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METHOD DEVELOPMENT AND METHOD VALIDATION: AN OVERVIEW

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ABSTRACT

Analytical method development and validation are ongoing processes integral to research and development activities. Method development typically involves defining method specifications and selecting appropriate instrumentation. Analytical methods are crucial for various purposes, including material testing and providing data to support specifications. High-performance liquid chromatography (HPLC) is widely recognized as one of the most accurate methods for both qualitative and quantitative analysis of pharmaceutical drugs. Method validation is the process of demonstrating that an analytical method is appropriate for its intended purpose, a critical step for ensuring analytical reliability. Validation ensures the reliability, consistency, and quality of analytical data, with parameters evaluated including accuracy, linearity, limits of detection and quantification, ruggedness, and robustness. This review highlights different methodologies for pharmaceutical development and discusses various validation parameters.

KEYWORDS: Method development, Validation, Limit of quantitation, Limit of detection, Linearity, Robustness, Ruggedness.

INTRODUCTION

Analytical method development and method validation is an important step in the process of drug discovery. Analysis plays a critical role in the development of products or services, and its importance is magnified in the pharmaceutical industry because it directly impacts human lives.[1]

Analytical chemistry involves the separation, quantification, and identification of chemical

components in both herbal and synthetic materials, which may contain one or more compounds or elements. This field is divided into two main categories: qualitative analysis, which identifies the chemical constituents present in a sample, and quantitative analysis, which measures the quantity of specific elements or compounds in the substance, i.e., the sample.[2]

The primary objective of an analytical procedure is to produce reliable, accurate, and reproducible data. The implementation of validated analytical techniques is crucial in meeting this objective. The results obtained from method validation help in assessing the precision, accuracy, and reproducibility of analytical outcomes, marking it as a fundamental aspect of sound analytical practices. Furthermore, the validation of analytical methods is a requirement under many regulatory guidelines and quality standards that govern laboratory operations.[3]

Analytical method development

In the absence of established techniques, innovative methods are being advanced for the assessment of new products. To detect both pharmacopoeial and non-pharmacopoeial products, novel techniques are being developed to enhance accuracy and robustness while reducing cost and time. These methods are refined and validated through initial experiments. Alternative approaches are devised and implemented to replace existing processes, with a comprehensive evaluation of their advantages and disadvantages in the context of laboratory data comparison.[4]

Importance of method development

Drug evaluation involves identifying, characterizing, and separating drugs in combinations, such as dosage forms and bodily fluids. During the manufacturing process and drug development, the primary goal of analytical methods is to produce information about potency (Which is directly linked to the required dosage), impurity (Associated with the drug's safety), bioavailability (Which includes critical drug properties like crystal form, drug uniformity, and release), stability (Indicating degradation products), and the impact of manufacturing parameters to ensure consistent drug production.[5]

Basic criteria for new analytical method development

a) The specified drug or combination might not be recognized by any official pharmacopeia.

b) Due to patent restrictions, there may be no available analytical methods for the drug documented in existing literature.
c) The presence of formulation excipients might hinder the development of analytical methods for the drug in its formulated state.
d) There might be a lack of analytical techniques for measuring the drug in biological fluids.
e) Analytical procedures for the drug when combined with other substances might not exist.
f) Current analytical methods might demand costly reagents and solvents, involve intricateextraction and separation techniques, and prove to be unreliable.[6,7]

Steps involved in analytical methoddevelopment

Various steps involved in the development of an analytical method are as follows:

Characterization of Analyte and Standard

- All essential information regarding the analyte and its structure, including its physicaland chemical properties like solubility and optical isomerism, is compiled.
- A standard sample of the analyte, assumed to be of 100% purity, is obtained. Appropriate measures are taken for its correct storage, which may include refrigeration, use of desiccators, or freezing.
- In cases where multiple components within a sample matrix need to be quantified, the quantity of each element is carefully determined, along with the presentation of this information and the assessment of available standards.
- Analytical techniques such as spectroscopy (UV-Visible, FTIR, atomic absorption spectroscopy, etc.), high-performance liquid chromatography, and gas chromatography, among others, are employed. These techniques are selected based on their compatibility with the stability of the samples.[8]

Requirement of the technique

The establishment of an analytical methodology is crucial for developing key performance characteristics such as linearity, selectivity, specificity, range, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), and others, which must be clearly defined.[9]

Review of Literature and Prior methods

A comprehensive examination of existing literature related to the drug is conducted, focusing on its physical and chemical attributes, production processes, solubility, and applicable analytical methodologies. This review includes consulting relevant sources such as books, academic journals, the United States Pharmacopeia/National Formulary (USP/NF), the Association of Official Agricultural Chemists (AOAC), and publications by the American Society for Testing and Materials (ASTM). Utilizing the Chemical Abstracts Service's automated computerized literature search is highly beneficial for this purpose.[10]

Method selection

- Based on the information gathered from literature reviews, the methodology is continuously developed and adapted as necessary. Occasionally, it may be crucial to acquire new instruments to develop, modify, or replicate and validate existing methods for analyzing specific analytes and conducting tests.
- In cases where suitable existing methods for analyzing the targeted analyte are not available.[11]

Proper instrumentation and Preliminary investigations

The process involves examining the standard methodology relevant to the research by appropriately setting up instruments through Installation Qualification (IQ), Operational Qualification (OQ), and Performance Qualification (PQ).[12]

Optimization process

During optimization, parameters are adjusted one at a time, and various conditions are systematically compared before resorting to a trial and error method. This task requires a structured and scientific approach, taking into account all pertinent details and thoroughly documenting the process, including any unsuccessful attempts.[12]

Accurate Record-Keeping of analytical characteristics

It is essential to meticulously document the accurately determined analytical characteristics, including limit of detection (LOD), limit of quantification (LOQ), cost, linearity, evaluation time, sample preparation procedures, and more.

Assessment of the developed method with real samples

The sample solution should enable precise and comprehensive identification of the target

drug peak, distinct from all other components in the matrix.

Quantitative Analysis and Percentage recovery estimation of actual samples

The process involves calculating the percentage recovery of actual standard drugs, spiked into a sample matrix devoid of the analyte. It's crucial to demonstrate consistent recovery rates across different tests, although achieving 100% recovery is not mandatory, provided that the results are reliably findings.[13] reproducible, ensuring a high degree of confidence in the.

Validation

Validation plays a critical role in quality assurance, encompassing a thorough examination of systems, facilities, and processes to ascertain whether they fulfil their intended functions effectively and consistently as specified.[14]

Types of validation

Validation is classified into following types:[15]

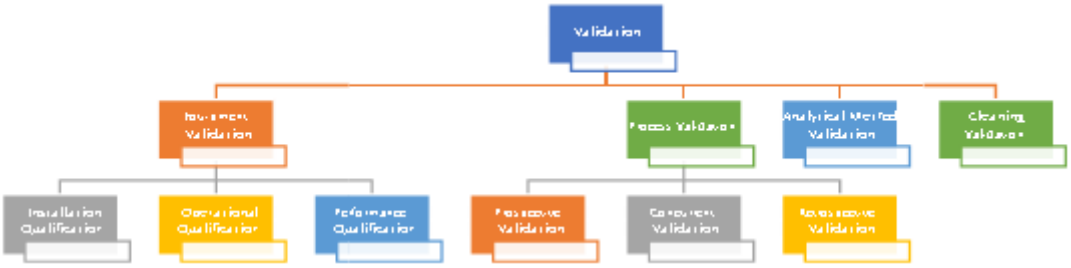

Figure 1: Types of validation.

Analytical method validation

The validation of an analytical method is confirmed through laboratory studies, ensuring that the method's performance characteristics align with the needs of the intended analytical application. Validation is essential for any new or modified method to ensure it can provide consistent and reliable results when used by different operators, with similar instruments, in the same or different laboratories.

Method validation is a documented process that ensures the processing method delivers a high degree of confidence in meeting its predetermined specifications.[16]

It comprises primarily five distinct stages, outlined as follows

1. Qualification of the system

It ensures that the instrument is suitable for the intended analysis, the materials are fitfor use in analytical assessments, the analysts possess the necessary training, skills, and prior documentation, including analytical methods and duly authorized protocols with pre-established criteria, are thoroughly evaluated. If the overall qualifications of a system are not addressed and problems occur, pinpointing the root cause of the issue becomes challenging.[17]

2. Sampling

Sampling involves selecting a portion of the material that accurately represents the whole, which is then analysed. Choosing the right sampling method is crucial as it ensures the selected sample truly reflects the entire batch, allowing for valid statistical conclusions. The field of statistical literature offers a vast array of sampling techniques. However, it's important to consider and evaluate the relative costs and time required for each method beforehand.

3. Sample preparation

Sample preparation is a critical step in successful method validation, accounting for 60 to 80% of the workload and operational costs in an analytical laboratory. There is a wealth of well-documented literature on sample preparation. However, researchers should bear in mind that the choice of a specific preparation technique depends on the analyte concentrations, the sample matrix, the sample size, and the analytical instrumentation to be used.[18]

4. Sample analysis

The analysis process involves using instruments to obtain qualitative or quantitative information from the samples at a suitable sensitivity level. Conceptually, the analysis can be viewed as a system with three integral components: input, converter, and output. The input (denoted as x) and output (denoted as y) correspond to the analyte concentration and the instrument response, respectively. The choice of a specific analytical method is influenced by several factors, such as the chemical characteristicsof the analytes, their concentrations within the sample, the sample matrix, as well as considerations of speed, cost, and others.

5. Data evaluation

The primary objective of data evaluation is to analyze and understand a given dataset through the application of mathematical and statistical methods. This process enables the extraction of meaningful information and facilitates the drawing of conclusions about the inputs and outputs, especially concerning the overall validation procedure.[19]

Significance of validation

- Assures superior quality.
- Adherence to timelines.
- Refinement and optimization of the method.
- Reduction in batch failures, leading to improved efficiency, manufacturing, andproductivity.
- Lowered quality-related costs.
- Reduced rejection rates.
- Enhanced yield.
- Minimized complaints regarding process-related problems.
- Swift and practical initiation of new equipment.
- Heightened awareness among workers about the process.(20)

Validation parameters

The primary goal of method validation is to demonstrate that the method performs its intended function in an accurate, reliable, and consistent manner. The validation parameters, according to ICH guidelines, are outlined as follows:

Accuracy

Accuracy is defined by the closeness of agreement between the obtained values and the known values. It can also be described as the proximity between the actual value and the measured value. Often referred to as trueness, accuracy can be assessed through a minimum of nine determinations across at least three different concentrations within the specified range.[21]

Precision

Precision reflects how closely a set of measurements from multiple samplings of a homogeneous sample align under specified conditions, indicating the degree of variation.[22]

Precision is considered at three levels

- **Repeatability**

This level of precision measures the consistency of outcomes under the same conditionsover a short period, also known as intra-assay precision. It involves a minimum of six replicate preparations of a uniform sample, each tested at 100% of the target concentration.[23]

- **Intermediate precision**

This measures the consistency of results within the same laboratory but under varying conditions such as on different days, by different analysts, or using different equipment, with each condition involving the preparation of six sample solutions following the designated method.[24]

- **Reproducibility**

This pertains to the consistency of results across different laboratories, where each lab prepares a total of six sample solutions in accordance with the analytical method.[25]

Specificity

At each development stage, the analytical method must exhibit specificity. It should be capable of unambiguously identifying the analyte of interest in the presence of all potential components, including degradants, excipients or sample matrix, and peaks from sample blanks.[26]

Limit of Detection (LOD)

The LOD refers to the smallest amount of an analyte that can be detected by the chromatographic method, although it may not be quantifiable as an exact figure. To determine the LOD, a blank sample is injected, and the signal-to-noise ratio is calculated based on the peak responses observed in blank chromatograms.[27]

Limit of Quantitation (LOQ)

The LOQ is defined as the smallest amount of an analyte that can be accurately and precisely quantified.[28]

Linearity

Linearity refers to the ability of an analytical method to generate results that are directlyproportional to the analyte's concentration within the sample.[29]

Range

The range is defined as the interval between the maximum and minimum amounts of an analyte that can be accurately determined in the sample. For assay tests, the specified range should span from 80% to 120% of the test sample's concentration.[30]

Ruggedness

Ruggedness refers to the ability of an analytical method to maintain reproducibility of results when subjected to variations in conditions, such as different laboratories, analysts, instruments, environmental factors, and operators.[31]

Robustness

Robustness describes the capacity of an analytical method to remain unaffected by small, deliberate variations in method parameters. In the context of high-performance liquid chromatography, such parameters that may be adjusted include the pH, flow rate, column temperature, and the composition of the mobile phase.[32]

System suitability parameters

The system suitability test assesses the chromatographic system's sensitivity, resolution, and reproducibility to ensure it is adequate for the intended analysis. Key metrics used to evaluate system suitability include the tailing factor, the number of theoretical plates, retention time, and resolution, among others.[33]

Table 1: Acceptance criteria of system suitability parameters.[34]

S. No.	Parameter name	Acceptance criteria
1.	Tailing factor	<2
2.	Number of theoretical plate	>2000
3.	Resolution	>1.5
4.	RSD	<2

Tailing Factor (T)

The tailing factor is calculated by dividing the distance from the peak's front edge to its backedge by twice the distance from the peak's front edge to the midpoint.[35]

$T=(X+Y)/2X$

Where,

X = Front edge of the peak and,Y = Back edge of the peak

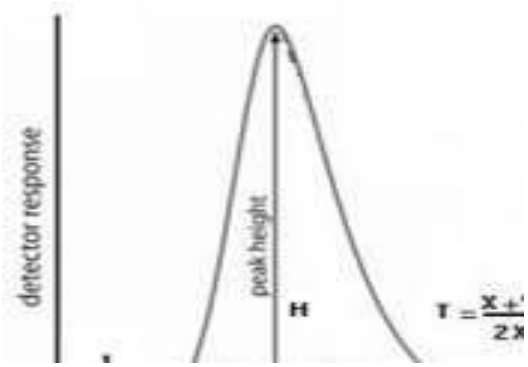

Figure 2: Tailing factor.

Capacity Factor (k)

The capacity factor represents the extent to which an analyte is retained in comparison to anon-retained compound. It is denoted by the symbol k.[36]

It can be calculated as:

K= (tR–t0)/ t0

Where,

tR is the retention time of the peak andt0 is the column dead time

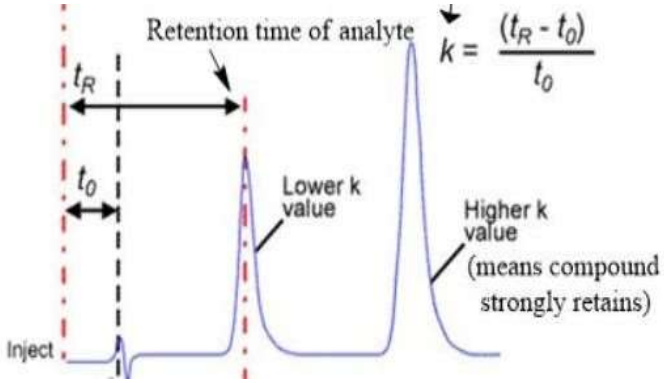

Figure 3: Capacity factor.

Retention Time (tR)

Retention time refers to the period elapsed from the injection of the compound to the elutionof its peak. The retention time for compounds can differ based on several factors, includingthe temperature of the column, the solvent composition, the characteristics of the stationary phase, and the applied pressure.[37]

Resolution (R)

Resolution indicates the separating capability of the entire chromatographic system. It is determined by the ratio of the difference in distance between the maxima of two peaks to the

average width of the peaks at their baseline.[38]

Rs= 2[(tR,A- tR,B]/WA+WB

Where,

tR1 and tR2 are retention time of second and first compounds, respectively.

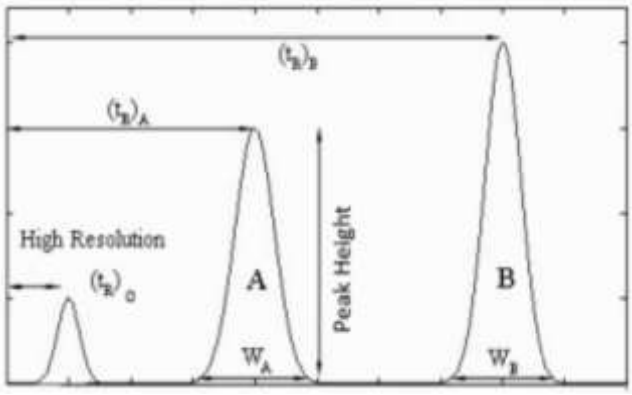

Figure 4: Resolution.

CONCLUSION

This article provides an overview of what validation entails, its various types, its importance, the development of methods, and the execution of the validation process to prove that the method is suitable for its intended use. It thoroughly defines all validation parameters, including linearity, limit of quantitation (LOQ), limit of detection (LOD), range, specificity, robustness, ruggedness, and system suitability, illustrating these concepts with drug examples. Validation is a critical process in the pharmaceutical industry, ensuring that qualityis integrated into the procedures that underpin drug development and manufacturing.

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