International Journal of Web of Multidisciplinary Studies
(Peer-Reviewed, Open Access, Fully Refereed International Journal)
website: https://ijwos.com
Volume 3 Issue 3 , March 2026
OPEN ACCESS
E-ISSN : 3049-2424
DOI: https://doi.org/10.71366/ijwos03032621664

An Overview of Developing and Validating HPLC Methods

ANITHA NALLAMOTHU[1], Dr. J. SAI CHANDRA[2], Dr K V L N Murthy[3]
[1] *Assistant Professor (c), UCEN-JNTUK Narasaraopet, Andhra Pradesh, INDIA- 522 601*
[2]*Assistant Professor, Dept. of Chemistry, JNTUH University College of Engineering Sultanpur, Sangareddy, Telangana, INDIA- 502273.*
[3]*Head, Department of Chemistry, Sri Harsha women's Degree College, Andhra pradesh, INDIA A522426*

Article Info

Article History:

Published:31 March 2026

Publication Issue:
Volume 3, Issue 3
March-2026

Page Number:
588-601

Corresponding Author:
Dr. J. SAI CHANDRA

Abstract:

High-performance liquid chromatography (HPLC) is the most commonly used technique for detecting, separating, and quantifying drugs. Various chromatographic parameters (sample pretreatment, mobile phase, column choice, detector type) are considered in the optimisation of HPLC methods. The objective of this article is mainly to describe the processes of method development, optimisation, and validation. The development and validation of analytical methods are critically important for the discovery, development, and manufacture of pharmaceutical drugs and research with humans and animals. HPLC is one of the leading techniques in separation science where analytes are separated by passing through a column packed with micrometre-sized particles. Pharmaceutical industries are required to develop a comprehensive validation policy in accordance with good manufacturing practices (GMP), which describes the approach to validation. This article is focused on the optimisation of HPLC conditions and gives an overview of the introduction, principles, types, instrumentation, method development, and validation processes in HPLC. Validation of HPLC methods provides important information regarding the range, linearity, specificity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), robustness, ruggedness, and system suitability. These validation processes must adhere to regulatory guidelines, for example, those from the International Council for Harmonisation (ICH).

Keywords: HPLC Methods

1. INTRODUCTION

High-performance liquid chromatography (HPLC) is a very useful technique in analytical chemistry that is widely used to separate, identify, and quantify compounds in any sample that can be dissolved in a liquid. It is regarded as one of the most precise methods for qualitative and quantitative analysis of drug products [1]. It works by pumping a sample solution onto a column packed with a porous stationary phase, and a liquid mobile phase is pumped through the column at high pressure. Separation takes place because of differences in migration rates due to different partitioning of sample components between the stationary and mobile phases. Each component elutes at a different time according to its partitioning behaviour [2]. In HPLC, a compound with a higher affinity for the stationary phase moves more slowly and travels a shorter distance, whereas a compound with a lower affinity moves faster and travels a longer distance [3]. The HPLC method was developed mainly to separate and quantitate the major drug, reaction impurities, synthetic intermediates, and degradation products. HPLC is one of the most advanced tools in analytical chemistry, capable of separating, identifying, and quantifying compounds in any liquid-dissolvable sample. It is recognised as one of the most reliable techniques for qualitative and quantitative analysis of drug products and stability evaluation [4].

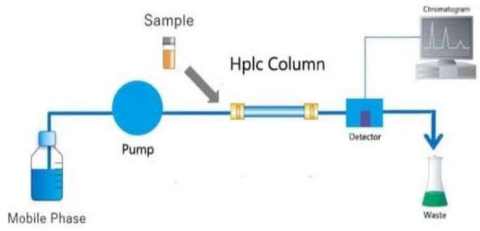

Figure 1: HPLC system

Principles:

The separation of the components is due to the adsorption separation principle in both normal-phase and reverse-phase HPLC modes. The components of a mixture injected into the column of an HPLC move at different rates depending on their relative affinities to the stationary phase. The components with more affinity to the adsorbent will move more slowly, and the components with less affinity will move faster. Each component has a different attraction to the stationary phase, so they are effectively separated.

Type of content:

1. **Normal phase mode. According to the mode of chromatography**, the stationary phase is nonpolar, and the mobile phase is polar.
2. **Reverse phase:** The stationary phase is non-polar, and the mobile phase is polar.
3. **Based on the principle of separation:** Adsorption chromatography: change of the affinity of the compounds to the stationary phase.
4. **Ion-exchange chromatography:** A mixture of like-charged ions is separated by an ion.
5. **Size exclusion or gel permeation Chromatography:** Gels act as a sieve that separates a mixture of components of different molecular sizes.
6. **Elution technique based: Isocratic separation:** In this method, the same combination of mobile phase is used consistently throughout the separation procedure.
7. **Gradient separation:** This method uses a mobile phase of lower polarity or elution strength initially, followed by a gradual increase in polarity or elution strength.
8. **Based on scale of operation: Analytical HPLC:** In this approach, only the analysis of the sample is performed; no sample retrieval.
9. **Preparative HPLC:** In this method, the individual components of a pure substance can be collected with the help of a fraction collector.
10. **Based on the type of analysis:**

a. **Qualitative analysis:** It is used to identify the compound, to detect the presence of impurities, and to determine the total number of components in the sample.
b. **Quantitative analysis:** This process is used to determine the quantity of each component in a mixture by comparing the area of the peaks of the standard and the sample [40].

HPLC instrumentation

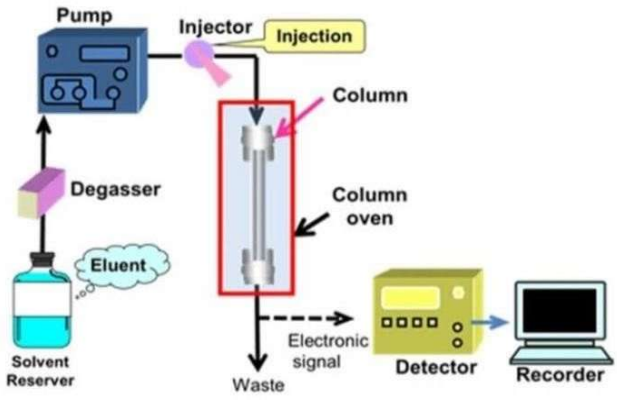

Fig. No. 2: HPLC instrumentation

Components of the HPLC System

1. Solvent reservoir, mixing and degassing system
2. High-pressure pump.
3. Sample injection needle
4. Editorial
5. Detector
6. System for recording data
7. Solvent Reservoir Mixing Systems Degas System

The container of the mobile phase is the solvent reservoir where the solvent is stored. These reservoirs are typically made from glass or stainless steel that will not change colour. The solvent reservoir is most commonly a glass bottle [5]. In addition to dispensing the mobile phase, the pump must mix solvents accurately and precisely. Mixing units are of two types: low-pressure mixing and high-pressure mixing [6]. The degassing mechanism is used to remove air bubbles from the solution, which is based on ultrasonication and filtration techniques for effective results [5].

High-pressure pump

A pump is used to move liquid at a certain rate. It is expressed in millilitres per minute (ml/min). A typical flow rate is 1-2 ml/min. The pump's pressure range is 6000-9000 psi (400-600 bar) [7]. Many applications use several types of pumps, including continuous pressure pumps, pumps incorporating syringe mechanisms, and pumps incorporating reciprocating piston systems [8].

Sample injector.

The sample injector is responsible for the delivery of the liquid sample to the mobile phase. A sample valve is located between the pump and the column [5]. The sample is introduced into the continuously moving stream of mobile phase by an autosampler or injector, which then carries it to the HPLC column. The standard sample volume is often from 5 to 20 microliters [7]. Handbook

There are two types of injectors: injectors and automatic injectors [8].

Column

The column is where the components are actually separated. Columns are usually made of stainless steel. It normally ranges in size from 5 to 25 meters long and 2 to 4.6 centimetres in diameter on the inside [7]. Detector. The detector is able to identify and convert the various elements coming out from the column into an electrical signal [7]. There

are two types of detectors in this case. Specific detectors and bulk property detectors. The specific detectors are: UV-VIS detector, Photodiode array detector, Fluorescence detector, Mass spectrometric detector. Refractive index detectors, electrochemical detectors, and light scattering detectors are examples of bulk property detectors [8].

Data Acquisition System

The results are displayed in a series of peaks, and the area under each peak is automatically computed by the computer connected to the screen [9].

2. Development of an HPLC method:

Analytical method development and validation are critical steps in the discovery, development, and manufacture of pharmaceuticals. They are used to establish the identity, purity, strength, and efficacy of pharmaceutical products. There are many factors to consider in developing methods. In UV detection, the first step is to gather data on the physicochemical properties of the analyte (pKa, log P, solubility, etc.) to define the best detection mode for analysis. Most analytical development work is focused on validating an HPLC method to demonstrate stability. The HPLC method has been developed for the separation and quantification of the major active drug along with any reaction impurities, synthetic intermediates, and any degradants [10,11]. Where no established methods exist, new products are analysed with new methods. The article discusses new approaches for improving the precision, ruggedness, and reducing the cost and time of analysis of existing pharmacopoeial and non-pharmacopoeial products. These methods have been tested and refined by experiment. Alternative methods are proposed and implemented to replace the current procedure. A comparison of laboratory data is given to point out the advantages and disadvantages of each [12]. The factors driving the development of new methods for the analysis of drugs are:

a. When a drug or a combination of drugs is not contained in the pharmacopoeias.
b. When a drug formulation is not available for analytical methods due to interference from formulation excipients.
c. Analytical methods do not exist for the quantification of the analyte in biological fluids [13].

Steps in HPLC method development are:

- Determination of physicochemical properties of drug molecules.
- Choose chromatographic conditions.
- Establishing an analytical method.
- Preparation of sample
- Method optimisation.
- Method validation.

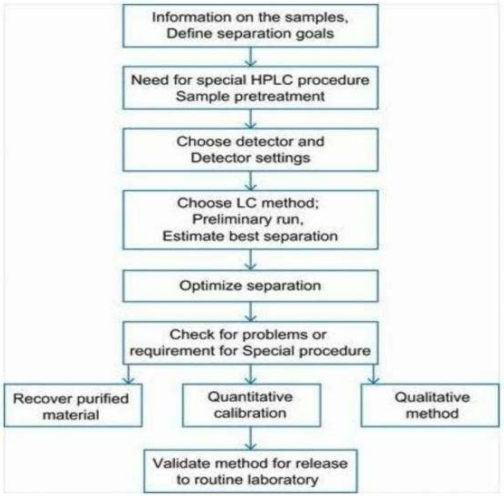

Figure No. 3 Steps in HPLC method development

Identification of physicochemical properties of the drug molecule:

Physicochemical properties of the drug molecule play a prime role in method development. To design an effective method, the physical characteristics of the drug must be considered first. These characteristics include solubility, polarity, pKa, and pH. Polarity is a physical property of a compound that helps an analyst decide on the appropriate solvent and mobile phase composition. The molecular polarity allows for explaining the solubility behaviour. Water (polar solvent) and benzene (nonpolar solvent) are immiscible. As a rule, "like dissolves like," and substances with similar polarities tend to be soluble in each other. The choice of mobile phase or diluents may be influenced by the solubility of the analyte. Diluents should dissolve the analyte and should not react with any of its components. The pH and pKa values are very important in the development of HPLC methods. The pH is defined as the negative base 10 logarithm of the hydrogen ion concentration.

pH = −log 10 [H 3 O +] .

For ionisable analytes, selecting a suitable pH often results in symmetrical sharp peaks in HPLC. Such sharp symmetric peaks are a requirement for the attainment of low detection limits, low relative standard deviation between injections, and reproducible retention times in quantitative analysis [14,15].

Selection of Chromatographic Conditions

In the first steps of method development, a series of starting conditions (detector, column, and mobile phase) is selected to obtain the first "Scouting" chromatograms of the sample. Reverse-phase separations on a C18 column with UV detection are usually employed. At this point, a decision has to be made as to whether to proceed with the development of an isocratic or a gradient method. Column selection:

The column is unquestionably the most critical part of a chromatographic system. The appropriate column selection can provide good chromatographic separation as well as accurate and reliable analysis. In contrast, improper use of a column may lead to unclear, poor, and inadequate separations and provide invalid or hard-to-interpret results [16]. The core element of an HPLC system is the column. The most significant effect on the resolution of analytes in method development is obtained by changing the column. the perfect column for a particular

The application should consider the chemistry of the stationary phase, retention capacity, particle size, and column dimensions. An HPLC column consists of three main parts, which are the stationary phase and hardware matrix. The stationary phase is supported by matrices such as silica, polymers, alumina, and zirconium. The stationary phase most commonly used is silica. Silica matrices are robust, easily derivatised, manufactured with uniform spherical sizes, and resistant to compression under pressure. The choice of the right column for an application involves the stationary phase chemistry, retention capacity, particle size, and column dimensions. The HPLC column is made up of three primary components: the hardware, the matrix, and the stationary phase. The stationary phase is supported on a matrix such as silica, polymers, alumina, or zirconium. The most common matrix is silica due to its strength, ease of derivatisation, ability to produce uniform spheres, and non-compression under pressure. Silica is chemically stable in most organic solvents and in low-pH systems. The downside of using silica as a solid support is that it starts to dissolve at pH values above 7. Recently, silica-supported columns for high pH applications have been developed. The structure, shape, and particle size of silica are properties that are useful in effective separation. Smaller particle sizes give more theoretical plates. Whether a column is suitable for normal-phase or reverse-phase chromatography depends on the nature of the stationary phase. Normal phase chromatography uses a polar stationary phase and a non-polar mobile phase. Generally, polar compounds elute more slowly than non-polar compounds. Here is a list of the most common reverse-phase columns and their applications. Propyl (C3), butyl (C4), and pentyl (C5) phases for ion-pairing chromatography (C4), peptides with hydrophobic residues, and other large molecules. In general, C3-C5 columns will have lower retention for non-polar solutes than C8 or C18 phases. Some examples are Zorbax SB-C3, YMC-Pack C4, and Luna C5. Generally, these columns are less stable to hydrolysis than those with longer alkyl chains. Octyl (C8, MOS) phases have diverse applications. They are somewhat less retentive than C18 phases but are still useful for the

analysis of pharmaceuticals, nucleosides, and steroids. The first and most important stage in the development of a method is the selection of the stationary phase or column. In an effort to develop a robust and reproducible method, a stable, high-performance column is needed. For method development, the stability and reproducibility of the column must be demonstrated to avoid problems with inconsistent sample retention. Separation selectivity for certain components can vary not only between columns from different manufacturers, but also between batches from the same manufacturer. The main factors affecting this variability are the column dimensions, the silica substrate properties, and the bonded stationary phase characteristics. Today, silica-based packaging is the choice for most HPLC columns due to its diverse physical properties [17].

Choice of chromatographic mode. The chromatographic mode is influenced by the molecular weight and polarity of the analyte. All case studies will be based on reversed-phase chromatography (RPC), the most common mode of chromatography used for small organic molecules. RPC is often used to separate ionisable compounds (acids and bases) either using buffered mobile phases (to avoid the analyte ionising) or ion-pairing reagents [18].

Mobile phase optimisation:

Choice of the buffer:

System suitability parameters and overall performance were evaluated for various buffers, including potassium phosphate, sodium phosphate, and acetate. chromatographic efficiency. Sequential trials were performed with these buffers, and potassium dihydrogen phosphate was found to be the optimum buffer to obtain optimum peak separation. We tested 0.02M, 0.05M, and 0.1M buffer concentrations, and they did not significantly affect the elution pattern or resolution. However, the 0.05M concentration significantly improved the sensitivity of the method [19].

Influence of pH:

The relative mobile phase pH relative to the pKa of the analyte is important for ionisable analytes to keep the target analyte in the ionised or neutral form. Mobile phase pH adjustment is a very valuable tool in the chromatographer's toolbox as it allows the simultaneous optimisation of retention and selectivity for important pairs of components [19].

Organic modifier effect:

The organic modifier used in reverse-phase HPLC is simple and popular, with acetonitrile and methanol being the most popular (and tetrahydrofuran, or THF, rarely). For complex multicomponent samples, gradient elution is generally preferred as it can be difficult to achieve elution of all components with a single solvent strength, in the desired retention factor (k) range of 1 to 10 under isocratic conditions [19].

Detector and wavelength selection:

Chromatographic separation is followed by the detection of the analyte of interest by suitable detectors [20]. Commonly used commercial detectors in liquid chromatography (LC) include UV detectors, fluorescence detectors, electrochemical detectors, refractive index (RI) detectors, and mass spectrometry (MS) detectors. The choice of detector depends on the characteristics of the sample and the purpose of the analysis. In multicomponent analysis, absorption spectra may be shifted to longer or shorter wavelengths than the compound. It is therefore essential to obtain and overlay the UV spectra of the target analyte and impurities, normalising them to account for differences in concentration in the mixture. Wavelength must be chosen to give sufficient response for most analytes [19,21].

Developing an analytical approach:

The first step in the development of an analytical method for RP-HPLC is the selection of the main chromatographic parameters such as the mobile phase, the column, the mobile phase flow rate, and the pH of the mobile phase. A series of trials is performed to determine the parameters, which are then tested against system suitability criteria. The typical system suitability parameters are a retention time of more than 5 minutes, a theoretical plate count of greater than 2000, a tailing factor of less than2, a resolution of more than 5, and a percent.

R.S.D. of analyzed peak areas in standard chromatograms less than 2.0%. When two components are simultaneously estimated, the wavelength of detection is usually fixed at an isobestic point. The linearity of the drug is then evaluated to determine the concentration range in which it exhibits a linear relationship. The developed method is also applied to a mixture prepared in the laboratory for the feasibility of simultaneous determination. Finally, the marketed formulation was diluted and analysed within the established linear concentration range [22,23]

Sample preparation:

Sample preparation is one of the important steps of HPLC analysis to make the solution uniform and consistent for the injection onto the column. The objective is to prepare a sample aliquot that is free from significant interferences, will not damage the column, and is compatible with the HPLC method to be used. Such compatibility requires the sample solvent to be soluble in the mobile phase without affecting retention or resolution. The process begins with the collection of the sample and ends with the injection of the sample onto the HPLC column [24].

Optimising the method:

Identify weaknesses of the method and optimise it via experimental design. Evaluate its performance in different conditions, different instrument configurations, and different sample types [25].

Validation of method:

Validation is the confirmation by examination and provision of objective evidence that the specific requirements for a defined intended use are fulfilled. This involves evaluating the performance of the method and proving that it satisfies the defined criteria [26].

Validation of the Analytical Method:

The International Organization for Standardization (ISO) defines validation as the process of confirming that the specified requirements are adequate for the intended purpose. In this context, verification is defined as the provision of objective evidence that a specific product satisfies the specified requirements [27]. Analytical method validation is the documented proof that a particular procedure will consistently produce articles that conform to their predetermined specifications and quality attributes. Validation of an analytical method is the process of demonstrating by laboratory studies that the performance characteristics of the method meet the requirements of the intended analytical application. Any new or modified approach must be validated to show its effectiveness by providing reliable and reproducible results regardless of the operator or laboratory equipment used. The type of validation program needed is completely dependent on the specific method and its intended use. Results of method validation are important for the evaluation of the quality, reliability, and consistency of analytical results and constitute an essential part of good analytical practices. Properly working equipment is important for the validation process in terms of calibration and maintenance. Analytical methods should be validated initially or revalidated as appropriate [28]. It usually consists of 5 different steps, which are described below:

3. System quantification :

System quantification ensures the instrument is fit for the intended analysis. Materials are fit for the purpose of making analytical decisions. The analysts have the required training, skills, and prior documentation. This includes the whole analytical methods, duly authorised protocols, and pre-set standards that have been fully analysed. If the general quantifications of a system are ignored, then it will be difficult to trace the root cause of any problems that arise [41].

Sampling:

Sampling is the process of choosing a representative sample of the fabric for evaluation. The sampling technique is important, as it will ensure that the sample selected is representative of the whole material, thus allowing for significant statistical conclusions. [41] The statistical literature contains extensive studies of sampling methods, but the costs and requirements of each method must be carefully evaluated beforehand.

Sample Preparation:

Sample preparation is an essential component for successful method validations. It is noted that sample preparation comprises 60 to 80% of the workload and operational costs in an analytical laboratory. Sample preparation has examples and well-documented literature. However, the researchers should consider that the choice of a particular preparation method is dependent on parameters such as analyte concentrations, sample matrix, sample size, and chosen instrumental technique [41].

Sample Analysis:

Analysis of samples is the process of obtaining qualitative or quantitative information about samples at an acceptable level of uncertainty by means of instruments.

The process can be seen as comprising three interconnected components: input, converter, and output. The input (x) is the concentration, and the output (y) is the response. The choice of a particular analytical technique depends on several factors, including the chemical nature of the analyte, concentration of the analyte in the sample, sample matrix, speed, cost, and other relevant factors [41].

Evaluation of data:

Data assessment is to put it all together and to gain insight into a given data set using mathematical and statistical methods. This process allows to extract meaningful information and helps to draw conclusions on the inputs, outputs, and, more importantly, the overall validation process [41].

Cleaning validation: Cleaning validation is documented proof that a system or piece of equipment can be consistently cleaned to predetermined specifications and criteria with a high degree of assurance. It is a systematic process that demonstrates the efficacy and reproducibility of cleaning pharmaceutical production equipment. The main objective of cleaning validation is to demonstrate that the cleaning process can adequately remove product residues, degradants, additives, excipients, and cleaning agents to control any possible microbial contamination.

Reasons Why Cleaning Is Validated

1. **Avoiding Contamination:** To prevent cross-contamination of pharmaceutical products and active pharmaceutical ingredients (API) with other products or microbes
2. **Regulatory adherence:** Ensures compliance with regulatory requirements for cleanliness, safety, and quality in pharmaceutical manufacturing.
3. Internal quality control: It guarantees that internal control of manufacturing processes is consistent to keep the quality of the product.
4. Product Integrity: Ensures the quality and integrity of pharmaceutical products.
5. Equipment Reusability: Enables safe and effective re-use of manufacturing equipment [42,43].

Cleaning necessity validation

For detergents [42,43]. To ensure effective cleaning methods and to remove the risk of cross-contamination of APIs. Cleaning Validation Protocol: 1)

A cleaning validation protocol usually includes the following:

Goals of the Validation Process

Validation goals clearly specified

Duties: The roles and responsibilities of those conducting and approving the validation study.

Equipment description:

Characteristics and specifications of the equipment concerned

Timing of cleaning:

Definition of the time between the end of production and the start of cleaning procedures.

Cleaning procedures:

specific cleaning for each product, manufacturing device, or equipment

Cleaning Cycles:

Number of cleaning cycles to perform in series.

Regular monitoring:

procedures for routine checks of equipment cleanliness.

Sampling Method:

methods of sample collection, including the rationale for the technique used

Areas for sampling specified:

Specific areas to sample and why.

Recovery studies:

Information on recovery studies, where appropriate.

Analytical Methods:

Analytical methods: Limit of detection (LOD) and limit of quantification (LOQ)

Acceptance Requirements:

Defined boundaries with justification of choice [44]

Importance of validation

- Provides consistent product quality.
- It makes processes more time-efficient.
- Improves the manufacturing process.
- Enhances productivity and reduces product failures.
- Reduces reject rates and quality costs.
- Increases process yields and reduces complaints.
- Allows for faster and smoother integration of new hardware.
- Raises worker awareness and understanding of the process [45].

The following standard parameters are recommended by the FDA, USP, and ICH.

1. Range
2. Linearity of the
3. Specificity.
4. Correctness
5. Accuracy
6. Solution stabilities
7. Limit of detection
8. LOQ, limit of quantitation

9. Resilience
10. Hardness
11. Suitability of the system.

Range:

The range of an analytical technique is the interval between the highest and lowest analyte concentrations sampled for which the method has shown acceptable precision, accuracy, and linearity [29].

Linearity:

Linearity of a method is the ability of the method to produce test results that are directly proportional to the concentration of analyte in samples within a given range. For HPLC methods, this linear relationship between the detector response (e.g., peak area or height) and analyte concentration is assessed. The relationship for the drug substance can be established by direct dilution of a standard stock solution or by independent weighing of the drug substance. Test this by preparing the sample components according to the suggested procedures. Linearity should be evaluated visually by inspection of a plot of signals versus analyte concentration or content. If a linear relationship is observed, the results should be analysed by appropriate statistical techniques, e.g., regression analysis. The regression line can provide mathematical estimates to measure the degree of linearity, often expressed as the variance around its slope. In some cases, the analytical response needs to be described by the proper function of the analyte concentration. Several pharmaceutical methods employ common linearity ranges and acceptance criteria [30].

Specificity:

In the approval of strategy, the terms selectivity and specificity are often used interchangeably to refer to the same idea. Specificity is the ability to identify and measure the analyte distinctly in the presence of other components that may be required to be detected. The specificity of a testing system is determined by the comparison of results obtained from samples with impurities, degradation products, or placebo compounds with results obtained from samples that do not contain these interfering substances [31,32].

Accuracy:

Accuracy is the degree of closeness of a measured value to a true value or an accepted value. In practice, accuracy is measured as the difference between the mean measured value and the true value. This is the It is usually assessed by applying the technique to samples containing known amounts of analyte, with no interference, and comparing the results to the standard and blank solutions. The accuracy is then expressed as a percentage of the analyte recovered in the assay based on results, often called the recovery of added known amounts of analyte [20].

Correctness:

Precision is the degree of agreement or dispersion of a series of measurements of the same homogeneous sample, made under the same conditions on different samples. It evaluates the reproducibility of the analytical method and is divided into two parts: repeatability and intermediate precision.

Repeatability – variation observed when a single analyst uses a single instrument. It does not differentiate between variations due to the instrument or system and those due to the sample preparation process. In method validation, repeatability is determined by analysing multiple replicates of a composite sample by the analytical method as written and determining recovery values.

Intermediate precision is the variation within a laboratory, due to factors such as different days, different instruments, or different analysts [33].

Stability of solution:

During validation, the stability of standards and samples is tested under typical conditions, standard storage conditions, and sometimes in the instrument itself. This procedure aids in determining the need for any special storage requirements such as refrigeration or protection from light [34].

Limit of detection (LOD) :

The detection limit of an individual analytical method is the lowest amount of analyte in a sample that can be detected but not quantitated accurately [35].

Limit of Quantitation (LOQ):

The quantitation limit of an individual analytical system is the minimum amount of analyte in a sample that can be measured with a stated level of confidence and accuracy. This limit is an important parameter for the quantification of small amounts of the substance in test samples and is especially useful for the detection of contaminants or impurities [36].

Robustness:

The robustness of an analytical procedure is the ability to remain unaffected by small but deliberate changes in method parameters, which provides an indication of its reliability and consistency during normal usage [37].

Robustness:

Ruggedness of an analytical method is defined as the consistency and reproducibility of test results when the same samples are analysed under varying conditions. Such conditions can include differences between laboratories, analysts, instruments, reagent lots, assay times, temperatures, or days. It evaluates the method's capability to deliver trustworthy results despite the variations that generally appear between different laboratories or analysts [38].

System suitability:

Liquid chromatographic methods contain system suitability tests as a routine part of the method to ensure that the detection sensitivity, resolution, and reproducibility of the chromatographic system are adequate for the analysis. These tests are based on the principle that the equipment, electronics, analytical methods, and sample form constitute a system that can be assessed as a unit. The method's suitability is assessed by determining the important parameters such as peak resolution, theoretical plate count, peak tailing, and capacity [39].

4. CONCLUSION

In recent years, much attention has been devoted to the development of analytical techniques for the identification, purity assessment, and quantification of drugs in pharmaceutical analysis. The review gives a broad overview of HPLC method development and validation with a simple approach for HPLC method design for compound separation. Before you start developing an HPLC method, you must have a good knowledge of the physicochemical properties of the main compound. Furthermore, the selectivity of separation is strongly affected by the composition of the buffer and mobile phase, including its organic content and pH. Validation is a critical step in the pharmaceutical industry to ensure that quality is built into the processes that support drug development and manufacturing. The optimised method is validated by different parameters like range, linearity, specificity, accuracy, precision, limit of detection, robustness, and system suitability as per ICH guidelines.

References

1. Rao BV, Sowjanya GN, Ajitha A, Rao Uma MV. A review on stability-indicating HPLC method development, World Journal of Pharmacy and Pharmaceutical Sciences. 2015; 4(8):405-423.
2. Rajan HV. Development and validation of an HPLC method – A Review. International journal of current research

in pharmacy. 2015;1(2):55-68.

3. Kumar V, Bhardwaj R, Gupta G, Kumar S. An Overview on HPLC Method Development, Optimization and Validation process for drug analysis. The Pharmaceutical and Chemical Journal. 2015; 2(2):30-40.
4. B.V. Rao, G.N. Sowjanyal, A. Ajitha, V.U.M. Rao, Review on stability-indicating HPLC method development, World Journal of Pharmacy and Pharmaceutical Sciences, 4(8) (2015) 405-423.
5. Jena Ak. HPLC: a highly accessible instrument in the pharmaceutical industry for effective method development. Pharm Anal Acta 2011;3.
6. Agilent Technologies, the LC Handbook guide to LC Columns and Method Development. Agilent Technologies. 2016; 16.
7. Chawla G, Chaudhary KK. A Review of HPLC technique covering its pharmaceutical, environmental, forensic, clinical, and other applications. Int J Pharm Chem Anal. 2019; 6(2): 27-39.
8. Hamilton RJ, Sewell PA. Introduction to high-performance liquid chromatography. Springer. 1982; 1-12.
9. Malviya R, Bansal V, Pal OP, Sharma PK. High-performance liquid chromatography: a short review. J Glob Pharma Technol 2010; 2(5): 22-26.
10. United States Pharmacopoeia and National Formulary, (24th) Asian edition, The United States Pharmacopoeia Convention Inc. U.S.A.2126.
11. Sankar SR, Textbook of Pharmaceutical Analysis. 5th Edition 2006. Rx publications, Triunelveli. 2006;13-1,2.
12. Santosh Kumar Bhardwaj, a.b. K Dwivedi and D. D. Agarwala, A Review: HPLC Method Development and Validation, International Journal of Analytical and Bioanalytical Chemistry 2015; 5(4): 76-81.
13. Skoog, West, Holler, Crouch, "Fundamentals of analytical chemistry", eighth edition, 2009 (Indian edition), Cengage Learning India Pvt. Ltd., New Delhi, page no. 271-280.
14. http://www.scribd.com/doc/9508765/Physica l-Properties-of-Drug.
15. Buffers and pH Buffers: available from: www.xtremepapers.com.
16. Sánchez MLF. Chromatographic techniques, European RTN Project, GLADNET, Retrieved on 05-09-.
17. M.S. Charde, A.S. Welankiwar, J. Kumar, Method development by liquid chromatography with validation, International Journal of Pharmaceutical Chemistry, 04 (02)(2014) 57-61.
18. M.W. Dong, Modern HPLC for practicing scientists, John Wiley and Sons, New Jersey, 2006.
19. C.K. Kaushal, B. Srivastava, A process of method development: A chromatographic approach, J. Chem. Pharm. Res. 2(2) (2010) 519-545.
20. Mohamad T, Mohamad MA, Chattopadhyay

M. Particle size role, importance, and Strategy of HPLC analysis: An update. International Archives of BioMedical and Clinical Research. 2016; 2(2): 5-11.

21. N.Toomula, A. Kumar, S.D. Kumar, V.S. Bheemidi, Development and validation of Analytical Methods for Pharmaceuticals, J Anal Bioanal Techniques. 2(5) (2011) 1-4.
22. Sethi PD. Introduction – High Performance Liquid Chromatography, 1st edn, CBS Publishers, New Delhi.2001; 1-28.
23. Julia T, Mena AJ, Aucoin MG, Kamen AA. Development and validation of a HPLC and validation of a HPLC

method for the quantification of baculovirus particles. J Chromatogr B. 2011;879: 61-68.

24. Santosh G, Nagasowjanya G, Ajitha A, Uma Maheswara Rao Y. HPLC method development and validation: an overview. International Journal of Pharmaceutical Research and Analysis. 2014; 4(2): 274-280.
25. Kayode J, Adebayo. Effective HPLC method development, Journal of Health, Medicine and Nursing.2015:12: 123-133.
26. Gad S. Pharmaceutical manufacturing handbook of regulations and quality, John Wiley and Sons; 2006.
27. ICH harmonised tripartite guideline validation of analytical procedures: text and methodology Q2(R1), 2005.
28. P. M. Deshmukhe, M. S. Charde, R. D. Chakole, A Review on HPLC Method Development and Validation, Ijppr.Human, 2021; Vol. 21 (4): 66-82.
29. Validation of analytical procedures: Text and Methodology, International Conference on Harmonization, draft Revised (2005), Q2 (R1).
30. Harshad V. Paithankar, HPLC METHOD VALIDATION FOR

PHARMACEUTICALS: A REVIEW,

International Journal of Universal Pharmacy and Bio Sciences 2(4): July-August 2013.

31. Nevado JJB et.al. Reliable and sensitive SPE-HPLC-DAD Screening of Endocrine Disruptors Atrazine, Simazine and their Major Multiresidues in Natural Surface Waters: Analytical Validation and Robustness study Performance. J Chromatograph Separate Technique 2014; 5:215.
32. Nia Y et al. al Determination of Ti from Tio2 Nanoparticles in Biological Materials by Different ICP-MS Instruments: Method Validation and Applications. J Nanomed Nanotechnol.2015; 6:269.
33. Ngwa G. Forced Degradation Studies. Forced Degradation as an Integral Part of HPLC Stability-Indicating Method Development. Drug Delivery Technology. 2010; 10(5).
34. V. Kumar, R. Bhardwaj, 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.
35. Subramanian Natesan et al. Improved RP-HPLC Method for the Simultaneous Estimation of Tranexamic Acid and Mefenamic Acid in Tablet Dosage Form. Pharm Anal Acta 2011, 2:115.
36. Laxman Sawant et al. al Quantitative HPLC analysis of Ascorbic Acid and Gallic acid in Phyllanthus Emblica. J Anal Bioanal Techniques 2010, 1:111.
37. ICH Q2A. Text on Validation of Analytical Procedures, International Conference on Harmonization. Geneva, 1995.
38. Validation of Analytical Procedures: Methodology. ICH-Guidelines Q2B, Geneva. 1996, 11. (CPMP/ICH/281/95).
39. Validation of Compendial Procedures, United States Pharmacopoeia, USP 36 NF, 27 (2)

(2010)

40. Dr. Kavitha P.N: A Textbook of Instrumental Methods of Analysis, Nirali Prakashan, pg. no.

13.2 to 13.3.

41. Araujo P. Key aspects of analytical method validation and linearity evaluation. J Chromatogr B 2009;877:2224-34.

42. Goyal D, Maurya S, Verma C. Cleaning validation in the pharmaceutical industry: an overview. Pharma Tutor 2016;4:14-20.

43. Lodhi B, Padamwar P, Patel A. Cleaning validation for the pharmaceuticals, biopharmaceuticals, cosmetic and neutraceuticals industries. J Innov Pharm Biol Sci 2014;1:27-38.

44. Murthy DN, Chitra K. A review article on cleaning validation. Int J Pharm Sci Res 2013;4:3317.

45. Lavanya G, Sunil M, Eswarudu MM, Eswaraiah MC, Harisudha K, Spandana BN, et al. Analytical method validation: an updated review. Int J Pharm Sci Res 2013;4:1280