

# *Validation of Analytical Methods for Biological Products – A CBER Reviewer's Experience*

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## *Presentation Outline*

- ◆ Regulations, guidelines and guidances
- ◆ Method validation characteristics
- ◆ Application of statistical methods
- ◆ Validation of a previously validated method for a different product
- ◆ Robustness

## Regulations

- ◆ 21 CFR 211.194(a)(2):
  - The statement shall indicate the location of data that establish that the methods used in the testing of the sample meet proper standards of accuracy and reliability as applied to the product tested.
  - “(If the method employed is in the current revision of the United States Pharmacopeia, National Formulary, AOAC INTERNATIONAL, Book of Methods, or in other recognized standard references, or is detailed in an approved new drug application and the referenced method is not modified, a statement indicating the method and reference will suffice).”
  - The suitability of all testing methods used shall be verified under actual conditions of use.

## *Guidelines and Guidances*

- ◆ FDA Guidance : Analytical Procedures and Methods Validation for Drugs and Biologics – issued July 2015
- ◆ ICH Q2(R1) Guideline : Validation of Analytical Procedures: Text and Methodology – November 2005
- ◆ FDA Guidance: Bioanalytical Method Validation
- ◆ USP General Chapter <1225> : Validation of Compendial Procedures
- ◆ USP General Chapter <1226> : Verification of Compendial Procedures
- ◆ USP General Chapter <1224> : Transfer of Analytical Procedures



## Validation Characteristics

Validation Characteristics	Type of Analytical Procedures			
	Identification	Impurities: Quantitative	Impurities: Limit	Assay
Specificity	+	+	+	+
Accuracy	-	+	-	+
Linearity	-	+	-	+
Repeatability	-	+	-	+
Int. Precision	-	+	-	+
Detection Limit	-	-	+	-
Quantitation Limit	-	+	-	-
Range	-	+	-	+
Ruggedness	+	+	+	+

## ***Method Validation - General***

- ◆ Experiments for assessing validation characteristics should follow the procedure to be validated (including, **number of replicates, calculations**), unless justified otherwise (e.g., spiking, orthogonal method)
- ◆ Validation experiments must be conducted using the article(s) (DS or DP) for which the method validation is conducted.
  - Matrix effect
    - A standard (e.g., WHO, CBER, etc.) may have the same activity, which may be modified by excipients, which may affect validation results
- ◆ Sometimes standards may be used in lieu of DS/DP
  - If matrix is not significantly different from that of DS/DP
- ◆ Addition of standard (spiking) should not alter matrix significantly

## *Method Validation - General*

- ◆ Validation characteristics should be evaluated in terms of reportable results
  - Exception – linearity : Response vs reportable result (conc., activity, peak percent)
- ◆ Representative Lots of Appropriate Materials
  - Intermediates, Bulk, Final Container
  - Multiple lots recommended for Biological Product Assays
  - Exceptions possible in certain cases
- ◆ Concentrations/potency covers at least the specification range - Data above and below the specification range are not relevant
- ◆ Experimental Design to Evaluate Various Validation Parameters Simultaneously

## *Specificity*

- ◆ ICH Q2(R1) – Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present [p. 8]
- ◆ Implications
  - Identification: ensure the identity of an analyte.
  - Purity Tests: ensure that all the analytical procedures performed allow an accurate statement of the content of impurities of an analyte, i.e. related substances test, heavy metals, residual solvents content, etc.
  - Assay (content or potency): provide an exact result which allows an accurate statement on the content or potency of the analyte in a sample.



## *Specificity*

- ◆ Accuracy, linearity and precision are part of Specificity for Assay (content) and Quantitative tests for purity/impurity.
  - This is an often misunderstood point
- ◆ Specificity should show:
  - Lack of matrix interference, including suppression or enhancement of response by matrix components
    - If there is matrix interference, the procedure must be able to subtract or correct for the interference (e.g. correction factor)
  - Resolution: Ability to resolve analyte from other components
    - Ensure that there is no peak underneath an apparent single peak (peak purity analysis)



## *Specificity*

- ◆ Unique response from the analyte under test conditions
  - Positive response from a positive control, and none or negligibly small response from a negative control
  - Chromatographic method – retention time, peak resolution, elution profile, comparison with an authentic standard
  - Spectroscopic method – unique wavelength, unique pattern
  - Unique reagents (e.g., Karl Fischer method) supported by available knowledge
  - Recovery (expected vs actual) unaffected by the matrix
    - Issue: How to know what is expected? Same method is circular. An orthogonal method may be the answer
    - Difficult to get an orthogonal method for biological assays

## *Specificity*

- ◆ Representative matrix vs sample
  - Matrix without analyte may not be available for biological products
  - Mock/laboratory formulation of representative matrix may be acceptable in some cases
- ◆ Residual from sample linearity study
  - Random distribution around 0 response at 0 conc./potency
  - This is not possible for many biological products – e.g., the standard curve is a log-log or a semi-log plot
- ◆ Stability indicating assays
  - Analyze in the presence of all actual and potential degradants
  - Samples subjected to stress conditions (heat, humidity, light)

## *Accuracy*

- ◆ ICH Q2(R1) – 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 [p. 8]
  - How do we know what the true value is?
  - How do we know the reference value in a drug substance or a drug product?
- ◆ Potential Approaches
  - Orthogonal method – “comparison of the results of the proposed analytical procedure with those of a second **well-characterized procedure**” [ICHQ2(R1), p. 13]

# Accuracy

## ◆ Potential Approaches

- Spike recovery – addition of known amount of an analyte to the actual drug substance/product by spiking with a reference standard (assigned value)
- Spike recovery is also possible by spiking to a representative lab scale formulation – difficult for biological samples due to their complexity
- Recovery should be calculated based on **known quantity spiked**, not total (amount present in the unspiked sample plus spiked amount)
  - Problem - How do you determine amount present in the unspiked sample
- For methods that have digestion or extraction step, spiking should be done before such steps

## *Accuracy*

- ◆ Here is a confusing statement in ICH Q2(R1)
  - “Accuracy may be **inferred** once precision, linearity and specificity have been established” – [ICHQ2(R1), p. 13]
  - This line is often misinterpreted – we receive many BLAs or Supplements in which accuracy is not assessed but “inferred” citing ICHQ2(R1)
  - **Determination of accuracy is necessary to “establish” specificity**
  - This line simply says that one does not have to do accuracy twice – once as part of specificity and again as stand-alone

## Case Study # 1

- ◆ A polysaccharide, which is an active component of a vaccine, is quantitated by hydrolyzing the sample and quantitating one of its constituent monosaccharides by HPAEC-PAD
- ◆ An OOS investigation shows that the bacteria have another polysaccharide, which is not an active component but has the similar monosaccharide composition – all but one constituent monosaccharide is the same
- ◆ A small amount of the second polysaccharide co-purifies with the first polysaccharide during manufacturing
- ◆ Validation was done using an immunopurified material, which did not contain the second polysaccharide – not a representative DS
- ◆ Specificity study did not look into the complete matrix (representative formulation minus the active polysaccharide) because the manufacturer believed that it did not contain any other sugar component

## *Linearity*

- ◆ **ICH Q2(R1)** – 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 [p. 9]

In some cases, ... the test data may need to be subjected to a mathematical transformation prior to the regression analysis [p. 12]

**Examples:** (1) most of the biological assays, including immunoassays,

(2) Chromatographic assays with electrochemical detection, when the analyte is a weak electrolyte and ELSD



# *Linearity*

## **A few questions:**

- ◆ Why do we need to evaluate linearity for method validation?
- ◆ What to plot against what? Why?
- ◆ What is the role of standard curve in linearity study
- ◆ Is it necessary to study linearity if no standard curve is used?  
For example, Molecular Size Distribution assay using SEC
- ◆ Typically parallelism should be evaluated as part of linearity for biological assays.
  - Why?
  - Is it necessary to evaluate parallelism for all assays?

## *Linearity*

- ◆ Linearity shows that the response is related to attribute (e.g., concentration or reportable result) by a mathematical function. That is, if you know the response, you can measure the attribute
  - Simplest and most common mathematical function is straight line ( $y = mx + c$ )
  - In general :  $R = Af(c) + B$  [R = response]
  - Over the assay range
  - Weighting or use of complex regression should be adequately justified
- ◆ For standard curve-based assays, it is a test to show that the standard curve is adequate
  - Parallelism
- ◆ For assays that do not involve standard curve, linearity shows that the results are independent of the analyte conc.

## *Linearity*

- ◆ Parallelism between standard curve and sample dilution curve
  - Does the analyte in the standard behave in the same way as the analyte in the drug substance/product.
  - Is the response modified by the matrix?
- ◆ Do we need to demonstrate parallelism for chromatographic or other separation-based methods?
  - Depends – **no**, if the analyte is the same chemical in both standard and samples
    - Actual product matrix is replaced by the eluent, which is same for the analyte in standard and sample – eluent is the matrix
  - Parallelism should be demonstrated when that is not the case – retention times of analyte in standard and samples are different or analyte in standard is different from that in sample (surrogate standard)

## *Linearity*

- ◆ What if a drug substance/product lot is qualified as the standard?
  - Matrices of the standard and samples are the same
- ◆ How is the standard qualified? – generally against an authoritative standard (e.g., FDA, USP, WHO/NIBSC standard)
  - Matrices are likely not the same
  - If not, parallelism should be demonstrated at the time of qualification of the standard, which shows that the analyte in the authentic standard behave in the same way as that in the to be qualified standard (secondary standard)
    - Demonstrate that the qualified values are adequate and linked to the authentic standards

# Linearity

- ◆ Plot of expected (theoretical) conc. vs measured conc.
  - Seen frequently in submissions to demonstrate linearity
  - Such plots show correlation between expected and measured concentrations
  - Such correlation is a measure of **accuracy** – correlation between measured and true/reference/expected values
  - A slope of  $\sim 1.0$  shows good correlation/accuracy
  - How is the expected concentration determined – by the same method (**circular**) or a different method (**acceptable**)?
    - Hence the concept of orthogonal method for accuracy
- ◆ “Linearity should be evaluated ... a plot of signals as a function of analyte concentration or content.” – [ICH Q2(R1), p. 12]
  - At the minimum 5 conc. levels

## Range

- ◆ It is established by confirming that the analytical procedure provides an acceptable degree of linearity, accuracy and precision – [ICHQ2(R1) p. 12]
- ◆ Expressed as reportable results, same units as the specification
  - Exception – e.g., when the specification is based on results of two tests
- ◆ Should be evaluated from sample data, **not from standard data**
- ◆ Minimally,
  - For assays: 80 – 120% of the target test concentration
  - For the determination of an impurity: from the reporting level of an impurity to 120% of the specification
    - Linearity, accuracy and precision should be determined over this range
- ◆ Why 80 – 120%?
  - To allow for manufacturing, formulation and analytical variations

## Precision

- ◆ Repeatability
  - A minimum of 6 determinations at 100% of the test concentration, or
  - a minimum of 9 determinations covering the specified range for the procedure (e.g., 3 concentrations/3 replicates each) – my preference to cover the assay range.
- ◆ Intermediate Precision
  - Impact of controllable variables – days, analysts, equipment, etc.
    - **Difference from robustness** – impact of normal variations – small variations of temp., flow rate, reagent concentration, etc.
  - Use DOE to minimize number of experiments to evaluate the effects of each variable
- ◆ We often see data above the assay/specification range during validation of assays for impurities – not relevant

## *Detection and Quantitation Limits*

- ◆ The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated
- ◆ The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.
  - Range (and linearity) should include QL as its lower limit
  - QL should be supported by precision and accuracy data
  - ICH Q2(R1) provides methods for estimation of DL and QL (pp. 15-16) but **estimated values must be verified experimentally**
    - For DL: actual detection of response/signal at DL
    - For QL: demonstration of accuracy and precision at QL
  - DL and QL should be in the same unit in which the test result is expressed (not in terms of the unit of the response)





## ***Case Study # 2 : Quantitation Limit for MSD Analysis by SEC***

- ◆ Molecular size distribution analysis (MSD) by SEC
- ◆ A critical test for the determination aggregates and fragments of proteins as impurities of proteins
  - Required quality control tests for IgG, blood coagulation factors and their antidotes, monoclonal antibodies
- ◆ Measures percents of aggregates and fragments from peak area percent
- ◆ It is not possible to estimate QL using the methods described in ICH Q2(R1) [part 7.3] :  $QL = 10\sigma/S$ 
  - σ = the standard deviation of the response
  - S = the slope of the calibration curve
  - There is no standard curve
  - Peak percent does not change with concentration
  - Relevance of peak percent vs conc. (a flat line) plot in this assay?

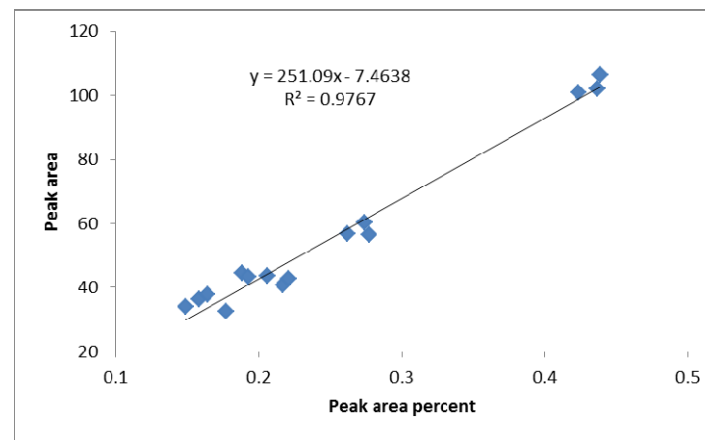
## Case Study # 2 : Quantitation Limit for MSD Analysis by SEC

- ◆ Solution?
- ◆ A plot to obtain meaningful values of  $\sigma$  and  $S$
- ◆ For most of the assays, calibration curve is constructed by plotting concentration vs response (signal)
- ◆ For most of the assays, the reportable result is concentration
- ◆ Thus, a plot of reportable result vs response – for MSD assay peak area percent (reportable result) vs peak area (response)

For IgG aggregates:

Mix two solutions that differ significantly in aggregate percent

$$\text{LOQ} = 10\sigma/S = 0.12\%$$



## *Limit Test*

- ◆ Not appropriate simply because of a “less than” specification
- ◆ Should be justified – a limit test is often used to establish clearance of process-related impurities
- ◆ Depends upon
  - How much is present in DS/DP
  - Capability of your method (< method LOQ)
  - Nature of the analyte – toxicity; is the method adequate?
  - Reporting requirement (ICH Q3)
- ◆ Quantitative determination of impurities preferred when technologically feasible

## ***Robustness***

- ◆ Not part of method validation; should be evaluated during Method Development – ICHQ2(R1)
  - Impact of normal, expected, uncontrollable variations (method optimization)
- ◆ Capacity of a method to remain unaffected by **small, but deliberate variations** in method parameters and provides an indication of its reliability during normal usage [ICHQ2(R1)]
- ◆ **OFAT**: One-factor-at-a-time
  - While keeping others at nominal level
- ◆ **Factorial** (full or fractional) – DOE approach
  - Multifactor design
  - Allows evaluation of **interaction effects** of multiple changes
  - Significant reduction in number of experiments

## *Statistical Considerations*

- ◆ Widely used in method validation to draw conclusions
- ◆ Statistics can give correct conclusions only if,
  - There is adequate statistical power, which requires large number of data points
  - Correct null hypothesis is chosen
- ◆ A null hypothesis may not be rejected for many reasons, including lack of adequate statistical power; that does not mean that the null hypothesis is acceptable
  - One cannot conclude that two data sets are equivalent just because a null hypothesis of equivalence cannot be rejected.
- ◆ It is only when a null hypothesis can be rejected conclusively that the reverse of null hypothesis is acceptable
  - An equivalence conclusion can be accepted only if a non-equivalency null hypothesis can be rejected conclusively

## *Frequent Comments on Method Validation*

- ◆ Method Validation not performed citing that the method is compendial (USP-NF or EP). However,
  - There is no USP-NF monograph for the article (DS or DP)
  - No method in USP-NF or USP-NF procedure is substantially altered
- ◆ What is a compendial method?
  - A method described in a monograph is compendial for that article
  - A method described in a USP-NF General Chapter becomes compendial when it is referenced in a USP-NF monograph and only for the monograph article in which it is referenced (USP General Notices and Requirements: General Chapters)
- ◆ Most of the biological products have no monograph in USP-NF. Thus, methods employed for quality control of biological products are not compendial even if they are described in USP-NF General Chapters
- ◆ EP, JP, etc. are not compendia in US



## *Frequent Comments on Method Validation*

- ◆ Improper selection of test category
- ◆ Not all validation characteristics addressed (for the test category)
- ◆ Impurity that should be quantified and monitored categorized as a limit test
- ◆ Failure to include all data – only final data provided in a table; no description/explanation how the data were obtained
- ◆ Samples used in method validation were not clearly described
- ◆ Conclusions regarding validation characteristics are made based on a related study, which is referenced in the validation report but is not included in the submission
- ◆ Validation conducted at concentration levels above the specification limit
- ◆ Linearity evaluated with standard only; not with the product

## *Frequent Comments on Method Validation*

- ◆ Linearity plot actual vs expected (**accuracy**)
- ◆ Parallelism between standard and sample dilution curves not provided for non-chromatographic methods
- ◆ Method LOQ is above the proposed specification limit
  - Should be a limit test and validated accordingly.
- ◆ LOQ/LOD estimated, not experimentally verified
- ◆ LOQ not evaluated for a quantitative impurity test
- ◆ Compendial methods are not verified citing that they are verified for another article (which was approved before)
  - Methods should be verified under actual condition of use
- ◆ Results from different column lots, reagent lots, **analysts**, etc., not included for intermediate precision



## Case Study # 3 : Accuracy

- ◆ In a BLA submission, accuracy was inferred because specificity, linearity and precision data met acceptance criteria
- ◆ No accuracy study performed

R<sup>2</sup>: Acceptance criteria  $\geq 0.95$ ; Result = 0.975

Repeatability and intermediate precision at the target test conc.

Acceptance criteria  $\leq 10\%$ ; RSD = 4.2%, 7.6%

Specificity: Acceptance criteria = No interference by matrix

Result: A<sub>570</sub> of matrix without analyte = 0.005

A<sub>570</sub> of 10  $\mu\text{g/mL}$  (QL) in matrix = 0.23

Linearity based on standard only – linear regression analysis of A<sub>570</sub> vs analyte concentration; Intercept = 0.003

**Q: What is the accuracy of this method?**

## *Frequent Comments on Method Validation*

- ◆ Critical assay parameters not evaluated during robustness evaluation – only solution stability data provided
- ◆ Evaluation of Accuracy as recovery percentage of total amount of analyte after addition, not the recovery of the added quantity
  - The concentration of unspiked sample determined using the same assay method for which accuracy is being evaluated? – Circular
- ◆ Accuracy evaluated at the target concentration only – not across intended range of assay
- ◆ Assay range established based on linearity only; accuracy was demonstrated for a narrower range
- ◆ Assay range reported in terms of response/signal, not in terms of reportable results
- ◆ Repeatability and Accuracy were studied from response (e.g., peak area), not from reportable results



***Thank you***

*Questions?*