

# Common mistakes in demonstrating analytical method suitability

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**Regulatory Education for Industry (REdI) Annual Conference**

**June 9, 2022**

# Learning Objectives



- Describe FDA Regulations related to product testing methods & suitability
- Describe major Guidelines & Guidance documents related to development and validation of analytical methods (FDA, ICH, USP)
- Identify types of analytical methods & evaluation of validation characteristics (performance parameters)
- Provide examples of some common issues (mistakes) in method validation

# FDA Regulations



**Current Good Manufacturing Practice (cGMP) regulations [21 CFR 211] require test methods must meet proper standards of accuracy and reliability:**

## **21 CFR 211.165(e):**

- “The accuracy, sensitivity, specificity, and reproducibility of test methods employed by the firm shall be established and documented.”

## **21 CFR 211.194(a)(2):**

- “The suitability of all testing methods used shall be verified under actual conditions of use.”

# ICH/USP/FDA Validation Expectations



Validation Characteristics/parameters	Type of Analytical Procedure/method			
	<i>Identification (ID)</i>	<i>Impurities: Quantitative</i>	<i>Impurities: Limit</i>	<i>Assay</i>
Specificity	+	+	+	+
Accuracy	-	+	-	+
Linearity	-	+	-	+
Repeatability	-	+	-	+
Int. Precision	-	+	-	+
Detection Limit (DL)	-	-	+	-
Quantitation Limit (QL)	-	+	-	-
Range	-	+	-	+
<i>Robustness*</i>	+	+	+	+

# Compendial & non-compendial method validations



- Compendial: Standard methods in Pharmacopeias (USP, JP, Ph. Eur.)
  - Only verification required; Follow USP <1226>
  - Verify suitability under actual conditions of use
- Noncompendial: methods not in Pharmacopeia
  - Follow ICH Q2(R1) for validation
  - Assess validation characteristics as appropriate
- Stability indicating methods
  - Specificity
    - Analyze with all actual & potential degradants (impurities)
    - Use stressed samples (agitation, heat, light, pH, etc.)

## Common mistake: There isn't a need for data to support compendial methods



- Compendial methods that are 'read' alone, e.g., pH or osmolality: Verify repeatability (must prepare SOP)
- Compendial methods that require culture or further analysis, e.g., sterility or endotoxin
  - USP method should be followed; if not followed, the method is considered an alternative method and should be fully validated, with data provided to demonstrate sensitivity equivalent or greater than the compendial method
  - Demonstrate the matrix does not interfere with the measurement
    - Interference demonstrated from the recovery of a positive control
    - If low recovery, sample needs additional sample dilution or wash steps



- An approved validation protocol should be followed
  - Common mistake: Protocol not followed & acceptance criteria are not defined
- The procedure (SOP) to be validated must be followed (including, number of replicates, calculations, etc.)
  - Common mistake: Method not adequately described in the regulatory filing
- Actual product not used in validation (*common mistake*)
  - Validation studies should be conducted with representative material used during testing (ex. drug substance (DS) or drug product (DP))
- Sample concentration used in validation should cover the specification range.
- Spiking of standard (into DS/DP) for accuracy study, should not alter sample characteristics

# Challenge/Poll Question #1

“The accuracy, sensitivity, specificity, and reproducibility of test methods employed by the firm shall be established and documented.”

The above statement is taken directly from:

- a) ICHQ2(R1)
- b) 21 CFR 211.165
- c) USP <1225>
- d) Albert Einstein's lab notebook

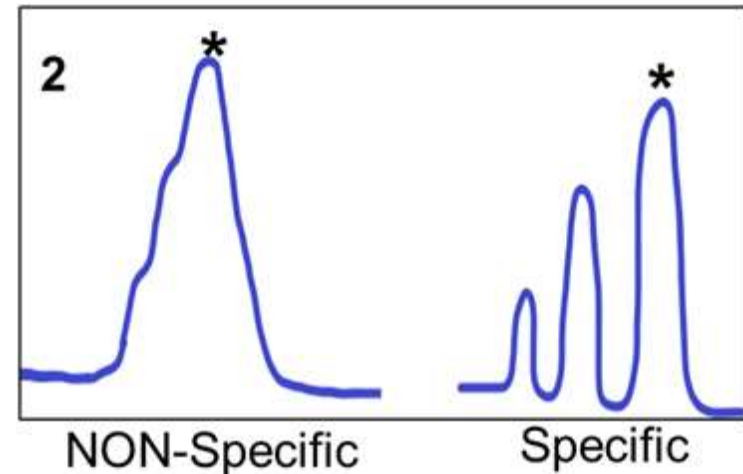
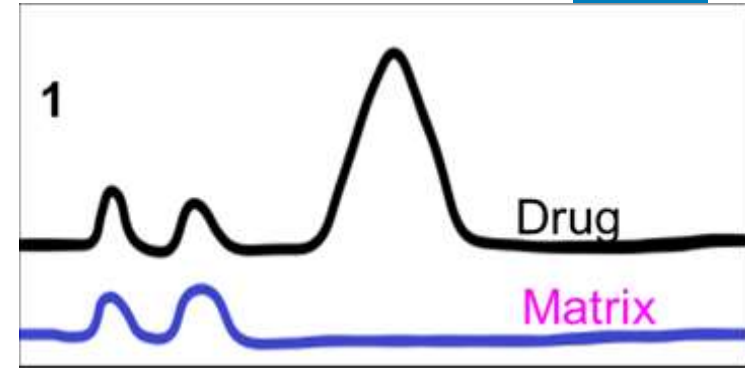


# Performance characteristics:

## **Specificity**



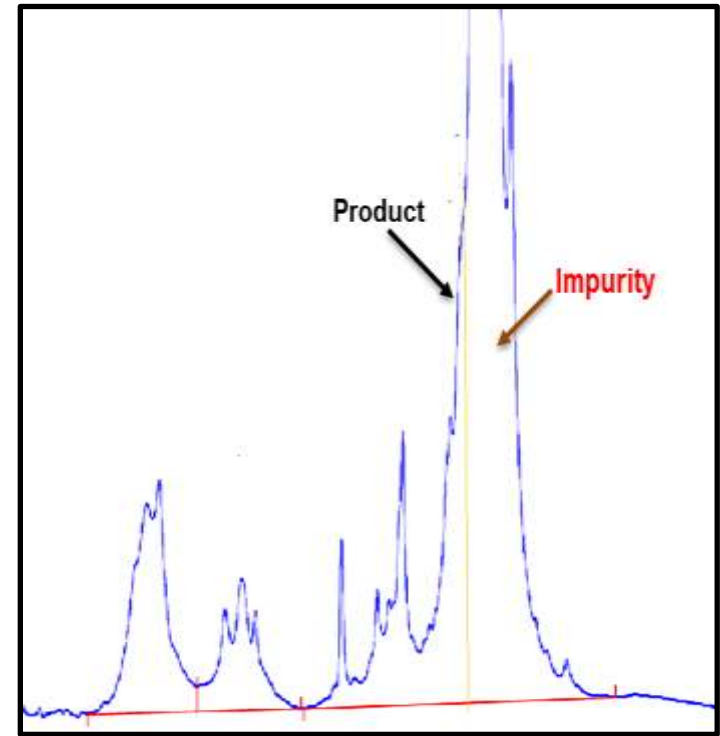
- ICH Q2(R1): "Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present"
- Required for all types of analytical procedure
- Specificity should show:
  1. Absence of matrix (placebo) interference, including suppression or enhancement of response
    - Common mistake of not providing overlays
  2. Resolution: Ability to resolve analyte from other components
    - Perform peak purity analysis to demonstrate specificity



# Common mistake: chromatographic peaks poorly resolved – method specificity not demonstrated

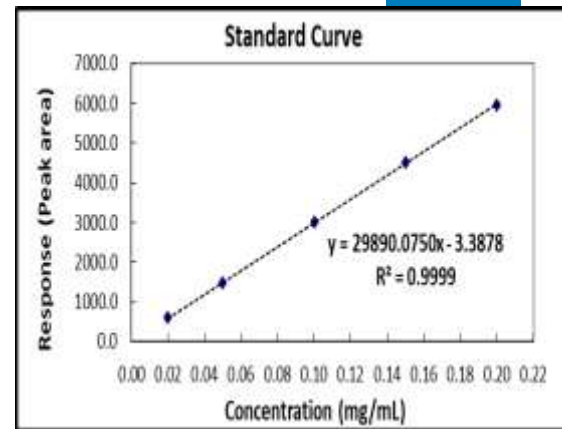


- HPLC method submitted for product identity (ID) & % composition
- Insufficient resolution between product & impurity peaks; hence, method can not reliably ID or measure product.
- ICH Q2(R1): “Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present”
- Method is not suitable for use; hence not approvable
  - Further method development needed



# Linearity

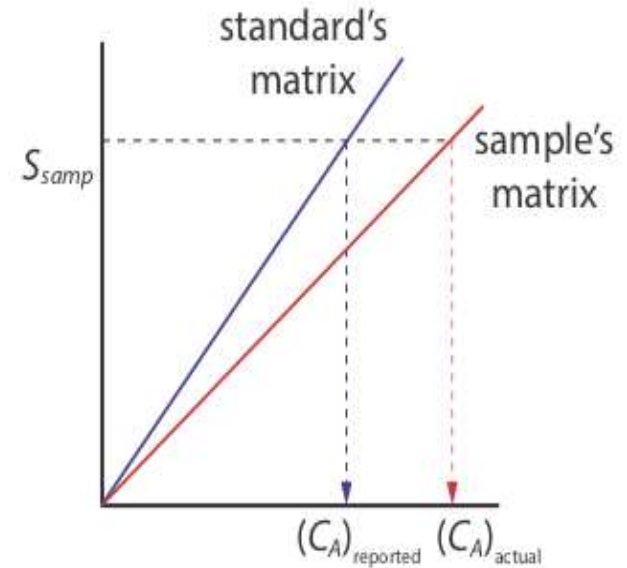
- ICH Q2(R1) – “The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample”
  - “Linearity should be evaluated ... a plot of signals as a function of analyte concentration or content.” ICH Q2(R1)
  - Minimum of 5 concentration levels recommended
- Common Mistake: Plot of expected (theoretical) conc. vs measured conc.
  - Seen frequently in submissions to demonstrate linearity
  - Such correlation (measured Vs expected) is accuracy



# Common mistake: standard used to demonstrate linearity



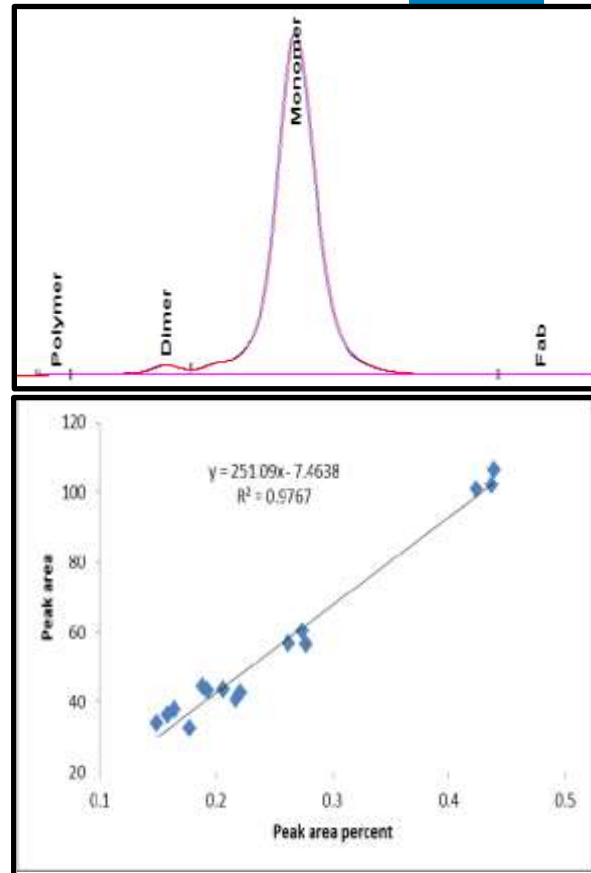
- Suitability of the method should be demonstrated with product itself; parallelism (slope) between sample and standard curve could be different due to matrix differences
  - Sample concentration determined using standard curve could be lower than actual concentration; due to matrix (suppression) effect.
  - Need matrix matching of standards & samples
  - May not be significant issue for separation-based methods



Note: this is also a common problem when determining method accuracy

# Common mistake: Linearity of MSD methods

- Molecular Size distribution (MSD) analysis by SEC
  - A critical QC test for protein and oligo therapeutics
  - A purity/impurity method to determine the proportion of product & impurities using % Peak area
  - **Not possible to determine linearity using concentration vs response plot**
  - Linearity is demonstrated by plotting % peak area (reportable) vs peak area (response), e.g., %aggregate, %main (product), %fragment
  - This principle applies to similar methods that report the result as a percentage composition



# 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.”
  - Evaluates the correctness of the method
  - **NOT** Required for qualitative (ID) and limit tests
  - Accuracy should be established across the specified range (min. of 3-concentrations/3-replicates)
  - Usually determined from linearity data



Common mistake: Accuracy study uses a standard that was quantified by the SAME method as is being validated



- How is accuracy determined?
  - Orthogonal method – “comparison of the results of the proposed analytical procedure with those of a second well-characterized procedure” (ICHQ2(R1))
- Alternative approaches, from % recovery
  - Spike known amount of the analyte (standard – from reputable source or in-house) into the sample (or placebo) & calculate spike recovery:  
$$\% \text{Recovery} = (\text{amount found} / \text{amount added}) * 100$$
  - Dilute known amount of the sample and calculate recovery
- Mistake: Accuracy may be inferred from precision, linearity & specificity...without determining accuracy

# Range



- ICHQ2(R1)-“Range is established by confirming that the analytical procedure provides an acceptable degree of linearity, accuracy and precision”
- Expressed as reportable results & has same units as specification
- Should be evaluated from sample data, not from standard data
- Determination,
  - For assays: 80 – 120% of the target test concentration
  - For impurity: From the reporting level of an impurity to 120% of the specification
    - Linearity, accuracy and precision should be determined over this range



# Common mistakes: setting wrong Range

- Assay range was established based on linearity only
  - Range should be demonstrated based on precision, accuracy and linearity throughout the range; including at the quantitation limit
- Assay range reported in terms of response/signal, not in terms of reportable results
  - For MSD and purity assays range should be in %peak area; not in concentration units
- Range don't cover product specification

# Precision



- ICHQ2(R1)-“The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions”.
  - Measured from %RSD
$$RSD = \left(\frac{s}{\bar{x}}\right)100$$
 s-standard deviation &  $\bar{x}$ -mean of measurements
- Repeatability: intra-assay precision
  - 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: 6 replicates @ each of the 6 concentration levels to cover the assay range, tot. of 36 determinations.
- Intermediate Precision: inter-assay precision
  - Impact of controllable variables – days, analysts, equipment, etc.

## Challenge/Poll Question #2

Which of the following studies is not expected for method validation?

- a) Linearity
- b) Specificity
- c) Calibration
- d) Range
- e) Limit of quantitation

# Detection and Quantitation Limits

- *ONLY expected for quantitative impurity methods*
- *ICH Q2(R1)*: Detection Limit (DL) is the lowest amount of analyte which can be detected but not necessarily quantitated
- *ICH Q2(R1)*: Quantitation Limit (QL) is the lowest amount of analyte which can be quantitatively determined with suitable precision and accuracy.
  - Range 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 but estimated values must be verified experimentally
  - DL and QL should be in the same unit as the reportable value

# Robustness



- Mostly considered during method development phase
- ICH Q2 (R1): Measure of method's capacity to remain unaffected by small, but deliberate variations in (method) parameters and provides an indication of its reliability during normal usage.
  - E.g.: (HPLC: minor changes in temperature, buffer composition/pH, flow rate, etc.....), solution stability, extraction time, etc....
  - If measurements are susceptible to slight parameter variations, then the parameter should be suitably controlled, or a precautionary statement should be included in the procedure.
  - system suitability parameters should be established to ensure minor (unintentional) changes don't affect the validity of the method whenever it's used.

# Summary



- What is the purpose of analytical method validation?
  - To demonstrate method suitability (regulatory requirement)
    - Validation is the proof that the method works appropriately & only thru valid methods can one generate valid data.
- Validation studies should be performed via a properly designed study with pre-set acceptance criteria.
- Suitability of the method should be demonstrated using the product itself
- All validation parameters should be evaluated for quantitative methods

# References:



## Regulatory Guidance & Guidelines

- FDA Guidance : Analytical Procedures and Methods Validation for Drugs and Biologics – updated July 2015
- ICH Q2(R1) Guideline : Validation of Analytical Procedures: Text and Methodology – updated September 2021
- FDA Guidance: Bioanalytical Method Validation – updated May 2018
- USP General Chapter <1225> : Validation of Compendial Procedures
- USP General Chapter <1226> : Verification of Compendial Procedures
- USP General Chapter <1224> : Transfer of Analytical Procedures

