METHOD DEVELOPMENT AND VALIDATION: A REVIEW

[1]Khushbu Makwana, [2]Priyanshi Sharma

[1]Assistant Professor, [2]Research Scholar
Department of Chemistry
Shree Swaminarayan Science College
Swaminarayan University, Kalol, Gujarat.

Abstract- **Pharmaceutical analysis is a very important in quality control as well as quality assurance of drugs and pharmaceutical formulations. Analytical method development has the basic need for analysis of drugs. The analytical instruments used for analytical method development and validation have reduced the time of analysis, increased precision, accuracy and reduced costs of analysis. As a consequence, most of pharmaceutical organizations are investing huge amount of money for the establishment of advanced analytical laboratories. For the testing of API, excipient and final product, an analytical procedure should be developed. Such well-developed procedure should essential assure that it will consistently produce the intended and precise result with high degree of accuracy. To gain such specific result, analytical method should be validated.**

Key words: **Analytical method development, validation, ICH guideline.**

INTRODUCTION:

Drug development is the process of bringing a new pharmaceutical drug to the market once a lead compound has been identified through the process of drug discovery. It includes preclinical research on microorganisms and animals, filing for regulatory status, such as via the United States Food and Drug Administration for an investigational new drug to initiate clinical trials on humans, and may include the step of obtaining regulatory approval with a new drug application to market the drug.[1][2] The entire process – from concept through preclinical testing in the laboratory to clinical trial development, including Phase I–III trials – to approved vaccine or drug typically takes more than a decade. Clinical trials involve three or four steps: [3]

- Phase I trials, usually in healthy volunteers, determine safety and dosing.
- Phase II trials are used to get an initial reading of efficacy and further explore safety in small numbers of patients having the disease targeted by the NCE.
- Phase III trials are large, pivotal trials to determine safety and efficacy in sufficiently large numbers of patients with the targeted disease. If safety and efficacy are adequately proved, clinical testing may stop at this step and the NCE advances to the new drug application (NDA) stage.
- Phase IV trials are post-approval trials that are sometimes a condition attached by the FDA, also called post-market surveillance studies.

Basically the common method for the development and validation of the analytical method is completed by the following process [4].
1) Planning the appropriate method that must be developed.
2) The information related to the work should be collected.
3) Qualitative and quantitative analytical method that can be performed in the lab should be developed.
4) The procedure for testing the sample should be created.
The well-developed method should be easily validated which is the basic criteria of analytical process.

ANALYTICAL METHOD DEVELOPMENT:

Method development consists of three main stages: feasibility—where you determine if the method will work with your sample; development—where you optimize the method; and validation—where the optimized method is validated to the relevant regulatory requirements. Method development is a process that arises in parallel accompanied by a development of the pharmaceutical products. When talking about the cost, time, productivity and effectiveness of the drug product, the idea of suitable method development is essential. The characteristic of the API was concentrated in the initial phase of development of drug. The safety evaluation for the pre-clinical trial is the vital step. After that pre formulation studies should be performed. This is followed by the stability studies. Analytical method validation is reasonable to help these parameters. After that by studying the nature and characters of API, the analytical testing method are clarified and extended during the process of progressing the development of the drug. The method must not be complicated. It must be robust. The suitable regulatory guidelines must be followed [5].

A well-developed method guarantees the goal which is essential during every step of development of the drug. It ensures the improved equipment in the lab. For the accomplishment of excellent quality and accurate testing outcomes, significant job is performed by a validated analytical process. Subsequently guarantee of assurance of QC instrument is focused by everyone in the chemical lab. Analysis method can be of different types such as electrochemical analysis, chromatography analysis, spectral analysis etc [6].

A well-developed method helps in drug testing against specification during manufacturing and quality release operations; similarly it promotes the studies regarding characters of chemical, safety examination and analysis of activities of the medicine. The development of analytical process is utilized for assisting the procedure of synthesis of drug. The formulation studies assist to screen the drug with potential activities. The finished pharmaceutical drug should be stable from the phase of raw material to final formulation. So stability studies should be regularly monitored. The identification, purification, physical specification and potential activities of the medicine are set up by these methods [7].

(1)The purpose behind the analytical method from development of product to the manufacture of product is to give information on the point given below product degradation which indicate the stability

2) Analyzing and evaluating the properties of API for instance uniformity of drug, crystalline property of chemical, release of API, etc. It helps in the study of bioavailability.

3) The study of impurities which in the identification of drug profile for the safety.

4) The study of the potential activities of the finished product which deals with the calculation of the correct dose. [8]

Steps involved in method development:

1. Analyte standard characterization
2. Method requirements
3. Literature search and prior methodology
4. Choosing a method
5. Instrumental setup and initial studies
6. Optimization
7. Documentation of analytical figures of merit
8. Evaluation of method development with actual samples
9. Determination of percent recovery of actual sample and demonstration of quantitative sample analysis

VALIDATION

Validation is the process of establishing documentary evidence demonstrating that a procedure, process, or activity carried out in testing and then production maintains the desired level of compliance at all stages. Effective process validation contributes significantly to assuring drug quality. The basic principle of quality assurance is that a drug should be produced that is fit for its intended use. [9] This principle incorporates the understanding that the following conditions exist:

• Quality, safety, and efficacy are designed or built into the product.

• Quality cannot be adequately assured merely by in-process and finished-product inspection or testing.

• Each step of a manufacturing process is controlled to assure that the finished product meets all quality attributes including specifications.[10]

Drug validation is important because [11]

(1)It is mandatory condition for enrollment of any pharmaceutical item or pesticide plan.

(2) It supports to accomplish the scope of "legitimate/reference technique" endorsed by administrative offices.

(3) It ensures high caliber of the outcomes.

(4) It improves the money related main concern of the research facility.

(5) It is obligatory necessity for accreditation of the research center by ISO 17025 rules.

(6) It helps in arriving at acknowledgment of the drugs by worldwide organizations

ANALYTICAL METHOD VALIDATION

For creating the trustworthy analytical data from the competent laboratory, a proper standard method should be set up. It can only be possible from validation of analytical method. The total information about chemical should be studied for the set-up of the method and its validation. Reproducible data should be given by the analytical procedure even when performed by different analyst in various lab centers utilizing distinctive reagent, different instruments and

equipment. For the validation of the analytical method, certain parameters should be followed such as linearity, accuracy, precision, specificity and reproducibility of the result of the sample. The number of medication presented for consumers has been increasing every day at higher rate. These medications might contain totally fresh element that is not yet seen in the market or there might be small basic alteration or modification in the structure from current medication.
Essential measures for method validation of drug analysis are given below:

1) For biological fluids, assay may be difficult to perform by using analytical method.
2) There is presence of many excipients in the formulation which can interfere with the formulated drug and expected results are difficult to gain.
3) For the combine product of two or more active pharmaceutical ingredient, analytical method may not be found.
4) The complete literature about the analytical methods of the drug cannot be gained because of the patient guidelines.
5) Requirement of costly reagents and solvents in the existing analytical procedures may not be suitable. It may likewise include difficult extraction and separation procedures which is not suitable.
6) Absence of the drug or drug combination in any pharmacopoeias.
Validation of analytical procedure is the legal requirement and is mandatory to perform. ICH guidelines [Q2 (R1)] have set the guidelines for the validation of analytical method. They are listed below.

TYPES OF ANALYTICAL METHODS TO BE VALIDATED

The validation of the analytical methods must be performed for the following test:
1) Identification tests
2) Analysis of the impurities for its quantification and its limit test
3) Analysis of active pharmaceutical ingredient for is quantification

1) Identification Tests: For the identity of chemical or ingredient, identification testis planned. It can be done by various type of analytical method. Examination of various properties such as reaction with other substances, spectral evaluation, properties of chromatogram and so on. In this test, comparison of sample is done with the reference standard.
2) Analysis of the impurities for its quantification and its limit test: Impurities can be quantified and identified. Almost all raw materials contain the impurities. Total removal of the impurities is very difficult task. So regulatory body has set certain criteria for the limit in the presence of the impurities. Percentage purity of the chemicals is reflected by this test. Following the various parameters of the validation in limit test is less essential whereas it is utmost criteria for quantification analysis.
3) Analysis of API for its quantification: Quantification of API or other chemical is the most essential part of the analytical test. It reflects the accurate presence and proper action of the API in the drug product. With regard to such archive, assay can be defined as estimation of active pharmaceutical ingredient in the product quantitatively. The quantification of API should follow certain procedure which has same parameters of validation. In the same way, dissolution which also deals with the release of API should follow the same guidelines of the validation.

ANALYTICAL METHOD VALIDATION CHARACTERISTICS [12][13][14]

An ICH guideline has set certain criteria for the validation of analytical method. The parameters are listed below:
1) Specificity
2) Accuracy
3) Precision
✓ Repeatability
✓ Intermediate Precision
✓ Reproducibility
4) Limit of Detection
5) Limit of Quantification
6) Linearity
7) Range
8) Robustness
The level of revalidation requires relies upon the alteration type. Validation is also required for more other alteration.[10]

Characteristics	Kinds of Analytical Procedure

Test of Identification		Impurities test		Assay
Quantification		**Limit**		Test of Dissolution (measurement only) Content/Potency
Accuracy	**-ve**	**+ve**	**-ve**	**+ve**
Precision Repeatability Intermediate Precision	-ve -ve	+ve +ve(1)	-ve -ve	+ve +ve
Specificity(2)	+ve	+ve	+ve	+ve
Limit of Detection	-ve	-ve(3)	+ve	-ve
Limit of Quantification	-ve	+ve	-ve	-ve
Linearity	-ve	+ve	-ve	+ve
Range	-ve	+ve	-ve	+ve

Indication of sign
-ve= not essential parameter to performed
+ve=essential parameter to performed
(1) = the test of intermediate precision is not necessary to performed if reproducibility test id performed
(2) Another supporting method can cover the specificity if specificity is unable to perform
(3) For certain condition it might be required (ICH harmonized tripartite guideline, 2005

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

(1) Specificity

Normally, the impurities, chemical of degradation etc. are present in raw materials. Evaluation of such things that has very high potency of presence in the raw material is defined as specificity. It incorporates:

***Identification*:** It assures the identification of the ingredient.

Purity Tests: The removal of the impurities is almost impossible. So certain limits are set for impurities. Impurities can be present in the form of content of residual solvent, heavy metals, related substances etc. The test of such substances can be done by purity test.

Assay (Content or Potency): It is the quantitative determination of the API. API shows the potency of the drug. (ICH harmonized tripartite guideline, 2005)

(2) Linearity

Linearity is generally indicated by the calibration curve, which shows that the testing chemical in the sample is directly proportional with measurement or data of the testing substance. It should be performed within the range. Samples are prepared either by diluting the standard stock solution or weighing different amount of sample as per the protocol. At least five concentrations must be prepared for analysis. (ICH harmonized tripartite guideline, 2005)

(3) Range

Range is the interval within which the concentration of API must lies. It is one of the parameter of validation. It provides the idea of upper limit and the lower limit of the concentration of API. Between that interval, API can show the good efficacy. Range should be set in that interval which can show linearity, accuracy and precision in acceptable level. Range should be set in such a way which does not affect the result of linearity, precision and accuracy. Even at the extreme level should be suitable.

The criteria of ranges that should be followed are given below:

1) The normal range of assay for good drug lies between 80 to 120 percent of label claim.
2) While performing the content uniformity, it should be within the range of 70 to 130 percent of label claim.
3) ± 20 percent is recommended in case of dissolution test. (ICH harmonized tripartite guideline, 2005)

(4) Accuracy
Accuracy is the closeness of understanding between the worth which is acknowledged either as an ordinary genuine quality or an acknowledged reference quality, and the quality discovered[15]. Trueness is another term for the accuracy.

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

(6) Limit of Detection
The detection limit in a particular analytical procedure is the most minimal measure of chemical in a sample which could be identified yet not really evaluated as the accurate measurement i.e. quantification cannot be exact.

(7) Quantification Limit
The quantification limits in the particular analysis methods are the most minimum quantity of sample chemical from which quantitative assay of component can be calculated. The result should be within the acceptable range.

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

CONCLUSION:
This review mainly focus on development of analytical 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. Therefore the silent points enumerated in the above review article are immense use to an analyst while estimating the pharmaceutical formulations as well as bulk drugs. Optimized method is validated with various parameters like accuracy, precision, linearity, limit of detection, robustness etc. as per ICH guideline.

REFERENCES:

(1) Strovel J, Sittampalam S, Coussens NP, Hughes M, Inglese J, Kurtz A, et al; "Early Drug Discovery and Development Guidelines: For Academic Researchers, Collaborators, and Start-up Companies". Assay Guidance Manual. Eli Lilly & Company and the National Center for Advancing Translational Sciences. . (2016).
(2) Jump up to Taylor D "The Pharmaceutical Industry and the Future of Drug Development". Issues in Environmental Science and Technology. Royal Society of Chemistry: 1–33; (2015)..
(3) Ciociola AA, Cohen LB, Kulkarni P "How drugs are developed and approved by the FDA: current process and future directions". The American Journal of Gastroenterology. 109 (5); (2014).
(4) Swartz, M., & Krull, I.; Analytical Method Development and Validation; CRC press. (1997).
(5) Azim, S., Mitra, M., & Bhasin, S.; HPLC Method Development and Validation: A Review *International Research Journal of Pharmacy*, 4(4), 39-46(2013).
(6) Behera, S.; UV-Visible Spectrophotometric Method Development and Validation of Assay of Paracetamol Tablet Formulation. *Journal of Analytical and Bioanalytical Techniques*. (2012).

(7) Bhandarkar, F. S., & Vavia, P. R.; A Stability indicating HPLC Method for the Determination of Meloxicam in Bulk and Commercial Formulation. *Tropical Journal of Pharmaceutical Research*, 8(3): 257 (2009).

(8) P. Ravisankar, S. Gowthami, G. Devlala Rao; A review on analytical method development; *Indian Journal of Research in Pharmacy and Biotechnology*; 2(3);1183-1195(2014)

(9) Neera Chikanbanjar, Nidhi Semwal, Ureena Jyakhwa; A Review Article on Analytical Method Validation; *Journal of Pharma Innovation*;1(1);48-58(2020)

(10) *FDA (1987). "Guidelines on General Principles of Process Validation*

(11) Boggula, N., Kasturi, J., Reddy, P., & Bakshi, V.; Non-steroidal anti-inflammatory drugs:an overview. *Journal of Drug Delivery & Therapeutics*, 9(1-s), 442-448(2019).

(12) ICH, Q2A, Text on Validation of Analytical Procedures, *International Conference on Harmonization*, October 1994, Geneva.

(13) ICH, Stability testing of new drug substances and products (QIAR) I*nternational Conference on Harmonization* IFPMA, 2000; Geneva.

(14) ICH, Q2B, Validation of Analytical Procedures, Methodology, *International Conference on Harmonization*, November, 1994, Geneva

(15) Neera Chikanbanjar, Nidhi Semwal, Ureena Jyakhwa*;* A Review Article on Analytical Method Validation; *Journal of Pharma Innovation;1(1), 48–58(2020)*