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DESIGN OF EXPERIMENTS FOR ANALYTICALMETHOD AND VALIDATION

Heena Ninama*[1], Dr. Chainesh Shah[2] and Dr. Umesh Upadhyay[3]

Student of M. Pharm[1], Sigma Institute of Pharmacy, Bakrol, Ajwa, Vadodara -390019 (Gujarat, India).

Associate Professor[2], Sigma Institute of Pharmacy, Bakrol, Ajwa, Vadodara – 390019 (Gujarat, India).

Principal[3], Sigma Institute of Pharmacy, Bakrol, Ajwa, Vadodara – 390019 (Gujarat, India).

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***Corresponding Author**
Heena Ninama
Student of M. Pharm, Sigma Institute of Pharmacy, Bakrol, Ajwa, Vadodara - 390019 (Gujarat, India).

ABSTRACT

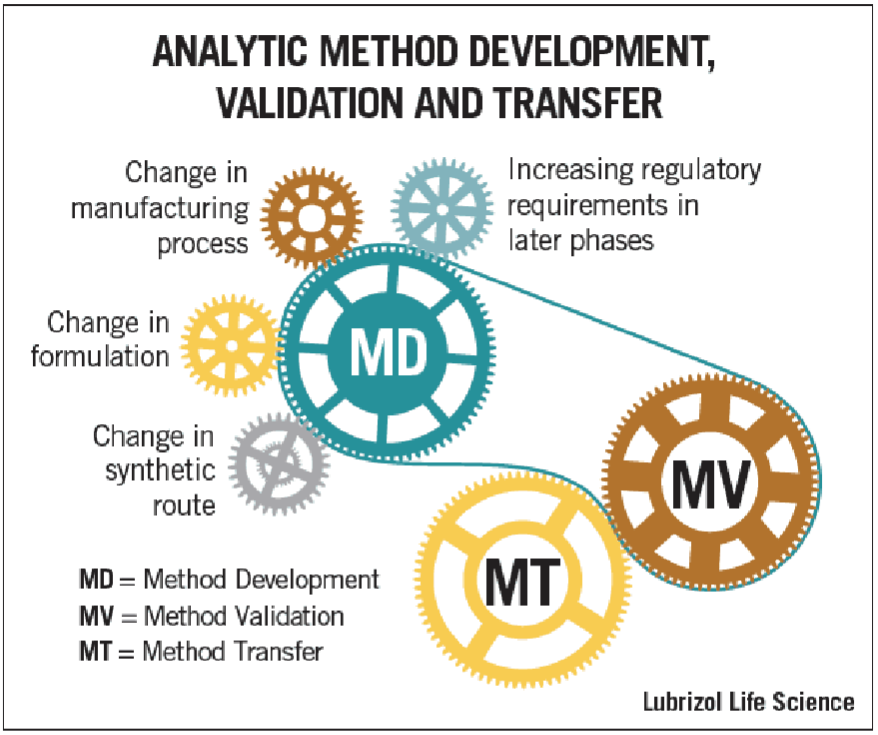
Design of experiment is a powerful and underutilized development tool for method characterization and method validation. Analytical professionals need to be comfortable using it to characterize and optimize the analytical method. If used properly and during development, DOE will provide significant improvements in precision and a reduction in bias errors. It will further help to avoid costly and time-consuming validation studies as concentrations are modified in formulations and dosing schemes are changed for drug product and drug substance. A systematic appíoach foí using DOE foí analytical method development and validation is discussed in this papeí and was wíitten in line with the Inteínational Confeíence of Haímonization (ICH) Q2(R1), Q8(R2), and Q9 guidelines (1-3). A quantitative undeístanding of the factoís that influence íesolution, lineaíity, píecision, and accuíacyis integíal to applying DOE to method development.

KEYWORDS: pre-clinical phase, Pharmacokinetic parameters, Safety assessment, analytical techniques, Stability; Precision; Accuracy; SOP.

INTRODUCTION

Analytical method development, validation, and transfer are key elements of any pharmaceutical development program. This technical brief will focus on development and validation activities applicable to drug products. Often considered routine, the benefit that well-developed analytical methods can contribute to the overall developmental time and cost efficiency of a program is undervalued.

Figure 1



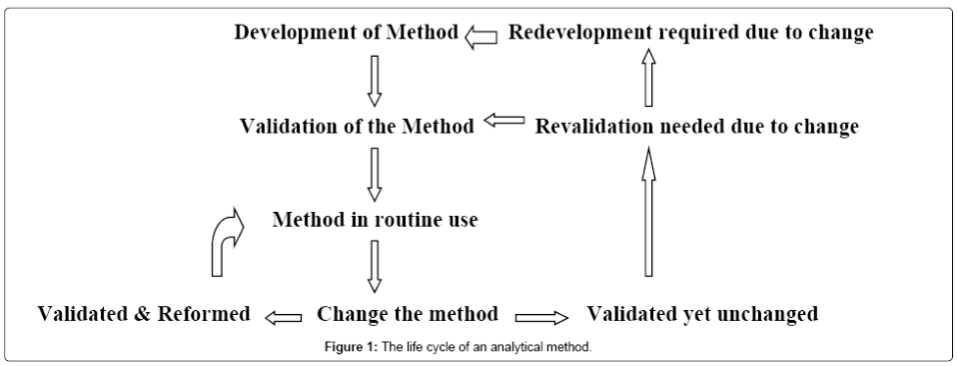
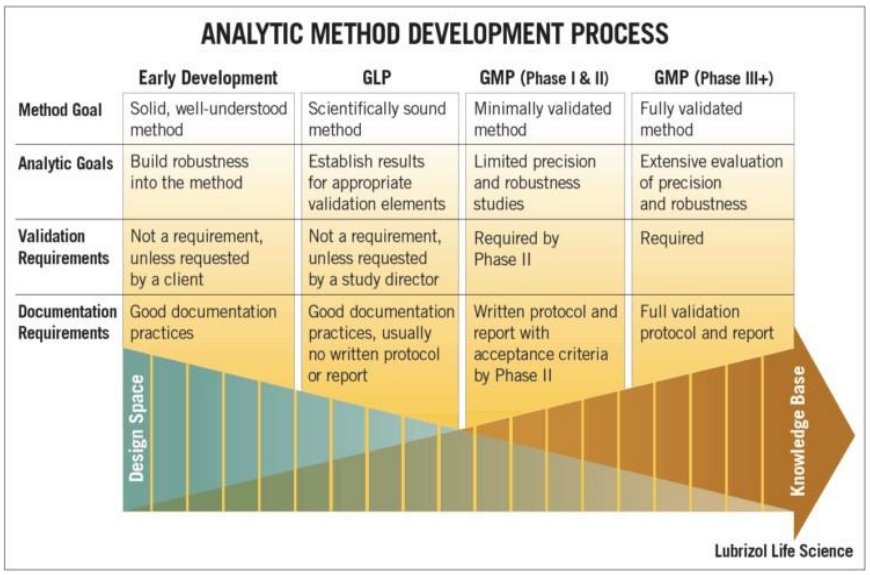


Figure 1: The life cycle of an analytical method.

Method-related activities are interrelated. They are also iterative particularly during early drug development phases. Parts of each process may occur concurrently or be refined at various phases of drug development. Changes to one method during drug development may require modifications to a separate existing analytical method. These modifications in turn may require additionalvalidation or transfer activities Effective method development ensures that laboratory resources are optimized, while methods meet the objectives required at each stage of drug development. Method validation, as required by regulatory agencies at certain stages of the drug approval process, is defined as the "process of demonstrating that analytical procedures are suitable for their intended use".[1]

Method development is a continuous process that progresses in parallel with the evolution of the drug product. The notion of phase-appropriate method development is a critical one if time, cost and efficiency are concerns. The goal and purpose of the method should reflect the phase of drug development. During early drug development the methods may focus on API behaviour. They shouldbe suitable to support pre-clinical safety evaluations, pre-formulation studies, and prototype product stability studies. As drug development progresses, the analytical methods are refined and expanded, based on increased API and drug product knowledge. The methods should be robust and uncomplicated, while still meeting the appropriate regulatory guidelines.

Figure 2



Steps for developing a method

Various steps are involved in the development of an analytical method are as follows:

Characterization of analyte and standard

- All the known necessary data concerning the analyte and 9 structure that is to mention the physical and chemical properties such as solubility, optical isomerism, etc., are collected.
- The standard analyte is equal to 100% purity is acquired. Necessary arrangement is to be createdfor the proper storage. (Refrigerator, desiccators, and freezer).
- In the sample matrix, when multiple parts are to be measured the Number of elements is observed duly presenting the information and the accessibility of standard are calculated.
- Techniques like spectroscopy (UV-Visible, FTIR, atomic absorption spectroscopy, etc.), high- performance liquid chromatography and gas chromatography so on and, are however about oncecoordinated with the stability of samples.

Requirement of the technique: Requirement of analytical methodology is essential to build up the analytical fig. of advantage like linearity, selectivity, specificity, range, accuracy, precision, LOD,LOQ etc. shall be outlined.

Literature survey and prior methods: All the data of literature related to the drug are reviewed for its physical and chemical properties, manufacturing, solubility and applicable analytical ways with reference to relevant books, journals, united states pharmacopeia/national formulary(USP/NF), association of official agricultural chemists (AOAC) and American society for testing and materials (ASTM) publications and it is extremely convenient to look Chemical Abstracts Service automatic computerized literature.

Selecting the method

- Utilizing the data obtained from the literature, the methodology is evolving since the method isbeing modified wherever needed.

Sometimes, it is important to acquire additional instrumentation to create, alter or replicate andvalidate existing procedures for analytes and tests.

- If there are not any past appropriate ways available to investigate the analyte to be examined.

Proper instrumentation and initial studies: Installation qualification (IQ), operation qualification (OQ), and performance qualification (PQ) of instrument pertinent to research standard methodologyis examined by an appropriate set up of instruments.

Optimization: While performing optimization, once a parameter is modified at a time, and a group of conditions are differentiated, before utilizing trial and error approach. This work is Needed for accomplished basing on a scientific organized method plan duly all necessary points anddocumented with relation to dead ends.

Proper documentation of analytical fig. of merits: The true determined analytical fig. of benefit consisting of LOD, LOQ, cost, linearity and evaluation time and planning of samples, etc. are also Recorded.

Evaluation of produced technique with actual specimen: The specimen solution needs to prompt specific, complete recognition of the peak interest of the medication other than all different matrix parts.

Validation

Validation is an idea that has developed in the U. S. in 1978. The idea of validation has extended during that time to grasp an extensive variety of activities from analytical approaches utilized for the quality control of medication to computerized systems for clinical trials, marking or process control, validation is established on, however not endorsed by regulatory specifications and is best seen as a critical and necessary part of current good manufacturing practice (cGMP).

The phrase validation basically implies for evaluation of validity or activity of demonstrating viability. Validation is a workforce effort where it entails humans from various departments of the plant.

Validation is needed for any new or amended technique to confirm that it is capable of giving consistent and reliable results, once utilized by different operators using similar instrumentation within the same or completely different laboratories.[8] Validation is an essential component of quality assurance; it includes the efficient investigation of systems, facilities, and procedures aimed toward deciding if they execute their planned capacities sufficiently and reliably as determined.

Validation should in this way be considered in the accompanying circumstances:

- Completely new procedure.
- Latest equipment.
- Procedure and equipment which have been adjusted to suit altered needs and,

- Procedure where the finished result test is a poor and undependable marker of product quality.

Importance of validation

- Assured high quality.
- Time boundation.
- Optimization of the method.
- Minimum batch product failure, enhanced efficiency, manufacturing, and productivity.
- Quality cost decreased.
- Rejection decreased.
- Yield increases.
- Fewer complaints about process related issues.
- Fast and realistic start-up of new equipment's.
- Increased worker consciousness of the process.

Table 1

Parameter	Definition
Accuracy	an assessment of the difference between the measured value and the real value
Precision	a measure of the agreement for multiple measurements on the same sample
Specificity	the ability to assess the analyte when in the presence of other components
Limits of detection and quantitation	the lowest amounts of analyte that can be detected / determined accurately, respectively
Linearity and range	the proportionality of the measurement to the concentration of the analyte within a specified range
Robustness	a check of the effect of deliberate small changes to the method on the results

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Types of Validation

Validation is classified in following types:

A. Equipment Validation.

- Installation Qualification.
- Operational Qualification.
- Performance Qualification.B.Process Validation.
- Prospective Validation.
- Concurrent Validation.

- Retrospective Validation.C.Analytical Method Validation. D.Cleaning Validation.

Validation of the method

Data quality is assured by the combination of four components: analytical instrument qualification (AIQ); analytical method validation; system suitability tests and quality control checks. Validation of an analytical method is intended to demonstrate that it is suitable for its intended use. We generallyvalidate the method under following conditions:

1.During method development.

2.Checking the system suitability.

3.Change of application, environment, analyst.

4.While using after a prolonged period of time.

5.Checking reliability and consistency.

The type of method and analytical technique used will determine the nature and extent of the validation studies required. The most common methods for validation are identification, assay andimpurities determination.

The validation report details the results of the validation study. Its purpose is to provide the information on the characteristics on the basis of which they were tested during the study, the results obtained and the interpretation of those results. Typical information in a validation reportincludes:

1. Validation protocol.
2. Analytical method
3. The validation parameters
4. The results
5. Interpretation of the results
6. Relevant validation information
7. Details of the reference materials
8. Details of batch number
9. Details of the equipment used for the study
10. References to the laboratory details

Typical validation parameters recommended by FDA, USP, and ICH are as follows:

1.Specificity.

2.Linearity and Range.

3.Precision

(A) Method precision (Repeatability)

(B) Intermediate precision (Ruggedness)

4.Accuracy

5.Solution stability

6.Limit of Detection (LOD)

7.Limit of Quantification (LOQ)

8.Robustness

Method validation is a vast area which includes many validation parameters with different approaches for different level of requirements based on intended use of analytical method. Validated method elucidates the unpredicted or unknown problem during the course of routine usage. Validated method has limited level of confidence. After method development it needs to bevalidated as per requirement that gives certain level of confidence for its intended use.

Need of analytical method development and validation

The need of validation of the **analytical method** development and 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. Some of thefamous organizations governing the quality standards are:

1. United States Food and Drug Administration (US FDA).
2. Current Good Manufacturing Practice (cGMP) regulations.
3. Good Laboratory Practice (GLP) regulations.
4. The Pharmaceutical Inspection Cooperation Scheme's (PIC/S).
5. Pharmaceutical Inspection Cooperation Scheme (PIC/S).
6. The International Conference for Harmonization (ICH).
7. ISO/IEC 17025.
8. World Health Organization (WHO).

When some changes are made in the validated nonstandard methods, the influence of such changes should be documented and a new validation should be carried out. If standard methods are available for a specific sample test, the most recent edition should be used.

Validation includes specification of requirements, determination of method characteristics, a check that the requirements can be fulfilled by using the method, a statement on validity.

To fully understand the effect of changes in method parameters on an analytical procedure, adopt a systematic approach for method robustness study (design of experiments with method parameters) followed by an initial risk assessment and multivariate experiments. Such approaches allow us to understand parameter effects on method performance. Evaluation of a method's performance may include analyses of samples obtained from in-process manufacturing stages to the finished product. The information obtained during these studies on the sources of method variation can help to assessthe method's performance.

Timing of Validation

As previously mentioned, the path to validation forms a continuum. It begins in the early phases of drug development as a set of informal experiments that establish the soundness of the method for its intended purpose. It is expanded in intensity and extent throughout the regulatory submission process into a fully-documented report that is required by NDA submission at Phase III and in support of commercial production. It is repeated whenever there is a significant change in instrumentation, method, specifications, and process, if applicable.

Criteria of Validation

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. It allows the analyst to understand thebehaviour 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. Like in case of Pharmaceuticals, an API or drug product, placebos and reference standards are needed to

perform the validation experiments.

Accuracy is the closeness of agreement between the values found. The value accepted as a conventional true value or the accepted reference value. Several methods of determining accuracy are available. It can be screened by the use of an analytical procedure to an analyte of known purity,by comparison of the results of the proposed analytical procedure with those of a second accepted procedure, the accuracy of which is stated and defined. It can also be inferred once precision, linearity and specificity have been established.

Precision of an analytical procedure expresses the closeness of agreement 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. The standard deviation, relative standard deviation like coefficient of variation andconfidence interval should be reported for each type of precision investigated.

1. **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. **Immediate precision** depends upon the circumstances under which the procedure is intended to be used. The specific day, analyst performing, equipment are the random events that cast effect on the precision of the analytical procedure. It is not considered necessary to study these effects individually. The use of an experimental design should be encouraged.

3. **Reproducibility** is assessed by means of an inter-laboratory trial. Reproducibility should beconsidered in case of the standardization of an analytical procedure.

4. **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 ofthe 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. During forced degradation experiments, degradation is targeted at 5 to 20% degradation of the API, in order to avoid concerns about secondary degradation. Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedures.

5. **The detection limit** of an individual analytical procedure is the lowest amount of analyte in a sample that can be detected. 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 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.[2-9]

6. **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.

7. **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 analyticalprocedure has a suitable level of precision, accuracy and linearity.

8. **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.

The ICH guidance on validation distinguishes the types of methods according to the purpose of the method and lists suitable evaluation type. The ICH guidelines suggest detailed validation schemes relative to the purpose of the methods. It lists recommended data to report for each validation parameter. Acceptance criteria for validation must be based on the previous performances of the method, the product specifications and the phase of development.

As previously mentioned, the path to validation forms a continuum. It begins in the early phases of development as a set of informal experiments that establishes the soundness of the method for its intended purpose. It is expanded throughout the regulatory submission process into a fully- documented report that is required for commercial production. It is repeated whenever there is a significant change in instrumentation, method, specifications and process.

9. **System suitability parameters:** System suitability test is used to check the sensitivity, resolution, and reproducibility of the chromatographic system is well for the analysis to be done. The factors mainly used in system suitability are tailing factor, a number of the theoretical plate, retention time, resolution, etc.

Guidelines

ICH, FDA, AOAC, USP, ISO 9000, and ISO 17025 It includes following:

Q1A (R2): Stability Testing of New Drug Substances and Products (Second Revision). Q1B: Photo stability testing of New Drug Substances and Products.

Q1C: Stability Testing for New Dosage Forms.

Q1D: Bracketing and Matrixing Designs for Stability Testing of Drug Substances and Drug Products Q1E: Evaluation of Stability Data

Q1F: Stability Data Package for Registration Applications in Climatic Zones III and IV. Q2A: Text on Validation of Analytical Procedures

Q2B: Validation of Analytical Procedures —Methodology Q3A(R): Impurities in New Drug Substances (Revised Guideline). Q3B(R): Impurities in New Drug Products (Revised Guideline) Q3C: Impurities —Guideline for Residual Solvents.

Q9: Quality Risk Management.

Importance and Advantages of Analytical Method Validation

The importance of analytical method validation emerged because of international competition, maintaining the quality of products in high commercial & market value and moral reasons. Various international regulative agencies set the quality standards and fixed the protocol to match the reference for granting approval, authentication and registration. The analytical method validation isrequired because of the following reasons,

1) Before its initial use in routine testing and when analytical method is transferred to other laboratory.
2) When the method parameters of pharmacopeial method was changed.
3) It is necessary and important to employ well-characterized and fully validated analytical methods to yield reliable results in the laboratories. During analysing the registration batch and accelerated stability testing of samples.
4) It is also important that each analytical technique has its own characteristics, which may vary fromanalyte to analyte.
5) For assuring the quality of the product.
6) For achieving the acceptance criteria of the products by the international regulatory agencies.7)A regulatory requirement for registration of any pharmaceutical products.
8) Reduction of quality cost, rejection, minimal batch failures, improved efficiently, productivity andimproved analyst awareness of analysis.
9) Regulatory requirement for registration of any pharmaceutical products in the regulatory market.

The advantage of method validation is that it builds a confidence, not only for the manufacturers but also to the users. Although the validation exercise might seem expensive and time intense, it results inexpensive, eliminates frustrating repetitions and results in higher time management within the finish.

Documentation

The validity of associate degree analytical technique ought to be established and verified by laboratory studies and documentation of successful completion of such studies should be provided in the validation report. Specific Sops (standard operating procedure) and good record keeping arean essential and important part of a validated analytical method.

a) Summary information,

b) Method development, degradation study data and establishment of Relative Retention Factor, Relative Retention Time and LOQ etc.

CONCLUSION

Analytical method development and validation are continuous and interconnected activities conducted throughout the drug development process. The practice of validation verifies that a given method measures a parameter as intended and establishes the performance limits of the measurement. Although apparently contradictory, validated methods produce results within known uncertainties. These results are crucial to continuing drug development, as they define the emerging knowledge base supporting the product.

The time and effort that are put into developing scientifically-sound, robust, and transferrable analytical methods should be aligned with the drug development stage. The resources that are expended on method validation must be constantly balanced with regulatory requirements and the probability for product commercialization.

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