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Development of Analytical Methods for Drug Discovery, Development, and Evaluation: A Comprehensive Review

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ABSTRACT
The main goal is to create goods consistently and affordably with the required qualities and characteristics. For the research, development, and assessment of drugs in pharmaceutical formulation, a technique must be developed. Examining the development and validation of the process utilised for the medication from the start of formulation to the last commercial batch of product was the main objective of this review paper. When an analytical procedure is used to draw conclusions about the caliber of medicines samples, the conclusions must be trustworthy. A written validation policy is used in the pharmaceutical sector. Types of validation and validation policies adhere to good manufacturing practice (GMP) laws' requirements. The creation of analytical methods helps to understand the crucial process variables and minimize their impact on precision and accuracy. Consistent, dependable, and accurate data are provided via a proven systematic approach. These parameters—accuracy, precision, specificity, limit of detection, limit of quantitation, linearity, range, and robustness—are shown in accordance with ICH guidelines. Validating a method ensures that it will produce results that are repeatable, reliable, and suitable for the intended use. Therefore, it is essential to specify in detail both the circumstances under which the process is to be used and the intent behind it. Therefore, method validation is a crucial part of the policies that a lab should implement to produce accurate analytical data.

Keywords: Validation, Method development, quantitation, Limit of detection, Linearity, Robustness

INTRODUCTION

The process of proving that analytical techniques are appropriate for their intended purpose is known as method validation. In more detail, analytical technique validation entails generating substantiated proof that the recommended approach will reliably produce correct test results that assess a product in comparison to its set specifications and quality attributes. Key elements in drug development and manufacturing include the creation, validation, and transfer of analytical methods [1]. Analytical chemistry is the foundation for method development and comprises techniques for identifying, separating, and quantifying the chemical components of pharmaceutical substances [2]. Drug innovation, a research lab trial, preclinical testing, clinical testing, and regulatory registration are all steps in the development of a pharmaceutical. Numerous administrative organizations, such as the United States Food and Drug Administration (USFDA), also demand that the drug product is evaluated for its identification, potency, characteristics, quality, stability, and purity before it can be released for use to further improve the sufficiency and protection of the medication after acceptance. In order to avoid such problems, pharmaceutical validation and process controls are crucial [3]. The release onto the market and the date it is taken into consideration for inclusion in pharmacopoeias often occur at different times. This occurs as a result of potential weaknesses in the ongoing and extensive use of these treatments, reports of ongoing toxicity (leading to their removal from the market), the emergence of patient resistance, and the advancement of more advanced medicine in an effort to increase competition. In such cases, the pharmacopoeia for certain drugs may not have the necessary specifications or testing methods. It becomes vital to create fresher analytical methods for such drugs [4]. The development and validation of analytical approaches play crucial roles in the research, development, and production of pharmaceuticals. Obtaining accurate, realistic, and consistent data is the main

goal of an analytical measure. Validated analytical techniques are crucial to reaching this objective. The results of the validation of thetechnique can be used to determine the quality, consistency, and reliability of the analytical findings that are related to any sane analytical procedure. The majority of regulations and quality standards that affect laboratories also call for the validation of analytical methods. [5] In the context of early-phase clinical trials, this paper provides a practical introduction to the creation and validation. For daily operations in the quality control environment, the method should be validable, transferable, robust, dependable, accurate, and exact. If the method has not been fully created, it should not move on to the validation phase. Validation tests must be carried out using accredited and calibrated instruments and equipment, and they must be adequately recorded [6].

Analytical method development

When definitive techniques are present, new methodologies are being developed for evaluation of the novel product. To investigate the presence of either pharmacopoeial or non-pharmacopoeial product novel techniques are developed to reduce the value besides time for higher precision and strength [7]. These methodologies are optimized and valid through preliminary runs. Alternate ways are planned and implemented to exchange the present procedure within the comparative laboratory information with all accessible merits and demerits.The purpose of analytical method development is to establish the identity, purity, physical characteristics, and potency of drugs, including the drug's bioavailability and stability [8].Analytical method development and validation can be understood as the process of showing that analytical procedures are adequate for the purpose of assessing drugs, and particularly the active pharmaceutical ingredient (API).Analytical procedures are developed to test specific characteristics of substances against the predefined acceptance criteria for such characteristics [9].Therefore, the development of analytical methods involves the evaluation and selection of the most precise assay procedures to determine the composition of a drug.

Necessity of method development

Drug evaluation exhibits the identification characterization and resolution of drugs in combination like dosage forms and organic fluids. At some point of producing technique and development of drug the principal purpose of analytical strategies is to generate data regarding efficiency (which might be directly connected with the need of a identified dose), impurity (related to safety of the medication), bioavailability (consists of key drug traits like crystal kind, uniformity of drug and release of drug), stability(that shows the degradation product), and effect of manufacturing parameters to verify that the production of drug product is steady [10].The development and validation is of great importance for any drug development program.We can identify at least three main reasons why analytical method development is critical for any biotechnology company developing new drug candidates.Firstly, the quality of a drug is obviously at the core of the success possibilities of a pharmaceutical development program, so that biotech companies developing innovative compounds must take analytical method development very seriously [11]. Secondly, analytical method validation is required by regulatory authorities worldwide for both clinical trial applications and marketing authorizations.For example, a biotech company having an Investigational Medicinal Product Dossier (IMPD) for its drug, with a low-quality chemistry, manufacturing, and controls (CMC) section will have a difficult time to have its clinical trials approved by European drug agencies. Then, method development is key to have clinical studies approved [12].
Finally, after all, patients will eventually receiving the investigational medicinal product (IMP) in early phase clinical trials (first in human / phase 1 studies), so the development and manufacturing quality of a medicine is vital to ensure patient safety and to hopefully see promising efficacy in the new treatments [13].
The figure below provides an overview of the analytic method development process, including the method goals, the the analytic goals, validation requirements, and the documentation requirements at the different stages of drug development.

Characterization of analyte and standard

- All the known necessary data concerning the analyte and its structure, which includes physical and chemical properties such as solubility, optical isomerism, etc., are collected.
- The standard analyte is equal to 100% purity and is acquired. Necessary arrangement is to be created for the proper storage (refrigerator, desiccators, and freezer).
- In the sample matrix, when multiple parts are to be measured the amount of elements is observed duly presenting the information and the accessibility of standard are calculated [14].
- Techniques like spectroscopy (UV-vis, FTIR, atomic absorption spectroscopy, etc.), high-performance liquid chromategraphy and gas chromatography etc., are, however, approximately once coordinated with the stability of samples [15].

- When talking about analytical methods in drug development, qualitative and quantitative methods should be differentiated.

Methods for compound testing include validation parameters such as specificity, limit of detection, limit of quantitation, linearity, accuracy, range, precision (under laboratory repeatability conditions), and stability. In addition, revalidation may be required if changes are introduced in the synthesis of the drug substance, in the composition of the drug product, or if modifications are made to the analytical procedure [16].

The Method Development and validation processes

The steps of methods development and method validation depend upon the type of method being developed. However, the following steps are common to most types of projects:

- method development plan definition
- background information gathering
- laboratory method development
- generation of test procedure
- Methods validation protocol definition
- laboratory method validation
- validated test method generation
- validation report. A well-developed method should be easy to validate. A method should be developed with the goal to rapidly test preclinical samples, formulation prototypes, and commercial samples. As the method development and validation processes advance, the information gathered is captured in the design and subsequent improvement of the method. Ideally, the validation protocol should be written only after a thorough understanding of the capabilities and intended use of the method. The validation protocol will list the acceptance criteria that the method can meet. Any failure to meet the criteria will require a formal investigation be conducted. The validation parameters, also termed analytical performance characteristics, depend on the type of analytical method [17].

Pharmaceutical analytical methods are categorized into five general types:

- identification tests
- potency assays
- impurity tests: quantitative
- impurity tests: limit
- specific tests. The first four tests are universal tests, but specific tests such as particle-size analysis and X-ray diffraction, are used to control specific properties of the active pharmaceutical ingredient (API) or the drug product. Validation requirements depend on the type of test method, including
- specificity: ability to measure desired analyte in a complex mixture
- accuracy: agreement between measured and real value
- Linearity: proportionality of measured value to concentration
- precision: agreement between a series of measurements
- range: concentration interval where method is precise, accurate, and linear
- detection limit: lowest amount of analyte that can be detected
- Quantitation limit: lowest amount of analyte that can be measured
- robustness: reproducibility under normal but variable laboratory conditions [18]. Only specificity is needed for an identification test. However, the full range of specificity, accuracy, linearity, range, limit of detection (LOD), limit of quantitation (LOQ), precision, and robustness testing is needed for more complex methods, such as quantitative impurity methods. The validated test method is included in the validation report that summarizes the results of the validation studies. Both the validation report and the test method aresubmitted as part of the NDA or ANDA [19].

Advances in technology and equipment

Recent progress in methods development has been largely a result of improvements in analytical instrumentation. This is especially true for chromatographs and detectors. Isocratic and gradient reverse phase HPLC have evolved as the primary techniques for the analysis of non-volatile APIs and impurities [20]. The HPLC detector of choice for the development of many types of methods is the photodiode array (PDA) detector, because it can be used for both quantitative and qualitative analysis. The use of a PDA detector to determine peak purity of the active ingredient in stressed samples greatly facilitates the development of stability-indicating assays. The emphasis on the identification of trace impurities and degradants has led to the increased use of hyphenated techniques such

as liquid chromatography–mass spectrometry (LC–MS) and liquid chromatography–nuclear magnetic resonance spectroscopy (LC–NMR). This trend will continue with the need to better define degradation pathways. The ultraviolet (UV) absorbance detector remains the most common HPLC detector for potency and impurity analysis [21]. Once specificity has been demonstrated, the PDA detector is replaced with a variable wavelength detector and the HPLC effluent is monitored at fixed wavelengths. Stability-indicating andimpurity methods areoften required to measure analytes within a wide concentration range. For example, process impurities and/or degradation products can be present at levels of 0.1%, and the main active ingredient istypically present at the nominal concentration (100%). This amount is well within the linear range of a fixed-wavelength detector, but not within the linear range for LC–MS detectors. Recent FDA and ICH guidance on chiral drug products and impurities has presented new challenges for methods development scientists [22]. However, recent advances in the use of chiral HPLC columns have greatly facilitated progress in this area. The advances are primarily a result of the introduction of chiral stationary phases (CSPs) prepared by the reaction of amylose or cellulose derivatives with silica. The new CSPs allow trace levels of enantiomeric impurities to be measured. Gas chromatography remains the method of choice for the analysis of volatile compounds [23]. Gas chromatography with mass spectrometry detection (GC–MS) is increasingly being used to identify impurities and to determine active ingredient peak purity in stressed samples. Advances in laboratory robotics and automation arealso beginning to be applied to methods development and validation. Development teams are using laboratory robotics to develop automated methods for high-volume tests. An in-depth review of all the recent advances in analytical instrumentation is beyond the scope of this article. However, several methods should be noted. Advances in the use of nondestructive infrared (IR) and near-infrared spectroscopy (near IR) and NMR techniques are particularly promising for methods development scientists [24].

Issues and Challenges

For a method development and validation program to be successful, a holistic approach is recommended. A common challenge encountered during method development and validation is that methods are typically developed by the R&D department, whereas validation is typically the responsibility of a validation group. It is important that the R&D and validation groups work as one team. Various groups also may be responsible for ensuring the suitability of the methods to support early clinical phases and commercial manufacturing. The transfer of analytical methods from one group to another then becomes an important step for ensuring that the proper validation is in place to justify its intended use [25]. Because the method will be run by several groups during its progression from development to validation, the method must be robust. This means that the method should provide reliable data, both on a wide range of equipment and in the hands of several chemists. A common weakness in the development and validation of methods is that the methods are not robust enough. If robustness is not built into methodsearly in development, then the result most likely will be loss of efficiency during routine QC testing and a lengthy and complicated validation process as well. Another challenge encountered early in the development of methods intended to support stability studies is ensuring that the method indicatesstability [26]. This process is typically achieved by conducting forced-degradation studies. The design and execution of these studies require a thorough understanding of the product being tested, as well as a good understanding of the analysis technique. As mentioned previously, new regulatory guidelines are being published that govern the expectations of regulatory agencies throughout the world regarding methods development and validation. Another challenge is that many pharmaceutical companies must improve their methods to meet current regulatory standards. From a simple method improvement to complete redevelopment and subsequent cross-over to an older method, the upgrade of analytical methods can be a daunting task. For this reason, one must be aware of current trends in regulatory guidelines and adopt a proactive approach to changes that may affect development and validation programs. Finally, one of the key requirements for method validation (which is also one of the key challenges) is that only well-characterized reference materials with well-documented purities should be used during method validation activities [27]. The challenge stems fromthe fact that, in some cases, the tools used to characterize reference standard materials are being developed and validated at the same time as the reference standard itself.

Analytical method validation

Validation of an analytical approach is established through laboratory research that the execution attributes of the procedure meet the requirements for the proposed scientific application. Validation is required for any new or altered procedure to verify that it is fit to provide predictable and dependable outcomes, once used by various administrators by usingcomparable instrumentation in similar or absolutely distinct laboratories [14]. Method validation is a reported program that offers the assurance that the processing system will give a high level of affirmation to meet its predicated acceptance basis [9]. It consists of mainly five different steps which are as follows:

Qualification of the system: System qualifications permit to check that the instrument is appropriate for the planned investigation, the materials are appropriate to be used in analytical judgments, the analysts have the correct instruction, capabilities, and foregoing documentation such as analytical inclusive of analytical approaches, proper authorized protocol with pre-set up standards have been reviewed. On the occasion that the general qualifications of a device are overlooked and trouble arises, the source of the problem will be hard to recognize [15].

Sampling: Sampling assists in the choice of a representative part of the fabric that is along these lines subjected to evaluation. The selection of a suitable sampling technique is of significant importance since it gives assurances that the sample chose is really illustrative of the material as a whole for the purpose of important statistical inferences. In the statistical literature, there is a considerable collection of work on sampling techniques, in any case, the relative expenses and time involved with every technique should be assessed ahead of time [15].

Preparation of the sample: Preparation of the sample is a key component to effective method validation. Sample planning has been mentioned to represent 60 to 80% of the work action and working expenses in an investigative lab. The literature on the preparation of the sample is enough and is adequately documented. In any case, the investigator should recall that the choice of a particular preparation technique is based on the concentrations of analytes, the sample matrix, the size of the sample and the instrumental method [15].

*Analysis of sample:*Evaluation is associated with the instrument used to extract qualitative or quantitative data from samples with an adequate vulnerability level. The investigation could be predictable, in a great sense, as the device has 3 interconnected fundamental components, namely input, converter, and output. The input and output are assigned by the letters x and y, and they represent the concentration and response individually. The selection of a specific analysis depends on many considerations, for example, the chemical properties of the analytical species, the concentration of the analytes in the sample, the sample matrix, speed, cost, etc. [15].

Assessment of data: The essential reason behind information assessment is to outline and pick up knowledge into a specific informational index by utilizing numerical and statistical techniques. Data evaluation allows extracting valuable data and inferring from inputs and outputs, and in particular from the validation procedure in general [15]. Cleaning validation cleaning validation is a reported proof with a high level of confirmation that can uniformly clean a system or equipment to already determined and specification criteria. Cleaning approval is a reported procedure that demonstrates the efficacy and consistency in cleaning pharmaceutical production equipment. The goal of cleaning approval is to verify the viability of the cleaning system for the expulsion of product deposits, degradants, additives, excipients or cleaning agents and in thecontrol potential microbial contamination.

Validation parameters

The main aim of method validation is to produce proof that the method will what it is supposed to do, accurately, reliable and consistent [9]. The validation parameters as per ICH guidelines are described below:

Accuracy: Accuracy is expressed as the nearness of agreement between the values found and values that are already available. It can also be defined as the closeness between the true value and the observed value. It is sometimes called trueness, and it could be determined by using at least 9 determinations on a minimum of 3 concentration over the specified range [19]. The precision of prochlorperazine maleate (PRO) and betahistine hydrochloride (BET) was studied using the standard addition method at three different levels (50%, 100%, and 120%). A known amount of drug was added to the preanalyzed sample, and percentage recovery calculated. When this method was used for accuracy, the recovery was found to be 99.38% for betahistine hydrochloride and 99.11% for prochlorperazine maleate [20]. For the concurrent determination of nitazoxanide and ofloxacin accuracy was studied by the standard addition method at five different levels (50%, 75%, 100%, 125%, and 150%). The results indicate that the recoveries were observed to be in the range of 80% to 120%, therefore, the method is accurate [21]. Accuracy of paracetamol was studied by preparing standard solution of different concentrations (10, 35, 55 µg/ml) and injected to check the % recovery. The percent recovery of the drug was found in the range of 98.8 to 102.0%, respectively, for all concentrations [22].

*Precision:*The exactness of an analytical procedure expresses the nearness of agreement (degree of scatter) between a group of measurements obtained from different sampling of a uniform sample underneath the prescribed conditions [23]. Precision may be taken into consideration at 3 levels:

- Repeatability: It expresses the exactness below a similar operating condition over a brief interval of time and also referred as intra-assay precision. A minimum of six replicates of the preparation of thetest of a similar or consistent sample ready for 100% check [24].
- Intermediate precision: It expresses the exactness under inside research laboratories, in distinct days, through distinct analyst, on distinct instruments/equipment. Two different analysts each preparing six sample solutions, as per specified method [25].
- Reproducibility: It refers to the precision between different analytical laboratories. Every research facility set up an aggregate of six sample solutions, according to the analytical technique [24]. The precision of the prochlorperazine maleate (PRO) and betahistine hydrochloride (BET) method was determined by interday and intraday variation (% RSD). Intraday precision was performed by analyzing standard drug solutions within the calibration range, three times on the same day.

The precision was obtained by analysing drug solutions within the calibration range on three different days over a period of seven days. The low % RSD values of interday (1.02 to 1.48% for BET at 252.9 nm and 0.67 to 0.82% for PRO at 260.15 nm) and intraday (0.77 to 1.09% for BET at 252.9 nm and 0.27 to 0.61% for PRO at 260.15 nm variation for BET and PRO, revealed that the method is precise [28]. For simultaneous estimation of the precision of nitazoxanide and ofloxacin, injection of six replicates of a sample prepared from commercial tablets was performed and assay was calculated to determine the repeatability of the retention time and a peak area of the standard and samples. The percentage values of the relative standard deviation (% RSD) for the area of nitazoxanide and ofloxacin were 0.44 and the 0.2% and RSD values for a retention time of nitazoxanide and ofloxacin were 0.44% for both drugs [21]. The precision of paracetamol was checked by injecting a solution of 80 µg/ml for six times on the same days, on different days, and in a different time interval on the same day. The % RSD was found to be less than 3%, which showed good precision [22].

Specificity: For every stage of development, the analytical technique should demonstrate specificity. The technique should have the power to unequivocally assess the analyte of interest whereas within the presence of all expected parts, which can encompass degradants, excipients/sample matrix, and blank sample blank peaks [26]. Specificity was determined to determine the retention time of each drug in the mixture and in the sample. The retention time of standard drugs was determined, and it was found that it was 3.750 min and 1.533 min for nitazoxanide and the ofloxacin and retention time of both drugs in the standard mix was found to be 3.760 min for nitazoxanide and 1.542 min for ofloxacin, respectively [29].

Conclusion

This article provides information on what validation is, its different sorts, why it is important, how to build a method, and how to do the validation procedure to show that the approach works as intended. The definitions of all validation criteria, including linearity, LOQ, LOD, range, specificity, robustness, ruggedness, and system applicability, are clear and provide examples of specific medications. One of the most important steps in the creation of pharmaceuticals is the effective development and validation of analytical procedures. Several crucial elements that contribute to success in these fields will also help with regulatory compliance. One of these criteria is experience, both the aggregate experience of the development and validation department and the level of experience of the individual scientists. Another key element in ensuring successful method development and validation is a solid mentoring and training program. To develop pharmaceuticals that are safe and effective, companies must maintain a suitable degree of competence in this crucial area.

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