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REVIEW ON: ANALYTICAL METHOD VALIDATION REVIEW

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ABSTRACT
The development of sound Analytical methods is of crucial importance during the process of drug discovery, release to market and development Pharmaceutical analysis plays a very prominent role in quality assurance as well as quality control of bulk drugs and pharmaceutical formulations. Analytical method development has become the basic activity of analysis. Recent development in analytical methods has been resulted from the advancement of analytical instruments. The improvement of the analytical method development and analytical instruments have reduced the time of analysis, increased precision and accuracy and reduced costs of analysis most of pharmaceutical organizations are investing huge amount of money for the establishment of advanced analytical laboratories. Analytical techniques are developed and validated for active pharmaceutical ingredients (API), excipients, drug products, degradation studies and related substances, residual solvents etc. Drug approval by regulatory authorities requires the applicant to prove control of the entire process of drug development by using validated analytical methods. There is a great need for development of new analytical methods for quality evaluation of new emerging drugs. Analytical method development provides a high degree of assurance and is an important process in the drug discovery

KEYWORDS: Analytical method development, validation, Quality control.

INTRODUCTION

The objective of any analytical measurement is to obtain consistent, reliable and accurate data. Validated analytical methods play a major role in achieving this goal. There is a great need for development of new analytical methods for quality evaluation of new emerging drugs. The results from method validation can be used to judge the quality, reliability and consistency of analytical results, which is an integral part of any good analytical practice. Validation of analytical methods is also required by most regulations and quality standards that impact laboratories.

The validation of an analytical method should be established and verified by laboratory studies, and documentation of successful completion of such studies should be provided in the assay validation report. General and specific SOP and good record keeping are an essential part of a validated analytical method. The data generated for bio analytical method establishment and the QCs should be documented and available for data audit and inspection. Documentation for submission to the agency should include:

Basic criteria for new method development for drug analysis

- The drug or drug combination may not be official in any pharmacopoeias
- A proper analytical procedure for the drug may not be available in the literature due to patent regulations
- Analytical methods may not be available for the drug in the form of a formulation due to the interference caused by the formulation excipients.
- Analytical methods for the quantitation of the drug in biological fluids may not be available.
- Analytical methods for a drug in combination with other drugs may not be available.
- The existing analytical procedures may require expensive reagents and solvents. It may also involve cumbersome extraction and separation procedures and these may not be reliable.

Method Validation

The need to validate an analytical method is encountered by analysis in the pharmaceutical industry on an almost routine basis, because adequately validated methods are a requirement for approvable regulatory filings. Method validation has received considerable attention in literature and from industrial committees and regulatory agencies. The International Conference on

Harmonization (ICH) of technical requirements for the registration of pharmaceuticals for human use has developed a consensus text on the validation of analytical procedures. The document includes definition of different validation parameters. The United States Environmental Protection agency (US EPA), Resource Conservation and Recovery Act (RCRA), The American Association of Official Analytical Chemist (AOAC), United States Environmental Protection Agency (USP) and other scientific organizations provide methods that are validated through multi-laboratory studies The United States Food and Drug Administration (US FDA) has proposed guidelines on submitting sample and analytical data for methods validation. The United States Pharmacopoeia (USP) has published specific guidelines for method validation and compound evaluation.

The objective of validation of analytical procedures is to demonstrate that it is suitable for its intended purpose.
The symposium of the validation of analytical procedures is directed to the four most common types.

- Identification tests.
- Quantitative tests for impurities content.
- Limit tests for the control of impurities.
- Quantitative tests of the active moiety in samples of drug substance or drug product or other selected components in the drug product.

Methods need to be validation and revalidation

- Before their introduction into routine use.
- Whenever the condition change for which the method has been validated e.g. instrument with different characteristics.
- Whenever the method is changed and the changes are outside the original scope of the method.
- Validation analytical method require the following
- Assuring quality
- Achieving acceptance of products by the international agencies.
- Mandatory requirement purposes for accreditation as per ISO 17025 guidelines.
- Mandatory requirement for registration of any pharmaceutical product or pesticide formulation.
- Validation methods are only acceptable for under taking proficiency testing.
- Validated/Evaluated method undergoes quality control procedures for further evaluation

Method development

Analytical method development and validation play important roles in the discovery development and manufacture of pharmaceuticals. These methods used to ensure the identity, purity, potency, & performance of drug products. There are many factors to consider when developing methods. The initially collect the information about the analyte's physicochemical properties (pKa, log P, solubility) and determining which mode of detection would be suitable for analysis (i.e., suitable wavelength incase of UV detection). The majority of the analytical development effort goes into validating a stability indicating HPLC–method. The goal of the HPLC-method is to try & separate quantify the main active drug, any reaction impurities, all available synthetic inter-mediates and any degraded products or metabolites.

Steps involve in method development are

1. Understand the physicochemical properties of drug molecule.
2. Set up HPLC conditions.
3. Preparation of sample solution for method development.
4. Method optimization.
5. Validation of method

Need of Analytical Method Validation

It is essential to employ well-characterized and fully validated analytical methods to yield reliable results in the laboratories while analysing the registration batch and accelerated stability testing samples. It is also important to emphasize that each analytical technique has its own characteristics, which will vary from analyte to analyte. In these instances, specific validation criteria may need to be developed for each analyte. Moreover, the appropriateness of the technique may also be influenced by the ultimate objective of the study. When sample analysis for a given study is conducted at more than one site and commercial batch for people consumption, it is necessary to validate the analytical method(s) as per ICH guidelines and to provide proper validation information for different sites and different parameter and to establish inter and intra laboratory reliability

Role of Validation in the Pharmaceutical Industry

Analytical method validation is require during drug development and validation to comply with current good manufacturing practices. Pharmaceutical industries should have an overall validation policies which documents how validation will be performed .This will include the validation of production process, cleaning procedures, computer system validation and analytical methods in process control test procedures .The purpose of this validation is to show that processes involved in the development and manufacture of drugs .

The cause that validation is included in cGMP in this way is to ensure that quality is built in every step and not just tested for at the end.

Components of method validation

Analytical method validation is defined as the process of proving (through scientific studies) that an analytical method is acceptable for its intended use or it is the process used to confirm that the analytical procedure employed for a specific test is suitable for The following are typical analytical performance characteristics which may be tested during methods validation.

- Accuracy
- Precision
- Repeatability

- Intermediate precision
- Linearity
- Detection limit
- Quantitation limit
- Specificity
- Range
- Robustness
- System suitability determination
- Forced degradation studies

Accuracy

The accuracy of an analytical method is the extent to which test results generated by the method and the true value agree. Accuracy can also be described as the closeness of agreement between the value that is adopted, either as a conventional, true or accepted reference value, and the value found. Accuracy is determined by replicate analysis of samples containing known amounts of the analyte. Accuracy should be measured using a minimum of five determinations per concentration. A minimum of three concentrations in the range of expected concentrations is recommended. Accuracy should be reported as percent recovery by the assay of known added amount of analyte in the sample or as the difference between the mean and the accepted true value, together with the confidence intervals.

Precision and reproducibility

The precision of an analytical method describes the closeness of individual measures of an analyte when the procedure is applied repeatedly to multiple aliquots of a single homogeneous mixture. Precision should be measured using a minimum of five determinations per concentration. A minimum of three concentrations in the range of expected concentrations is recommended. Precision is expressed as relative standard deviation. Precision is further subdivided into intraday precision means within same day run, interday precision means different day run and repeatability which measures precision with time and may involve different analysts, equipment, reagents, and laboratories. At least 6 determinations of 3 different matrices at 2 or 3 different concentrations should be performed, and the RSD calculated. The ICH (4) requires precision from at least 6 replications to be measured at 100 percent of the test target concentration or from at least 9 replications covering the complete specified range.

Linearity and range

The linearity of an analytical method is its ability to elicit test results that are directly proportional to the concentration of analytes in samples within a given range. Linearity is determined by a series of 3 to 6 injections of 5 or more standards whose concentrations span 80–120 percent of the expected concentration range. The response should be directly proportional to the concentrations of the analytes. A linear regression equation applied to the results should have an intercept not significantly different from 0. If there is a linear relationship test results should be evaluated by appropriate statistical methods.

- ✓ Correlation coefficient (r)
- ✓ Y-intercept
- ✓ Slope of regression line

The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

Detection Limit (LOD)

The limit of detection is the point at which a measured value is larger than the uncertainty associated with it. It is the lowest concentration of analyte in a sample that can be detected but not necessarily quantified. It can be determined by following ways.

Based on visual inspection

The detection limit is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected.

Based on signal to noise ratio

This method is applied to those procedures which shows baseline noise. This ratio is determined by comparing measured signals from samples with low concentration of analyte with those of blank sample and establishing the minimum concentration at which the analyte can be easily detected. For LOD this ratio is 3:1 or 2:1.

Based on Standard deviation of the response and the slope

Each sample is assayed repetitively and SD of the response is calculated and LOD is calculated as

$$LOD = 3.3 \times \sigma / S$$

Where (σ) SD and S is slope of regression equation.

For calculating standard deviation of the response based on the standard deviation of the blank measurement of the magnitude of analytical background response is performed by analyzing an appropriate number of blank samples and calculating the standard deviation of these responses.

For calculating standard deviation of the response based on the slope of the calibration curve a specific calibration curve is studied using samples containing an analyte in the range of the limit of detection. The residual standard deviation of a regression line, or the standard deviation of y-intercepts of regression lines, may be used as the standard deviation.

Based on the relative standard deviation (RSD)

For LOD RSD= 0.3 x LOQ

Based on plot
SD v/s concentration is plotted. LOD is three times the value of y-intercept.

Quantitation Limit (LOQ)
The LOQ is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is used particularly for the determination of impurities and/or degradation products. It can be determined by following ways.

Based on visual inspection
This is mostly used for non-instrumental methods but may be used for instrumental methods also. The detection limit is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected.

Based on signal to noise ratio
This method is applied to those procedures which shows baseline noise. This ratio is determined by comparing measured signals from samples with low concentration of analyte with those of blank sample and establishing the minimum concentration at which the analyte can be easily quantitated. For LOQ this ratio is 10:1.

Based on Standard deviation of the response and the slope
Each sample is assayed repetitively and SD of the response is calculated and LOQ is calculated as

$$LOQ = 10 \times \sigma / S$$

Where σ is SD and S is slope of regression equation.

Based on the relative standard deviation (RSD)

For LOQ RSD < 5%

Based on plot
SD v/s Concentration is plotted. LOQ is ten times the value of y-intercept.

Specificity
Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present (impurities, degradants, and matrix). It includes

Identity testing
To ensure the identity of an analyte

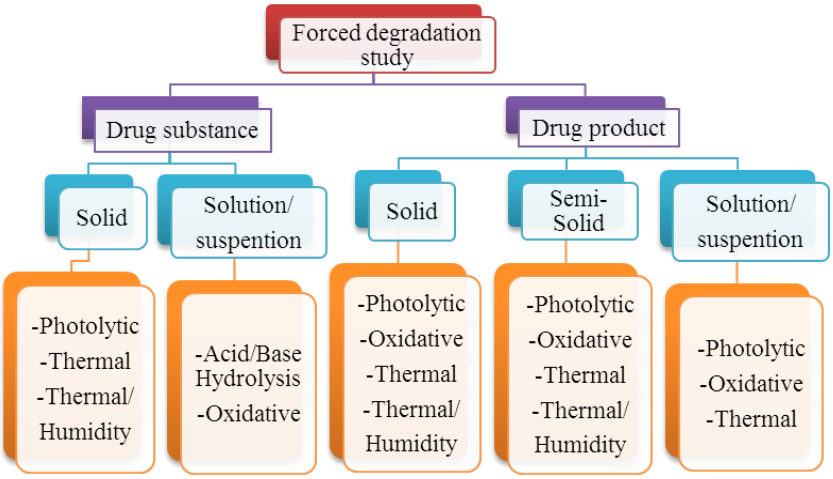
Purity testing
To ensure accurate statement on the content of impurities of an analyte

Assay
To allow an accurate statement on the content of an analyte in a sample**.**

Force Degradation Studies
The stability of a drug product or drug substance is a critical parameter which may affect purity, potency and safety. Changes in drug stability can risk patient safety by formation of a toxic degradation products or a lower dose than expected .therefore it is essential to know the purity profile and behaviour of a drug substance under various environmental conditions. Study design to intentionally degrade a drug substance or drug product by exposing to heat, light, moisture, and pH

Advantage of Force Degradation Studies-
- To facilitate salt selection or formulation optimization
- Developing stability-indicating analytical methods
- Provides information about degradation mechanisms and potential degradation products
- Preparing reference material of identified degradation products
- To understand the drug molecule chemistry and to generate more stable formulation

Force degradation carried out for following reasons
- ✓ To develop and validate stability indicating method.
- ✓ To determine degradation pathway of drug substance and drug product(e.g during development phase)
- ✓ To identify impurities related to drug substances or excipients
- ✓ To understand the drug molecule chemistry
- ✓ To generate more stable formulation
- ✓ To generate a degradation profile that mimics what would be observed in a formal stability study under ICH conditions
- ✓ To solve stability related problems(e.g. mass balance)**.**



An illustrative flow diagram is showing the different forced degradation conditions to be used for drug substances and drug products.

a) Acid and Alkali Hydrolysis

The hydrolytic degradation of a new drug in acidic and alkaline condition can be studied in 0.1 N HCl / 0.1 N NaOH. If reasonable degradation is seen, testing can be stopped at this point. However in case no degradation is seen under these conditions degradation should be tried in HCl / NaOH of higher strength and for longer duration of time. Alternatively if total degradation is seen after subjecting the drugs to initial condition, acid/alkali strength can be decreased along with decrease in reaction temperature.

b) Oxidative Degradation

To test for oxidation, it is suggested to use hydrogen peroxide in the concentration range of 3 to 50 %. In some drugs, extensive degradation is seen when exposed to 3% of hydrogen peroxide for very shorter time period at room temperature. In other cases exposure to high concentration of hydrogen peroxide, even under extreme condition does not cause any significant degradation. The behavior is on expected lines, as some drugs are in fact oxidisable, while others that are not. The latter are not expected to show any change even in the presence of large amount of oxidizing agent.

c) Photolytic Degradation

UV light: The photolytic studies should be carried out by exposure to light using either a combination of cool white & UV fluorescent lamp. Exposure energy should be minimum of 1.2 million lux hrs fluorescent light and if decomposition is not seen the intensity should be increased by 5 times. In case still no decomposition takes place, the drug can be declared photo stable.

d) Heat degradation

Heating the drug powder at higher temperature in oven can carry out stress testing for dry heat degradation. The heating time can be increased up to 12 hrs, if no sufficient degradation is seen in initial studies.

Table Conditions of Force Degradation

STUDY	CONDITIONS
Acidic pH	0.1 N HCl
Neutral pH	pH 7.0 Phosphate Buffer
Basic pH	0.1 N NaoH
Oxidation	O_2 Atmosphere, or H_2O_2
Photolysis (UV)	1000 watt h/M_2
Photolysis (Fluorescence)	6×106 lux h

CONCLUSION

The aim of this article is to provide a simple way to use approaches with a correct scientific background to improve the quality of the analytical method development and validation for identification, purification and finally to quantification any required drug etc. The main activities involved in the analytical development of a method are separation and characterization of impurities as well as degraded products, analytical investigations, studies for identification and finally setting up of parameters optimization to specific requirements.

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