World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 6.129

METHOD DEVELOPMENT VALIDATION OF HPLC IN ANALYTICAL CHEMISTRY

Dr. V. Sreejanardhanan*

Professor and Head, Shri Venkateshwara College of Pharmacy, Puducherry Shine Sudev, Research Scholar, Shri JJT University, Rajasthan.

***Corresponding Author: Dr. V. Sreejanardhanan**

Professor and Head, Shri Venkateshwara College of Pharmacy, Puducherry Shine Sudev, Research Scholar, Shri JJT University, Rajasthan.

Article Received on 31/03/2023 **Article Revised on 20/04/2023** **Article Accepted on 11/05/2023**

ABSTRACT

The development of novel techniques for non-pharmacopoeial products is crucial as it can significantly reduce the time and cost required to achieve better and more distinctive outcomes. In order to guarantee the quality, dependability, and consistency of these newly developed methods, it is essential to validate them in accordance with the International Conference on Harmonization (ICH) guidelines. Different types of validation processes, including Prospective, Concurrent, Retrospective, and Revalidation, should be employed to ensure reliability. This study also addresses the prioritization of validation, protocols, and the use of High-Performance Liquid Chromatography (HPLC) instruments. Additionally, the advantages and limitations of HPLC are emphasized.

KEYWORDS:- Method Development, Process Validation, HPLC etc.

INTRODUCTION

Validation of new techniques is crucial in ensuring that they are trustworthy and produce consistent results. Prospective validation involves the validation of the method before its use, while concurrent validation involves the validation of the method during its use. Retrospective validation, on the other hand, involves the validation of an already established method. Revalidation is carried out when a significant modification is made to the method or instrument. The use of these validation processes can guarantee the reliability of newly developed methods. Prioritizing the validation process is vital to ensure that the method being validated has a significant impact on the product. The validation protocols used must be detailed and comprehensive to ensure that the validation process is carried out efficiently. The study also highlights the significance of using HPLC instruments, which are widely used in the pharmaceutical industry to analyze complex mixtures. HPLC provides high resolution, accuracy, and reproducibility in the separation and analysis of complex mixtures. Despite its numerous advantages, HPLC has its limitations. For example, it requires specialized personnel to operate, and the cost of equipment and maintenance can be high. In conclusion, the development of novel methods for non-pharmacopoeial products can significantly reduce costs and improve outcomes, but it is crucial to validate them using reliable processes and instruments like HPLC.

Method Development

When there are no official methods available for analyzing new products, alternate methods are introduced. These methods are also useful for existing non-pharmacopoeial products as they can reduce the time and cost required to achieve better precision and potency. However, it is important to compare laboratory data between the alternate and existing procedures to identify the advantages and limitations of using alternate methods. High-Performance Liquid Chromatography (HPLC) is a widely used method for analyzing complex mixtures in the pharmaceutical industry. The primary goal of HPLC methods is to separate and quantify the main active drug, reaction impurities, synthetic intermediates, and degradants. This can provide valuable information on the quality, purity, and potency of the product. While HPLC has many advantages, such as high resolution, accuracy, and reproducibility, it also has limitations. For example, it can be costly and requires specialized personnel to operate. Additionally, the method may not be suitable for all types of compounds and mixtures, and it may not be able to detect low levels of impurities or degradants, alternate methods can be valuable in the absence of official methods, and HPLC can provide accurate and reliable data on the quality and purity of products. However, it is important to consider the advantages and limitations of using these methods to ensure that the most appropriate approach is chosen for each situation.

Steps Involved in method development are

Understanding the physicochemical properties of drug molecules
Selection of chromatographic conditions
Development in the approach of analysis
Sample preparation
Method optimisation
Method validation

Method validation

Method validation is a crucial process used to verify that an analytical procedure is appropriate and effective for its intended use. It is essential to ensure the quality, reliability, and consistency of analytical data. The results obtained from the method validation process can be used to assess the suitability of the method and its applications. Method validation is an integral part of analytical practice, and it varies depending on the particular method and its applications. The validation process is performed to demonstrate that the method is specific, accurate, precise, and reliable. It is also used to ensure that the method is free from interferences and can detect the analyte of interest at the required levels. Method validation involves several steps, including defining the validation characteristics, determining the specificity, accuracy, precision, linearity, range, and limit of detection and quantification of the method, and evaluating the robustness and ruggedness of the method. These steps ensure that the method is optimized and validated for its intended use. The validation process must be carefully designed and executed to ensure that the results obtained are reliable and can be used to support the quality and safety of the product being analyzed. The validation protocol must be clearly defined and include all relevant parameters, acceptance criteria, and procedures for evaluating the results. Method validation is a crucial process for ensuring the quality and reliability of analytical data. It is an integral part of analytical practice and must be carefully designed and executed to ensure that the method is validated for its intended use. The results obtained from the validation process can be used to support the quality and safety of the product being analyzed.

Scope of process validation

Validation is one of the processes which engaged in all levels of product manufacturing the scope of validation in different fields are

Analytical

Instrument calibration
Process utility service
Raw materials
Equipment
Facilities
Product Design
Manufacturing Operations
Cleaning
Operators.

Importance of Validation

1. Validation can reduce costs by reducing-
Rejects
Reworks
Reliance on in-process controls
Down Time.
2. Help timely corrective action
3. Assure consistent production performance
4. Ensure achievement of quality goals
5. Allow parametric release, etc

Basic Concept of process validation

Calibration, Verification and maintenance of equipment
Prequalification
Establishing specifications and performance characteristics
Selection of methods, process and equipment to yield product
Qualification of process and equipment
Testing the final product by validated analytical methods to meet specification

Significance of process validation

The validation of a process involves the examination of data collected during the design and manufacture of a product to ensure that the process is capable of consistently producing products that meet predetermined standards. This process is critical for ensuring the safety and quality of drug products and is an essential component of the quality assurance system. The fundamental principle of quality assurance is that any drug product produced should be suitable for its intended use. The validation of a process provides assurance that the product is manufactured according to specific standards and requirements. It ensures that the process is reliable, consistent, and produces products that meet the intended specifications. The validation process involves the identification of critical process parameters, the establishment of appropriate acceptance criteria, and the development of a plan to monitor and control the process. The validation of a process is particularly important in the pharmaceutical industry, where drug products are subject to strict regulations and quality standards. The validation process ensures that the manufacturing process is capable of producing products that meet the required safety and quality standards. It also helps to identify any potential issues or risks associated with the manufacturing process and provides an opportunity to implement appropriate corrective measures. In conclusion, the validation of a process is critical for ensuring the safety and quality of drug products. It is an essential component of the quality assurance system and ensures that the manufacturing process is reliable, consistent, and capable of producing products that meet the predetermined standards.

Process validation contribute

Quality safety and efficacy are designed into the product
Quality of drug can be determined by inprocess and testing of finished product

The main aim of manufacturing process is that to produce final product meets all desired characteristic and quality attributes include specifications

Types of process validation

1. Prospective validation
2. Concurrent validation
3. Retrospective validation
4. Revalidation

Prospective validation

It is a method of establishing documented evidence prior to process implementation which that a system does for what it is proposed to do based on pre planned protocols. Validation protocol is executed before the process is put into commercialuse, it is normally used for the introduction of new drug products into a routine pharmaceutical production, and objective of this method is to prove that the process will work in accordance with the validation protocol.

It is done during the product development stage and it is not limited to
List of analytical method as appropriate
Proposed in process controls with acceptance criteria
Additional testing and analytical validation are carried out
Sampling plans
Methods for recording and evaluating results
Functions and responsibilities

Concurrent validation

It is similar to the prospective validation but the operating firm will sell the products during the qualification runs to the public with market price. It includes in process monitoring of steps and end product testing of current production that is running, this can provide documented evidence that the manufacturing process is in a state of control.

Retrospective validation

This type of validation is based on archives and the steps involved in this validation are

- Preparation of a specific protocol
- Reporting of the result of data review
- Conclusion
- Recommendation

The basics steps for retrospective validation are unique process specifications which characterise and should be constant with drug product final specifications and which are derived from acceptable process average and process variability.
Some most essential elements are
Batch size
Strength
Master manufacturing
Current specifications for active materials/finished products
Changes in manufacturing

Revalidation

It is a type of validation which includes the repetition of a validation process and changes in revalidation are source of active raw material manufacturers, packaging material, process and facility.

Order of priority in process validation

It mainly depends upon how much the product needs quality guarantee, accuracy, precision, cleanliness etc.
Quality and stability of parenteral products should be more and in the order of priority
1. Sterile product
2. Non sterile product

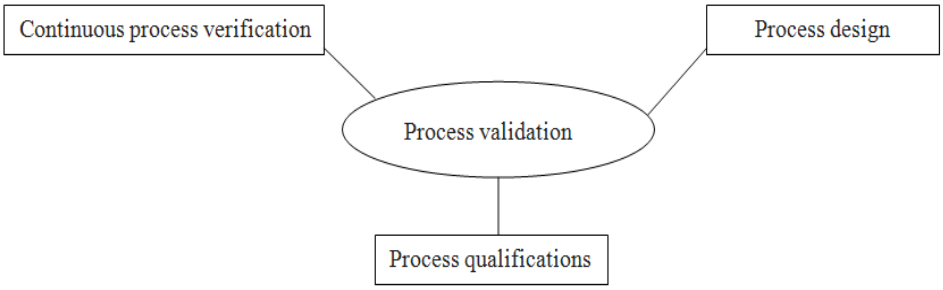
Sterile products and their processes

LVPS - Large volume parenterals
SVPS –Small volume parenterals
Ophthalmicand medical devices

Non sterile products and their processes

Low dose/ high potential tablets and capsules and its delivery
Drugs have stability problems
Other tablets and capsules
Oral liquids,topicals and Diagnostic aids

Various approaches in process validation



Process design

The aim of this stage is to design a process suitable for routine commercial manufacturing to deliver a product with desired qualities

Process qualification

It has two elements
Design of unique facility and qualification of equipment and utilities

PPQ - Process performance qualification

Continuous process verification
Goal of this stage is to provide continual assurance i.e., the process remains in state of control during manufacture

Three stages of life cycle approaches are
1. Design and development of products / process
2. Qualification of commercial manufacturing equipment
3. Maintenance of the process during production

Strategy for method validation
Process of preparation and execution should follow a validationprotocol andthe steps are
1. develop a protocol for operating procedure
2. define the application purpose and scope of methods
3. define the performance parameters and criteria
4. define validation experiments which includes equipment characteristics, selected quality materials (standard and reagents)
5. Pre- validation experiments
6. perform full internal and external validation experiments

Validation protocol
It is a written form which includes how the validation should conduct
Protocol as following
General Information
Objective
Background
Developmentand technical transfer (to justify in process testing and controls)
Previous Validation
Qualification status of equipments and facilities
Process flow chart
Manufacturing procedure

Applications
- HPLC has applications in the field of Pharmacy, Environmental, Clinical, Forensic and Food industry
- HPLC provide the information of compound about its resolution, identification and quantification
- It also plays role in chemical separation, molecular weight determination and purification of compounds

Chemical separation
- It is based on the fact that certain compounds have different migration rates and phases
- It is used for the separation of individual components

Purification
- It is the process of separation of desired compounds from a mixture of compounds or contaminants

Molecular weight determination
- It is used to determine the molecular weight of chemical substance, Proteins, etc.

Identification
- It is the process of assay of a compounds that are carried using HPLC since the retention time and separation process of desired component are different from the other compounds, hence we can identify the pure compound easily

Other applications of HPLC includes
Pharmaceutical applications
Dissolution study of Pharmaceutical dosage forms
Stability studies (shelf life determination)
Identification of Pharmaceutical ingredients of dosage forms
Assay of Pharmaceutical formulation
Quality control
Research and development

Environmental applications
Detection of phenolic components in drinking water
Identification of diphenhydramine in sedimented samples
Bio monitoring of pollutant

Forensics
Quantification of the drug in biological sample
Identification of anabolic steroids in serum, urine etc
Determination of presence of cocaine and metabolites in blood
Forensic analysis in textile industry

Clinical
Quantification of ions in the human urine
Analysis of antibiotics in plasma
Estimation of biliverdin in blood plasma (hepatic disorders)
Detection of endogenous neuropeptides in extra cellular fluids

Food and flavours
Quality of soft drink and drinking water
Analysis of alcohol and its derivatives
Sugar analysis in fruit juices
Analysis of polycyclic compounds in vegetables
Analysis of explosives in agricultural crops
Screening for pesticides and insecticides in fruits

Advantages
HPLC has advantages they are
- Growth of analytical science and applications
- High specific, reasonably precise and analytical method for complicated samples
- Tackling macromolecules
- Analysis of labile natural products
- Analysis of products including biochemical, polar, metabolic products etc.
- Preparation of sample is simple in HPLC

- Resolution of compounds and speed of separation is high
- Report precise and accurate results
- Sensitivity of detectors uses is high
- Stationary phases and columns are used
- Recording and storage of information is easy
- Columns operated can be reused for significant period of time
- HPLC coupled with mass spectrophotometers and FTIR system have efficiency
- HPLC coupled with hyphenated techniques can be used to analyse impurities in pharmaceutical products

Disadvantages

It also has certain limitations they are

- Price of column and solvents
- Lack of long term reproducibility
- Complexity of separation of certain antibodies specific to protein
- Cost of developing an HPLC apparatus for assay is tremendous
- Speed of the HPLC are different on the different compounds with different polarities
- Low sensitivity of compounds
- Compounds get absorbed by chemicals present in the packing material of column
- The variation in the pressure of column leads to the instability of column (separation may not take place)
- Qualitative analysis may be limited when HPLC is combined with mass spectrometry
- Resolution only occur in some complex samples
- Newer trends with better efficiency have been established

CONCLUSION

After reviewing the literature, it was discovered that HPLC systems are widely utilized in various analytical fields of chemistry. Method development and validation processes are implemented to guarantee the quality of analytical procedures, which results in the acquisition of rapid, dependable, and reproducible data at a lower cost and in less time. HPLC is a highly advanced technique that has been employed for several decades, and it involves sophisticated technologies. The review primarily centered on the utilization of different types of HPLC techniques, their advantages and limitations, and how to ensure quality by adhering to the ICH guidelines for newly developed methods.

REFERENCE

1. Panchumarthy. R. etal A review on step by step Analytical Method validation IOSR Journal of pharmacy, 2015; 5(10): 07-19(e). ISSN 2250-3013,(p)-ISSN: 2319-4219
2. Vibha G. Development and Validation of HPLC method, International Research Journal of Pharmaceutical and Applied Sciences, 2012; 2(4): 17-25. ISSN-2277-4149
3. Azim. M.S HPLC Method development and Validation, International Research Journal of Pharmacy, 2012; 2012; 4(4): 39-46. ISSN 2230-8407
4. N. Udupa and D Sreedhar Quality in Pharmaceutical Education Research and Practice Vision 2020, Manipal University Press(MUP), 2016. ISBN 978-93-82460-42-8
5. Current Good Manufacturing practice for Finished Pharmaceutical, 21 CFR part 210 and 211, US Food and Drug Administration.
6. Validation of analytical procedures Text and Methodology (1994) International Conference on Harmonization (ICH) on Technical.Q2(R1)
7. Quality Management System- Process validation Guidance/GHTF-SG3-N99-10,2004(2)
8. Sudev S. Review on HPLC method development Validation and optimization, *Int. J. Pharm. Sci. Rev. Res.*, 2019; 56(2): 25-43. ISSN 0976-044X
9. USP 31 (2009) General Tests, chapters 621-Chromatography System Suitability, USP convention, Rockville, MD.
10. Maxwell W etal Applying the Validation time line to HPLC System Validation. LC/GC, 1994; 12(9): 678-682.
11. Shewiy D.H etal, Journal Pharmaceutical Biomedical Analysis, 2012; 66: (11-33).
12. Haber L. Validation of HPLC methods, *Biopharm. Int*., 1999; 12: 64-66.
13. Carr. G.P etal. A Practical Approach to Method validation in Pharmaceutical Analysis. *J. pharm. Biomed. Anal*., 1990; 8(8-12): 629.