Comprehensive Review on Equipment and Analytical Method Validation Parameters

Shine Sudev[1], Sujith Thomas[2], Bidah Mohamed Iqbal[3]
Moulana College of Pharmacy, Kerala, India.
email:shinesudev@gmail.com

Abstract

Validation is a vital and recognized parameter within the cGMPs. The method validation involves verification of the documents that's submitted for authorization. As per preset protocols, validation is a necessity being an essential and an integral a part of cGMP. Quality assurance is a major element underneath this and careful attention to important factors like choice of quality method through in -process and end-product testing is to be done. Validation as a process was evolved in US in 1978.The idea of validation has evolved throughout the years to embrace a good variety of activities from analytical ways used for the quality management of drug substances to processed systems for clinical trials, labeling and method management. The importance extended to validation has staggeringly augmented over the previous few years.

Key words: validation, validation of equipment, validation of analytical procedures, HPLC.

Introduction

Validation is that act of documentation to confirm that any procedure, process, equipment, material, activity or system actually do provide the expected and required result. They are concerned with the maintenance of a high degree of quality. As outlined by the Food and Drug Administration (FDA), the validation is done to: “establish documented proof that provides a high degree of assurance that a particular method can compatibly manufacture a product meeting its predetermined specifications and quality attributes.”

According to ISO, it may be outlined as " validation is the confirmation by examination and the provision of objective evidence that the particular requirements for a specific intended use can be consistently fulfilled."

As explicit by ICH it may be outlined as follows;
“Process Validation is the means of ensuring and providing documentary evidence that processes within their specified design parameters are capable of repeatedly and reliably producing a finished product of the required quality.”

The WHO definition is as follows;“The documented act of proving that any procedure, process, equipment, material, activity or system actually leads to expected result.”

VALIDATION

Validation may be accurately outlined as;"establishing documented proof that a system or a method once operated at intervals within established parameters can perform effectively and reproducibly manufacture a product meeting its predetermined specification and quality attributes."

VALIDATION OF EQUIPMENT

Equipment validation is a term generally used to describe "a set of independent procedures that are used to verify if a product meets the specification, requirements and quality attributes for its intended purpose."Equipment validation helps ensure that the product will perform consistently within a given range.

Some protocols for successive equipment validation include;

- Document availability-all the documentation that is required, should be verified and made available.
- Component and design verification-they are to be verified in accordance with specification and verification norms.
- Electrical supply and connections-ensured that all connections are checked thoroughly.
- Safety compliance-safety checks are to be done and ensure that the noise levels are within applicable standards.
- Environmental verification-instruments checked for their operation in various environments.

VALIDATION PROCESS

Categorized into 5 separate qualification categories as follows;

- Design Qualification(DQ);
  The first step is to demonstrate at the proposed design of the instrument can cope with functional requirement of the end user. All proposed design must satisfy the DQ before construction and procurement of parts.
- Installation Qualification(IQ)
  The instrument, with all its components and documentation is placed correctly and checked for performance according to the requirement.
- Operational Qualification(OQ)
  All the major parts of the instrument are tested to ensure they all perform correctly and are in sync with entire system.
- Performance Qualification(PQ)
  The instrument is monitored over an amount of time to visualize if it systematically delivers the expected results at intervals within the desired parameters.
- Component Qualification(CQ)
  Those parts that are s ourced from a third party manufacturer and the auxiliary equipments are to be subjected to random tests for their performance and quality to ensure they are manufactured to the right specifications and do not hamper the performance of the instrument.

BENEFITS OF EQUIPMENT VALIDATION

- Improvement of overall production reliability and availability
- Increases safety
- Lower repair costs
- Elimination of premature replacements.
- Greater confidence in reliability of results
- Reduction of variation in results
- Identification of variation in results

Eg: for validation of sterilization equipment's; involves the use of indicators

1. Physical indicators
2. Chemical indicator
3. Biological indicators

Physical Indicators

Device	Parameter Monitored
Temperature recording charts	Temperature
Recording chart	Radiation dose
Bubble point pressure test	Pressure

Chemical indicators

Device	Parameter monitored
Brownie's tube	Temperature
Indicator paper impregnated with reactive chemical which undergo a distinct colour change on reaction with Ethylene oxide in presence of a heat and moisture	Gas concentration, temperature, time
Plastic device impregnated with radio sensitive chemicals which undergo colour change at relative low radiation dose	Indicates exposure to radiation
Dosimeter device (acidified ferric ammonium sulphate solution responds to related changes in their optical densities)	Accurately measures radiation dose

Biological indicators

Method	Microorganism required
Moist method (Autoclave)	Bacillus stearothermophillus

Dry heat (Hot air oven)	Clostridium tetani / Bacillus subtilis
Filtration (membrane)	Pseudomonas diminuta
Radiation (non-ionizing)	Bacillus pumilus

VALIDATION OF ANALYTICAL PROCEDURES

The objective of validation of analytical procedure is to demonstrate that the procedure is suitable for its indented purpose. Typical validation characteristics to be considered are as follows;

- Specificity
  The ability to access a specific analyte in the presence of components which are expected to be presented (Impurities, degradents, matrix, etc.)
- Accuracy
  The accuracy of an analytical procedure typically expresses the closeness of agreement between a value that is accepted as a standard true or an accepted reference and the value that was found.
- Precision
  The preciseness of an analytical procedure typically expresses the closeness of agreement between a series of measurements that's obtained from multiple sampling of the unvaried sample under a nominal and prescribed condition.
- Repeatability
  It expresses the preciseness below an equivalent operative condition over a brief interval of your time, also called intra-assay precision.

Intermediate precision; express within the laboratory variations: different days, different analysis, different technique.

- Reproducibility;
  It expresses the precision between the laboratories (collaborative studies, usually applied to standardization of methodology).
- Detection limit
  It is the lowest amount of a substance in a sample which can be just detected and not necessarily quantities as an exact value.
- Quantization limit
  It is the lowest amount of analyte which can be quantitatively determined in a sample with the required degree of precision and accuracy. It is used particularly useful in the determination of impurities and degradation products.
- Linearity
  It is said to be the ability to obtain test result which is directly proportional to the concentration or amount of analyte present in the sample.
- Range
  It can be defined as the interval between the upper and lower limits of concentration or amount of analyte present in the sample for which it can be demonstrated that the analytical procedure includes a appropriate level of preciseness, accuracy and dimensionality.
- Robustness

It is the measure of its capacity to remain relatively unaffected by small, but deliberate variation in method parameters and this indicates the reliability of the process during its normal usage.

METHODOLOGY

A. Specificity

The investigations of specificity is to be conducted during the validation of processes like identification test, determination of impurities and the assay.

• Identification test;

Suitable identification test should have the capacity to discriminate between compounds of closely related structures which are likely to be present in the sample. The procedure is confirmed by obtaining positive result from the sample containing the analyte and negative result from that sample not containing the analyte.

• Assay and impurity test;

Assay should be involving the practical demonstration of the presence of analyte in the presence of impurities. This is usually done by spiking pure substance with appropriate levels of impurities and demonstrates that the assay result is unaffected even in the presence of these impurities.

In impurity test, it is usually established by spiking drug substance with appropriate levels of impurities or some other foreign substance and practically proving the separation of these impurities individual and/or from other components in the sample matrix can be done.

B. Linearity

A linear relationship should be evaluated across the range that these analytical procedures are said to be valid. The linearity should be evaluated by visual inspection of a plot of signals as functions of analyte concentration. If there is a linear relationship, test result is to be evaluated by appropriate statistical method.

C. Range

It is established by confirming that the analytical procedure provides an acceptance degree of linearity, accuracy and precision when applied to the samples.

D. Accuracy

Accuracy is established across the specified range of the analytical procedures.

Eg: accuracy should be assessed on sample spiked with known amount of impurities.

E. Precision

Validation of test for assay and quantitative determination of impurities basically includes an investigation of precision. Standard deviation, relative standard deviation and confidence interval are usually reported for each type of precision investigation.

F. Detection Limit

Several approaches are possible;
• Based on visual evaluation
Analysis of sample with known concentration of analyte and established the minimum level at which the analyte can be reliably detected.
• Based on signal to noise
Determination is performed by examining the measured signal from sample with known low concentration of analyte with those of blank sample and establishing the minimum concentration at which the analyte is reliably detected.

G. Qualification Limit:
Several approaches can be possible
• Based on visual evaluation
The quantization limit is mostly determined using analyte of already known concentration and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision
• Based on signal to noise approach
Determination of the signal to noise ratio Is performed by comparing measured signal from sample with known low concentration of analyte with those of blank sample and establishing the minimum concentration at which the analyte can be dependably quantified.

H. Robustness:
The evaluation of robustness should be considered during the development phase and it depends on the type of procedure under study.

Method Validation of HPLC

It needs to be established that the performance characteristics of the procedure meet the requirements for its intended use and this is where validation comes to play. It begins with the planned and systematic collection of the validation data to support analytical procedures. The validation of analytical methods is to be done as per ICH guideline.

Validation parameters to be verified:
As described above, the following analytical performance characteristics are typically tested.
· Accuracy
· Precision
· Repeatability
· Intermediate precision
· Linearity
· Detection limit
· Quantization limit
· Specificity
· Range
· Robustness
· System suitability determination
· Forced degradation studies

· Stability studies

Accuracy, precision, linearity, repeatability, robustness, range and specificity can be verified as mentioned earlier.

- Detection Limit

  The limit of detection is nothing but the lowest amount of analyte that is present in a sample that can be detected but not necessarily quantities as an exact value. In analytical procedures that exhibit baseline noise, the LOD can be determined based on a signal-to-noise (S/N) ratio, which can expressed as the concentration of analyte in the sample.

The signal-to-noise ratio is determined by:

$$s = H/h$$

Where H = height of the peak corresponding to the component.

h = definite quantity of the biggest noise fluctuation from the baseline of the chromatogram of a blank resolution.

- Quantization Limit

  The limit of Quantization (LOQ) simply said is the lowest amount of analyte present in a sample that can be quantitatively determined with suitable precision and accuracy. For analytic al procedures such as HPLC that exhibit baseline noise, the LOQ is generally estimated from a determination of S/N ratio.

In addition to the above general validation parameters the following one are also included in the method validation of HPLC;

- System Suitability Parameters

  System Suitability determination is nothing but the evaluation of the components of an analytical system to prove that the performance of a system meets the standards required. These parameters are often calculated by experimentation to produce a quantitative system quality check report, these include number of theoretical plates (efficiency), capacity factor, separation (relative retention), resolution, tailing factor, relative standard deviation (precision).

System suitability (SST) parameters are as follows:

- Resolution (Rs)

  Resolution is the parameters describing the power of separation of the chromatographic system relative to the components of the mixture. The resolution of 2 neighboring peaks is outlined because of the magnitude relation of the space between 2 peak maxima. It is the difference between the retention times of two components divided by their average peak width. The ideal value of RS is 1.5 for a baseline separation.

$$RS=2[(tR)B-(tR)A] / WB+WA$$

Here, tR(A) and tR(B) are the retention times of components 1 and 2 and WA and WB are peak width of components 1 and 2.

- Capacity factor (K')

Capacity factor, k', is the ratio of the number of molecules of solute in the stationary phase to the number of molecules of the same in the mobile phase. A measure of how well the sample molecule is retained by a column or TLC plate during an isocratic separation is the capacity factor. The ideal value of k' ranges from 2-10
Capacity issue are often determined by exploitation the formula,

$$k` = \frac{V_1 - V_0}{V_0}$$

Where, V_1 = retention volume at the apex of the peak (solute)
V_0 = void volume of the system.

- Column efficiency (N)
  It is simply the measure of band spreading of a peak. Smaller the band spread and higher is the number of theoretical plates, better the column and system performance.For a decent system columns with N starting from 5000 to a hundred thousand Plates/meter are ideal.

Efficiency is calculated by using the formula:

$$N=16(tR/W)^2$$

Here, tR is the retention time and W is the peak width.

- Peak asymmetry factor (As) and tailing factor:
  It can be used as criteria of column performance. The peak asymmetry is measured at 10 % of full peak height and is then divided by corresponding front half width

Asymmetry factor is calculated by,

$$\text{Asymmetry factor} = B/A$$

B= Peak half width, A= Front half width
Columns that produce peaks with as values of 0.95 to 1 % are considered good.

- Peak Purity
  Also known as peak homogeneity.It is the analysis of the main peak done in order to assess the presence of impurities under the main peak. It forms an essential part of the method validation.

In a nutshell, HPLC method is to be validated with various parameters (e.g. accuracy, precision, specificity, linearity, detection limit etc.) as per ICH guidelines.

GC method validation:

In the case of analytical procedures like GC, several steps are involved in method validation; these might include review of information on samples to be analyzed, definition of separation goals; assurance of special procedure necessities, sample pretreatment if any; detector selection and setting, separation conditions optimization, check for problems or special procedure requirements, recovery of purified material, quantitative calibration and

qualitative method development. The experimental conditions ought to be optimized to urge desired separations and sensitivity once obtaining applicable separations. This will be achieved through planned and systemic examination of parameters.
During improvement one parameter is modified at a time and set of conditions are isolated instead of employing a trial and error approach.

Analytical ways ought to be validated or revalidated before their introduction into routine use or whenever the conditions of the method are amended.

Much like HPLC the typical parameters recommended by FDA, USP, and ICH that are to be validated are as follows;
1. Specificity
2. Linearity & Range
3. Precision
   - Method precision (Repeatability)
   - Intermediate precision (Reproducibility)
4. Accuracy (Recovery)
5. Solution stability
6. Limit of Detection (LOD)
7. Limit of Quantification (LOQ)
8. Robustness
9. System suitability

As previously mentioned in HPLC the parameters listed above are to be verified and ensured that they follow the required specifications. The various parameters (e.g. specificity, precision, accuracy, detection limit, linearity, etc.) are thus validated as per ICH guidelines.

Method validation of IR spectroscopic analysis

Method validation of IR spectroscopy also follows ICH specifications for dimensionality, property, accuracy, precision, robustness, detection limit and quantization limit.

- Linearity
  Linearity is evaluated by multivariate analysis of normal or standard mixture at concentration points in triplicate starting from 1.5 to 3.5 mg that's on 3 consecutive days (n = 3). The values are the reported as the mean ± S.D. of the calibration curves. Coefficient of correlation and analysis of variance (ANOVA) are to calculated and given. The analytical curves are generated on 3 consecutive days (n = 3), by plotting the mean absorbance values of spectra at 1757-1671 cm^{-1} against concentration yielded correlation coefficients. To boot, the data were validated by means of analysis of variance, which showed significant linear regression.

- Selectivity
  It is evaluated by analysis of the spectra of the mixture of placebo and the working normal mixture at the concentration of 2.5 mg. The spectra analyses ought to show that formulation excipients of the pharmaceutical product failed to interfere considerably within the infrared spectroscopic analysis technique.

- Accuracy

  The accuracy is set by measuring the reference normal recovery in triplicate at 3 levels starting from 80 to 120% of the method concentration (2.5 mg),then continuing in keeping with ICH recommendations. The accuracy of the strategy or the process was confirmed by determing the average recoveries from the samples by applying the quality addition method. The mean percentage recoveries of product ought to be in accordance with the mounted limits of 98.0% up to 102.0%, indicating the quality of the developed technique in quantifying the concentration of analyte in pharmaceutical tablets.

- Precision

  Both repeatability and intermediate precision is to evaluated. Repeatability was studied by analyzing work normal solutions at identical concentration and through identical day. Intermediate exactitude was evaluated by repetition of the assays on 2 completely different days by 2 analysts.

- Robustness

  The hardiness of the strategy is additionally to be evaluated. This is done by analyzing knowledge when checking the time of compression, pressure and therefore the mark of restrainer. Applied mathematics analysis is that the performed to gauge the influence of variation in time, compression pressure and alternative parameters. The hardiness is additional confirmed by F check (Snedecor) homo geneity of variance and t (Student) to match the mean, that is given below; Fcalculated < Fcritical, P = five-hitter and tcalculated < tcritical, P = 5%. Thus, the means are equivalent.

- Limits of Detection and Quantification

  LOD and LOQ values were found and therefore the values near to zero that indicate the sensitivity of the strategy.

- Assay of Pharmaceutical product

  The IR spectroscopic analysis technique was validated and then applied for quantization in prescribed drugs like tablets. The results of the sample spectroscopic analysis measurements were obtained that was then compared with those obtained from normal mixtures at identical concentration levels.

Method validation of UV spectroscopy

The proposed technique was validated for varied parameters like accuracy, precision, limit of detection (LOD), limit of quantization (LOQ), robustness, ruggedness, sensitivity and specificity in keeping with ICH guideline and USP pointers.

- Linearity and range

The linearity or dimensionality of an analytical procedure is its ability (within a given range) to get result that are directly proportional to the concentration of an analyte within the sample. The range of an analytical procedure is that the interval between the higher and lower concentration of an analyte in the sample that it's been incontestable that the analytical procedure includes a appropriate level of precision, accuracy and dimensionality. The linearity of the analytical technique was established over the concentration range investigated by triplicate analysis (n = 3) at a concentration range of 2-14 µg/ml. The absorbance obtained at various concentrations was recorded, and therefore the graph is premeditated as concentration (µg/ml) versus absorbance. The rectilinear regression equation and the coefficient correlation were obtained from the UV probe Software.

- Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between price or the worth that is accepted either as a traditional true worth or an accepted reference worth and the value found. This can be generally termed as trueness. The accuracy of proposed technique was determined on the premise of recovery study. Recovery study was carried out by spiking normal operating resolution to sample resolution (formulation) at 3 completely different levels 80%, 100 % and 120%.The final concentration of the sample was determined at every levels of the amount; 3 determinations were performed. The percentage recovery was calculated as mean±standard deviation.

- Precision

It is the exactitude of an analytical procedure.Simply put it expresses the closeness of agreement or degree of scatter between a series of measurements obtained from multiple sampling of the consistent sample underneath the prescribed conditions. The exactitude of the strategy was incontestable by intra-day and inter-day variation studies. In the intra-day precision study, 3 completely different solutions of same concentration were made and analyzed within the same day (morning, noonday and evening), whereas in the inter-day exactitude study, the solutions of same concentration were prepared and analyzed, for 3 consecutive days, and therefore the absorbance were recorded. All study was performed in triplicates. The result was indicated by machine RSD.

- Limit of detection (LOD)

The detection limit of a particular analytical procedure is the lowest quantity of analyte in a sample, which may be detected, however not essentially quantified as a definite worth. The limit of detection (LOD) was calculated by making solutions of various concentrations from 2-14µg/ml.

$LOD = 3.3\ \sigma/S$

Where,

σ=Standard deviation
S= Slope

- Limit of quantification (LOQ)

The detection limit is that the lowest quantity of analyte in a sample which may be detected and quantified to an appropriate degree of precision and accuracy.The LOQ was calculated using the formula involving the standard deviation of response and the slope of the calibration curve.
LOD = 10 σ/S
Where,
σ=Standard deviation
S= Slope

- Sensitivity

The sensitivity of the strategy was determined by calculating the various parameter like molar absorption factor and Sandell's sensitivity.

- Robustness

The robustness of an analytical procedure is the measure of its capability to remain unaffected by small, however deliberate variations in technique parameters and provides a sign of its reliableness throughout traditional usage. For determining the robustness of the projected technique, normal resolution was prepared and analyzed by a change in wavelength. The wavelength was to be chosen λmax ± 1.

- Ruggedness

It could be a degree of reliableness or reproducibility of test result undern verification of condition like a completely different analyst, instruments and different days. To establish ruggedness of the projected technique, standard solution was prepared and analyzed with the change in the different analyst.

- Specificity

Specificity is that the ability to assess the analyte unambiguously within the presence of elements which can be expected to be present. Generally these would possibly embody impurities, degradents, matrix, etc. The 3 completely different concentrations at 3 levels say, 80%, 100%, 120% respectively of the normal is prepared which is then spiked. At every level of the number, the triplicate study was performed to examine the result of the spiked impurity.

CONCLUSION

Validation is concerned with providing a high degree of assurance for the method potency and it's strength. It is an quality attributing tool employed in the pharmaceutical industries. It additionally helps with the reduction of price related to method assuring, sampling and testing. It ensures the consistency and reliableness of a valid method to provide a top quality product, which is the inspiration of any trade. It builds confidence, not just for the developer but also to the user. Though the validation exercise might seem pricey and time

overwhelming, it proves to be economical by eliminating errors and breakdowns and results in higher productivity.

REFERENCE

1.Shine Sudev, Sreejanardhanan.V,; Review on HPLC method Development Validation and Optimization;Int.J.Pharm.Sci.Rev.Res.2019,56(2) 28-42.

2.Shine Sudev, Farsana,Mohamed.P, Ameer Suhail.A.K,Anju Jose; comprehensive study and review on Pharmaceutical validation;jetir;June 2019 volume 6 issue 6 pg no:90-102

3.Azim Md. Sabir, Mitra Moloy, Bhasin Parminder S; review on HPLC metho development and validation;Azim Md. Sabir et al. Int. Res. J. Pharm. 2013, 4 (4)

4.Singh SP, Dhaker K, Namdev A, Khinchi MP, Bhatnagar S; A Review on Process Validation; PharmaTutor; 2016; 4(12); 9-19.

5.http://www.ofnisystems.com/services/validation/validation resources/. Overview of validation documents and projects.2019.

6.Dr.keyur B,Ahir, Khushboo D. Sing, Sushma P, Yadav, Hetal S, Patel,Chetan B, Poyahari; Overview of Validation and Basic Concepts of Process Validation.2014:3(2):178-190

7.Monika Sharma,Shikha Agarwal,Shweta Agarwal,Manoj Sharma; A Review on prospective validation;the pharma innovation journal2015;4(3):01-07.

8.Kaur Harpreet*,Singh Gurpreet,Seth Nimrata;A Review on Pharmaceutical Process Validation; Journal of Drug Delivery and Therapeutics;2013, 3(4),189-194.
9.Dr.keyur B,Ahir, Khushboo D. Sing, Sushma P, Yadav, Hetal S, Patel,Chetan B, Poyahari; Overview of Validation and Basic Concepts of Process Validation; Scholars Academic Journal of Pharmacy;2014;3(2):178-190

10.Elsie Jattoand Augustine O.Okhamafe; An Overview of Pharmaceutical Validation and Process Controls in Drug Development.;Tropical Journal of Pharmaceutical Research,December 2002;1(2):115-122.

11.Lindholm J, Development and Validation of HPLC Method for Analytical and Preparative Purpose, Acta Universities Upsaliensis Uppsala, 2004; 13-14.

12.V. Kumar, R. Bharadwaj, G.G., S. Kumar, An Overview on HPLC Method Development, Optimization and Validation process for drug analysis, The Pharmaceutical and Chemical Journal, 2(2) (2015) 30-40.

13.Validation of Compendial Procedures, United StatePharmacopeia, USP 36 NF, 27 (2) (2010). Validation of Analytical Procedures: Text and Methodology, International Conferences on Harmonization, Draft Revised .

14.ICH-Guidelines Q2 (R1), Validation of Analytical Procedures: Text and Methodology. 2005

15.Ahuja S, Scypinsk S. 2001. Handbook of modern pharmaceutical analysis. 5th ed., London: Academic Press. p 345-442.

16.Lindsay S. 1987. HPLC Analytical chemistry by open learning, 4th ed., New York: John Wiley &Sons. p 54-72.

17.Snyder LR, Kirkland JJ, Glajch JL. 1997. Completing the method: Validation and transfer. In Snyder LR, Kirkland JJ, Glajch JL, editors. Practical HPLC method development, 2nd ed., New York: John Wiley & Sons. p 233-312.

18.Quality Management System – Process Validation Guidance GHTF/SG3/N99-10, 2004 (Edition 2).

19.Kumar's pharmaceutical process validation: A CGMP concept. Pharmatutor, 3(1), 2011, 1-9.

20.Chows. Pharmaceutical validation and process control in drug development. Drug information journal, 31, 1997,1195-1201

21.Guidelines for Process Validation of Pharmaceutical Dosage Form – Saudi Food & Drug Authority, Version 2, February, 1992.