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A BRIEF REVIEW ON: METHOD VALIDATION

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ABSTRACT

Validation is a crucial element in meeting the standards of current good manufacturing practices (CGMP) and good laboratory practices (GLP). In every pharmaceutical company, it is essential to carry out testing on raw materials, in-process materials, final packaging, and excipients effectively. Validation of analytical methods is regarded as a fundamental requirement for testing these pharmaceutical items. For testing active pharmaceutical ingredients (APIs), excipients, and final products, a clear analytical procedure must be established. Such a well-structured procedure should guarantee that it consistently delivers accurate results with a high level of precision. To achieve these specific outcomes, it is necessary to validate the analytical method. The process of analytical method validation involves assessing accuracy, precision, limits of detection (LOD), limits of quantification (LOQ), linearity, and range. The outcomes of method validation can be utilized to oversee the quality, dependability, and consistency of analytical outcomes, which is a core component of any robust analytical practice. Furthermore, the validation of analytical methods is mandated by the majority of regulations and quality standards that govern laboratories. The primary purpose of this review article is to provide guidance to emerging researchers on enhancing the quality of analytical method development and the validation process.

KEYWORDS: Analytical chemistry, CGMP, GLP, Linearity, Method validation.

1. INTRODUCTION

Analytical chemistry can be defined as the study of the identification, measurement, and separation of natural and synthetic materials that contain one or more chemicals or elements. There are two primary categories of analytical chemistry: qualitative analysis, which involves

identifying the chemical components present in the sample, and quantitative analysis, which calculates the quantity of a certain element or compound in the sample. When examining pharmaceutical formulations and bulk medications for quality control and assurance, pharmaceutical analysis is extremely important.[1]

As a result, the fundamental task of analysis is now developing analytical methods. The development of analytical devices has led to breakthroughs in scientific and concrete analytical approaches. The time and expense of analysis have decreased and accuracy and precision have increased due to advancements in analytical instrumentation and analytical method development. For active pharmaceutical components, excipients, associated chemicals, drug products, degradation products, residual solvents, etc., analytical techniques are developed and verified resulting in it becoming a crucial component of the requirements for regulatory organizations Official test methods are ultimately the outcome of analytical method development. As a result, quality control labs employed these techniques to verify the drug's products' performance, safety, efficacy, identification, and purity.[2-3]

The In order for regulatory bodies to approve a medicine, the applicant must demonstrate control over the whole drug development process using approved analytical techniques. The number of new medications being introduced is always increasing.[4] Therefore, developing new techniques and introducing them to control their quality is vitally necessary. The following criteria are necessary for contemporary pharmaceutical analysis.

1. Analysis must to be economical and time-efficient.
2. The analysis's correctness must comply with Pharmacopoeia requirements.
3. The selected approach needs to be selective and accurate.[5]

1.2 Significance of validation

- Guarantees quality.
- Enhances process efficiency.
- Lowers costs related to quality.
- Decreases batch failures, leading to greater efficiency and productivity.
- Decrease in rejection rates.
- Enhanced production levels.
- Diminished testing requirements during processes and for end products.
- Quicker and more dependable initiation of new machinery.[6]

1.3 Departments responsible

1.3.1 Site validation committee (SVC)

Formulate the Site Master Validation plan, prepare, carry out, and approve validation studies.

1.3.2 Manufacturing department

Produces the batches as part of routine production.[7]

1.3.3 Quality assurance

Ensures adherence to standards, verifies that documentation and procedures are established, and approves protocols and reports.

1.3.4 Quality control

Conducts testing and reviews protocols and reports as necessary.

Department/Designation	Responsibility
Manager Production	Responsible for manufacturing of batches and review of protocol and report
Manager QC	Responsible for analysis of samples collected
Executive QC	Responsible for samples collection and submission to QC
Manager Maintenance	Providing utilities and engineering support
Executive Production	Responsible for preparation of protocol and manufacturing of validation batches
Manager QA	Responsible for protocol authorization and preparation of summary report.[8]

Types of method validation

Validations are of different types which are given below: (Fig.1)

- Process validation.
- Analytical method validation.
- Cleaning validation.
- Software validation.

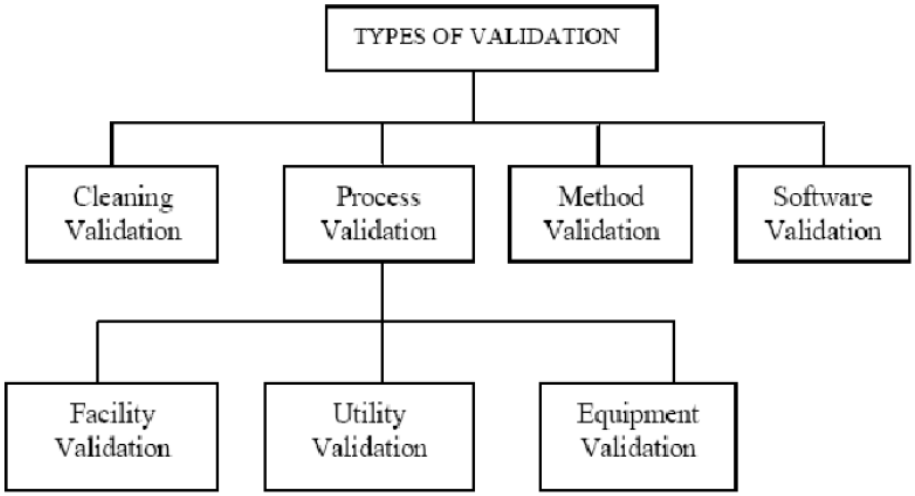

Fig. 1. Type of validation.

2. Analytical method validation

The validation process of analytical methods is implemented to ensure that the analytical procedures used for specific tests fulfill the desired criteria. Frameworks from organizations such as the USP, ICH, and FDA can guide the validation of pharmaceutical techniques. The outcomes from method validation can be utilized to assess its quality, dependability, and uniformity related to analytical findings.[9] In the pharmaceutical sector, the key justifications for validating an assay are, firstly, that assay validation is essential to the quality-control system, and secondly, regulatory requirements for proper manufacturing practices necessitate assay validation. Laboratory studies are employed to verify analytical techniques by illustrating that the execution properties of the procedure are suitable for the intended scientific application. Any new or revised methodology must be confirmed to ensure it can deliver reliable and consistent outcomes.[10-11] Results that were previously utilized by certain administrators involve the use of similar instruments in either the same or entirely different laboratory settings.

2.1 Key parameters of validation characteristics

- ➢ Precision.
- ➢ Accuracy.
- ➢ Specificity.
- ➢ Linearity.
- ➢ Range.
- ➢ Detection Limit.

- ➢ Quantitation Limit.
- ➢ Ruggedness.
- ➢ Robustness

2.1.1 Accuracy

The accuracy of a measurement refers to "It is the closeness of agreement between the actual value and measured value". In a highly accurate method, when analyzing a sample with a known "true value," the measured value will match the true value exactly. Generally, accuracy is represented and assessed through recovery studies.[12]

There are three methods for determining accuracy

1. Comparing to a reference standard.
2. Measuring the recovery of an analyte that has been added to a blank matrix.
3. Performing standard addition of the analyte.

It should be clearly defined how individual or total impurities will be assessed. The ICH document suggests that accuracy should be evaluated using at least nine determinations across three concentration levels.[13]

Acceptance criteria

The average result should be within 15% of the expected value, except at the lower limit of quantitation (LLOQ), where it should not exceed a 20% deviation. The difference of the mean from the nominal value is used to assess accuracy.

2.1.2 Precision

The degree of consistency among individual test outcomes when a method is repeatedly applied to multiple samples from a homogeneous source.

Three levels of precision can be considered:

- Repeatability: the precision measured over a short time frame using the same analyst and operational conditions.
- Intermediate precision: the procedure has been evaluated using different instruments, analysts, and testing days.
- Reproducibility: studies conducted between different laboratories.

The criteria for accepting precision are usually represented as %RSD (Percentage relative standard deviation) for repeatability and intermediate precision assessments, with values expected to stay within set limits according to the intended application of the methods.[14]

% RSD = (Standard Deviation / Mean) x 100.

2.1.3 Specificity

Specificity pertains to the effectiveness of the analytical technique in distinguishing and measuring the analyte within complex mixtures. An examination of specificity is essential during the analysis of impurities and the validation of identification procedures. An ICH guideline describes specificity as the capacity to accurately evaluate the analyte in the presence of other substances that could potentially be found. These might typically include impurities, degradation products, the matrix, and others.

The definition carries several implications

- **Identification test:** Identification tests must be capable of distinguishing between compounds that have closely related structures which are anticipated to be present, thereby confirming the identity of an analyte.
- **Purity test:** It is essential for the analytical procedure conducted to provide an accurate assessment of the impurity content in an analyte, including related substances, residual solvents, heavy metals, and so forth.
- **Assay:** Achieving an accurate result is necessary for generating a reliable report on the potency or concentration of the analyte in a sample.[15]

2.1.4 Linearity

The calibration curve illustrates that the measurement or data for the testing substance correlates directly with the quantity of the testing chemical present in the sample, often showing a linear relationship. This property is known as linearity. To evaluate linearity, one should visually examine a graph plotting concentration (on the x-axis) against mean response (on the y-axis). It is important to calculate the correlation coefficient, Y-intercept, and regression equation.(Fig.2) The data from the regression line can be utilized to calculate the strength of linearity. At least five different concentrations are needed to assess the linearity of the relationship between absorbance and concentration.[16]

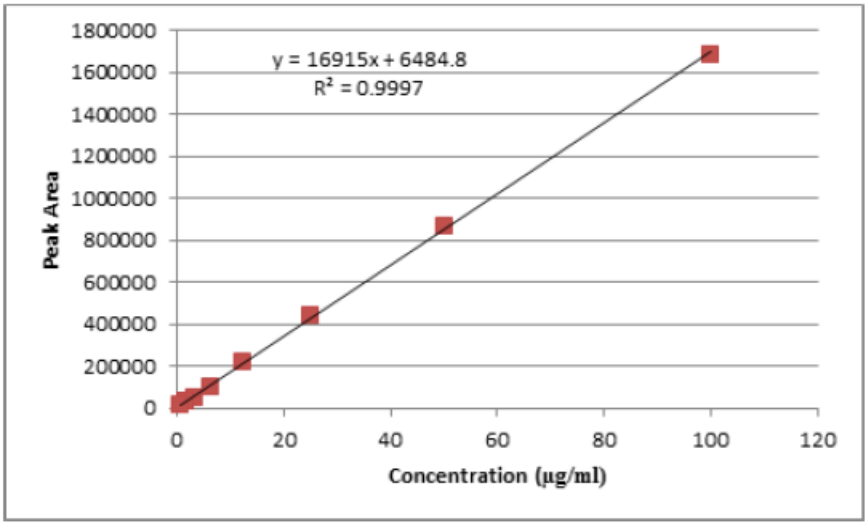

Fig. 2: Linearity.

2.1.5 Limit of detection

The limit of detection (LOD) for an analytical method refers to the smallest quantity of an analyte within a sample that can be identified, although it doesn't necessarily mean it can be quantified, under specified experimental conditions. In essence, it indicates whether the sample is above or below a particular threshold.[17] The LOD is influenced not only by the analytical method used but also by the type of equipment employed.

Measurement relies on

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

Visual assessment

The LOD is established by analyzing samples with known concentrations of the analyte and determining the minimum level at which the analyte can be detected. This method applies to both instrumental and non-instrumental techniques.

Signal-to-noise ratio

This method can only be utilized in analytical procedures that exhibit baseline noise. It involves comparing the signals measured from samples with known low analyte concentrations to those from blank samples. Signal to noise ratio 2:1 or 3:1 is generally accepted.[18]

The standard deviation of the response and the slope

LOD =3.3σ/S

σ = Standard deviation of the response.

S = Slope of the calibration curve of the analyte from regression line.

2.1.6 Limit of quatification

It refers to the smallest amount of an analyte in a sample that can be accurately and precisely quantified.

Visual assessment method

Visual assessment may be applicable for non-instrumental techniques, but can also be utilized alongside instrumental methods. The quantitation limit is typically established by analyzing samples with known analyte concentrations and determining the lowest level at which the analyte can be quantified with satisfactory accuracy and precision.

Signal-to-noise ratio approach

This method is applicable to analytical procedures that show baseline noise. The signal-to-noise ratio is determined by comparing the measured signals from samples with known low analyte concentrations to those of blank samples, and subsequently establishing the minimum concentration at which the analyte can be reliably quantified.[19] A common signal-to-noise ratio is 10:1.

2.1.7 Range

The range of an analytical procedure refers to the span between the highest and lowest concentrations of an analyte in the sample where it has been established that the analytical method offers adequate precision, accuracy, and linearity. This range is typically obtained from studies on linearity and is specifically dependent on the intended application of the method.

The following minimum specified ranges should be taken into account

- For the assay of a drug substance or a finished drug product: 80 to 120% of the test concentration.
- For content uniformity: 70 to 130% of the test concentration.
- For dissolution testing: ±20% around the specified range

2.1.8 Robustness

The robustness assessment method evaluates the ability to remain unaffected by minor but intentional changes in method parameters. This assessment aids in establishing the reliability of methods under standard laboratory conditions.

Comparison results can be accurately determined under different conditions in typical scenarios. Instances of common variations in analytical methods include:

- The effect of pH changes in a mobile phase.
- The influence of composition variations in a mobile phase.
- Use of different columns (From varying vendors or batches).
- Changes in temperature.
- Variations in flow rate.[19]

2.1.9 Rugdeness

The degree to which test results can be reproduced when examining the same sample under various standard test conditions, including different analysts, instruments, days, reagents, columns, and TLC plates, reflects the method's resilience.[20] This indicates that environmental variables do not significantly affect the method. Evaluating the reproducibility of test results in relation to assay precision serves as a direct assessment of the method's ruggedness.[21]

3. CONCLUSION

The validation of analytical methods plays a crucial role in the pharmaceutical sector. This review article aims to assist novice researchers in enhancing the quality of the analytical method development and validation process. This article provides a comprehensive overview of analytical method validation. The outcomes of method validation can be utilized to monitor the quality, reliability, and consistency of analytical results, which are essential components of effective analytical practices. The optimized reverse phase HPLC method for paracetamol demonstrates linearity, accuracy, precision, robustness, simplicity, rapidity, and selectivity. It can be readily employed for routine quality control assessments of raw materials and formulations.

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