Analytical Method Development and Validation: A Review

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Abstract: The main objective of analytical method development and validation are the incessant and inter-reliant task related with the research and expansion in quality control & quality assurance departments. Analytical methods play a critical role in manufacturing of pharmaceuticals and evaluation of medicines in pharmaceutical formulation and it also helps in establishment of product-specific acceptance criteria and constancy of results. In pharmaceutical industries, validation process is very essential for the effective running of pharmaceutical firms which includes performance of validation, types of validation and validation policy are comply with the requirements of Good Manufacture Practice (GMP) regulations. A novel, safe and susceptible methods like UV-Visible spectrophotometric, HPLC and Chromatography methods has been discussed in this article. A method will be developed which will give information about validation parameters like accuracy, precision, linearity, LOD, LOQ, specificity, range, robustness, ruggedness, repeatability and reproducibility. Validation provides major improvements in precision and a reduction in errors. It can further help to avoid expenses and time-consuming exercises.

Key words: Chromatography method, Error, Precision, Spectrophotometric, Validation.

1. INTRODUCTION

1.1 Analytical chemistry

Analytical chemistry is a branch of chemistry which deals with recognition of components (qualitative) and determination of quantity of components (quantitative) of substances or samples or mixture. There are two types of analysis, one is qualitative analysis and one more is quantitative analysis. In qualitative analysis, there is recognition of components or analyte of mixture or sample is passed out. In quantitative analysis, there is determination of quantity of components or analyte of mixture or sample is passed out [1, 2]. Analytical data is not mandatory just in chemistry although also in other sciences like biology, zoology, arts such as painting and sculpture, archaeology, space investigation and clinical analysis. Significant areas of application of analytical chemistry are quality control in manufacturing industries, monitoring and control of pollutants, clinical and biological studies, and geological assays, fundamental, biomedical science, forensic science and applied research [2, 3].

Analytical techniques also help in determining the levels of toxic waste in the body like uric acid, cholesterol, drugs and some salts.

1.2 Analytical methods development

The principle of analytical method development is to set up the identity, purity, physical characteristics, and strength of drugs, as well as the drug's bioavailability and stability.

Analytical method development and validation can be understood as the procedure of viewing that analytical procedures are sufficient for the purpose of assessing drugs, and mainly the active pharmaceutical ingredient (API).

Analytical procedures are developed to check specific characteristics of the substances beside the predefined acceptance criteria for such characteristics.

Thus, analytical method development involves the evaluation and selection of the most precise assay procedures to determine the composition of a drug.

Analytical method could be spectral, chromatographic, electrochemical, hyphenated or miscellaneous. Analytical method development is the method of selecting correct assay procedure to determine the composition of a formulation. It is the process of proving that an analytical method is suitable for use in laboratory to determine the concentration of

subsequent samples Analytical methods should be used inside GMP and GLP environments and must be developed using the protocols and acceptance criteria set out in the ICH guidelines Q2(R1). The prerequisite for method development are as follows [4-8].

1. Qualified and calibrated instruments
2. Documented methods
3. Reliable reference standards
4. Qualified analysts
5. Sample selection and integrity
6. Change control

1.3 Analytical Method Development is also required for:-

1. Herbal products and their effectiveness
2. New procedure and reactions
3. New molecules development
4. Active ingredients (Macro analysis)
5. Residues (Micro analysis)
6. Impurity profiling
7. Component of interest in different proportion
8. Degradation studies

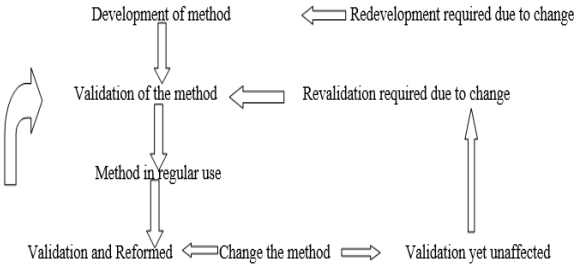

Figure1: The life cycle of analytical method [8, 9].

The life cycle of an analytical method is brief as shown in Figure 1. The common steps followed in the method development are as follows:

1. Standard analyte characterization
2. Method requirements
3. Literature search
4. Selecting the method
5. Instrumental setup and preliminary studies
6. Optimization of parameters
7. Documentation of analytical figure
8. Evaluation of the method development with the sample
9. Determination of percent recovery of the sample
10. Demonstration of quantitative sample analysis.

1.4 Necessities of method development

Method development is the keystone of medical device and pharmaceutical development. It is often one of the first steps in identifying and understanding the chemical nature of a target molecule or material. It can also be used to identify potential impurities or degradants in a formulation.

Steps for developing a method a variety of steps are involved in the development of an analytical method are as follows:

1.5 Characterization of analyte and standard

• All the known necessary data relating to the analyte and its structure that is bring up 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 formed for the proper storage (refrigerator, desiccators, and freezer)

• In the sample matrix, when several parts are to be measured the amount of elements is observed duly presenting the information and the accessibility of standard are calculate.

• Techniques like spectroscopy (UV-Visible, FTIR, atomic absorption spectroscopy, etc.), high-performance liquid chromatography and gas chromatography so on and, are however about once coordinated with the stability of samples.

1.6 Requirement of the technique:

Requirement of analytical methodology is necessary 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 valid analytical ways with reference to applicable 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 growing since the method is being

modified wherever desired. Sometimes, it is significant to obtain additional instrumentation to create, change or replicate and validate existing procedures for analytes and tests.

• If there are not any past appropriate ways available to examine 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 methodology is examined by a proper 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 required for accomplished basing on a scientific organized method plan duly all necessary points and documented with relation to dead ends [9, 10].

The instrumentation techniques can be classified in three principal areas:

1. Spectroscopy
2. Electrochemistry
3. Chromatograph.

Analysis can be performed in two ways

1. Classical Chemical Methods (wet techniques)
2. Instrumental Methods

2.INTRODUCTION TO SPECTROSCOPY

Spectroscopy is the study of interaction of electromagnetic radiation with matter. These interactions involve absorption and emission of radiation (energy) by the matter. Spectroscopy is of two types, absorption spectroscopy and emission spectroscopy. The study of electromagnetic radiation absorbed by the sample, in the form of spectra is called absorption spectroscopy (UV-visible, IR, NMR, microwave and Radio wave spectroscopy). The study of electromagnetic radiation emitted by the sample, in the form of spectra is called emission spectroscopy (flame photometry and fluorimetry). Spectroscopy is helpful for the study of atomic and molecular structure and used in the analysis of a wide variety of samples. Atomic spectroscopy is the study of interaction of electromagnetic radiation with atoms, changes in energy takes place at atomic level (e.g. atomic absorption spectroscopy and flame photometry). Molecular spectroscopy is the study of interaction of electromagnetic radiation with molecules, changes in energy takes place at molecular level (e.g. ultraviolet and infrared spectroscopy) [2, 11].

2.1 UV-VIS spectroscopy

In UV-visible spectroscopy, the amount of light absorbed at each wavelength of UV and visible region of electromagnetic spectrum is considered. This absorption spectroscopy uses electromagnetic radiations among 200 nm to 800 nm and is divided into the ultraviolet (UV, 200-400 nm) and visible (VIS, 400-800 nm) regions.

The principle of UV-Visible spectroscopy is based on the absorption of ultraviolet light or visible light by sample or chemical substance which results in the production of different spectra. When a molecule absorbs UV radiation, the electron present in that molecule undergo excitation, this causes transition of electron within a molecule from a lower level to a higher electronic energy level and the ultraviolet emission spectra occur from the reverse type of transition. Most frequently used solvents in UV spectroscopy are water, methanol, ethanol, ether, chloroform, carbon tetrachloride, cyclohexane and dichloromethane. Applications of UV spectroscopy are detection of functional groups, detection of conjugation, detection of geometrical isomers and detection of impurities.

1.2 Instrumentation of UV-visible spectroscopy:

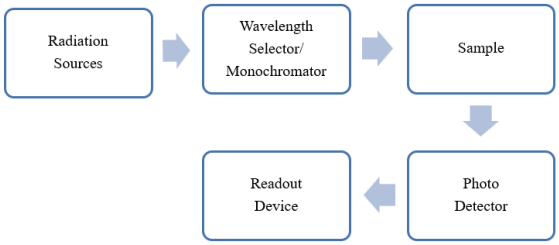

Figure 2: UV- Visible spectroscopy

- Radiation Source

Hydrogen-discharge lamp is the most frequently used source of radiation in the U.V region (200-400 nm) while a deuterium-discharge lamp is used when more intensity (3-5 times) is preferred. A tungsten-filament lamp is used when absorption in the visible region (400-800 nm) is to be determined.

- Monochromator

It helps to split the radiations into separate wavelengths that are it only allows passing a specific wavelength through it. Monochromators are usually made up of prism or grating which is made up of quartz. This is so because quartz does not absorb the radiations thus ensuring no loss of intensity and exact results.

- Beam separator

As the name suggests, beam separators help to split the single radiation into two different paths/chambers that is the reference chamber and the sample chamber. The former is called the reference beam and the later is known as the sample beam.

- Detectors

Detectors have photocells or photomultiplier tubes that produce a voltage proportional to the radiation energy that strikes them.

- Amplifier

The spectrophotometer has a balancing electronic amplifier that subtracts the absorption of the solvent from that of the solution electronically.

- Recorder

A recorder automatically records the spectrum as a plot of the wavelengths of absorbed radiations against absorbance (A) or molar absorptivity (e) [2, 11-12].

2.3 Working of U.V-Visible spectroscopy

When the U.V-Visible range electromagnetic radiation is emitted by the source, it passes through a monochromator which separates the electromagnetic radiations into separate radiations of different wavelengths.

Then the desired wavelength electromagnetic radiations components are passed through a beam separator which divides the radiations into two chambers that is reference chamber containing reference sample and sample chamber containing actual sample which is to be analyzed.

Then, the radiations penetrate both the samples, and some radiations are absorbed by the sample while some different wavelength is transmitted without any absorbance. These transmitted radiations fall on the amplifier which subtracts the absorption of the solvent from that of the solution. Then finally, the transmitted electromagnetic radiations fall on the detector then recorded by a recorder.

The most significant thing is that the U.V-Visible spectrometer does not plot the graph between the transmittance and wavelength instead it plots the graph between absorbance and the wavelength because it is easy for the expert to analyze the graph as it is linear and not inverted.

3.INTRODUCTION OF CHROMATOGRAPHY

Chromatography is a physicochemical method for separation of mixture of compounds. Chromatography is a method of separation of mixture of compounds into individual components among two phases, a stationary phase and a mobile phase.

3.1 Chromatography is classified as follows:

1. Based on interaction of solute to stationary phase
   - Adsorption chromatography
   - Partition chromatography
   - Ion exchange chromatography
   - Molecular exclusion chromatography
2. Based on chromatographic bed shape
   - Column chromatography
   - Planar chromatography
   - Paper chromatography
   - Thin layer chromatography
   - Displacement chromatography
3. Techniques by physical state of mobile phase
   - Gas chromatography
   - Liquid chromatography
   - Affinity chromatography [2, 13].

4. INTRODUCTION OF HPLC

High performance liquid chromatography (HPLC) or high-pressure liquid chromatography. HPLC be capable of separation, reorganization and determination the chemical mixtures and quantitation of component [3, 13]. The key principle of liquid chromatography is adsorption. It is a chromatographic process in which mobile phase is liquid. Sample is in the form of liquefied solution. Sample is injected into column of a spongy material (stationary phase) and a liquid phase (mobile phase). Sample goes through the column with mobile phase via high pressure delivered through a pump. Sample components move according to their attraction towards the immobile phase. The component which has more affinity towards the immobile phase travels slowly. The component which has less affinity towards the stationary phase moves

more rapidly. The components are separated from each other [2, 14]. The most familiar solvents used for HPLC are n-hexane, methylene chloride, choloform, methyl-t-butyl ether, Tetrahydrofuran (THF), Isopropanol (IPA), Acetonitrile (MeCN or CAN), and Methanol. Basic chromatographic parameters are efficiency (number of theoretical plates), retention factor, selectivity, resolution and pressure. Application of HPLC is chemical separation, purification and detection. Additional applications of HPLC consist of pharmaceutical applications, environment application, forensics, clinical, food and flavor.

Instrumentation of HPLC:

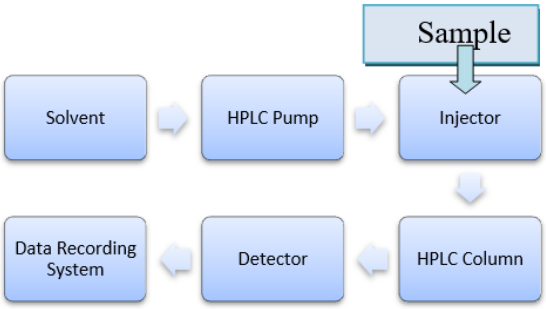

Figure3: HPLC System

Components of the HPLC system:

- Solvent reservoir, mixing system and degassing system
- High pressure pump
- Sample injector
- Column
- Detector
- Data recording system [2, 15].

Solvent reservoir, mixing system and degassing system:

Solvent reservoir supplies the solvent (mobile phase). These are glass or stainless-steel containers. The most common type of solvent reservoir is glass bottle. In addition to delivery of mobile phase, the pump must mix solvents with high accuracy and high precision. Two types of mixing unit are low pressure mixing and high pressure mixing. Degassing system removes entrapped air bubbles from the solvent. Degassing is done by degasser techniques are ultra-sonication and filtration [2, 16].

High pressure pump:

The role of pump is to force a liquid and give a specific flow rate. Flow rate is expressed in milliliters per minute (ml/min). Normal flow rate is 1-2 ml/min. Pump pressure range is 6000-9000 psi (400-600 bar) [2, 17] .Commonly used pump types are constant pressure pump, syringe type pump and reciprocating piston pump [2, 18].

Sample injector:

The liquid sample is introduced into the mobile phase by sample injector. Sample valve come between the pump and the column. An injector (auto sampler) is able to inject the sample into the continuous flowing mobile phase stream that carries the sample into the HPLC column. Typical sample volumes are 5-20 micro liters (µl) [3, 20]. Two types of injector are manual injector and automatic injector [2, 19].

Column:

Column is a place where definite separation of components takes position. Column is made up of stainless steel. It is 5-25 cm elongated and 2-4.6 cm inner diameter [2, 20].

Detector:

The detector can detect the individual component that elute from the column and convert the data into an electrical signal. Types of detector used are of two types, specific detectors and bulk property detectors. Specific detectors include UV-VIS detector, photo diode array detector, fluorescence detector and mass spectrometric detector. Bulk property detectors include refractive index detector, electrochemical detector and light scattering detector [2, 19-20].

Validation

Validation: 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 required for any fresh 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. Validation is an important component of quality assurance; it includes the efficient investigation of systems, facilities, and procedures aimed toward

deciding if they carry out their planned capacities satisfactorily 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.

Important stages in validation
The action identifying with validation studies can be categorized mainly into three stages:
Stage 1: This includes pre-validation qualification stage which covers all exercises identifying with product studies and improvement, formulation pilot batch testing, scale-up research, exchange of innovation to business scale groups, setting up stability conditions, and managing of in-process, finished pharmaceutical formulations, qualification of equipment, master documents, and process limit.
Stage 2: This involves process validation phase. It is intended to check that every installed limit of the vital process parameter is substantial and that satisfactory products can be created even below the worst situations.
Stage 3: It is also called as the validation maintenance stage, it requires constant review of all procedure related archives, including validation of the review reports, to guarantee that there have been no modifications, departure, failures, and alteration to the production procedure and that all standard operating procedures (SOPs), involving change control procedures, had been observed. At this phase, the approval team involving people representing all essential departments also guarantees that there have been no modifications/deviations that ought to have brought about requalification and revalidation [9, 21].

Importance of validation
• Assurance of quality
• Minimal batch failure
• Reduction in rejections
• Improved efficiency and productivity
• Increased output
• Reduced testing in process and in finished goods.
• 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 [2, 22].

Types of validation:

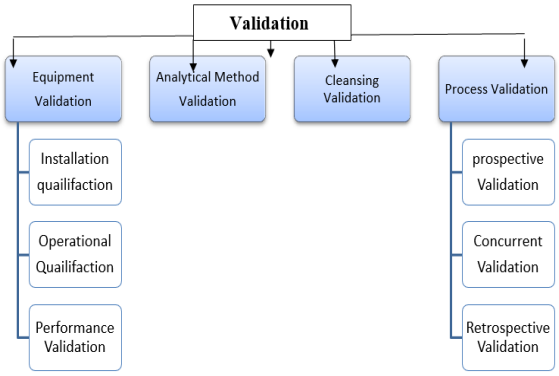

Figure: 4 Types of validation [9, 22].

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.

Equipment Validation includes the following:
Installation qualification (IQ): IQ guarantees all crucial processing, packaging system, and ancillary items are in compliance with the installation. It checks that the equipment has been established or installed as per the manufacturer's suggestion in a systematic way and positioned in surrounding appropriate for its meant purpose.

Installation qualification points include
• Equipment layout character that is the material of construction cleanability and many others.
• Installation situations like wiring, functionality, utility and so forth.
• Calibration, preventative protection, cleansing plans.
• Safety characteristics.

• Supplier documentation, prints, illustrations, and hand operated.
• Software documentation.
• Enlist the spare components.
• Environment-related conditions like clean room necessities, humidity, and temperature.

Operational qualification (OQ): OQ performed to give a high level of degree of affirmation that the equipment works as proposed.
OQ concerns consist of:
• Process control limits like temperature, time, and stress, line velocity, set up conditions, and so on.
• Software parameters.
• Crude material details.
• Process operating methods.
• Material managing necessities.
• Process change control.
• Training.
• Short-term balance and capability of the technique.
• The use of statistically valid procedures inclusive of screening examinations to optimize the technique can be utilized throughout this stage.

Performance qualification (PQ): PQ checks that the device is repeatable and it is uniformly producing a quality item.
PQ concern consists of:
• True product, procedure parameters, and process set up in OQ.
• Adequacy of the product.
• Guarantee of technique ability as built up in OQ.
• Process repeatability, prolonged process stability [9, 23-24].

Process validation
The process validation is a component of the coherent prerequisites of a quality management system. Process Validation is the most essential and perceived parameters of current good manufacturing practices. The objective of a quality system is to produce items that are matched with their proposed use uniformly. Process approval is a key component in guaranteeing that these standards and objective are met. Process validation is reported evidence which gives a high level of affirmation that a particular procedure will produce a product meeting its determined prerequisites. It mainly involves the following [9, 25].

Prospective validation: It is described as the well-known reported program that a device does what it indicated to do base on pre-planned protocols. This validation is normally performed previously for distribution both of a newer item or item made under a revised production process. In this validation, the protocol is accomplished before the procedure is placed into industrial use. Prospective validation ought to incorporate, however, not be limited to the subsequent:
• Short depiction of the procedure.
• Summary of the important processing steps to be evaluated.
• Equipment/facilities list is to be utilized (involving calculation, observing/recording equipment) collectively with its calibration status.
• Finished dosage forms for discharge.
• List of analytical techniques, as suitable.
• Proposed in-process controls with specification criteria.
• Additional testing to be completed, with specification limits and analytical approval, as suitable.
• Sampling design.
• Techniques for recording and assessing outcomes.
• Functions and obligations.
• Proposed timetable [9, 26].

Concurrent validation: It is same as prospective validation with the exception of the working firm, will offer the product at the time of qualification runs, to the society at its market cost, and furthermore like retrospective validation. This type of validation includes in-process observing of vital processing steps and product checking out. This helps to produce and reported proof to demonstrate that the manufacturing technique is in a condition of control. This approval includes in-process observing of essential processing steps and product testing. This creates and recorded proof to demonstrate that the production procedure is in a condition of the control.
• In remarkable conditions, it might be acceptable not to finish the validation program before routine manufacturing begins.
• The choice to complete simultaneous approval must be supported, archived and accepted by authorized personnel.
• Documentation prerequisites for simultaneous validation are similar as designated for prospective validation [9, 25-26].

Retrospective validation: It is characterized by the established reported confirmation that a system does what it implies to do on the audit and investigation of historical data. This is accomplished by the survey of the ancient manufacturing testing information to demonstrate that the procedure has always remained in control. This kind of approval of a procedure for an item already in distribution. Retrospective validation is adequate for well established procedures and will be wrong where there have been current modifications within the composition of the product, working methods or device.

Few basic components of retrospective validation are:
• Batches are produced for a definite duration (last 10 successive batches). The number of lots discharged every year.
• Batch size/strength/producer/year/period.
• Master manufacturing/packaging files.
• Current particulars for active ingredients/finished materials.
• List of process deviations, corrective actions, and modification to production archives
• Data for stability study for a few batches [9, 25-26].

Revalidation: Revalidation gives the proof that modifications in the procedure, as well as the procedure condition that are presented don't unfavorably influence process attributes and product quality. Organizations, facilities, equipment and methods which include cleaning, ought to be periodically assessed to affirm that they stay valid. Where no remarkable modifications have been made to the approved status, a review with proof that facilities, organizations, equipment and procedures address the recommended necessities satisfies the need for revalidation.
Revalidation becomes vital in specific circumstances. Few of the modifications that require validation are mentioned below:
• Modifications in crude materials.
• Modifications in the equipment.
• Modifications in the source of active crude material producer.
• Alteration of packing material.
• Modification of the procedure.
• Modifications inside the plant/facility.
• A selection is no longer to carry out revalidation studies have to be completely justified and reported.

Analytical method validation:
Validation of an analytical approach is established through laboratory research, that the execution attributes of the procedure meet the requirements for the proposed scientific application. Validation is required for any new or altered procedure to verify that it is fit for giving predictable and dependable outcomes, once used by various administrators by usage of comparable instrumentation inside the similar or absolutely distinct laboratories.
Method validation is a reported program that offers with that the processing system will give a high level of affirmation to meet its predicated acceptance basis [9, 26-27].

It consists of mainly five different steps which are as follows:
Qualification of the system: System qualifications permit to check that the instrument is appropriate for the planned investigation, the materials are appropriate to be used in analytical judgments, the analysts have the correct instruction, capabilities, and foregoing documentation such as analytical inclusive of analytical approaches, proper authorized protocol with pre-set up standards have been reviewed. On the off chance that the general qualifications of a device are overlooked, and trouble arises, the source of the issue will be hard to recognize.

Sampling: Sampling assists in the choice of a representative part of the fabric which is along these lines subjected to evaluation. The selection of a suitable sampling technique is of significant importance since it gives assurances that the sample chose is really illustrative of the material as a whole for the purpose of important statistical inferences. Inside the statistical literature, there is a considerable collection of work on sampling techniques, anyway the relative expenses and time engaged with every technique ought to be assessed ahead of time.

Preparation of sample: Preparation of the sample is a key component to effective method validation. It has been mentioned that sample planning represents 60 to 80% of the work action and working expenses in an investigative lab. The literature on the preparation of the sample is enough and properly documented. In any case, the investigator ought to recall that the choice of a particular preparation technique relies upon

concentrations of analytes, sample matrix, size of the sample and the instrumental method.

Analysis of sample: The evaluation is associated with the instrument utilized to extract qualitative or quantitative data from the samples with an adequate vulnerability level. The investigation could be predictable, in a great sense, as the device has 3 interconnected fundamental components, namely input, converter, and output. The input and output are assigned by the letters x and y, and they represent the concentration and response individually. The selection of a specific analysis depends on many considerations, for example, the chemical properties of the analytical species, the concentration of the analytes in the sample, sample matrix, speed, cost, and so forth.

Assessment of data: The essential reason behind information assessment is to outline and pick up knowledge into a specific informational index by utilizing numerical and statistical techniques. Data assessment permits extracting valuable data and reaching inferences about the inputs and outputs, and in particular about the validation procedure in general [9, 28].

Cleaning validation

Cleaning validation is a reported proof with a high level of confirmation that can uniformly clean a system or equipment to already determined and specification criteria. Cleaning approval is a reported procedure that demonstrates the efficacy and consistency in cleaning pharmaceutical production equipment. The goal of cleaning approval is to check the viability of the cleaning system for the expulsion of product deposits, degradants, additives, excipients, or cleaning agents and in a the control of potential microbial contamination.

It is vital to validate cleaning techniques for the following motives:

• Pharmaceutical products and active pharmaceutical ingredient (API) can be contaminated by other products and microbes.

• It is an administrative prerequisite in pharmaceutical product manufacture the worry is the same-guarantee that the equipment is properly clean and safety and quality is kept up.

• It is likewise guaranteed from an inside control and consistency perspective the quality of manufacture.

• To protect product integrity.

• To reuse the equipment.

Necessity for cleaning validation

To check the viability of cleaning techniques and to make sure that no risks are related to cross-contamination of API or detergents [9, 29-30].

Cleaning validation protocol:

• The goal of the validation procedure.

• Obligations regarding performing and endorsing the validation study.

• Equipment details.

• The interval between the end of production and the start of the cleaning techniques.

• Cleaning methods to be utilized for every product, each manufacturing device or each piece of equipment.

• The quantity of the cleaning cycle to be performed continuously.

• Routine checking equipment.

• Sampling techniques, including the basis for why a specific sampling technique is utilized.

• Clearly defined sampling areas.

• Information on recovery studies, where suitable.

• Analytical techniques including LOD and LOQ.

• The acceptance criteria, along with including the method of reasoning for getting specified limits [9, 31].

Validation parameters:

The main aim of method validation is to produce proof that the method will what it is supposed to do, accurately, reliable and consistent [10, 21].

The validation parameters as per ICH guidelines are described below:

1) Accuracy
2) Precision
3) Linearity
4) Limit of detection
5) Limit of quantitation
6) Specificity
7) Range
8) Robustness [2, 16].
9) Ruggedness
10) Repeatability
11) Reproducibility [9, 32].

1) Accuracy: Accuracy is defined as the closeness of the test results to the true value.

2) Precision: Precision is defined as the measurement of closeness of agreement for multiple measurements

on the same sample. The precision is expressed as the relative standard deviation. %RSD = Standard deviation/Mean ×100.

3) Linearity: Linearity is the ability of analytical procedure to obtain a response that is directly proportional to concentration (amount) of analyte in the sample. Linearity is expressed as the confidence limit around the slope of the regression line.

4) Limit of Detection (LOD): LOD is defined as lowest amount (concentration) of analyte in a sample that can be detected or identified, not quantified. LOD is expressed as a concentration at a specified signal: noise ratio, usually 3:1. LOD = 3.3 × S/ SD.

5) Limit of Quantitation (LOQ): LOQ is defined as lowest amount (concentration) of analyte is a sample that can be quantified. For LOQ, ICH has recommended a signal: noise ratio 10:1. LOQ = 10 × S/SD.

6) Specificity: Specificity is defined as the ability of an analytical method to measure the analyte clearly in the presence of other components.

This definition has following implications:

a. Identification

b. Purity tests

c. Assay

7) Range: The range of the method is the interval between upper level and lower level of analyte that have been determined with acceptable accuracy, precision and linearity. It is determined on either a linear or nonlinear response curve and expressed in the same unit as the test results are expressed.

8) Robustness: Robustness is defined as the measurement of capacity of analytical procedure to remain unaffected by small variations in method parameters [2, 16].

9) Ruggedness: Ruggedness is the degree or measure of reproducibility under different situations such as in different laboratories, different analyst, different machines, environmental conditions, operators etc.

10) Repeatability: It expresses the exactness below a similar operating condition over a brief interval of time and also referred as intra-assay precision.

11) Reproducibility: It refers to the precision between different analytical labs [9, 32].

5.CONCLUSION

The main objectives of development of analytical methods are for identification, purification and eventually to qualification any necessary drug etc. The development of analytical methods helps in accepting the critical process parameters and to decrease their effects on precision and accuracy. This article also gives information about validation, its types, why it is essential, how to build up a method and how to carry out the validation procedure to demonstrate that the technique is able for its proposed reason. All validation parameters such as linearity, LOQ, LOD, Range, specificity, robustness, ruggedness. Validation is a important technique in the pharmaceutical industries and it is used to assure that the quality is worked into the procedures supporting the development of drug and production.

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