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A review article on analytical method validation

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Abstract:

The development and validation of analytical methods play an essential role in the discovery, development, and manufacturing of pharmaceuticals. Validation is one of the key elements to fulfill the requirement of current good manufacturing specifications (CGMP) and good laboratory specifications (GLP). Every year, several drugs entered the market; hence it is mandatory to develop newer analytical methods for such drugs. After the development, it becomes necessary to validate the new analytical method. Method development is the process that proves that the analytical method is acceptable for use. The validation of the analytical method gives information about various stages and parameters like accuracy, precision, linearity, Limit of Detection, Limit of Quantification, specificity, range, and robustness. Validation should be done as per regulatory guidelines such as ICH guidelines. This article was prepared with the aim to review analytical method development and validation.

Key Words: CGMP, GLP

INTRODUCTION

The part of chemical science which studies and provides the knowledge about the separation of chemical compounds which are then identified after that is known as the chemistry of the analytical process. Quantification and qualification are examined in the analysis of chemicals. The different mixtures of chemical compounds or samples are firstly separated. These are then identified which is called the qualitative process. The amount of specific chemical components is determined by an analytical process which is known as the quantification process. The decision of analysis methodology depends on numerous considerations, for instance; sample matrix, the concentration of analysts, its physical and chemical properties, the expenses and rate of the analysis, quantity of sample, and quantitative or qualitative measurements of the sample. If the data deals with chemical identification and characterization, it is referred to as a qualitative analytical method. Similarly, if the method deals with the numerical data and determines the amount of the sample ingredient, it is known as the quantitative analytical method. Based on the developed method, different processes are followed for the development of the method then validation of the method. The common method for the development and validation of the analytical method is completed by the following process [1].

1) Planning the appropriate method that must be developed.
2) The information related to the work should be collected.
3) Qualitative and quantitative analytical methods that can be performed in the lab should be developed.

4) The procedure for testing the sample should be created.
The well-developed method should be easily validated which is the basic criteria of the analytical process. From the initiation of the discovery of the drug, the development of analytical methods and their validation is very essential. And it is also responsible to manufacture and develop the drug. These processes provide official test methods. The testing labs choose that process that monitors the drug activities [2]. The identification, purification, and potential

Analytical Method Development

The process of confirming the analytical testing strategy utilized for a particular test that is reasonable for its expected use is referred to as method validation. The results which are gained by the process of validation of the method are utilized for passing a judgment on the consistent outcomes of the analysis. Then ensure for the quality and reliable product. For the reliable testing procedure, the well-developed method is regarded as the fundamental process. The requirement of the testing method is characterized by this. The reliability for the requirement of the process is affirmed by the performance capacities of the considered The analytic testing process is proposed for the insurance of the identification, purification, and potential action of the medicine. The physical characteristics are also studied in this process. A stable study for a longer time is assured by the well-developed method. It also confirms the quality of the drug during the manufacturing of the drug.
Likewise, the developed process may bolster the evaluation of the performance of the drug.
It ensures the safety parameters and the study of physical characters [4].
Method development is a consistent procedure that arises in parallel accompanied by the development of pharmaceutical products. When talking about the cost, time, productivity, and effectiveness of the drug product, the idea of suitable method development is basic or essential. For the drug development phase, the objective and purpose of the method should be reflected. The characteristic of the API was concentrated in the initial phase of the development of the drug. The safety evaluation for the pre-clinical trial is a vital step. After that pre-formulation studies should be performed. This is followed by stability studies.
Analytical method validation is reasonable to help these parameters. After that by studying the nature and characters of API, the analytical testing method is verified and extended during the process of progressing the development of the drug. The method must not be complicated. It must be robust. The suitable regulatory guidelines must be followed [5].
A well-developed method guarantees the goal which is essential during every step of the development of the drug. It ensures improved equipment in the lab. For the accomplishment of excellent quality and accurate testing outcomes, a significant job is performed by a validated analytical process. Subsequently, the guarantee of assurance of QC instrument is focused on by everyone in the chemical lab. Analysis methods can be of different types such as electrochemical analysis, chromatography analysis, spectral analysis, [6]. A well-developed method helps in drug testing against specification during manufacturing and quality release operations; similarly, it promotes the studies regarding characters of chemical, safety examination, and analysis of activities of the medicine. The development of an analytical process is utilized for assisting the procedure of synthesis of drug. The formulation studies assist to screen the drug with potential activities. The finished pharmaceutical drug should be stable from the phase of raw material to the final formulation. So stability studies should be regularly monitored. The identification, purification, physical specification, and potential activities of the medicine are set up by these methods [7]. The purpose behind the analytical method from the development of a product to the manufacture of a product is to give information on the point given below
Product degradation indicates the stability
2) Analyzing and evaluating the properties of API for instance uniformity of drug, crystalline property of chemical, release API, etc. It helps in the study of bioavailability.
3) The study of impurities which in the identification of drug profiles for safety.
4) The study of the potential activities of the finished product deals with the calculation of the correct dose.

VALIDATION

Validation is defined as a demonstration of giving that any procedure, strategy, process, instrument, materials, action, framework or analyzer proceed as planned following a predetermined arrangement of criteria. The validated procedure guaranteed reliability and consistency in the planned outcome. Further, it focuses on the

compliance of the product and analysis of the final product. It is a significant thing in the pharmaceutical industry. The validation of the analytical method aims for the consistency accuracy and reliability of the results of the testing sample. Any method can show the problems, limitations, and interference by external materials during performing the testing. Hence, such problems should be resolved. It has a significant job in accomplishing such objectives [8].

Reasons for validation [9]

1) It is a mandatory condition for enrollment of any pharmaceutical item or pesticide plan.
2) It supports to accomplish the scope of legitimate/reference technique" endorsed by administrative offices.
3) It ensures a high caliber of the outcomes.
4) It improves the money-related main concern of the research facility.
5) It is an obligatory necessity for accreditation of the research center by ISO 17025 rules.
6) It helps in arriving at the confession of the drugs by worldwide organizations.

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

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 [10].

Goals of 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 time and money from retesting and reprocessing.
4) For the process with the fulfillment of the criteria of robust.
5) For consistent manufacture of the product and the process reproducibility.
6) Declination of expenses due to product defect.
7) For regulatory compliance.
8) For the higher quality confirmation of the medicines.

2. Analytical Method Validation

Validation that deals with the analytical method are a basic necessity to play out with the chemical evaluation. Method validation is the method of playing out various appraisals intended to check whether a method of analysis shows appropriate expected explanation and that are equipped for giving gainful, acceptable measurement as per the regulation. As per the regulation and guidelines, the method should provide valuable data that assure the quality of the product. Multiple testing of the sample is used for the determination of such results. A well-validated method should fulfill all the criteria. The validation of the analytical method should include the testing of the excipients and should focus on the typical testing conditions. All these conditions prove that the validation of the analytical method is specific to the product

The objective of Analytical Method Validation

1) When there are changes in the formulation or if changes are done in the concentration, further validation is not required if and only if the method validation of the analytical method is performed.
2) It decreases the risk of regulatory non-compliance.
3) Critical parameters of the process can be fully understood due to the analytical method.
4) Minimization of interference on accuracy and precision
5) It is used in authorization of product and marketing licenses for new products which are non-pharmacopeia.

Cleaning Validation

The product must be free from contamination which can only be influenced by the validation of the cleaning process. Removal of unwanted substances from the facilities and equipment used during the process be guaranteed by the technique of the cleaning. The unwanted contamination should be less than that of a regulatory requirement. In the drug factory, Cleaning validation is fundamentally a process. The validation of the cleaning process can be done by different analytical processes. The swab test is the most common test for checking the cleanliness of the equipment. The validation of the cleaning process ought to likewise clarify the development of acknowledgment measures. The correct method for the sampling should be followed. Free from microbial contamination and chemical contamination are vital requirements. The impurities should be less than the detection limit [12].

The purposes of the cleaning validation are listed below:

1) The grantee of the drug to be safe and pure can be gained
2) The requirement of clients and their satisfaction can be fulfilled.
3) The contamination due to microbes, chemicals including the cross-contamination of API can be minimized.
4) The consistency of the product and the API can be gained.

The cleaning of the facilities and the equipment should be effective. The residues of the previous products are removed from the equipment which preserves the product from degradation.

The chemical stability and the microbial stability of the API including excipients are confirmed by this process.

Computerized System Validation

Nowadays computer system is collecting the excellent fame in the world. Pharmaceutical industries are not separated from this computer system. From the R and D phase to the development of the production computer system is an inseparable profit in the pharmaceutical sector. For the operation of machines and equipment computer system can be used. The meaning of validation talks about the suitability of the validation in all sectors of the pharmaceutical industry such as documentation, production quality control, and storage. Computerized system validation not only refers to the program of the computer and the system of the computer it also relates to the process of the method. The achievement of the required specification during the production of the medicine is known as validation. Computerized system validation refers to the process rather than the application of the system of the computer. The validation must cover its relation with other systems and the system management. It should be user-friendly. Documentation of all the processes, training, validation, method operation of the machines, the equipment, and system, etc. should be safe from the use of this system. It is related to computer systems. For the validation activities, much effort is expected within the industry.

Analytical Method Validation

For creating trustworthy analytical data from the competent laboratory, a proper standard method should be set up. It can only be possible from the validation of the analytical method. The total information about chemicals should be studied for the set-up of the method and its validation. Reproducible data should be given by the analytical procedure even when performed by the different analysts in various lab centers utilizing distinctive reagents, different instruments, and equipment. For the validation of the analytical method, certain parameters should be followed such as linearity, accuracy, precision, specificity, and reproducibility of the result of the sample. The number of medications presented for consumers has been increasing every day at a higher rate. These medications might contain a fresh element that is not yet seen in the market or there might be small basic alterations or modifications in the structure from the current medication. Essential measures for method validation of drug analysis are given below.

1) For biological fluids, the assay may be difficult to perform by using the analytical method.
2) There is the presence of many excipients in the formulation which can interfere

The complete literature about the analytical methods of the drug cannot be gained because of the patient guidelines.

3) Requirement of costly reagents and solvents in the existing analytical procedures may not be suitable. It may likewise include difficult extraction and separation procedures that are not suitable.

4) Absence of the drug or drug combination in any pharmacopeias. 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 the analytical method. They are listed below.
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 a quantification
1) Identification Tests: For the identity of chemical or ingredient, an identification test is planned. It can be done by various types of analytical methods. Examination of various properties such as reaction with other substances, spectral evaluation, properties of a chromatogram, and so on.
In this test, a comparison of the sample is done with the reference standard
2) Analysis of the impurities for its quantification and its limit test:
Impurities can be quantified and identified. Almost all raw materials contain impurities. Total removal of the impurities is a very difficult task. So regulatory body has set certain criteria for the limit in the presence of the impurities. The percentage purity of the chemicals is reflected in this test. Following the various parameters of the validation in a limit test is less essential whereas it is the utmost criteria for quantification analysis.
3) Analysis of API for its quantification: Quantification of API or other chemicals is the most essential part of the analytical test. It reflects the accurate presence and proper action of the API in the drug product. Concerning such archive, the assay can be defined as the estimation of an active pharmaceutical ingredient in the product quantitatively. The quantification of API should follow a certain procedure that has the 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.
Analytical Method Validation
Characteristics
An ICH guideline has set certain criteria for the validation of the analytical method.
The parameters are listed below:
1) Specificity
2) Accuracy
3) Precision
- Repeatability
- Intermediate Precision
- Reproducibility
4) Limit of Detection
5) Limit of Quantification
6) Linearity
7) Range
8) Robustness
Besides, revalidation may be essential for the following conditions:
1) Alteration in the process of product manufacturing
2) Alteration in the ingredients in the final product of the drug
3) Alteration in the steps of the analytical method (ICH harmonized tripartite guideline, 2005).
The level of revalidation requires relies upon the alteration type. Validation is also required for other alterations.
Characteristics
Kinds of Analytical Procedure
Test of Identification
Impurities test Assay
Quantification Limit
Intermediate Precision
Evaluation of such things that has a very high potency of presence in the raw material is defined as specificity. It incorporates:

Identification: It assures the identification of the ingredient.

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. 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)

Linearity

Linearity is generally indicated by the calibration curve, which shows that the measurement or data of the testing substance is directly proportional to the quantity of the testing chemical in the sample. Such capacity is known as linearity. It should be performed within the range. The value of R2 is studied in the linearity. It must be within the range i.e near to one. Samples are prepared either by diluting the standard stock solution or weighing the different amounts of sample as per the protocol. Solution of different concentrations should be prepared. At least five concentrations must be prepared for analysis. (ICH harmonized tripartite guideline, 2005) Linearity is the property of a mathematical relationship or function that can be graphically represented as a straight line.

Regression line, y= a + BX

Where b is the slope of the regression line and A is the y-intercept.

Correlation coefficient, $r = \frac{\Sigma^n (x_i - Xm)(Yi - Ym)}{[\Sigma (x_i - XM)\Sigma (Yi - Ym)]^{1/2}}$

where x_i is an individual measurement in a set of n measurements and XM is the arithmetic mean of the set, Yi is an individual true value in a set of n true values and y is the arithmetic mean of the set.

Limit: $r^2 \geq 0.98$

Range

The Range is one of the parameters of validation. The Range is the interval within which the concentration of API must lie. It provides the idea of the upper limit and the lower limit of the concentration of API. Between that interval, API can show good efficacy. Range should be set in that interval which can show linearity, accuracy, acceptable precision level. Usually, the extraction of the appropriate range is done by the result of linearity which must be favorable for the procedure. Range should be set in such a way that does not affect the result of linearity, precision, and accuracy. Even at the extreme level should be suitable.

The criteria of ranges that should be followed are given below:

1) Normally the range of assay of the finished good of drug lies between 80 to 120 percent of label claim.

2) While performing the content uniformity, it should be within the range of 70 to 130 percent of label claims. A Proper justification should be given if a wide range has to be set for example metered-dose inhalers.

3) Plus minus 20 percent is recommended in case of the dissolution test (ICH harmonized tripartite guideline, 2005)

1. Accuracy/Recovery:

The accuracy of an analytical procedure expresses the closeness of agreement between the value, which is accepted either as conventional true value or an accepted reference value and the value found, i.e. analytical result. The accuracy of an analytical method is indicated by the recovery of analytical results.

It is done by either spiking or taking linear conc. of samples over the range of 80% to 120% of the target concentration with triplicate samples in each concentration.

The recovery is determined by the equation:

$$Recovery = \frac{Analytical\ Result}{True\ Value}\ X\ 100\%$$

Limit: The recovery should be in the range of 98.0% to 102.0%.

2. Precision:

Precision is the closeness among analytical results of an analytical method. It expresses within laboratories variations i.e. different analysts in different days and carried out under the same conditions of measurement. It is indicated by relative standard deviation, RSD, which is determined by the equation:

$$RSD\ (\%) = \frac{100}{XM}\left[\frac{\Sigma (x_i - Xm)^2}{n-1}\right]^{1/2}$$

where x_i is an individual measurement in a set of n measurements and XM is the arithmetic mean of the set.
Limit: The RSD should not be more than 2%.

3. Specificity:
Specificity is the ability to assess unequivocally the target pathogen or analytic in the presence of
The component that might be expected to be present.
It is investigated by injecting the blank (solvent)/placebo (matrix solution), standard solution, and sample solution to demonstrate the absence of interference with the solution of analytics.
If there is any peak occurrence in the HPLC chromatogram then its resolution should be NLT 1.5 with the primary peak.

4. Limit of Detection:
The Limit of Detection (LOD) is defined as the lowest concentration of an analytic in a sample that can be detected, not quantified. The Limit of Quantitation is the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated operating conditions of the method.
The terms are expressed as:
$LOD = 3.3\sigma / s$
Where σ the standard deviation of the response is s is the slope of the calibration curve?

5. Limit of Quantification
Quantitation limits based on the visual inspection are determined by establishing the minimum level of analyses that can be measured with acceptable accuracy and precision.
The values associated with the terms “acceptable accuracy and precision” should be explicitly defined in the validation protocol.
Quantitation limits based on signal-to-noise ratio can only be applied to procedures that exhibit baseline noise. Generally, a signal-to-noise ratio of 10:1 is considered acceptable.
LOQ: $= 10\sigma / s$
Where σ is the standard deviation of the response and S is the slope of the calibration curve?
7. Robustness:
The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and indicates its ability during normal range.

Robustness tests examine the effect that operational parameters have on the analysis results. For the determination of a method’s robustness, several method parameters, such as pH, flow rate, column temperature, injection volume, detection wavelength, or mobile phase composition, are varied within a realistic range, and the quantitative influence of the variables is determined. If the influence of the parameter is within a previously specified tolerance, the parameter is said to be within the method’s robustness range. Obtaining data on these effects helps to assess whether a method needs to be revalidated when one or more parameters are changed, for example, to compensate for column performance over time. In the ICH document5, it is recommended to consider the evaluation of a method’s robustness during the development phase, and any results that are critical for the method should be documented.

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