control the flow of materials in the production of the drug substance and drug product.

5. Define Systems Suitability, Controls and Validity Criteria

Positive and negative controls need to be determined for the method, systems suitability testing evaluates the equipment, analytical operations and samples to be analyzed meet specific minimum criteria and assures the method is working correctly prior to evaluation of test samples. What materials will be used for the control or reference standards? Validity criteria are also added to make sure the quantitation or identification is valid and can be depended on to be a correct assessment of the product.

6. Perform a Risk Assessment

The analytical method risk assessment is used to identify areas/steps in the analytical method that may influence robustness, precision, accuracy, linearity, specificity, signal to noise, stability etc. Specifically the risk question is “Where do we need characterization and development for this assay relative to the assay risks?”

FMEA and or other risk assessment methods may be used when performing a risk assessment. In addition to the traditional FMEA approach of failure mode, severity, probability and detectability, we need to add Influence on CQA and uncertainty to the risk ranking. Specific questions of what may influence precision and or what may influence bias or accuracy need to be examined. Each step in the analytical method should be looked at from this point of view.

ICH Parameter	CQA/assay name (release)	USL	Target	LSL	Assay/Test name for Characterization Only
Safety					
Identify					
Purity/impurity					
Potency					
Stability					
Yield					

Analytical Method Process Step and or Process Changes					Risk Analysis					
Unit Operation Number	Unit Operation Name	Baseline (optional)	Change (optional)	Difference (optional)	Potential Risk, Influence or Failure Mode	Severity and/or Influence (1,3,5,7,10)	Probability and/or Uncertainty (1,3,5,7,10)	Detectability (optional) (1,3,5,7,10)	Risk Score (RPN) Severity x Probability Only	Risk Score (RPN) Severity x Prob x Detect
	Description			Unit Operation Delta (Δ)						
1						5	3	5	0	75
2									0	0
2									0	0
2									0	0
3									0	0
3									0	0
3									0	0
4									0	0

Figure 3. Analytical Method Risk Assessment Example

7. Characterize the Method

Based on the risk assessment define the development/characterization plan for the assay. Determination of sample size and sampling method are key considerations. Assay development can be broken into three steps 1) system design, 2) parameter design and 3) tolerance design. System design is making sure we have the right chemistry, right materials, right technology, and right equipment. Parameter design is usually done by running DOEs and making sure we have the right parameters selected at their optimal design set point. Characterization of the design space for precision and accuracy is a key assay development outcome. Finally the allowable variation for key steps in the assay must be defined to assure a consistent outcome. Partition of variation (POV) (Little, T.A.) analysis is recommended to further breakdown precision variation into the factors that influence it. Plate variation for example must be considered when developing analytical methods. Failure to understand plate variation and other sources of assay error will directly mix into the total variation and will be linearly added to product variation and will increase limits of quantitation and detection effectively reducing the assay range and will add to out of specification rates for product acceptance testing.

Determination of the design space and edge of failure are good extensions of proper assay characterization.

8. Complete Method Validation and Transfer

Define the method validation requirements. There are many measures of measurement performance (for example amount of API, activity of API and impurities) that may be used in method validation (see figure 4). Make sure there is a clear identification of the requirements for each method when organizing the validation plan. Figures 4, 5 and 6 are adapted from Q2(R1) and identify the requirements to complete a method validation.

Representative DS and DP materials should be used during method validation. Representative materials and standards will assure the limits of detection and quantitation have been correctly calculated and validated and will perform well when measuring and testing actual product. Maturity of the DS/DP is also a consideration.

Conduct all method validation tests with the correct sample size and sampling method as defined in the method SOP. Achieve acceptable results for method validation of all analytical methods. Make sure acceptance criteria have been defined for each validation

method variable, modify/improve aspects of the assay so it will pass the validation testing criteria. Finally, it is necessary to determine whether the analytical method is fit for use and ready to transfer to other internal organizations or to external CRO/CMOs. This is determined by meeting all acceptance criteria for precision, bias, linearity etc. Equivalence tests are typically used for method transfer.

Method Validation List	
Specificity	
Linearity	
Range	
Accuracy	
Precision	Repeatability (Intra Assay)
	Intermediate Precision (Inter)
	Reproducibility (Inter Lab)
Detection and Quantitation Limits	Visual
	S/N
	LOD, LOQ (RMSE*K)/Slope
Robustness	
System Suitability	
Method Transfer Equivalence	
ATP OOS Impact	

Figure 4. Method Validation List

Method Validation Quick Reference Guide

Standard: VALIDATION OF ANALYTICAL PROCEDURES Q2 R1, Nov 2005

	Assay Characterization	Specificity	Linearity	Range	Accuracy
Definition	Understanding of the factors that influence the mean and standard deviation/CV of the assay.	To provide an exact result which allows an accurate statement on the content or potency of the analyte in a sample.	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.	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.	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.
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, Ad Rsquare, 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 5. Method Validation Quick Reference Guide

Type of analytical procedure characteristics	IDENTIFICATION	TESTING FOR IMPURITIES quantitat.	 limit	ASSAY - dissolution (measurement only) - content/potency
Accuracy	-	+	-	+
Precision				
Repeatability	-	+	-	+
Interm.Precision	-	+ (1)	-	+ (1)
Specificity (2)	+	+	+	+
Detection Limit	-	- (3)	+	-
Quantitation Limit	-	+	-	-
Linearity	-	+	-	+
Range	-	+	-	+

- signifies that this characteristic is not normally evaluated

+ signifies that this characteristic is normally evaluated

(1) in cases where reproducibility (see glossary) has been performed, intermediate precision is not needed

(2) lack of specificity of one analytical procedure could be compensated by other supporting analytical procedure(s)

(3) may be needed in some cases

Figure 6. What is Required for Method Validation and When to Use It (Q2(R1))

9. Define the Control Strategy

Once the assay has been developed and validated a clear control strategy needs to be put in place. How do you know the standards are stable? What will be used for tracking and trending the assay so the true assay/plate variation is known over time? What will be used to adjust/correct the assay once drift is detected? How will you transition from one set of reference materials to another?

10. Determine the Impact of the Analytical Method

Total variation is expressed in the following equation:

Stdev Total = SQRT (Product Variance + Assay Variance)

As the assay error rises the total standard deviation also rises. Using the 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. CV calculation is a good measure of assay error; however, it is not scaled to the acceptance limits, it is scaled to the mean. Rescaling the variation to the release limits helps to clarify if the variation in the assay is fit for use. The number 5.15 is used in the equation to represent 99% of the assay error. Generally a percent of tolerance of less than 20% is considered an acceptable result; more than 20% will result in a high level of out of specification release failures and should be considered for further development.

% Tolerance Measurement Error= (Stdev Measurement Error*5.15)/(USL-LSL)
where USL=Upper specification limit and LSL = lower specification limit

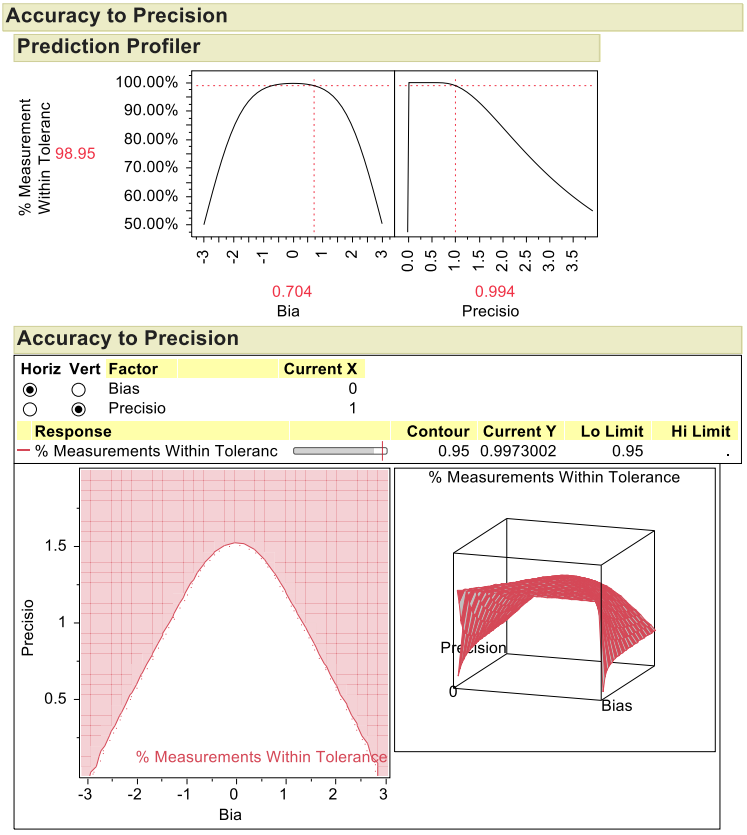

Figure 7. Accuracy to Precision Modeling

The attention paid to method development, validation and control will greatly improve the quality of drug development, patient safety and predictable, consistent outcomes.

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.

PAT — A Framework for Innovative Pharmaceutical Development, Manufacturing, and Quality Assurance, 2004