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RECENT UPDATE IN ANALYTICAL METHOD VALIDATION

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ABSTRACT

Analytical method validation is a critical step in ensuring the accuracy, precision, and reliability of analytical results. This review article provides an overview of the Current practices and regulatory requirements for analytical method validation. The Article discusses the importance of method validation, the different types of Validation (full, partial, and cross-validation), and the performance characteristics That need to be evaluated (accuracy, precision, specificity, sensitivity, linearity, Range, robustness, and ruggedness). The regulatory requirements for method Validation from various agencies such as the US FDA, ICH, and ISO are also Discussed. The article highlights the challenges and limitations of method validation And provides guidance on best practices for validating analytical methods. Additionally, the article reviews the different statistical tools and techniques used in Method validation, including regression analysis, analysis of variance, and Measurement uncertainty. The review concludes by emphasizing the importance of Method validation in ensuring the quality and reliability of analytical results.

KEYWORDS: *Validation, precision, specificity, accuracy, ICH guidelines*

1.INTRODUCTION

The chemistry of analytical process is the branch of chemical science that investigates and Disseminates information regarding the separation of chemical substances that are subsequently Recognised. In chemical analysis, quantification and qualification are looked at. First, the various chemical Ingredient or sample mixes are separated. The process of identifying these is referred to as Qualitative. The analytical procedure, also referred to as the quantification process, establishes the Quantity of a particular chemical component. factors influence the choice of analysis methodology, including the sample matrix, Analyte concentration, physical and chemical characteristics, analysis costs and rate, sample quantity, and quantitative or qualitative assessments of the sample. If the information pertains to the identification of chemicals

2. DEFINATION

According to FDA (FOOD AND DRUG ADMINISTRATION) VALIDATION is a procedure for Production and process control designed to assure that the drug products have their identity, Strength, quality and purity. According to FDA guidelines in May 1987, the validation packages Must provide the necessary information and test procedures required to prove that the system and process meet the specified requirements.

2.1) Importance of Validation

- Assurance of quality
- Time bound
- Process optimization
- Reduction of quality cost.
- Nominal mix-ups, and bottle necks
- Minimal batch failures,

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• improved efficiently and productivity
• Reduction in rejections.
• Avoidance of capital expenditures
• Fewer complaints about process related failures.
• Reduced testing in process and in finished goods.
• More rapid and reliable start-up of new equipment's
• Easier scale-up form development work.
• Easier maintenance of equipment.
• Improved employee awareness of processes.

2.2) When does Validation Begin?
Validation should ideally commence in the lab at the very beginning. In the laboratory, researchers Find out precisely how the product reacts and what conditions are needed to make it. They discover When the product breaks down or becomes unstable, useless, and when its quality starts to decline. Following the laboratory's establishment of the boundary processing criteria, the data can be Utilised to create validation requirements.

2.3) When does Validation Ends?
Validation of a system never truly ends. Once a new system and process have been validated the system still requires Maintenance, periodic calibrations and adjustment. Therefore, the process is always under scrutiny and constant evolution

2.4) Departments responsible
- ❖ Site validation committee (SVC) :- Develop site master validation plan, Prepare/execute/approve validation Studies.
- ❖ Manufacturing Department :- Prepares the batches as a routine production batch.
- ❖ Quality Assurance :- Ensure compliance, see that documentations/procedures are in place, approves protocols And reports.
- ❖ Quality Control :- Perform testing and reviews protocol and report as needed.

3) TYPES OF VALIDATION
Validations are of different types which are given below:
1. Process Validation
2. Analytical Method Validation
3. Cleaning Validation
4. Computerized System validation

3.1) Process Validation
The manufacturing process should be Flexible with some restrictions during the Process of Manufacture of the product. The Achievement of the alluring qualities should be ensured with the Prevention of Essential properties. For achieving these, Process validation is performed

3.1.1) Goals of the process validation
1) It provides the guarantee for the Assurance of the good quality which is Required for the Industry.
2) For diminishing different batches Variation.
3) For saving the time and money from Retesting and reprocessing.
4) For the process with fulfillment of the Criteria of robust.
5) For the consistence manufacture of the Product and the process Reproducibility.
6) Declination of expenses due to product Defect.
7) For the regulatory compliance.
8) For the higher quality confirmation

3.2) Analytical method validation
Analytical method validation is the process of verifying that an analytical method is suitable for it's Intended use. It involves evaluating the performance characteristics of the method to ensure that it can accurately and reliably measure the analyte of interest.

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3.2.1) Purpose of Analytical Method Validation :
To ensure the accuracy, precision, and reliability of analytical results.
To demonstrate that the method is fit for its intended purpose.
To comply with regulatory requirements.

3.2.2) Stages of Analytical Method Validation:
1. Method Development : Developing a new analytical method or modifying an existing one.
2. Method Optimization : Optimizing the method parameters to achieve the desired performance.
3. Method Validation : Evaluating the performance characteristics of the method.
4. Method Verification : Verifying the performance of the method in routine use.

Method validation is a reported program that offers with that the Processing system will give a high level of affirmation to meet its Predicated acceptance basis
The performance characteristics required to validate various methods by using various guidelines Such as USP, ICH, FDA, European guidelines etc.

3.3) Cleaning validation
Cleaning validation is the methodology used to assure that a cleaning process removes chemical And microbial residues of the active, inactive or detergent ingredients of the product manufactured In a piece of equipment, the cleaning aids utilized in the cleaning process and the microbial Attributes. All residues are removed to predetermined levels to ensure the quality of the next Product manufactured is not compromised by residues from the previous product and the quality of Future products using the equipment, to prevent cross-contamination and as a good manufacturing practice requirement.

The U.S. Food and Drug Administration (FDA) has strict regulations about cleaning validation. For example, FDA requires firms to have written general procedures on how cleaning processes will be validated. Also, FDA expects the general validation procedures to address who is Responsible for performing and approving the validation study, the acceptance criteria, and when Revalidation will be required. FDA also require firms to conduct the validation studies in Accordance with the protocols and to document the results of studies. The valuation of cleaning Validation is also regulated strictly, which usually mainly covers the aspects of equipment design , cleaning process written, analytical methods and sampling

Fig.1 Visual Inspection of cleaning validation

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3.4) Computerized system validation

This is widely used in the Pharmaceutical, Life Sciences and BioTech industries and is a cousin of software Testing but with a more formal and documented approach. The validation process begins With validation planning, system requirements definition, testing and verification activities, and validation reporting. The system lifecycle then enters the operational phase and continues until System retirement and retention of system data based on regulatory rules. Similarly, The Rules Governing Medicinal Products in the European Union, Volume 4, Annex 11: Computerised Systems applies to all forms of computerized systems used as part of a GMP Regulated activities and defines Computer System Validation Elements.

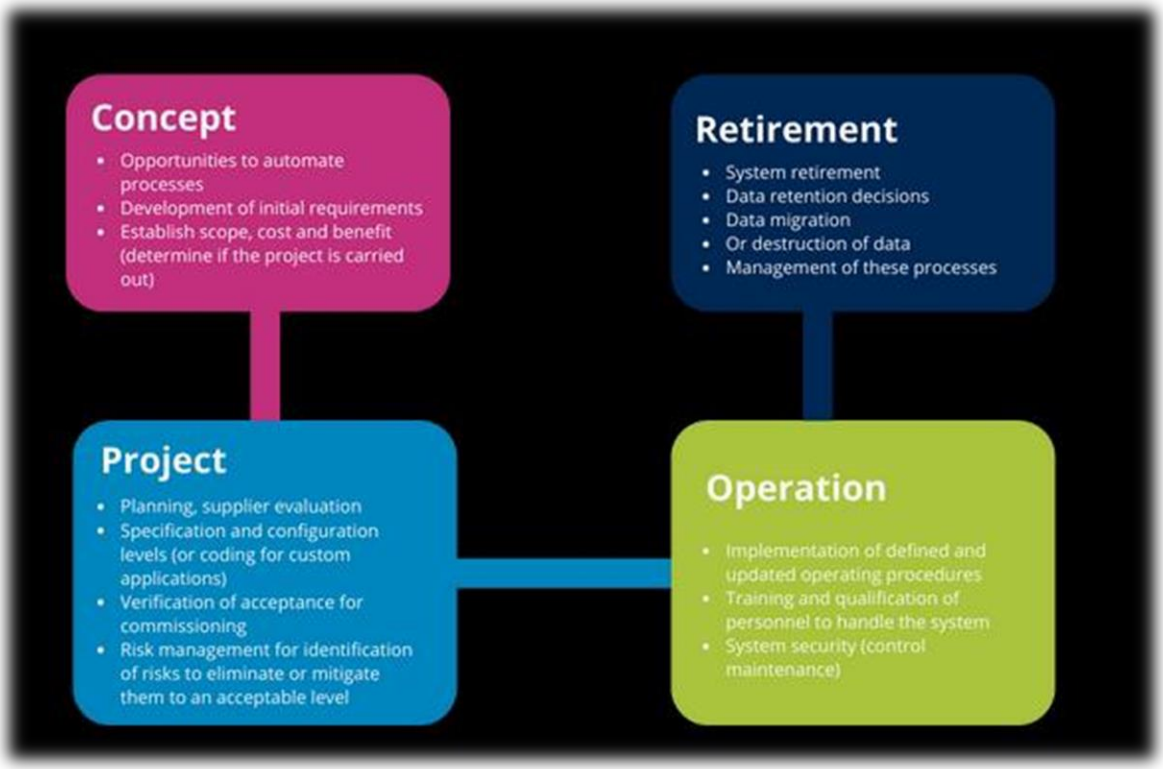

Fig .2 Complete guide to computerized system validation

Validation of analytical procedure is the Legal requirement and is Mandatory to Perform.
ICH guidelines [Q2 (R1)] have Set the guidelines for the validation of Analytical method.

4) TYPES OF ANALYTICAL METHODS TO BE VALIDATED

The validation of the analytical methods must be performed for
The following test:

1) Identification tests
2) Analysis of the impurities for its Quantification and its limit test
3) Analysis of active pharmaceutical Ingredient for is Quantification

4.1) Identification Tests

For the identity Of chemical or ingredient, Identification test is planned. It can be done by Various type of analytical Method. Examination of various Properties such as reaction with other Substances, spectral Evaluation, Properties of chromatogram and so on. In this Test, comparison of sample is done with the reference Standard.

4.2) Analysis of the impurities for its Quantification and its Limit test: Impurities can be quantified and identified. almost all raw materials Contain the impurities. Total removal of the impurities is very difficult task. So regulatory body has set certain Criteria for

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the limit in the presence of the impurities. Percentage purity of the Chemicals is reflected by this test. Following the various parameters of the validation in limit test is less essential whereas it is almost criteria For quantification analysis..

4.3) Analysis of API for its Quantification: Quantification of API or other chemical is the most essential Part of the analytical test. It reflects the accurate presence and proper action of The API in the drug product. With Regard to such archive, assay can be defined as estimation of active Pharmaceutical ingredient in the Product quantitatively. The quantification of API should follow certain procedure which has same Parameters of validation. In the same Way, Dissolution which also deals with the release of API should follow the Same guidelines of the validation.

5) ANALYTICAL METHOD VALIDATION CHARACTERISTICS

An ICH guideline has set certain criteria for the validation of Analytical method.

The parameters are listed below:

- Specificity
- Accuracy
- Precision
- Repeatability
- Intermediate Precision
- Reproducibility
- Limit of Detection
- Limit of Quantification
- Linearity
- Range
- Robustness

5.1) ACCURACY

Accuracy of an analytical method may be defined as, "Closeness of test results obtained by the method to true value".
i.e. measure the exactness of analytical method. It is expressed as percent recovery by the assay of known amount of analyte in the linearity range.

5.1.1) Determination methods

Application of analytical method to an analyte of known Concentration The accuracy may be determined by application of the analytical method to an analyte of known purity (example: Reference standard) and also by comparing the results of the method with those obtained using an alternate procedure that has been already validated..

5.1.2) Spiked – placebo recovery method

In this method, a known amount of pure active constituents Is added to formulation blank (sample that contains all Other ingredients except the active) and then perform the assay of resulting mixture and compare the obtained results With predictable results.

5.1.3) Standard addition method

In this method, perform the assay of given sample, then add a known amount of active constituent to that assayed Sample. After that this sample is again assayed. The Difference between the results of the two assays is compared With the expected results.

5.1.4) Acceptance criteria

The mean value should be within 15% of the supposed value Except at LLOQ, where it should not deviate by more than 20%. the deviation of the mean from the nominal value serves as the measure of accuracy

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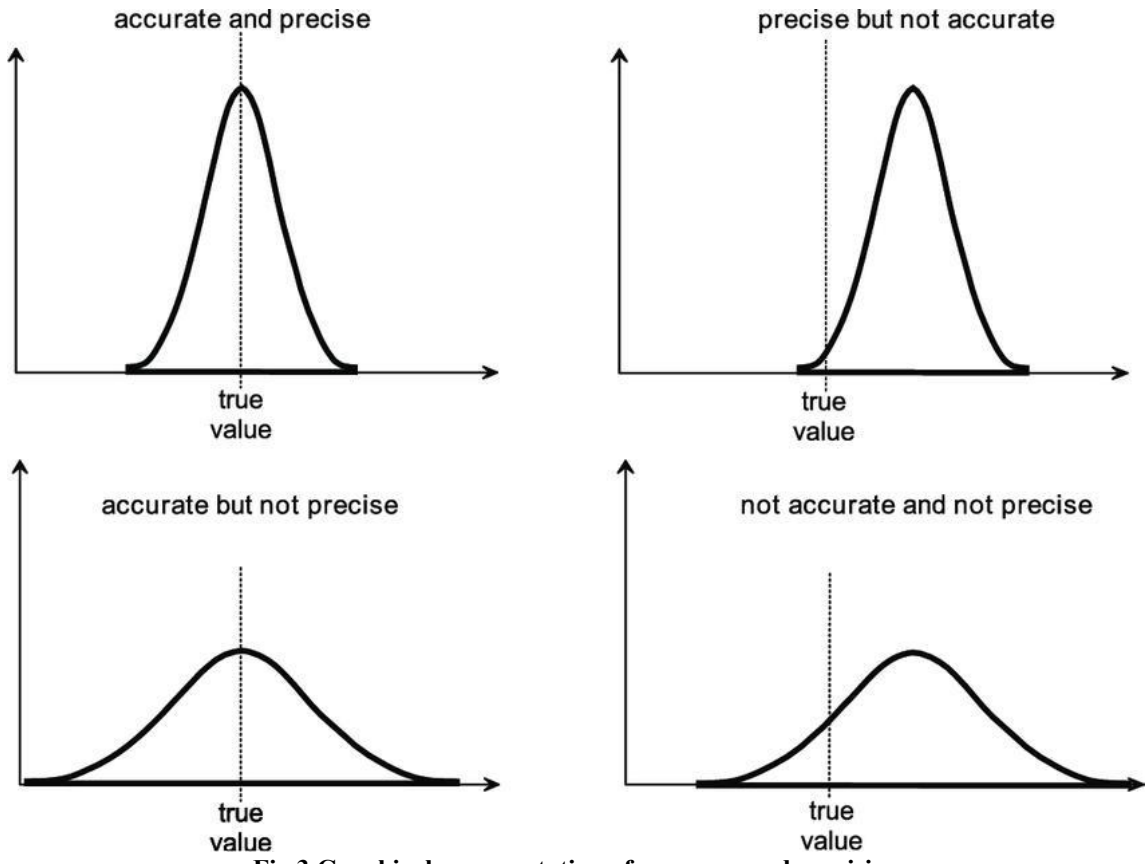

Fig.3 Graphical representation of accuracy and precision

5.2) PRECISION

The precision of an analytical method may be defined as, "Closeness of agreement between a series of measurements Obtained from multiple sampling of the same standardized Sample under the prescribed conditions. Should be investigated using homogeneous, authentic samples.

Expressed as SD/ % RSD = Standard Deviation ×100÷ Mean

Precision……. Considered at 3 levels,

5.2.1) Types of Precision

Repeatability Precision : The ability of an instrument or Method to produce consistent results when measuring the Same sample under the same conditions.

Reproducibility Precision*: The ability of an instrument Or method to produce consistent results when measuring the Same sample under different conditions, such as different Operators, instruments, or locations.

Intermediate Precision: The ability of an instrument or Method to produce consistent results when measuring the same Sample under different conditions, such as different days or Analysis

5.3) SPECIFICITY

Specificity refers to the ability of an analytical method to detect And measure a specific analyte or component in the presence of Other substances or impurities

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5.3.1) Identification test : It assures the identification Of the ingredient.

5.3.2) Purity Tests: The total removal of the Impurities is almost impossible. So certain Limits are set for impurities. Impurities can be present in the form of content of Residual solvent, heavy metals, related Substances etc. The test of such substances can be done by purity test.

5.3.3) Assay (Content or Potency): It refers to The quantitative determination of the API.API shows the potency of the drug. (ICH Harmonized tripartite guideline, 2005)

5.4) LINEARITY
Linearity refers to the ability of an analytical method to provide results that are directly proportional to the concentration of the Analyte.

5.4.1) Methods to Evaluate Linearity:
1. Calibration Curve: Plotting the analytical response against the concentration of the analyte.
2. Regression Analysis: Calculating the correlation coefficient (r) and coefficient of determination (r^2).
3. Residual Plot: Plotting the residuals against the concentration of the analyte.

5.4.2) Acceptance Criteria:
1. Correlation Coefficient (r) : ≥ 0.99
2. Coefficient of Determination (r^2) : ≥ 0.99
3. Residual Plot : Randomly distributed residuals

5.4.3) Identification tests : To ensure the identity of an analyte.

5.4.4) Purity tests
To ensure that all analytical procedures performed allow an accurate statement of the content of impurities of an Analyte, i.e. related substances test, heavy metals etc.

5.4.5) Assay
To provide an exact result which allows an accurate statement on the content or potency of an analyte in a sample?

5.5) LIMIT OF DETECTION
The limit of detection of an analytical procedure is the lowest amount of an analyte in a sample that can be detected, but Not necessarily quantify under stated experimental conditions. Simply indicates that the sample is below or above Certain level. The LOD will not only depend on the procedure of analysis but also on type of instrument.

5.5.1) Measurement is based on
• Visual evaluation.
• Signal to noise ratio.
• The standard deviation of the response and the slope.

Visual Evaluation
LOD is determined by the analysis of samples with known concentration of analyte and by establish the minimum level At which the analyte can be detected. It can be used for instrumental and non-instrumental procedure.

Signal to Noise Ratio
This approach can only be applied to analytical procedure which shows baseline noise. It is performed by comparing Measured signals from samples with known low concentration of analyte with those of blank samples and establishes The minimum concentration at which the analyte can be detected.
Signal to noise ratio 2:1 or 3:1 is generally accepted.the standard deviation of the response and the slope

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LOD =3.3σ / s
Σ= Standard deviation of the response.
S = Slope of the calibration curve of the analyte from regression line.

5.6) LIMIT OF QUANTITATION

The LOQ is the lowest amount of analyte in a sample which can quantitatively determine that may be measured with an acceptable level of accuracy and precision under the stated operational conditions of the method. LOQ can vary with the type of method employed and the nature of the sample.

5.6.1) Measurement is based on

- Visual evaluation.
- Signal to noise ratio.
- The standard deviation of the response and the slope.

Visual Evaluation

LOQ is determined by the analysis of samples with known concentration of analyte and by establish the minimum level at which the analyte can be detected. It can be used for instrumental and non-instrumental procedure.

Signal to Noise Ratio

This approach can only be applied to analytical procedure which shows baseline noise. It is performed by comparing Measured signals from samples with known low concentration of analyte with those of blank samples and establishes The minimum concentration at which the analyte can be detected. Signal to noise ratio 10:1 is generally accepted.
The standard deviation of the response and the slope,

LOD = 10σ / S
Σ = Standard deviation of the response.
S = Slope of the calibration curve of the analyte from regression line.

5.7) RANGE

Range of analytical procedure is the interval between the upper and lower concentration of analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy, and linearity. normally derived from linearity studies and specific range is dependent upon proposed application of the procedure.
The following minimum specified ranges should be considered:

❖Assay of a drug substance or a finished (drug) product: 80 to 120 % of the test concentration.
❖Content uniformity: 70 to 130 % of the test concentration.
❖Dissolution testing: +/-20 % over the specified range;

5.8) ROBUSTNESS

Ruggedness is the ability of an analytical method to resist changes in its performance due to variations in experimental conditions, instrument, or analyst.
Examples of typical variations are
❖Stability of analytical solutions;
❖Extraction time :- In the case of liquid chromatography, examples of typical variations are:
❖ Influence of variations of pH in a mobile phase;
❖ Influence of variations in mobile phase composition;
❖ Different columns;
❖ Temperature;
❖ Flow rate.
In the case of gas-chromatography, examples of typical variations are:
❖ Different columns;
❖ Temperature;

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❖ Flow rate.

5.9) RUGGEDNESS

Degree of reproducibility of test results obtained by analyzing the same sample under variety of normal test conditions such as different.

- Analysts
- Instruments
- Days
- Reagents
- Columns and TLC plates.

i.e. lack of influence of environmental variables on the method. Comparison of reproducibility of test results to the precision of assay is the direct measure of ruggedness of the method.

6) CONCLUSION

The conclusion of an analytical method validation review emphasizes the industries role of method validation in ensuring accurate, reliable, and regulatory-compliant results. It highlights key validation parameters such as accuracy, precision, and specificity, and stresses the need for ongoing development of validation techniques to meet emerging challenges in various industries.

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