A Comprehensive Review on Analytical Method Validation

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Abstract: Validation is one of the key elements to fulfill the requirement of current good manufacturing specifications (CGMP) and good laboratory specifications (GLP). Every pharmaceutical industry should efficiently carry out testing on source materials, in-process materials, finished containers, and excipients. Validation of analytical methods is thought to be a crucial prerequisite for testing such medicinal compounds. It is necessary to create an analytical technique for testing API, excipients, and finished goods. For the testing of API, excipient and final product, an analytical procedure should be developed. Such well-developed procedure should ensure that it will consistently produce the intended and precise result with high degree of accuracy. Validation of the analytical method is required to obtain such a precise result.

Keywords: Analytical method validation, CGMP, GLP.

INTRODUCTION
The chemistry of analytical process is the area of chemical research that examines and disseminates information on the separation of chemical compounds that are afterwards recognised. In the analysis of chemical, quantification and qualification are examined. The various chemical ingredient mixes or samples are first separated. These are then identified, a process known as qualitative process. The analytical process, also known as the quantification process, establishes the quantity of a certain chemical component. The choice of analysis methodology is based on a variety of factors, including the sample matrix, the concentration of the analytes, their physical and chemical properties, the costs and pace of the analysis, the amount of the sample, and whether or not it is measured quantitatively or qualitatively. A qualitative analytical method is one that deals with the identification and characterization of chemicals in data. Similar to this, a method is referred to as a quantitative analytical method if it calculates the quantity of an ingredient in a sample using numerical data. Different techniques are used for method development and validation depending on the generated method. Basically, the following procedure completes the usual technique for the creation and validation of the analytical method [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 method 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 analytical process. From the initiation of discovery of drug, development of analytical method and its 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 which monitors the drug activities [2]. The identification, purification and potential action of medicine are ensured by such process [3].

Analytical Method Development
The term “method validation” refers to the process of verifying that the analytical testing approach used for a certain test is appropriate for its intended usage. The results obtained from the technique validation procedure are used to make judgements about the analytical results that consistently follow. This guarantees a high-quality and dependable product. Well-developed method is regarded as the key process for the trustworthy testing procedure. This describes the testing method's need. The performance capabilities of the under consideration approach attest to its suitability for the process's requirements. For the purpose of ensuring the identification, purity, and potential effectiveness of medicines, an analytical testing procedure is presented. In this phase, the physical qualities are also researched. A well-developed method ensures the stability of investigations over a longer period of time. Additionally, it verifies the drug's quality as it is being manufactured. The new method may also help with drug performance evaluation. It guarantees the safety guidelines and the examination of physical characteristics [4]. The development of methods typically occurs concurrently with the creation of pharmaceutical products. The concept of appropriate technique development is fundamental or necessary when discussing the price, time, productivity, and efficacy of the therapeutic product. The goal and intent of the procedure should be evident for the medication development phase. In the early stages of development, emphasis was placed on the API's characteristics. The crucial step in the pre-clinical trial safety review. Pre-formulation investigations should then be carried out. The stability investigations follow this. Validation of the analytical technique is reasonable to support these parameters. The analytical testing procedure is then expanded upon and clarified as the drug development process moves forward by researching the nature and characteristics of API. The procedure must not be difficult. It needs to be strong. The suitable regulatory guidelines must be followed [5]. A well-developed method guarantees the goal which is essential during every step of development of the drug. It ensures the improved equipment in the lab. For the accomplishment of excellent quality and accurate testing outcomes, significant job is performed by a validated analytical process. Subsequently guarantee of assurance of QC instrument is focused by everyone in the chemical lab. Analysis method can be of different types such as electrochemical analysis, chromatography analysis,
spectral analysis etc [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 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 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 development of product to the manufacture of product is to give information on the point given below

1) Product degradation which indicate the stability.
2) Analyzing and evaluating the properties of API for instance uniformity of drug, crystalline property of chemical, release of API, etc. It helps in the study of bioavailability.
3) The study of impurities which in the identification of drug profile for the safety.
4) The study of the potential activities of the finished product which 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, frame work or analyzer proceed as planned following 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 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, limitation 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 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 high caliber of the outcomes. 4) It improves the money related main concern of the research facility. 5) It is obligatory necessity for accreditation of the research center by ISO 17025 rules. 6) It helps in arriving at acknowledgment 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

**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 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 of the medicines.

**Analytical Method Validation**

Validation that deals with the analytical method is a basic necessity to play out with the chemical evaluation. Method validation is the method of playing out various appraisals intended to check whether 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 determination of such results. A well-validated method should fulfill all the criteria. The validation the analytical method should include the testing of the excipients and should focus on the typical testing conditions. All these condition proves that the validation of the analytical method is specific to the product [11].

**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 risk of regulatory noncompliance.
3) Critical parameters of the process can be fully understood due to analytical method.
4) Minimization of interference on accuracy and precision.
5) It is used in authorization of product and marketing license for new products which are non-pharmacopeia.

**Cleaning Validation**

It is very essential that the product should be free from the 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 should be guaranteed by the technique of the cleaning. The unwanted contamination should be less as that of regulatory requirement. In the drug factory, Cleaning validation fundamentally process. The validation of cleaning process can be done by different analytical process. 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 the microbial contamination and the chemical contamination are the 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 client 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 R and D phase to the development of the production computer system is inseparable part 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 pharmaceutical industry such as documentation, production quality control and store. Computerized system validation not only refers to the program of computer and the system of computer it also relates to the process of method. The achievement of the required specification during the production of the medicine is known as validation [13]. 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 system and the system management. It should be user friendly. Documentation of all the process, training, validation, method operation of the machines, the equipment and system, etc. should be safe from by the use of this system. It is totally related to computer system. For the validation activities, much effort is expected within the industry [14].

**Analytical Method Validation**

For creating the trustworthy analytical data from the competent laboratory, a proper standard method should be set up. It can only be possible from validation of analytical method. The total information about chemical 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 different analyst in various lab centers utilizing distinctive reagent, 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 medication presented for consumers has been increasing every day at higher rate. These medications might contain totally fresh element that is not yet seen in the market or there might be small basic alteration or modification in the structure from current medication [15]. Essential measures for method validation of drug analysis are given below:

1) For biological fluids, assay may be difficult to perform by using analytical method.
2) There is presence of many excipients in the formulation which can interfere with the formulated drug and expected results are difficult to gain.
3) For the combine product of two or more active pharmaceutical ingredient, analytical method may not be found.
4) The complete literature about the analytical methods of the drug cannot be gained because of the patient guidelines.
5) Requirement of costly reagents and solvents in the existing analytical procedures may not be suitable. It may likewise include difficult extraction and separation procedures which is not suitable.
6) Absence of the drug or drug combination in any pharmacopoeias.

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. 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 its quantification

**1) Identification Tests:**

For the identity of chemical or ingredient, identification tests 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.

**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 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 utmost criteria for quantification analysis.

**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.

**Analytical Method Validation Characteristics**

An ICH guideline has set certain criteria for the validation of 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 drug
3) Alteration in the steps of analytical method (ICH harmonized tripartite guideline, 2005).

Explanations for the parameters of validation of analytical method are given below:

Specificity
Normally, the raw materials show the presence of impurities, chemical of degradation, etc. Evaluation of such things that has 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 with the quantity of the testing chemical in the sample. Such capacity is the knows 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 different amount of sample as per the protocol. Solution of different concentration should be prepared. At least five concentrations must be prepared for analysis. (ICH harmonized tripartite guideline, 2005)

Range: Range is one of the parameter of validation. Range is the interval within which the concentration of API must lies. It provides the idea of upper limit and the lower limit of the concentration of API. Between that interval, API can show the good efficacy. Range should be set in that interval which can show linearity, accuracy and precision in acceptable level. Usually the extraction of appropriate range is done by the result of linearity which must be favorable for the procedure. Range should be set in such a way which 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 claim. Proper justification should be given if broad range has to be set for example metered dose inhalers.
3) Plus minus 20 percent is recommended in case of dissolution test. (ICH harmonized tripartite guideline, 2005).

Accuracy
When the agreements of the data are close enough that is agreed either as a conventional true value or an accepted reference value. Then it is called the accuracy in validation of an analytical procedure. Trueness is another term for the accuracy. At least 9 conclusions of minimum 3 concentration levels could be performed which should cover the predetermined range is known as accuracy. For instance, three replications each from three concentrations could be performed for analytical procedure. Either the percent recovery or the difference between the mean and the accepted true value together with the confidence intervals should be recorded as the result of accuracy. (ICH harmonized tripartite guideline, 2005)

Precision
For the homogeneous sample, sampling should be done for the multiple time. Measurement series are gained. Precision are performed in predetermined condition. The result i.e. scattering of the result must be very close. The three types of precision which are repeatability, intermediate precision and reproducibility can be considered.
1) Repeatability: The type of precision which are performed within equivalent working environment and parameter. It must be completed in small interval of time. Intra-assay precision is another term given for it. Evaluation of this test is done from nine conclusions. It should cover the specific range while preparing the sample. For example, at least three replications each from three concentrations can be performed. Another way of analysis is examining minimum six 100% samples.
2) Intermediate Precision: This type of precision can be performed by variation in laboratory condition. The test can be done in alternate days, by another person, by other machine, etc.
3) Reproducibility: This type of precision is that which is done in between labs. It can be collaborative studies between different laboratories. Methodology standardization must be done for reproducibility. (ICH harmonized tripartite guideline, 2005)

Limit of Detection
The detection limit in a particular analytical procedure is the most minimal measure of chemical in a sample which could be identified yet not really evaluated as the accurate measurement i.e. quantification cannot be exact. From instrumental or non-instrumental process basis, a few methodologies for deciding the detection limit are possible.

Quantification Limit
The quantification limits in the particular analysis methods are the most minimum quantity of sample chemical from which quantitative assay of component can be calculated. The result should be within the acceptable range. Few methodologies in deciding the limit of quantitation can be performed based on non-instrumental or instrumental procedure. Some approaches for determination are
1) Visual Evaluation basis
2) Signal-to-Noise basis
3) Standard Deviation of the Response

Robustness
When little with deliberated changes is applied but still there is no difference in the result of the analytical method, the it is coin as robustness. During the normal uses, it gives a sign of its dependability. Appropriately or a preparatory explanation should be given in case of the susceptible results during the alteration in analysis process. Performing the large number of system suitability test is the tedious thing to do. But this process ensures the suitability if the test at any time of use. The list below is the changes that can be done for the validation.

- The test of stability in an analytical solution;
- The time of extraction.

**System Suitability**

It is considered as the fundamental piece of most of the analysis step. This test is the procedure specific. The setting of procedure for the system suitability depends upon the type of procedure to be validated. The tests depend on the idea that the equipment, electronics, analytical operations and samples to be analyzed establish a vital framework that can be examined as such. The parameters that should be notice for system suitability are listed below:

- Theoretical Plates (NLT 2000)
- Tailing Factor (NMT 2.0)
- Resolution if required (NLT 2.0)
- Reproducibility (%RSD of retention time, peak area) (NMT 2.0).

**REFERENCES**