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A Review on Analytical Method Validation

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ABSTRACT

Method development is a continuous process that progress in parallel with the evolution of the drug product. The notion of phase-appropriate method development is critical one if time cost and efficiency are concerns. The goal and purpose of the method should reflect the phase of drug development. Analytical methods plays vital role in the process of identification, separation and then quantification of chemical components in natural materials or synthetic materials based on their chemistry. The main purpose of the analytical method development and validation is to prove that proposed analytical method is accurate, specific, precise and robust in the pharmaceutical industry for analysis of a drug moiety. The analytical method validation is essential for analytical method development and tested for specificity, linearity, accuracy, precision, range, limit of detection, limit of quantification and robustness. In summary, analytical method development and validation confirms that the method is accurate, precise and can be used for the routine control analysis of drugs and drug product.

Key Words: *Method validation, Linearity, Accuracy, HPLC.*

INTRODUCTION

Validation is an integral part of quality assurance; it involves the systematic study of systems, facilities and processes aimed at determining whether they perform their intended functions adequately and consistently as specified. Validation means demonstration, by provision of objective evidence that consistently meets its predetermined requirements [1]. The word "validation" comes from Latin word term *valdius* meaning worth/strong, thus suggesting that something is true, useful, and reliable [2]. The most accurate definition of validation is provided by ISO 900:2000 as the conformation, by means of a thorough examination and obtaining realistic and unequivocal evidences, that the procedure is effectively applicable for its intended purposes [3]. It is the act of providing that any approach, strategy experimental procedure, process, instrumentation and room conditions selected for the method will function in a proper way under a fixed set of conditions. Besides it can be used to individually evaluate the appropriateness of these factors [4].

Types of Validation

1) Process Validation
2) Cleaning Validation
3) Equipment Validation

Process Validation

Process validation is a documented program which provides a higher degree of assurance that a specific process will produce a product meeting its predetermined specifications and quality attributes." It is a type of validation which establishes the flexibility and constraints in the manufacturing process controls in the attainment of desirable attributes in the drug product while preventing undesirable properties [5]. The main objectives of process validation are: to reduce variation between batches, reduction in utility cost,

reduction of rejection and reworks and consistent through output. It would normally be expected that process validation be completed prior to the release of the finished product for sale (prospective validation). Where this is not possible, it may be necessary to validate processes during routine production (concurrent validation). Processes, which have been in use for some time without any significant changes, may also be validated according to an approved protocol (retrospective validation). The Basic principles of Process Validation are Installation Qualification, Operational Qualification, Performance Qualification and Re-Qualification [6].

Cleaning Validation

Cleaning means to make any article, piece of equipment and area free from dirt, marks, or any unwanted matter. In pharmaceutical industry there is a great need of cleaning of equipment apparatus and processing area. The improper cleaning can lead to contamination and cross contamination. Pharmaceutical product can be contaminated by various materials such as residue of previously used active pharmaceutical ingredient, raw material, cleaning agents and dust particles. The main objective of GMP consist prevention of contamination and cross contamination of materials. Therefore a perfect cleaning method is required for avoiding the possibilities of contamination and cross contamination, for this a validated program is required, this program is known as cleaning validation. "Cleaning validation is documented evidence which assure that cleaning of equipment, piece of equipment or system will obtain pre-determined and acceptable limits". Cleaning validation helps in analytical investigation of a cleaning procedure [7,8].

Equipment Validation

The key concept of validation is to give a high degree of reported confirmation that the equipment and the procedure conform to the written guidelines. The degree (or intensity) is dictated by the complexity of the device or system. The validation should give the essential data and test methods required to give that the device and technique meet determined prerequisites [9,10]. Equipment Validation includes the following:

1) Installation Qualification
2) Operational Qualification
3) Performance Qualification

Analytical Method Validation

Method Validation means establishing documented evidence that a specific method and the ancillary instruments included in the method will consistently yield results that accurately reflect the quality characteristics of the product tested. It is the process of demonstrating that an analytical procedure is suitable for its intended purpose [11]. The method validation evaluates the range and conditions of applicability, and checks if every future measurement in routine analysis will provide a concentration of the analyte close enough to the true value [12]. In addition, it can also quantify the degree of coincidence of a measured concentration and the true value, by the calculation of the bias and the uncertainty associated with the result [13]. Therefore, the validation verifies if the method is suitable to be used as a quality control tool and for research support [14]. It is an essential step in method development, which must be implemented by laboratories to prove they can produce analytical data with high reliability [15]. Method Validation is an important requirement for any package of information submitted to international regulatory agencies to support new product marketing or clinical trials applications. Analytical method should be validated, including methods published in the

relevant pharmacopoeia or other organized standard references. The suitability of all test methods used should always be verified under the actual conditions of use and should be well documented [16].

IMPORTANCE OF VALIDATION

Every day, a high number of HPLC analyses, related to the monitoring of organic compounds, drugs and foods are performed in thousands of laboratories around the world. These measurements are very useful in many situations: quality control of drug, food and other consumer goods during manufacturing, processing, trading, and consumption, detection of deficient products or incorrect labeling, and clinical assistance.

In fact, every aspect of our life depends to some extent on analytical measurements. The methods should be validated because of the own professional duty of the analyst. It is recognized that a laboratory must take the adequate actions to ensure that it is capable of providing coherent, interpretable, and accurate results with a known uncertainty. Thus, the analytical methods must be reliable enough to guarantee that any decision based on it will be taken with high confidence [17].

Summarizing the Importance of Validation [17]

1) Process parameters and controls are determined during the validation of any process or system.
2) Validation helps to investigate the deviations caused during the process
3) It helps to determine the worse cases and risks that they arise during the manufacturing of the quality products
4) Assuring high quality of products
5) Deep Study and understanding of the system and equipment are made possible due to the validation
6) A validated process required less process control and the finished product testing.
7) Batch to batch variation is minimized due to validation of processes systems and equipment
8) Reduces the production cost of the product
9) Increases the production of manufacturing facility due to the minimized rework and rejection
10) Decreases the chances of failure of the batches
11) Achieving the range of "official/reference method" approved by regulatory agencies.
12) Mandatory requirement for accreditation of the laboratory by ISO 17025 guidelines.
13) Compulsory condition for registration of any pharmaceutical product or pesticide formulation.

Analytical Method Development

Method development is a continuous process that progress in parallel with the evolution of the drug product. The notion of phase-appropriate method development is 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 method may focus on API behavior. They should suitable to support pre-clinical safety evolution, pre-formulation studies and pro-type product stability studies [18].

When there are no authoritative methods are available, new methods are being developed for analysis of novel products. To analyze the existing either pharmacopoeial or non-pharmacopoeial products novel methods are developed to reduce the cost besides time for better precision and ruggedness. These methods are optimized and validated through trial runs. Alternate methods are proposed and put into practice to replace the existing procedure in the comparative laboratory data with all available merits and demerits [19,20,21].

The reasons for the development of novel methods of drug analysis are:

1) When there is no official drug or drug combination available in the pharmacopoeias.
2) When there is no decorous analytical process for the existing drug in the literature due to patent regulations.
3) When there are no analytical methods for the formulation of the drug due to the interference caused by the formulation excipients.
4) Analytical methods for the quantization of the analyte in biological fluids are found to be unavailable.
5) The existing analytical procedures may need costly reagents and solvents. It may also involve burdensome extraction and separation procedures.

Analytical Method Validation is carried out as per ICH guidelines [Q2(R1)].As per ICH guidelines, the validation of analytical procedures is carried in the follwong types of analytical procedures:

Types of Analytical Procedures to be Validated

The discussion of the validation of analytical procedures is directed to the four most common types of analytical procedures:

1) **Identification Tests:** Identification tests are intended to ensure the identity of an analyte in a sample. This is normally achieved by comparison of a property of the sample (e.g., spectrum, chromatographic behavior, chemical reactivity, etc) to that of a reference standard.[22]
2) **Quantitative Tests for Impurities Content:** Testing for impurities can be either a quantitative test or a limit test for the impurity in a sample.
3) **Limit Tests for the Control of Impurities:** Either test is intended to accurately reflect the purity characteristics of the sample. Different validation characteristics are required for a quantitative test than for a limit test.[22]
4) **Quantitative Tests of the Active Moiety in Samples of Drug Substance or Drug Product or Other Selected Component(s) in the Drug Product:** Assay procedures are intended to measure the analyte present in a given sample. In the context of this document, the assay represents a quantitative measurement of the major component(s) in the drug substance. For the drug product, similar validation characteristics also apply when assaying for the active or other selected component(s). The same validation characteristics may also apply to assays associated with other analytical procedures (*e.g.,* dissolution) [22].

Analytical Method Validation Characteristics

Following are the characteristics of Analytical Method Validation:

1) Specificity
2) System Suitability
3) Linearity
4) Range
5) Accuracy
6) Precision
   - ✓ Repeatability
   - ✓ Intermediate Precision
   - ✓ Reproducibility
7) Limit of detection
8) Limit of Quantification
9) Robustness
10) Solution Stability

Specificity

One of the significant features of HPLC is its ability to generate signals free from interference. Specificity refers to the ability of the analytical method to differentiate and quantify the analyte in complex mixtures. An investigation of specificity is to be conducted during the determination of impurities and validation

of identification tests. Specificity is the ability to assess unequivocally the analyte in the presence of other compounds that may be likely to be present [23].

System Suitability

System suitability test is used to check the sensitivity, resolution, and reproducibility of the chromatographic system are 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* [24].

Linearity

Linearity may be characterized as the capacity of an analytical technique to produce outcomes which are directly related to the concentration of an analyte in the sample. A linear relationship should be evaluated across the range of the analytical procedure. Linearity should be evaluated by visual inspection of a plot of signals as a function of analyte concentration. If there is a linear relationship, test result should be evaluated by appropriate statistical methods, by calculation of a regression line by the method of least squares [25].

Range

The Range of an analytical method is the interval between the upper and lower level 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. Minimum of the specified range to be 80% to 120% of the test sample for the assay test [26].

Accuracy

Accuracy is expressed as the nearness of agreement between the values found and values that are already available. It can also be defined as the closeness between the true value and the observed value. It is sometimes called as trueness. Accuracy may often be expressed as percent recovery by the assay of known, added amounts of analyte. Accuracy is a measure of exactness of the analytical method. The accuracy of an analytical procedure expresses the closeness of agreement between the value, which is accepted either as conventional true value or an accepted reference value and the value found, *i.e.* analytical result [27].

Precision

The precision of an analytical procedure represents the nearness of agreement between a series of measurements got from multiple sampling of the same homogenous sample under the similar analytical conditions and it is divided into 3 categories [28].

- Repeatability Precision under same operating conditions, same analyst over a short period of time [29].
- Intermediate precision method is tested on multiple days, instruments, analysts *etc* [30].
- Reproducibility is Inter-laboratory studies [29].

Limit of Detection

Lowest quantity of an analyte which may be detected by the chromatographically separation however it is not necessary that this quantity will quantify as a precise value. A blank resolution is injected and peak to peak quantitative noise relation we have to calculate from blank chromatograms. Then concentration is calculated at the signal to quantitative noise relation is concerning 3:1.

LOD can be expressed as LOD = 3.3SD/S
Where, SD = Standard deviation of response,
S = Slope of calibration curve [31].

Limit of Quantification

It is characterized by the least quantity of an analyte that can be quantified with exactness and precision.

LOQ can be communicated as LOQ = 10SD/S

Where SD = Standard deviation of response,
S = Slope of calibration curve [32].

Robustness

This refers to capacity to remain unaffected by small but deliberate variations in method parameter and provides an indication of its reliability during normal usage. If measurements are susceptible to variations in analytical conditions, the analytical conditions should be suitably controlled or a precautionary statement should be including in the procedure. The different technique parameters which can be modified in high-performance liquid chromatography are pH, flow rate, the temperature of the column and mobile phase composition [33].

Solution Stability

This refers to the stability of the analytical solution expressed as variation of the measured assay as a function of time. Stability of solution is evaluated from the standard preparation and the test preparation .Initially the solutions are prepared and anlyzed, further stored in 2-8 degree for 6 hrs and analyzed. The stock solution is further stored in refrigerator and tested after 24 hours.The responses for the aged solutions are evaluated [25].

CONCLUSION

Analytical method validation and method transfer data playing a fundamental role in pharmaceutical industry for releasing the commercial batch and long term stability data therefore, the data must be produced to acceptable scientific standards. For this reason and the need to satisfy regulatory authority requirements, all analytical methods should be properly validated and documented. This review article provides a structured way to perform method validation as per regulatory perspective for its intended purpose and to assure the capabilities of the test method.

REFERENCES

1) International Conference on Harmonization (ICH), Q2A; Text Validation of Analytical Procedures, March 195
2) Araujo, P. (2009). Key aspects of analytical method validation and linearity evaluation. *Journal of chromatography B*, *877*(23), 2224-2234.
3) ISO, I. (2004). 9000: 2000—Quality Management Systems—Fundamentals and Vocabulary. *International Organization for Standardization (ISO), Switzerland*.
4) Gowik, P. (2009). The validation of methods for regulatory purposes in the control of residues. *Journal of Chromatography A*, *1216*(46), 8051-8058.
5) Harisudha, K., Lavanya, G., Eswarudu, M. M., & Sunil, M. (2013). An overview on pharmaceutical process validation. *International Research Journal of Pharmaceutical and Applied Sciences*, *3*(1), 165-168.
6) Alam S.(2012) An overview on Pharmaceutical process validation, Journal of advanced pharmacy education and research.2(4).
7) Asgharian, R., Hamedani, F. M., & Heydari, A. (2014). Step by step how to do cleaning validation. *Int J Pharm Life Sci 2014; 5 (3): 3345*, *3365*.
8) Lodhi, B., Padamwar, P., & Patel, A. (2014). Cleaning validation for the pharmaceuticals, biopharmaceuticals, cosmetic and neutraceuticals industries. *Journal of Innovations in Pharmaceuticals and Biological sciences*, *1*(1), 27-38.
9) Verma,P.,Madhav,N.S.,7Gupta KR,V.(2012).A Review onPharmaceutical Validation and Process controls.The Pharma Innovation,1(7):51

10) Md Alamshoaib., (2012), An overview of Pharmaceutical process validation. *J Adv Pharm* .2:185-200.
11) Shabir, G. A. (2003),Validation of high-performance liquid chromatography methods for pharmaceutical analysis: Understanding the differences and similarities between validation requirements of the US Food and Drug Administration, the US Pharmacopeia and the International Conference on Harmonization. *Journal of chromatography A*, *987*(1-2), 57-66.
12) Hubert, P., Nguyen-Huu, J. J., Laurentie, M., Mercier, N., & MUZARD, G. (2003). Validation Des procédures analytiques quantitatives harmonisation des démarches. *STP Pharma pratiques*, *13*(3), 101-138.
13) González, A. G., Herrador, M. Á., & Asuero, A. G. (2005). Practical digest for evaluating the uncertainty of analytical assays from validation data according to the LGC/VAM protocol. *Talanta*, *65*(4), 1022-1030.
14) Us, M. A. (2005). ISO/IEC 17025: 2005, General requirements for the competence of testing and calibration laboratories.
15) Konieczka, P. (2007). The role of and the place of method validation in the quality assurance and quality control (QA/QC) system. *Critical Reviews in Analytical Chemistry*, *37*(3), 173-190.
16) International Conference on Harmonization (ICH), Q2B; Text Validation of Analytical Procedures: May 1997
17) Magnusson, B. (2014). The fitness for purpose of analytical methods: a laboratory guide to method validation and related topics (2014).
18) Ravisankar P.,and Rao, G. (2014).A review of analytical method development. Indian Journal of research in pharmacy and Biotechnology.2(3):236-247
19) Ravisankar, P., Gowthami, S., & Rao, G. D. (2014). A review on analytical method development. *Indian journal of research in pharmacy and biotechnology*, *2*(3), 1183-1195
20) Ravisankar, P., Rajyalakshmi, G., Devadasu, C., & Devala Rao, G. (2014). Instant tips for right and effective approach to solve HPLC trouble shooting. *Journal of chemical and pharmaceutical sciences*, *7*(3), 259-274.
21) Breaux J,Jones K, and Boulas P.(2003). Development services analytical method development and validation ,*Pharmaceutical Technology*.27(1): 6-13.
22) ICH, I. (1995). *Topic Q 2 (R1) Validation of Analytical Procedures: Text and Methodology. European Medicines Agency*. CPMP/ICH/381/95.
23) Validation of Analytical Procedures: Methodology ICH-Q2B
24) Gupta, V., Jain, A. D. K. J., Gill, N. S., & Guptan, K. (2012). Development and validation of HPLC method-a review. *International research journal of pharmaceutical and applied sciences*, *2*(4), 17-25.
25) Text on validation Analytical Procedures, ICH Guidelines Q2A
26) Shweta, K., & Anita, S. (2016). A review on analytical method validation. *Int J Pharm Res Rev*, *5*: 30-36.
27) Srivastava RK, Kumar SS. (2017).*An updated review: analytical method validation. Eur J Pharm Med Res* .4:774-7784.
28) Nayudu, S. T., & Suresh, P. V. (2017). Bio-analytical method validation–a review. *Int J Pharm Chem Res*, *3*:283-93.
29) .Sharma S, Goyal S, Chauhan K.(2018).A review on Analytical Method development and Validation. *International Journal Of applied Sciences*.10(6):8-15

30) LK, T., NT, R., & UN, M. (2016). A review on bioanalytical method development and validation. *Asian J Pharm Clin Res*, *9*(3), 6-10.
31) Pasbola, K., & Chaudhary, M. (2017). Updated review on analytical method development and validation by HPLC. *World J Pharm Pharm Sci*, *6*(5), 1612-1630.
32) Geetha G, Raju KN, Kumar BV, Raja MG.(2012). Analytical method validation: an updated review. Internatioal Jourrnal of advances in Pharmacy Biology and Chemistry.1:64
33) Pushpa Latha, E., & Sailaja, B. (2014). Bioanalytical method development and validation by HPLC: a review. *J Appl Pharm*, *1*, 1-9.