ejpmr, 2017,4(09), 774-784

SJIF Impact Factor 4.161

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Review Article

ISSN 2394-3211

EJPMR

AN UPDATED REVIEW: ANALYTICAL METHOD VALIDATION

Ritesh Kumar Srivastava*[1] and S. Senthil Kumar[1,2]

[1]Macleods Pharmaceuticals Limited, R and D Center, Andheri-400059, Mumbai, India.
[2]Pacific University, Faculty of Pharmacy, Udaipur-313004, Rajasthan, India.

***Corresponding Author: Dr. Ritesh Kumar Srivastava**

Macleods Pharmaceuticals Limited, R and D Center, Andheri-400059, Mumbai, India.

Article Received on 20/07/2017 **Article Revised on 10/08/2017** **Article Accepted on 31/08/2017**

ABSTRACT

The method validation is the process by which it is established that performance characteristics of the method meet the requirements for the intended analytical applications. Methods need to be validated or revalidated before their introduction into routine use at the manufacturing unit after the drug development. To comply with the requirements of GMP, pharmaceutical industries should have an overall validation policy which documents how validation will be performed. Method validation is defined as the process of proving that an analytical technique is acceptable for the intended use and this is an important requirement for analytical purpose. Result from method validation can be used to judge the quality, reliability and consistency of analytical data. It can further help to avoid costly and time consuming exercises in analytical technique. Validation is an act of proving that any procedure, process, equipment, material, activity or system performs as expected under given set of conditions and also give the required accuracy, precision, sensitivity, ruggedness, specificity degradation path and sensitivity of the analytical method. The review focused on the concept, strategy and importance of analytical method validation and applicability in the pharmaceutical industry.

KEYWORDS: Validation, API, USP, ICH guidelines, SOP, Impurity, Quality control.

INTRODUCTION

Method validation is an integral part of the method development; it is the process by which a method is tested by the developer or user for reliability, accuracy and preciseness of its intended purpose. In the development of pharmaceuticals, analytical technique is important part of finalization of results and its techniques should be validated as per quality performances.[1,2] The employed analytical method either from official pharmacopeia or In-house developed method to be evaluated the determination for quality of pharmaceuticals. If In-house developed method, need to challenge every part of chromatographic condition, standard and sample solution preparation to validate the method. The official pharmacopeia methods are generally validated method and the verification and suitability of the method may check before carried out the analysis.[2,3] The analytical method should be revalidated if any change or modification of the drug composition, route of synthesis, analytical procedure. The chromatographic methods are generally applied for the quantification and qualitative analysis of drug substances and drug products. The chromatographic method involve the analysis of assay content, residual solvents, related compounds like process impurities, degradation impurities, leachable and extractable from container and closure or manufacturing process.[4] Method validation activity is the process applied to confirm that suitability of analytical method for a specific procedure for its intended use.[5,6] The results obtained from method validation study, shall be used to evaluate to judge the quality, accuracy and reliability of analytical results. This is integral part in any analytical practice. Analytical method should be validated or revalidated in the following circumstances:

- Introduction of method into routine use in the manufacturing units.
- The change in the conditions of method which has been validated e.g. change in the different formulation matrix, manufacturing process, used of different starting material supplied by various industries.
- Any change in the chromatographic condition of the developed method
  Requirement of validation for new or modified method to ensure that it is capable of providing reliable and reproducible results, when various analyst operating the same instrument in same or different laboratories.

This review provides an overview for the validation of analytical methods and recommendation in documentation in the pharmaceutical environment.

REGULATORY AND INDUSTRY OVERVIEW

USP pharmacopoeia under the general chapter <1225> stated that "Validation is the process of providing documented evidence that the method does what it is intended to do" In other words the process of method validation ensures that the proposed analytical methodology is accurate, specific, reproducible and rugged for its intended use.[7] Japanese pharmacopoeia under chapter sixteen stated that the statement "The validation of an analytical procedure is the process of confirming that the analytical procedure employed for a test of pharmaceutics is suitable for its intended use". In other word, the validation of an analytical procedure requires us to demonstrate scientifically that risk in decision by testing caused by errors from analytical steps are acceptably small. The validity of a proposed analytical procedure can be shown by demonstrating experimentally that the validation characteristics of the analytical procedure satisfy the standard set up according limit of testing.[8] The FDA guideline guidance for industry at the point VI.A stated that the "Analytical method validation is the process of demonstrating that an analytical procedure is suitable for its intended purpose.[9] The ICH Q2 (R1) stated that the "The validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose".[10] The world health organization guideline on validation stated that the "Validation is an essential part of good practices including good manufacturing practices and good clinical practices. It is therefore an element of the pharmaceutical quality system. Validation as a concept incorporates qualification and should be applied over the life cycle of, e.g. the applicable product, process, system, equipment or utility.[11] The Brazil (ANVISA) include guideline for validation of analytical and bioanalytical methods with stated that the "Validation is to demonstrate that the method is appropriate for the intended purpose, that is, the qualitative, semi-quantitative and/or quantitative determination of drugs and other substances in pharmaceutical products".[12]

IMPORTANCE AND ADVANTAGE OF ANALYTICAL METHOD VALIDATION

The importance of analytical method validation emerged due to international competition, maintaining the standard of products in high commercial & market value and ethical reasons. Various international regulatory agencies have set the standard and fixed the protocol to match the reference for granting approval, authentication and registration. The analytical method validation is required due to following reasons,[13-15]

1. Before initial use in routine testing and when transferred the analytical method to another laboratory.
2. Whenever the conditions or method parameters of pharmacopeial method was changed.
3. It is essential to employ well-characterized and fully validated analytical methods to yield reliable results in the laboratories. While analyzing the registration batch and accelerated stability testing samples.
4. It is also important to emphasize that each analytical technique has its own characteristics, which will vary from analyte to analyte.
5. For assuring the quality of the product.
6. For achieving the acceptance of the products by the international agencies.
7. A mandatory requirement for registration of any pharmaceutical product.
8. Reduction of quality cost, rejection, minimal batch failures, improved efficiently, productivity and improved analyst awareness of analysis.
9. Mandatory requirement for registration of any pharmaceutical product in the regulatory market.

The biggest advantage of method validation is that it builds a degree of confidence, not only for the developer but also to the user. Although the validation exercise may appear costly and time consuming, it results inexpensive, eliminates frustrating repetitions and leads to better time management in the end. Minor changes in the conditions such as reagent supplier or grade, analytical setup are unavoidable due to obvious reasons but the method validation absorbs the shock of such conditions and pays for more than invested on the process. The analytical method should be validated when it is necessary to verify that its performance parameters are adequate for use for a particular analytical problem or method just developed with revised new acceptance criteria.[16, 17]

STRATEGY AND STEPS FOR ANALYTICAL METHOD VALIDATION

The possible steps for a complete of analytical method validation are listed below.[1,18]

1. Develop a validation protocol or operating procedure for the validation.
2. Define the application, purpose and scope of the method (The scope of the method should include the different types of equipment and the locations where the method will be run).
3. Define the performance parameters and acceptance criteria.
4. Define validation experiments.
5. Verify relevant performance characteristics of equipment.
6. Qualify materials, e.g. standards and reagents.
7. Perform pre-validation experiments.
8. Adjust method parameters or/and acceptance criteria if necessary.
9. Perform full internal (and external) validation experiments.
10. Define criteria for revalidation, Define type and frequency of system suitability tests.
11. Document validation experiments and results in the validation.

First the scope of the method and its validation criteria should be defined. These include: compounds, matrices, type of information, qualitative or quantitative, detection and quantitation limits, linear range, precision and accuracy, type of equipment and location. The scope of

the method should include the different types of equipment and the locations where the method will be run.

TYPES OF ANALYTICAL METHODS TO BE VALIDATED

The four most common types of analytical methods are directed to be validated:

1. Identification tests.
2. Quantitative tests for impurities content.
3. Limit tests for the control of impurities.
4. Quantitative tests of the active moiety in samples of a drug substance.

Identification tests are intended to ensure the identity of an analyte in a sample. This is achieved by comparison of a property of the sample (e.g., spectrum, chromatographic behavior, chemical reactivity, etc.) to that of a reference standard. Testing for impurities can be either a quantitative test or a limit test for the impurity in a sample. The test is intended to reflect the purity characteristics of the sample. Different validation characteristics are required for a quantitative test than for a limit test. Assay procedures are intended to measure the analyte present in a given sample. In the perspective of this document, the assay represents a quantitative measurement of the major component(s) in the drug substance.

ANALYTICAL METHOD VALIDATION PARAMETERS

The validation of an analytic method demonstrates the scientific soundness of the measurement or characterization. It is required to varying extents throughout the regulatory submission process. The validation practice demonstrates that an analytic method measures the correct substance, in the correct amount and in the appropriate range for the samples.[19-22] It allows the analyst to understand the behavior of the method and to establish the performance limits of the method. In order to perform method validation, the laboratory should follow a written standard operating procedure (SOP) that describes the process of conducting method validation. The laboratory should use qualified and calibrated instrumentation. There should be a well-developed and documented test method and an approved protocol prior to validation. The protocol is a systematic plan that describes which method performance parameters should be tested, how the parameters will be assessed with its acceptance criteria.[23] According the guidelines the following parameter should be captured to establish the rugged, reproducible, precise, accurate, specific, sensitive and stability indicating of analytical method.[24-27]

Specificity

Specificity is the ability to assess the analyte for the presence of various components that may be present. It can be established by a number of approaches, depending on the intended purpose of the method. The ability of the method to assess the analyte of interest in a drug product is determined by a check for interference by placebo. Specificity can be assessed by measurement of the API in samples that are spiked with impurities or degradants. If API-related compounds are not available, drug can be stressed or force-degraded in order to produce degradation products. In chromatographic separations, apparent separation of degradants may be confirmed by peak purity determinations by photodiode array, mass purity determinations by mass spectroscopy (MS) or by confirming separation efficiency using alternate column chemistry. Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedures.

Selectivity

The term selectivity is sometimes used interchangeably with specificity. Technically, however, there is a difference. Selectivity is defined as the ability of the method to separate the analyte from other components that may be present in sample, including impurities. Selectivity is separate and shows every component in the sample. Therefore, one could have a method that is specific, yet it may not be selective.[28]

Forced Degradation

Forced degradation or stress studies are undertaken to deliberately degrade the sample (e.g., drug product, excipients, or API). These studies are used to evaluate an analytical method ability to measure an active ingredient and its degradation products, without interference, by generating potential degradation products. During validation, samples of drug product (spiked placebos) and drug substance are exposed to heat, light, acid, base, and oxidizing agent to produce approximately 10% to 30% degradation of the active substance. The degraded samples are then analyzed using the method to determine if there are interferences with the active or related compound peaks. Forced degradation studies can be time consuming and difficult because it is often difficult to generate the proper level of degradation. Also, a certain amount of logic needs to be applied to extrapolate the results of these studies to what might be seen during actual stability studies.[29]

Detection Limit (LOD)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. It can be determined visually, by signal to noise ratio, standard deviation of the response and the slope. Detection limit signal to noise approach can only be applied to analytical procedures which exhibit baseline noise. Comparing measured signals from samples with known concentrations of analyte with those of blank samples and establishing the minimum concentration at which the analyte can be reliably detected. A signal-to-noise ratio between 3 or 2:1 is generally considered acceptable for estimating the detection limit. The detection limit (DL) may be expressed as: $DL=3.3\ \sigma/\ S$

where, σ is the standard deviation of the response, S is the slope of the calibration curve. The slope S may be estimated from the calibration curve of the analyte. The estimate of σ may be carried out in a variety of ways, based on the standard deviation of the blank and the calibration curve.

Quantitation Limit (LOQ)

The Quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products. It can be determined visually, by signal to noise ratio, standard deviation of the response and the slope. Quantitation limit signal to noise approach can only be applied to analytical procedures which exhibit baseline noise. Comparing measured signals from samples with known concentrations of analyte with those of blank samples and establishing the minimum concentration at which the analyte can be reliably detected. A signal-to-noise ratio between 10 or 10:1 is generally considered acceptable for estimating the quantitation limit. The quantitation limit (QL) may be expressed as: QL=10 σ/ S where, σ is the standard deviation of the response, S is the slope of the calibration curve. The slope S may be estimated from the calibration curve of the analyte. The estimate of σ may be carried out in a variety of ways, based on the standard deviation of the blank and the calibration curve. The LOQ level is usually confirmed by injecting standards which have an acceptable percent relative standard deviations (% RSD) not more than 10%.[8]

Linearity

The linearity of an analytical procedure is its ability to obtain test results that are directly proportional to the concentration of analyte in the sample. Test results should be evaluated by appropriate statistical methods, by calculation of a regression line like by the method of least squares. Correlation coefficient, y-intercept, slope of the regression line and residual sum of squares for which a minimum of five concentrations are recommended.[26]

Range

The range of an analytical procedure is the interval between the upper and lower concentration of analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity. Range is normally expressed in the same units as test results (e.g., percent, parts per million) obtained by the analytical method.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. It can be sub divided into repeatability, intermediate precision and reproducibility. Precision should be investigated using homogeneous, authentic samples. However, if it is not possible to obtain a homogeneous sample it may be investigated using artificially prepared samples or a sample solution. The standard deviation, relative standard deviation like coefficient of variation and confidence interval should be reported for each type of precision investigated.

1. **Repeatability:** Repeatability is also termed intra-assay precision. Repeatability is the variation experienced by a single analyst on a single instrument. Repeatability does not distinguish between variation from the instrument or system alone and from the sample preparation process. During the validation, repeatability is performed by analyzing multiple replicates of an assay composite sample by using the analytical method. Repeatability should be assessed using a minimum of 9 determinations covering the specified range for the procedure by 3 replicates or 6 determinations at 100% of the test concentration.

2. **Intermediate precision:** Intermediate precision expresses within-laboratories variations: different days, different analysts, different equipment, etc. Intermediate precision depends upon the circumstances under which the procedure is intended to be used. The applicant should establish the effects of random events on the precision of the analytical procedure. Typical variations to be studied include days, analysts, equipment, etc. It is not considered necessary to study these effects individually. The use of an experimental design (matrix) is encouraged. A statistical comparison is made to the first analyst's results.

3. **Reproducibility:** Reproducibility expresses the precision between laboratories (collaborative studies, usually applied to standardization of methodology). Reproducibility is assessed by means of an inter-laboratory trial. Reproducibility should be considered in case of the standardization of an analytical procedure, for instance, for inclusion of procedures in pharmacopoeias. These data are not part of the marketing authorization dossier.

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness and several methods available of determining the accuracy. Accuracy should be established across the specified range of the analytical procedure. Accuracy should be assessed using a minimum of 9 determinations over a minimum of 3 concentration levels covering the specified range (e.g., 3

concentrations/3 replicates each of the total analytical procedure). 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.

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 provides an indication of its reliability during normal usage. The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. It should show the reliability of an analysis with respect to deliberate variations in method parameters. Robustness is typically assessed by the effect of small changes in chromatographic methods on system suitability parameters such as peak retention, resolution and efficiency. Experimental factors that are typically varied during method robustness evaluations include: (i) age of standards and sample preparations (ii) sample analysis time (iii) variations to pH of mobile phase (iv)variation in mobile phase composition (v) analysis temperature (vi) flow rate (vii) column manufacturer (viii) type and use of filter against centrifugation. Robustness experiments are an ideal opportunity to utilize statistical design of experiments, providing data-driven method control.

Ruggedness

Ruggedness is measure of reproducibility test results under the variation in conditions normally expected from laboratory to laboratory and from analyst to analyst. The Ruggedness of an analytical method is degree of reproducibility of test results obtained by the analysis of the same samples under a variety of conditions, such as; different laboratories, analysts, instruments, reagents, temperature, time etc.

System Suitability

System suitability is the evaluation of the components of an analytical system to show that the performance of a system meets the standards required by a method. A system suitability evaluation usually contains its own set of parameters. For chromatographic assays, these may include tailing factors, resolution, and precision of standard peak areas, and comparison to a confirmation standard, capacity factors, retention times, and theoretical plates. During validation, where applicable, system suitability parameters are calculated, recorded, and trended throughout the course of the validation.

Solution Stability

During validation the stability of standards and samples is established under normal bench top conditions, normal storage conditions and sometimes in the instrument (e.g., an HPLC autosampler) to determine if special storage conditions are necessary, for instance, refrigeration or protection from light. Stability is determined by comparing the response and impurity profile from aged standards or samples to that of a freshly prepared standard and to its own response from earlier time points.

Filter retention

Filter retention studies are a comparison of filtered to unfiltered solutions during a methods validation to determine whether the filter being used retains any active compounds or contributes unknown compounds to the analysis. Blank, sample, and standard solutions are analyzed with and without filtration. Comparisons are made in recovery and appearance of chromatograms.

Extraction efficiency

Extraction efficiency is the measure of the effectiveness of extraction of the drug substance from the sample matrix. Studies are conducted during methods validation to determine that the sample preparation scheme is sufficient to ensure complete extraction without being unnecessarily excessive. Extraction efficiency is normally investigated by varying the shaking or sonication times (and/or temperature) as appropriate during sample preparation on manufactured (actual) drug product dosage forms.

The best practice to start validation of the stability indicating method for related substance in the following order is probably best i.e. Selectivity< LOD/LOQ< Forced degradation study. During analytical method validation if the problems occurred with selectivity, then analyst are dead before start the validation. At the beginning of the analytical method validation to ensure the selectivity. If method cannot detect a related compounds at the lowest level needed, then scientist won't be able to ensure to meet the specifications.

METHOD VERIFICATION

Method verification consists of partial validation. It should be performed for already validated analytical methods under the following circumstances:[30,31]

1. When an already validated method is used on a product for the first time (e.g. in case of a change in active pharmaceutical ingredient (API) supplier, change in the method of synthesis or after reformulation of a drug product).
2. When an already validated method is used for the first time in a laboratory (in some cases, method transfer may be preferable).
3. Method verification is suitable in lieu of method validation for pharmacopoeial methods.
4. Method verification may include only the validation characteristics of relevance to the particular change. For instance, in the case of a change in API supplier, the only expected difference would be in the impurity profile or solubility of the API, and therefore, for a related substances method, there should be an appropriate verification that the method is able to detect and quantitative all potential impurities, even the late eluting ones. Specificity should be among the tests considered.

A partial validation is typically allowed for a well-established analytical method, if only a few of the following parameters have been changed: analyst, instrument within the same company, reagent purity, species within matrix, change in concentration range, chromatographic conditions, detection conditions, and sample preparation. A partial validation may also be performed if dealing with limited matrix volume or rare matrix.[30]

METHOD REVALIDATION

Any of the validation parameters of the analytical method would be adjusted if the performances of the method fall outside the analytical acceptance criteria, or to boost the quality of the results. The revalidation is compulsory if a parameter is modified outside the operating range. Several analytical laboratories are reticent to improve their methods, to avoid performing a full revalidation, especially if they are surveyed by legal accreditation agencies. The revalidation degree depends on the extent of the influence of the changes on the performances of the method and decided on the basis of the fit of the results with the predefined acceptance criteria for each parameter. The parameters to be revalidated are determined by carrying out a system suitability test and analyses of control quality samples are carried out. Revalidation of an analytical procedure should be considered whenever there are changes made to the method, including changes to the mobile phase, column, temperature of the column, concentration/composition of the sample / standards and wavelength of detection. Periodic revalidation of analytical methods should be considered according to a period that is scientifically justifiable. It is acceptable for revalidation to include only the validation characteristics of relevance to the particular change and method. In the USP general chapter stated that In the case of compendial procedures, revalidation may be necessary in the following case: a submission to the USP of a revised analytical procedure; or the use of an established general procedure with a new product or new raw material.[7] The ICH guideline gives guidance on the necessity for revalidation in the following assets: changes in the synthesis, changes in the composition of the product; and changes in the analytical procedure.[10]

TRANSFERRING OF VALIDATED ANALYTICAL METHODS

Timely method transfer plays an important role in expediting drug candidates through development stages. Method transfer is not an easy task and requires careful planning and constant communication between the laboratory personnel involved in the transfer. Method transfer could occur within the same organization or between pharmaceutical companies and analytical service providers. To have a successful transfer, the analytical method itself must be robust and the equipment differences between the delivering and receiving parties should be carefully evaluated. Use of standardized automation equipment has shown to be advantageous during the method transfer.[32] Nowadays, there is no official document available that can be used as a guide for the receiving laboratory to estimate the success of the method transferring. The USP has published an article describing the most common practices of method transfer: comparative testing, covalidation between two laboratories or sites, complete or partial method validation or revalidation, and the omission of formal transfer, sometimes called the transfer waiver.[33,34] The transference of analytical method to the laboratory should be disciplined by a well-established procedure. It is important to note that the receiving laboratory has the entire responsibility to control and assess the validity of the transfer. However, the issuing laboratory should collaborate, as far as possible, during the process. The below mention point should consider during transfer of the method:

1. Appoint analytical method transfer project team, develop a transfer plan and transfer the scientific documentation to receiving location.
2. Train the scientist/chemist who receive the tasks related to the instrumentation, the method, the critical parameters, and the system suitability of problems.
3. Define and execute the tests to evaluate the success of the validation: some critical experiments of the method validation (at least two), and analysis of samples: type and number of samples (a minimum of three), replicates, and so on.
4. Generally, it should be performed by comparing a set of results obtained by an analyst in one laboratory to that obtained by another analyst at the laboratory to which the method is being transferred.
5. Mention the acceptance criteria (tolerated deviation from those obtained by the issuing laboratory, accepted bias, and uncertainty).
6. If the test results conform to the acceptance criteria, the analytical method transfer is successful. Otherwise, the transfer is unsatisfactory, and the reasons of failure must be investigated and corrected.
7. During method transfer, documented evidence should be established to prove that a method has equivalent performance when used in a laboratory different from that where it has been originally validated.
8. The two sets of results should be statistically compared and the differences between the two sets of test results should be within an acceptable range.
9. Method transfer should be performed before testing of samples for obtaining critical data for a dossier, such as process validation or stability studies or applied for routine use.
10. A predefined protocol should be followed which includes at least: a title, objective, scope, responsibilities of the research & development and the quality control lab; a specification of materials and methods; the experimental design and acceptance criteria; documentation (including information to be supplied with the results, and

report forms to be used, if any); procedure for the handling of deviations; references; and details of reference samples (starting materials, intermediates and finished products). The protocol should be authorized and dated.

DOCUMENTATION

The validity 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 validation report. General and specific standard operating procedures, good record keeping are an essential part of a validated analytical method.[35]

Method Validation Protocol

Depending upon of the practice of the industry, a method validation protocol could be simple or comprehensive and each parameter to be validated is described in detail with the acceptance criteria. If the acceptance criteria were not met, a deviation is added and the proper justification must be given. If it is deemed that the justification is not appropriate, then an action plan for the specific figure of merit in question is determined (i.e., repeat analysis, change of the analytical procedure, and revalidation). Also, if the analytical method has not been approved at the time of writing the validation protocol yet, it is recommended to attach a final draft of the method to the protocol.[35-38]

Before starting the experimental work, the protocols must be written by a qualified person and approved by a quality assurance department. The following points that are necessary to be specified in the validation protocol are listed below:

- The analytical method for a given product or drug substance.
- The test to be validated e.g. Assay, Related substances, Residual solvents, Dissolution, Identification and Solubility.
- The test parameters for each test, including type and number of solutions and number of tests
- The acceptance criteria for each parameter based on an internal standard operational procedure (product or method-specific adaptations may be necessary and are acceptable, if justified).
- List of batches of drug substance and/or drug products.
- For a drug product the grade/quality of the excipients used in the formulation.
- List of reference materials to be used in the validation experiments.
- Information of the instruments and apparatus to be used.
- Responsibilities should be captured in protocol i.e. scientist, chemists, analytical research project leader, quality assurance, quality control and regulatory etc.

Method Validation Report

The validation report must contain reference to the analytical methods (specific code) and name of the corresponding drug substance or product. In the validation reports the results may be filled in a predefined table and compared with the acceptance criteria. The list of reference materials (reference standards with the appropriate certificate of analysis) as well as the list of calibrated and qualified instruments used in the validation experiments should be documented in the report. The list of the batches of drug substances, drug product & excipients, laboratory notebook number, reference number of impurities should also be listed. The test parameters and acceptance criteria must be listed together with the results for each test, and the passed or failed results should be indicated. The validation report should also contain whether the method validation was successful and if any changes should be applied to the analytical method, and then the final analytical method must be resubmitted for quality assurance approval. The following points that are necessary to be specified in the validation report are listed below, [39-43]

- Objective and scope of the method (applicability, type) and Summary of methodology.
- All chemicals, reagents, reference standards, quality control samples with purity, grade, their source, or detailed instructions on their preparation.
- Procedures for quality checks of standards and chemicals used on that.
- Method parameters and critical parameters from robustness testing.
- Listing of equipment and its functional and performance requirements, e.g., cell dimensions, baseline noise, and column temperature range.
- Detailed conditions on how the experiments were conducted, including sample preparation. The report must be detailed enough to ensure that it can be reproduced by a competent technician with comparable equipment.
- Statistical procedures and representative calculations.
- Representative plots, e.g., chromatograms, spectra, and calibration curves.
- Method acceptance limits performance data and the expected uncertainty of measurement results.
- Approval with names, titles, date, and signature of those responsible for the review and approval of the validation report.
- Any deviations from SOPs, Protocols, GLP (If applicable), Instrument and acceptance criteria the justifications for deviations with the incident report to be captured.

A generalized flowchart of the analytical method validation detailed given in figure-1.

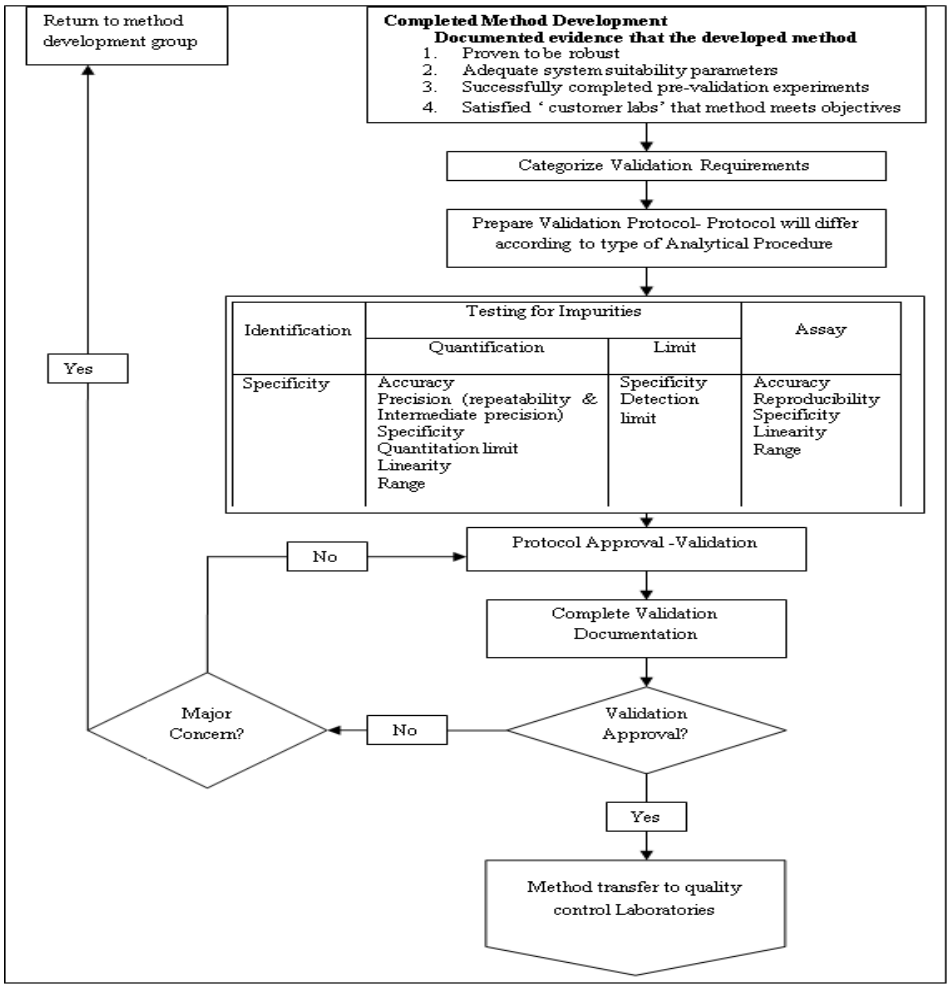

Figure1: Generalized analytical method validation flowchart.

PRACTICAL AND ECONOMICAL PARAMETER

The validated analytical with accomplished performance but expensive and contaminated will not be appropriate. The method is considered as "useful for routine analysis" if it achieves the acceptance criteria decided by the analytical laboratory. Additionally, the workplace safety and the waste of toxic compounds are strongly regulated and controlled by legal institutions, and strong penalties are proposed in case of breach. The following main parameters should be considered as practical and economical wise for analytical method validation in routine analysis at quality control laboratory.[44,45]

Cross contamination

The cross contamination is described as the modification of a sample during the analysis procedure, because of the previously analyzed sample. It can be caused by the matrix and the analyte itself and the cross contamination is also known as carryover.[46] Traces of an injected aliquot could remain in the chromatographic equipment, especially in the injection needle, and slightly accumulate. Thus, it can be dragged by the mobile phase and added to the next sample. The matrix compound can increase the noise and disturb the detection of the analyte. Besides, the remaining amount of analyte is added to the analyte from the next sample, thus falsely increasing the measured concentration. This problem is caused by highly concentrated samples. To evaluate the extent of the cross contamination, concentrated samples (near and over the LOQ) and a blank sample are successively analyzed. If a signal is detected in the blank chromatogram at the elution time of the analyte, the cross contamination is significant, and then an intermediate cleaning step or the reduction of the LOQ must be view. Anyway, the cleaning of the injection system after each analysis is recommended to avoid cumulative effects.[47]

Analysis time

The analysis time per sample can be calculated in several ways,[3,48]

1. Duration of the analysis of a single sample: As the sum of the duration of the sample preparation and the chromatographic analysis.
2. Simultaneous analysis of several samples: Applicable if a set of samples can be simultaneously processed. It is calculated as the time taken to analyze the whole set divided by the number of analyzing samples. The analysis time per sample normally decreases if the number of analyzed samples increases.

3. Successive analysis of several samples: If the analysis of the two sets of samples can be overlaid. For instance, a sample can be prepared while another is analyzed by the HPLC. In this case, the total analysis time is reduced. The analysis per sample is calculated as the number of samples analyzed during a specific time.

The reduction of the analysis time provokes a significant improvement in the financial bottom line of the analytical laboratory.

Simplicity

The simplicity of a procedure/ method is a subjective and qualitative parameter, which measures the probability of making a mistake. It depends on the skills of the worker and facilities of the laboratory.[49] The complexity is increased by the difficulty of each step and the number of steps. A step can be considered more complicated if there are more variables to establish (e.g., isocratic elution is easier than gradient elution), the intervention of the operator is increased (an automated injection is easier than a manual injection), and the solvents and equipment are stable and easy to handle. A complex method would provide less reliable results.[50]

Cost per analysis

The price of the analysis is most important for a commercial quality control laboratory. It should be reduced as much as possible, without losing analytical performances, in order to compete with other laboratories. It can be calculated considering the price and the amount of consumed chemicals, amortization of instrumentation (purchase and reparation), the salary of the workers, local taxes, and the management of toxic waste.[44, 51]

Safety for laboratory team

The concern about the workplace health and safety is nowadays high. This is especially important in a chemical laboratory, where hazardous compounds have to be handled. In fact, the use of the more toxic chemicals is being banned, and they are substituted by less harmful materials. The laboratories are expected to implement the mandatory safety protocols and facilities to protect the health of the laboratory staff. The risk for the worker is a qualitative parameter. It can be estimated considering the inherent toxicity and the manipulated amount of each chemical, volatility, flammability, and the probability of skin contact or inhalation.[52]

Environmental impact

The society is nowadays very conscious about the protection of the environment. Therefore, the current tendency in analytical chemistry points to the development of more eco-friendly methods. Besides, the laboratory must implement adequate waste treatment, following the legal rules. The environmental impact of the method can be reduced by prioritizing the use of innocuous solvents and reagents, reducing the amount of toxic compounds and diminishing the volume of the waste.[53]

POINT TO BE CONSIDER DURING VALIDATION

The following should consider during the analytical method validation.[1,10]

- Validation should be performed in accordance with the validation protocol. The protocol should include procedures and acceptance criteria for all characteristics. The results should be documented in the validation report.
- If the pharmacopoeial methods are available and used a non-pharmacopoeial methods justification should be required. Justification should include data such as comparisons with the pharmacopoeial or other methods.
- The test methods should be described in detail and should provide sufficient information to allow properly trained analysts to perform the analysis in a reliable manner. As a minimum, the description should include the chromatographic conditions (in the case of chromatographic tests), reagents needed, reference standards, the formulae for the calculation of results and system suitability tests.

ISSUES AND CHALLENGES

For a method development and validation curriculum to be successful, a comprehensive way is recommended. A common challenge encountered during methods development and validation is that methods are typically developed by the research and development division whereas analytical validation is typically the responsibility of a quality assurance and quality control. It is most important that the entire department work as one team. Each department may be responsible for assure the suitability of the methods to support various phases and commercial manufacturing units. 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. Because the method will be run by several groups during its progress from development to validation but the method must be robust precise and rugged.[54-57] A common weakness in development and validation of methods is that the methods are not robust enough. If robustness is not built into methods early in development then the results are likely to lack efficiency in quality testing and encounter lengthy and complicated validation process. It is achieved by conducting forced-degradation studies. The design and execution of these studies requires thorough knowledge of the product being tested as well as a good understanding of the analysis technique. The regulatory guidelines are being published that govern the expectations of regulatory agencies throughout the world for methods development and validation. There is need to meet current regulatory standards. From a simple method improvement to a complete redevelopment and subsequent implementation is boring target. For this reason, one must be alert to current trends in regulatory guidelines and to adopt a proactive approach to changes

that may affect development and validation programs.[58-60] Finally, one of the key requirements for methods validation is that only well-characterized reference materials with proper documented purities should be used during method validation.

CONCLUSION

In the recent years the introduction of new drug molecules are increased and day by day competition is more to satisfy the customer and regulatory market. There are no standards and analytical procedures in pharmacopeias for testing of the drug. So, analytical method development and validation is playing an important role in most of the pharmaceutical industries for testing, releasing of commercial batches and retest period of the drug. For this reason and the need to satisfy regulatory authority requirements by following GMP and GLP guideline, all analytical methods should be properly validated and documented inline with the acceptance criteria as per ICH guideline. In this review we discussed about validation parameters of analytical methods, strategy, importance, practical and economical parameter. Hence we concluded that the validation of developed analytical methods is critical elements in the development of pharmaceuticals. Success in these areas can be attributed to several important factors, which in turn will contribute to regulatory compliance.

REFERENCES

1. Pasbola K, Chaudhary M. Updated review on analytical method development and validation by HPLC. World J Pharm Pharm Sci., 2017; 6(5): 1612-30.
2. Toomula N, Kumar A, Kumar DS, Bheemidi VS. Development and validation of analytical methods for pharmaceuticals. J Anal Bioanal Technigues, 2011; 2(5): 2-4.
3. Ravisankar P, Navy CN, Pravallik D, Sri DN. A Review on step-by-step analytical method validation. IOSR J Pharm, 2015; 5(10): 07-19.
4. Uslu B, Lingeman H, Ozkan SA, Patil M, Dogan-Topal B. Analytical method development and validation of pharmaceutical analysis using chromatographic techniques. Chromatogr Res Int, 2012; 2012: 1.
5. Chandran S, Singh RSP. Comparison of various international guidelines for analytical method validation. Pharmazie, 2007; 62: 4-14.
6. Ermer J, Miller JHM. Method validation in pharmaceutical analysis: A guide to best practice. Wiley-VCH, Germany, 2005; 3-9.
7. United States Pharmacopeia. USP 39-NF 34, 2016.
8. Japanese Pharmacopeia. JP XVII Edition, 2016.
9. FDA guidance for industry, Analytical procedure and method validation for drugs and biologics, Food and Drug administrator, 2015.
10. International conference on harmonization; Validation of analytical procedure: Text and Methodology Q2 (R1); 2005.
11. WHO Draft guideline, Guidelines on validation-appendix4, analytical method validation 2016.
12. ANVISA, Guideline for bioanalytical method validation, 2012.
13. Pranshu T, Singh RP, Vikash J, Lakshmayya. Validation: A critical parameter for quality control of pharmaceuticals. J Drug Delivery Ther, 2012; 2(3): 34-40.
14. Bhagyasree T, Neelam I, Ajitha A, Uma Maheshwara Rao V. A review on analytical method development and validation. Int J Pharm Res Anal, 2014; 4(8): 444-8.
15. Ravichandran V, Shalini S, Sundram KM, Rajak H. Validation of analytical methods-strategies & importance. Int J Pharm Pharm Sci., 2010; 2(3): 18-22.
16. Bansal KS, Layloff T, Bush ED, Hamilton M, Hankinson EA, Landy JS, Lowes S, Nasr MM, St. Jean PA, Shah VP. Qualification of analytical instruments for use in the pharmaceutical industry: a scientific approach. AAPS Pharm Sci Tech, 2004; 5: 1-8.
17. Jenke DR. Chromatographic method validation: a review of current practices and procedures, II guidelines for primary validation parameters. J Liq Chromatogr Relat Technol, 1996; 19(5): 737-57.
18. Reddy VP, Rajan VST, Kumar NA, Angalaparameswari S, Chetty CM. A review on analytical method validation. Int J Rev Life Sci., 2011; 1(3): 141-4.
19. Campana SE. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. J Fish Biol., 2001; 59(2): 197-242.
20. Lavanya G, Sunil M, Eswarudu MM, Eswaraiah MC, Harisudha K, Spandana BN. Analytical method validation: An updated review. Int J Pharm Sci Res, 2013; 4(4): 1280-6.
21. Bhardwaj SK, Dwivedi K, Agarwal DD. A review: HPLC method development and validation, Int J Anal Bioanal Chem., 2015; 5(4): 76-81
22. Hema, Reddy GS. A review on new analytical method development and validation by RP-HPLC. Int Res J Pharmal Biosci, 2017; 4(3): 41-50.
23. Raman NVVSS, Mallu UR, Bapatu HR. Analytical quality by design approach to test method development and validation in drug substance manufacturing. J Chem., 2015; 1-8.
24. Rao G, Goyal A. An overview on analytical method development and validation by using HPLC. Pharma Chem J., 2016; 3(2): 280-9.
25. Pushpa LE, Sailaja B. Bioanalytical method development and validation by HPLC: a review. J Med Pharm Innovation, 2014; 1(6S): 1-9.
26. Patel RA, Shah CN. Method validation and method development by using analytical method-HPLC-review on current research. J Pharm Sci Bioscientific Res., 2016; 6(5): 728-32.
27. Prathapa B, Deyb A, Rao GHS, Johnsona P, Arthanariswaran P. A review-importance of RP-

HPLC in analytical method development, Int J Novel Trends Pharm Sci., 2013; 3(1): 15-23.
28. Shrivastava A, Gupta VB. HPLC: Isocratic or gradient elution and assessment of linearity in analytical methods. J Adv Scient Res., 2012; 3(2): 12-20.
29. Srivastava RK, Kumar SS. An updated review: forced degradation study. World J Pharm Pharm Sci, 2017; 6(6): 709-26.
30. Patel P, Patel N, Patel A, Patel A, Vyas A. Bio analytical method development and validation: A review. Int J Appl Pharm Biol Res., 2016; 1(2): 191-6.
31. Gholve SB, Ajgunde RR, Bhusnure OG, Thonte SS. Analytical method development and validation by QbD approach-A review. Der Pharmacia Sinica, 2015; 6(8): 18-24.
32. Rudaz S, Hubert P. Method validation, comparison and transfer. J Chromatogr B, 2009; 877(23): 2179.
33. Charde MS, Welankiwar AS, Kumar J, Chakole RD. Bioanalytical method development and validation. Int J Adv Pharm Anal, 2013; 3(4): 90-4.
34. Miller JM. Chromatography concept and contrasts. Second addition, Johan Wiley & Sons, Inc. New Jersey, Canada, 2005: 25-30.
35. Peters FT, Drummer OH, Musshoff F. Validation of new methods. Forensic Sci Int, 2007; 165: 216–24.
36. Bliesner DM. Validating chromatographic methods: A Practical Guide. Johan Wiley & Sons, Inc. Hoboken, New Jersey, 2005: 25-30.
37. Geetha G, Raju KNG, Kumar BV, Raja MGG. Analytical method validation: An updated review. Int J Adv Pharm Biol Chem., 2012; 1(1): 64-71.
38. Sudha T, Krishana Kanth V, Sainath NPC, Mishal, Saloman, Raja T, Ganesan V. Method development and validation-A review. J Adv Pharm Educ Res., 2012; 2(3): 146-76.
39. Braggio S, Barnaby RJ, Grosi P, Cugola MA. Strategy for validation of bio analytical methods. J Pharma Biomed Anal, 1996; 14: 375-88.
40. Breda CA, Breda M, Frigerio E. Bio analytical method validation: A risk based approach. J Pharma Biomed Anal, 2004; 35: 887-9.
41. Patil R, Deshmukh T, Patil V, Khandelwal K. Review on analytical method development and validation. Res Rev J Pharml Anal, 2014; 3(3): 1-10.
42. Kazusaki M, Ueda S, Takeuchi N, Ohgami Y. Validation of analytical procedures by high-performance liquid chromatography for pharmaceutical analysis. Chromatogr, 2012; 33(2): 65-73.
43. Azim MDS, Mitra M, Bhasin PS. HPLC method development and validation: A review. Int Res J Pharm, 2013; 4(4): 39-46.
44. Shavir GA, Lough WJ, Arain SA, Bradshaw TK. Evaluation and application of best practice in analytical method validation. J Liq Chromatogr Relat Technol, 2007; 30(3): 311-33.
45. Prthap B, Rao GHS, Devdass G, Dey A, Harikrishnan N. A review on stability indicating method development. Int J Innovative Pharm Res., 2012; 3(3): 229-37.
46. Riley CM, Rosanske TW. Development and validation of analytical methods. First edition, Elsevier Science LTD, New Yark, 1996; 200-201.
47. Pranshu T, Singh RP, Vikash J, Lakshmayya. Validation: A critical parameter for quality control of pharmaceuticals. J Drug Delivery Ther, 2012; 2(3): 34-40.
48. Breaux J, Jones K, Boulas P. Understanding and implementing efficient analytical methods development and validation. Pharma Technol Anal Chem Test, 2003; 5: 8-13.
49. Ahuja S, Scypinski S. Handbook of modern pharmaceutical analysis, separation science and technology. Edition third, Academic press, London, 2001: 415-42.
50. http://www.ivtnetwork.com.
51. Green JM. Peer reviewed: A Practical Guide to Analytical Method Validation. Anal Chemi, 1996; 68(9): 305A–9A.
52. Taverniers I, Loose MD, Bockstaele EV. Trends in quality in the analytical laboratory. II. Analytical method validation and quality assurance. Trends Anal Chem., 2004; 23(8): 535-52.
53. Betz JM, Brown PN, Roman MC. Accuracy, precision, and reliability of chemical measurements in natural product research. Fitoterapia, 2011; 82(1): 44-52.
54. Belouafa S, Habti F, Benhar S, Belafkih B, Tayane S, Hamdouch S, Bennamara A, Abourriche A. Statistical tools and approaches to validate analytical methods: methodology and practical examples. Int J Metrol Qual Eng, 2017; 8(9): 1-10.
55. Weitzel MLJ. The estimation and use of measurement uncertainty for a drug substance test procedure validated according to USP 1225. Accredit Qual Assur, 2012; 17(2): 139-46.
56. Raman NVVSS, Mallu UR, Bapatu HR. Analytical quality by design approach to test method development and validation in drug substance manufacturing. J Chem, 2015; 1–8.
57. Lung RK, Gorko MA, Liewelyn J, Wiggins N. Statistical method for the determination of equivalence of automated test procedure. J Autom Methods Manage Chem., 2003; 25(6): 123-7.
58. Nethercote P, Ermer J. Quality by design for analytical methods: implications for method validation and transfer. Pharm Technol, 2013; 36(10): 74–9.
59. Belouafa S, Habti F, Benhar S, Belafkih B, Tayane S, Hamdouch S, Bennamara A, Abourriche A. Statistical tools and approaches to validate analytical methods: methodology and practical examples. Int J Metrol Qual Eng, 2017; 8(9): 1-10.
60. Chauhan A, Mittu B, Chauhan P. Analytical method development and validation: A concise review. J Anal Bioanal Tech, 2015; 6(1): 1-5.