International Journal of Trend in Scientific Research and Development (IJTSRD)
Volume 7 Issue 4, July-August 2023 Available Online: www.ijtsrd.com e-ISSN: 2456 – 6470

A Review: HPLC Method Development and Validation

Ajay Sanjay Salvi, Mohini S. Khamkar, Dr. Lahu D. Hingane

Department of Quality Assurance, Aditya Pharmacy College, Beed, Maharashtra, India

ABSTRACT

Due to its very effective separations and often high detection sensitivity, HPLC is the most widely used separation method in contemporary pharmaceutical and biomedical analysis. The majority of medications in multiple component dosage forms can be examined using the HPLC method due to its many benefits, including speed, specificity, accuracy, precision, and ease of automation. The development and validation of HPLC procedures are crucial to novel discoveries, the creation of pharmaceutical medications, and numerous other investigations involving both humans and animals. To compare a defined characteristic of the drug substance or drug product to predetermined acceptance criteria for that characteristic, an analytical technique is designed. This review provides details on the numerous steps that go into developing and validating an HPLC technique. According to ICH Guidelines, validating an HPLC technique include testing for system appropriateness as well as accuracy, precision, specificity, linearity, range and limit of detection, limit of quantification, robustness, and other performance characteristics.

KEYWORDS: *HPLC, Method development, Validation*

How to cite this paper: Ajay Sanjay Salvi | Mohini S. Khamkar | Dr. Lahu D. Hingane "A Review: HPLC Method Development and Validation" Published in International Journal of Trend in Scientific Research and Development (ijtsrd), ISSN: 2456-6470, Volume-7 | Issue-4, August 2023, pp.34-39, URL: www.ijtsrd.com/papers/ijtsrd58595.pdf

IJTSRD58595

INTRODUCTION

High Performance Liquid Chromatography (HPLC) was derived from the classical column chromatography and, is one of the most important tools of analytical chemistry today. In the modern pharmaceutical industry, high performance liquid chromatography (HPLC) is the major and integral analytical tool applied in all stages of drug discovery, development, and production. The preferred approach for testing the peak purity of new chemical entities, keeping track of reaction changes during scale-up or synthesis processes, assessing new formulations, and performing quality control and assurance on finished pharmaceutical products is HPLC. The purpose of the HPLC approach is to attempt to quantify and separate the primary drug, any contaminants from reactions, all readily available synthetic intermediates, and any degradants. One of the most effective tools in analytical chemistry nowadays is high performance liquid chromatography. Every material that can dissolve in a liquid can have its constituents separated, identified, and quantified using this technique. HPLC is one of the most precise analytical techniques that is frequently used to analyse pharmacological products both quantitatively and qualitatively as well as to assess their stability. The stationary phase, or sample solution, is injected into a porous column, and the mobile phase, or liquid phase, is pumped through the column at a higher pressure. The adsorption of solute on stationary phase based on its affinity towards stationary phase is the separation principle that is used. The HPLC method has the following characteristics.

High definition
Quick analysis,
Stainless steel, glass column, and small diameter
Regulated mobile phase flow rate
Somewhat higher mobile phase pressure

HPLC Method Development:

When there are no official methods for a novel product, methods are devised. Reduce the cost and time for current (non-pharmacopoeial) items by using alternative methods. for increased robustness and precision. Comparative laboratory data with merits or demerits are made available when an alternative approach is suggested to replace the current procedure. The fundamental objective of the HPLC method is to attempt and quantify the separation and quantification of the main active medication, any reactive impurities, all readily available synthetic intermediaries, and any degradants.

Steps involved in Method development are.

- Understanding the Physicochemical properties of drug molecule.
- Selection of chromatographic conditions.
- Developing the approach of analysis.
- Sample preparation
- Method optimization
- Method validation

Understanding drug molecule physicochemical characteristics:

A drug's physicochemical characteristics are important for essential part in the development of methods. One must study the physical characteristics of the drug molecule, such as its solubility, polarity, pKa, and pH, in order to build a method. A compound's physical characteristic of polarity. An analyst can use it to choose the mobile phase's solvent and chemical component.[6] 6 The polarity of the molecules can be used to explain their solubility. Solvents that are nonpolar, like benzene, and polar, like water, do not combine. Like generally dissolves like, which means that substances with comparable polarities can be dissolved in one another. The choice of diluents depends on how soluble the analyte is. The pH value is often used to determine whether a substance is acidic or basic. In HPLC, choosing the right pH for ionizable analytes frequently produces symmetrical and sharp peaks.

Choosing the chromatographic parameters:

To get the first "scouting" chromatograms of the sample, a set of basic settings (detector, column, mobile phase) are chosen during initial technique development. On reversed-phase separations on a C18 column with UV detection, they are typically based. At this point, a choice should be taken regarding whether to develop an isocratic or a gradient methodology.

Column Selection:

A chromatograph's beginning point and centrepiece is a column, of course. An accurate and trustworthy analysis can be produced by a good chromatographic separation from a well-chosen column. A poorly used column can frequently produce unclear, insufficient, and poor separations, which can produce results that are unreliable or difficult to understand.

The column is the brains of an HPLC setup. During technique development, changing a column will have the biggest impact on the resolution of analytes. The stationary phase chemistry, retention capability, particle size, and column dimensions must all be taken into account when selecting the optimum column for an application. Hardware, a matrix, and a stationary phase are the three major parts of an HPLC column. Several types of matrices, including as silica, polymers, alumina, and zirconium, are used to support the stationary phase. The most typical matrix for HPLC columns is silica. Silica matrices have a low tendency to compress under pressure, are resilient, simple to derivatize, and are produced with uniform spherical sizes.

Most organic solvents and low pH solutions have little effect on the chemical stability of silica. A silica solid support's drawback is that it will dissolve above pH 7. For application at high pH, silica-supported columns have recently been created.

Separation is influenced by the silica support's composition, shape, and particle size. Increased or more theoretical plates are produced by smaller particles. Whether type of chromatography—normal phase or reverse phase—a column is best suited for depends on the characteristics of the stationary phase.

Normal phase chromatography utilizes a polar stationary phase and a non-polar mobile phase. Generally, more polar compounds elute later than non-polar compounds. Commonly used reverse phase columns and their uses are listed below. Propyl (C3), Butyl (C4), and Pentyl (C5) Phases are helpful for large molecules, hydrophobic peptides, and ion-pairing chromatography (C4). Comparing C3-C5 phases to C8 or C18 phases, non-polar solutes are often retained less well by C3-C5 columns. Zorbax SB-C3, YMC-Pack C4, and Luna C5 are a few examples. Compared to columns with longer alkyl chains, these columns are typically less resistant to hydrolysis. The applications of octyl (C8, MOS) phases are numerous. While less retention than the C18 stages, this phase is nonetheless very helpful for drugs, nucleosides, and steroids. 10 In developing a method, choosing the stationary phase or column is the first and most crucial step. Without the availability of a stable, high performance column, it is impossible to build a robust and reproducible procedure. The column production batches from the same manufacturer as well as columns from other manufacturers varied in the separation selectivity for specific components. The key ones include column dimensions, silica substrate parameters, and bonded stationary phase qualities. Due to a number of physical properties, silica-based packing is preferred in most current HPLC columns.

Chromatographic mode selection:

Polarity and molecular weight-based chromatographic modes. Reversed-phase chromatography (RPC), the most typical method for tiny organic compounds, will be the main topic of all case studies. Ion-pairing reagents or buffered mobile phases, which retain the analytes in a non-ionized state, are frequently used in

RPC to separate ionizable chemicals (acids and bases).

Mobile phase optimization:

Buffer Choice: The system appropriateness parameters and overall chromatographic performance of various buffers, including potassium phosphate, sodium phosphate, and acetate, were assessed.

Impact of pH:

If analytes can be ionised, the correct mobile phase pH must be selected based on the analyte's pKa so that the target analyte is in one predominant ionisation state, either ionised or neutral. One of the best techniques in the "chromatographer's toolbox" for changing both retention and selectivity between important pairs of components simultaneously is adjusting the pH of the mobile-phase.

Impact of organic modifier:

Choosing an organic modifier type in reverse phase HPLC is quite straightforward. Acetonitrile and methanol are often the options (rarely THF). Gradient elution is typically used with complicated multicomponent samples since it may not be viable to elute all components using a single solvent strength under isocratic conditions between k (retention factor) 1 and 10. 12

Choosing a wavelength and detector:

Following chromatographic separation, the desired analyte is identified using the appropriate detectors. Commercial detectors that are utilised in LC include mass spectrometry (MS) detectors, UV detectors, fluorescence detectors, electrochemical detectors, and detectors that measure refractive index (RI).

The sample and the goal of the analysis influence the detector selection. In the case of multicomponent analysis, the absorption spectra may have been altered from the parent chemical to longer or shorter wavelengths. Due to the various levels of contaminants in the combination, the UV spectra of the target analyte and the impurities must be collected and overlaid, and the spectra must be normalised. It is necessary to select a wavelength that will allow for a sufficient response for the majority of the analytes.

Creating the analytical strategy:

The selection of several chromatographic parameters, such as the mobile phase, column, flow rate, and pH of the mobile phase, is the initial step in the development of an analytical technique for RP-HPLC.

These parameters are all chosen through testing, and the system suitability parameters are taken into account afterwards. Retention time should be greater than 5 minutes, theoretical plates should be greater than 2000, the tailing factor should be less than 2, resolution between 2 peaks should be greater than 5, and the R.S.D. of the area of analyte peaks in standard chromatograms should not be greater than 2.0%, among other parameters that indicate a system is suitable.

When two components are estimated simultaneously, the detection wavelength is often at its isobestic point.

Sample preparation:

The analyst must look at the sample preparation phase of method development. For instance, if the sample contains insoluble components, the analyst should determine whether centrifugation (choosing the best rpm and time), shaking, and/or filtration of the sample are necessary. The purpose is to show that the sample filtration has no impact on the analytical outcome caused by leachable adsorption and/or extraction. Syringe filters' efficiency is largely dependent on its capacity to filter out impurities and insoluble substances without introducing unwanted artefacts (i.e.,extractables) into the filtrate. Whether using an actual in-process sample or a dosage form for a future HPLC analysis, the sample preparation process should be adequately specified in the applicable analytical technique. The manufacturer, type, and pore size of the filter media must be mentioned in the analytical technique. The goal of sample preparation is to transform a raw sample into a processed sample that yields superior analytical results to the raw sample. The prepared sample should be an aliquot that is compatible with the HPLC process and won't harm the column, and it should be reasonably clear of interferences.

Method optimization:

The improvement of HPLC conditions has received the majority of attention throughout HPLC method development optimization. It is necessary to consider the compositions of the fixed and mobile phases. Mobile phase parameter optimization is always prioritised since it is more practical and straightforward than stationary phase optimization. Only the parameters that are likely to have a significant impact on selectivity in the optimization must be looked at in order to reduce the amount of trial chromatograms required. The various elements of the mobile phase serve as primary control variables in the optimization of liquid chromatography (LC) techniques.

Calculating the gradient, flow rate, temperature, sample amounts, injection volume, and solvent type of the diluents. Following satisfactory selectivity, this is utilised to identify the ideal balance between resolution and analysis time. The variables include flow rate, column packing particle size, and column

dimensions. Changes to these parameters won't have an impact on selectivity or capacity factor.

Method Validation:

An analytical method is validated when it has been proven through laboratory tests that its performance characteristics are appropriate for the intended analytical application. Any new or modified method needs to be validated to make sure it can produce repeatable and reliable results when applied by various operators using the same equipment in the same or other laboratories. The specific approach and the applications it is intended for determine exactly what kind of validation program is necessary. A crucial component of any sound analytical procedure, method validation data can be used to assess the calibre, dependability, and consistency of analytical findings. The method validation process is fundamentally dependent on the use of equipment that is within specification, operating correctly, and having a sufficient calibration. It is necessary to validate or revalidate analytical techniques.

- Before they are used frequently
- Whenever the criteria for which the method has been approved change
- Whenever the technique is modified

The following are typical parameters that the FDA, USP, and ICH recommend.

1. Specificity
2. Range and Linearity
3. Precision

Technique specificity (Repeatability)
Intermediate accuracy (Reproducibility)

4. Accuracy (Recovery)
5. Stability of the solution
6. Limit of Detection (LOD)
7. Limit of Quantification (LOQ)
8. Robustness
9. System Suitability

Specificity: Selectivity of an analytical method is the capacity to detect an analyte accurately in the presence of interfering substances, such as synthetic precursors, excipients, enantiomers, and known or likely degradation products that may be anticipated to be present in the sample matrix.

Range and linearity: The capacity of an analytical technique to produce test results that are inversely proportional to the concentration of analyte in the sample is known as linearity. It is important to assess a linear relationship over the entire analytical procedure. By diluting a standard stock solution of the drug product's constituent parts according to the suggested process, it is directly proven on the drug substance. The confidence limit surrounding the slope of the regression line is typically used to express linearity. The ICH recommendation recommends using a minimum of five concentrations to establish linearity. The range between the upper and lower levels that can be determined using an analytical method with verified precision, accuracy, and linearity is known as the range of the method.

Precision: The degree of scatter between a set of measurements obtained from multiple sampling of the same homogeneous sample under the specified conditions is expressed as the closeness of agreement (precision) of an analytical procedure. There are three types of accuracy: repeatability, intermediate precision, and reproducibility. The standard deviation or relative standard deviation of a sequence of data is typically used to express the precision of an analytical technique. The degree of reproducibility or repeatability of the analytical technique under ideal circumstances might be referred to as precision. Ruggedness, or intermediate precision, expresses variability within laboratories, such as on different days or with different analysts or equipment within the same laboratory. By testing an adequate number of aliquots of a homogeneous sample, one can evaluate the precision of an analytical technique by calculating statistically accurate estimates of the standard deviation or relative standard deviation.

Accuracy (Recovery): The accuracy of an analytical technique expresses how closely the value found and the value accepted as either a conventional true value or an approved reference value agree. Applying the technique to samples that have known dosages of analyte added yields the result. To make sure there is no interference, these should be compared to both standard and blank solutions.

The accuracy is then computed as a percentage of the analyte recovered by the assay using the test findings. The recovery by the test of known, added amounts of analyte is a common way to represent it.

Stability of solutions: During validation, the stability of standards and samples is established under normal circumstances, normal storage circumstances, and occasionally in the instrument to ascertain whether special storage circumstances, such as refrigeration or protection from light, are required.

Limit of Detection (LOD): The lowest amount of analyte in a sample that can be detected but not necessarily quantitated as an accurate value is known as the limit of detection (LOD) of a specific method. The LOD can be predicated on a signal-to-noise (S/N) ratio (3:1), which is typically reported as the concentration of analyte in the sample, in analytical techniques that exhibit baseline noise. The signal-to-

noise ratio is calculated using the formula: s = H/h, where H is the height of the component-specific peak. The highest noise deviation from the chromatogram of a blank solution's baseline, expressed in absolute terms, is given by the formula h.

Limit of Quantification (LOQ): The smallest amount of analyte in a sample that can be quantitatively identified with adequate precision and accuracy is known as the limit of quantification (LOQ), also known as the quantitation limit of a specific analytical process. The LOQ is typically estimated from a determination of S/N ratio (10:1) for analytical processes like HPLC that exhibit baseline noise, and is typically confirmed by injecting standards that yield this S/N ratio and have an acceptable percent relative standard deviation as well.

Robustness: Robustness is a measure of an analytical method's capacity to stay unaffected by minute but intentional changes in method parameters (such as pH, mobile phase composition, temperature, and instrument settings), and it shows how reliable the method will be under typical conditions. Determining robustness is a systematic process that involves changing a parameter and evaluating the impact on the methodology through system suitability monitoring and/or sample analysis.

System Suitability: System compatibility evaluations are an essential component of liquid chromatographic techniques. They are used to confirm that the chromatographic system's detection sensitivity, resolution, and reproducibility are sufficient for the intended analysis. The tests are founded on the idea that the tools, electronics, analytical processes, and test samples make up a whole system that may be assessed as such. To assess the suitability of the employed approach, variables like peak resolution, theoretical plate count, peak tailing, and capacity have been assessed.

Conclusion:

Pharmaceutical analysis has paid a lot of attention lately to the development of analytical methods for drug identification, purity assessment, and quantification.

The development and validation of HPLC methods are generally covered in this article.

The creation of an HPLC method for the separation of substances was discussed using a general and very basic approach. Prior to the development of any HPLC process, understanding the primary compound's physiochemical characteristics is crucial. The choice of buffer and mobile phase (organic and pH) composition has a significant impact on separation selectivity. The gradient slope, temperature, flow rate, type, and concentration of mobile-phase modifiers can all be altered for the final optimization. According to ICH criteria, the optimised method is verified using a variety of factors (such as specificity, precision, accuracy, detection limit, linearity, etc.).

Abbreviations

HPLC	High Performance Liquid Chromatography
ICH	International conference on Harmonization
Id	Internal Diameter
LC	Liquid Chromatography
LOD	Limit of Detection
LOQ	Limit of Quantitation
m	Meter
mm	Mili meter
MS	Mass Spectrometry
ODS	Octyl decyl silane
RI	Refractive index
THF	Tetrahydrofuran
USP	United states Pharmacopeia
µm	Micron

Reference:

[1] Gurdeep R. Chatwal and Sham k. Anand, Instrumental Method of Chemical Analysis, Fifth Revised & Enlarged Edition 2002; 2.624-2.638.

[2] Dr. Shashikant D. Barhate, Dr. MD. Rageeb MD. Usman, Poonam A. Salunke, Shital S. Patil, Textbook of Modern pharmaceutical Analytical Techniques, Jan-2019; 258-277.

[3] Sethi PD, HPLC: Quantitative Analysis of Pharmaceutical Formulation, New Delhi, CBS Publishers and Distributors, 1996; 113-202.

[4] Santosh Kumar Bhardwaj a,b *, K. Dwivedia and D. D. Agarwala A Review: HPLC Method Development and Validation, International Journal of Analytical and Bioanalytical Chemistry, Nov 2015

[5] V. Gupta, A.D. K. Jain, N.S. Gill, K. Gupta, Development and validation of HPLC method - a review , Int. Res J Pharm. App Sci., 2(4) (2012) 17-25

[6] Y. Kazakevich, R. Lobrutto, HPLC for Pharmaceutical Scientists, John Wiley & Sons, New Jersey, 2007.

[7] S. Ahuja, H. Rasmussen, Development for Pharmaceuticals, Vol.8 Separation Science and Technology, Elsevier, New York 2007

[8] M.S. Azim, M. Mitra, P.S. Bhasin, HPLC method development and validation: A review, Int. Res. J. Pharm. 4(4) (2013) 39-46.

[9] B.V. Rao, G.N. Sowjanya1, 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.

[10] M.W. Dong, Modern Hplc for practicing scientists, John Wiley & Sons, New Jersey, 2006.

[11] Kumar V, Bharadwaj 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.

[12] HPLC – Chemiguide. May 2, 2007. www.chemguide.co.uk

[13] 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.