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Review On Hplc Method Development And Validation

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Abstract: This review outlines a plan for the methodical advancement of HPLC. One of the most powerful analytical chemistry technologies now in use is high performance liquid chromatography. Any liquid-soluble material can have its constituents separated, identified, and quantified by it. A number of chromatographic parameters, including as sample pretreatment, mobile phase selection, column selection, and detector selection, were examined in order to optimize the procedure. This article aims to discuss the process of developing, optimizing, and validating methods.The development of the HPLC method is influenced by the molecules' chemical structure, the synthetic pathway, solubility, polarity, pH and pKa values, and the activity of the functional groups, among other factors. The purpose of this essay was to review the development and validation of HPLC methods.

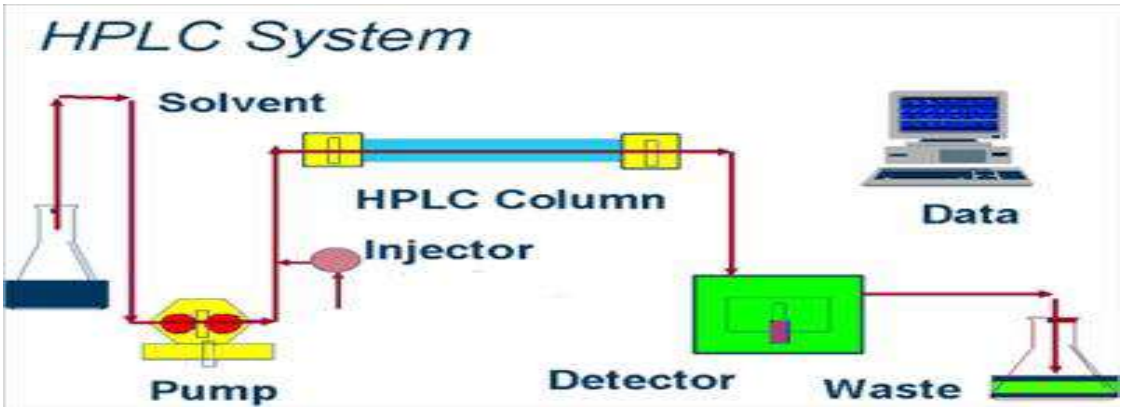
Keywords: HPLC, method development, validation, accuracy, specificity

INTRODUCTION : One of the most powerful analytical chemistry technologies now in use is high performance liquid chromatography. Any liquid-soluble material can have its constituents separated, identified, and quantified by it. High performance liquid chromatography is referred to as HPLC.The most accurate analytical techniques are frequently employed for both quantitative and qualitative drug product analysis.[1] The sample is divided according to variations in migration rates through the column brought about by various sample partitions. between the mobile and stationary stages. Elution happens at different times depending on how different components split. Chromatography was the process that the Russians invented first. In 1903, botanist M.S. Tswett created High Performance Liquid.OneCompared to gas chromatography, chromatography is more versatile. due to the fact that (a) it is not restricted to thermally stable volatile and non-volatile samples and (b) the selectionof stationary and movable phases is more extensive. An HPLC system's schematic diagram is shown in Figure 1. HPLC outperforms conventional LC methods in the following ways:

• Outstanding resolution. Stainless steel, glass, and a tiny (4.6 mm) diameter.or titanium columns.
• Column packing using 10 m-diameter tiny column particles (3, 5, and 6).
• Accurate mobile phase movement and relatively high inlet pressures.

• Continuous flow detectors that can handle small flow rates and detection of extremely small amounts.

• Quick analysis.[2]



•**HPLC Method Development:** Methods are created for new products when there aren't any formal ones. For current (non-pharmacopoeial) products, alternative approaches aim to increase precision and durability while cutting costs and time. Comparative laboratory data, including advantages and disadvantages, is provided whenever a different approach is suggested to replace an established practice. The primary active ingredient, any reaction impurities, all available synthetic intermediates, and any degradants are all separated and quantified using the HPLC method.
These are the steps involved in developing a method.

•Understanding the Physicochemical properties of a drug molecule.

•Choosing chromatographic conditions.

•Developing an analytical strategy. Sample preparation.

•Method improvement

•Method verification[3]

understanding the physicochemical properties of drug molecules:

When developing a method, the physicochemical characteristics of a medicinal molecule are crucial. The physical characteristics of the drug molecule, including its solubility, polarity, pKa, and pH, must be examined before developing a technique. The physical characteristic of a compound is its polarity. It helps an analyzer identify the mobile and solventphase makeup. 6 Molecule solubility can be explained by their polarity. Polar (like water) and nonpolar (like benzene) solvents do not mix. Materials with comparable polarity dissolve in one another because "like dissolves like." Diluents are chosen based on the solubility of the analyte. The acidity or basicity of a substance is often determined by its pH value.[3]

Choosing chromatographic conditions : A set of beginning settings (detector, column, and mobile phase) is selected during the early technique development process in order to produce the sample's first "scouting" chromatograms. Reversed-phase separations on a C18 column with UV detection are usually the basis for these. It is now necessary to decide whether to create a gradient approach or an isocratic one.[3]

Column selection :

The primary component of an HPLC system is the column. When developing a methodology, dynamical columns can yield the best analyte resolution results. Typically, spherical square-measuring colloid beads coated with polyester are packed into the column housing to generate stylish reverse part HPLC columns. The stationary component has a hydrophobic nature. A chlorosilane reacts with the hydroxyl radical teams on the colloid surface to introduce the stationary portion into the matrix. Generally speaking, the stationary part's characteristics have the most impact on Capability is a factor in extraction, potency, and property. There A wide variety of matrices, including silicon oxide, polymers, and aluminum oxide, are square measured to sustain the stationary component. For HPLC columns, silicon oxide is the most often utilized matrix.The nature, shape, and size of the silicon oxide particles help to separate the impacts. There are more theoretical plates where smaller particles gather. Potency of separation. On the other hand, using smaller particles causes backpressure to build up. The column more simply becomes occluded during action. In reverse part action, the stationary component is non-polar.Consequently, the mobile portion is polar, producing polar peaks. Previous non-polar peaks are usually washed. A stationary part is utilized for reverse part action in order to produce an On silicon oxide. Support, the free silanols' square measure responded with a To present the hydrophobic chlorosilane in a realistic manner Non-polar surfaceOnly because of steric limitations About one-third of the silanols on the

surface are derivatized. Analytes are responsible for peak tailing, which is the result of interactions between the remaining free silanols. The column is typically subsequently treated with chlorotrimethylsilane after derivatization with the appropriate stationary component.To increase the efficiency of the column and finish capping the remaining free silanols[4]

Buffer selection :

The buffer is usually chosen based on the desired pH. For reversed-phase on silica-based packing, the usual pH range is 2 to 8. Since buffers regulate pH best at their pKa, it is essential that the buffer's pKa be near the target pH. Generally speaking, a buffer with a pKa value of two units of the intended mobile phase Ph should be chosen [5].

Concentration of the Buffer :

A buffer value of 10–50 mM is typically sufficient for tiny compounds. Generally speaking, no more Less than 50% organic should be utilized with a buffer. The specific buffer and its concentration determine this. The sodium or potassium salt of phosphoric acid Potassium salts are the most popular buffer systems. for HPLC in reverse phase. Buffers containing sulfonate can be helpful. Replace the phosphonate buffers during analysis. substances that contain organophosphate.

Mobile Phase Selection:

Efficiency, selectivity, and resolution are all impacted by the mobile phase. It is crucial to consider the solvent's strength or the mobile phase's composition. This has an impact on RP-HPLC separation. Solvent-blocking solvents, such as tetrahydrofuran (THF), methanol (MeOH), and acetonitrile (ACN), are frequently employed in RP-HPLC.The wavelengths that are blocked off are 190, 205, and 212 nm, in that order. These Solvents are miscible with water. a mix of Acetonitrile with water is the ideal first option.You will be on the go while the method is being developed.[6]

Selection of detector :

One crucial element of HPLC is the detector. The choice of detector is influenced by a number of factors, including the chemical makeup of the analysts, possible interference, the necessary detection limit, and the availability and/or cost of the detector. The UV-visible detector has two wavelengths and is multipurpose.An absorbance detector is used for HPLC. This detector offers the high sensitivity needed for low-level impurity identification and quantification in routine UV-based applications. For Waters, the Photodiode Array (PDA) Detector offers sophisticated optical detection. solutions for LC/MS systems, analytical HPLC, and preparative HPLC. High performance is provided by its integrated software and optical advances. sensitivity to spectroscopy and chromatography. Because of its great sensitivity, stability, and reproducibility, the Refractive Index (RI) Detector is a perfect tool for analyzing substances that absorb little or no UV light. For measuring low quantities of target substances, the Multi-Wavelength Fluorescence Detector offers excellent sensitivity and selectivity in fluorescence detection.[7]

Collection and preparation of the sample:

It is ideal for the sample to dissolve in the first mobile phase. Formic acid, acetic acid, or salt can be added to the mixture if stability or solubility problems prevent this. Adding a sample can boost solubility. There is no damage from these additives. as long as the loaded sample's volume is minimal in relation to the column.volume. There may be one or two more peaks eluting in the only effect when high sample volumes are employed. The void volume is computed after the injection of the sample.
In order to create a consistent and repeatable solution that can be injected onto the column, sample preparation is a crucial step in HPLC analysis. The column. The goal of sample preparation is to produce an aliquot of the sample that,

• Is comparatively interference-free,

• Won't damage the column, and

• Does it work with the desired HPLC procedure, meaning that the solvent in the sample will dissolve in the mobile phase without compromising resolution or retention of the sample [8]

Method optimization :

Determine the "weaknesses" of the approach and make it better using experimental design. Find out how the Approach works in various contexts with differing levels of success. Samples and instrument configurations vary. Most of the research and optimization of HPLC techniques has been finished. The optimization of HPLC conditions has been the main focus. The compositions of the mobile and stationary phases They have optimized the mobile phase. Because it is considerably simpler and more laid-back, parameter optimization is always given priority over parameter optimization.[9]

Method validation :

The process of confirming through laboratory investigations that an analytical method's performance characteristics satisfy the needs for the intended analytical application is known as validation. To guarantee efficacy, any new or modified approach needs to be validated. that when utilized by several operators with the same or different laboratory equipment, it can yield reliable and repeatable findings. The type of validation software required depends only on the particular approach and how it is intended to be implemented.

Method validation is an essential part of any effective analytical practice and can be used to assess the quality, consistency, and dependability of analytical data. The method validation process requires the use of equipment that is operable, calibrated correctly, and within specification. It is necessary to validate or revalidate analytical methodologies. necessary.[10]

Components of method validation : Typical analytical performance traits that could be examined during techniques validation include the following: [11

Accuracy:

Accuracy is the degree to which a measured value resembles the genuine or accepted value. The discrepancy between the actual value and the mean value found is known as accuracy. It is determined by applying the technique to known samples. Different concentrations of the analyte have been introduced. To ensure that there is no interference, these should be compared to blank and standard solutions. From the test findings of the analyte retrieved by the assay, the precision is then computed as a percentage. The test recovery of known, extra analyte levels is a common way to describe it.[12]

Precision :

The degree of agreement between a set of measurements taken from several samples of the same homogenous sample is expressed as the precision of an analytical method.Gather a sample under the given circumstances. Three categories can be used to classify precision: precision in the middle, repeatability, andAll of these factors are crucial to take into account. Research on precision is necessary. use consistent, genuine samples. However, if you are unable to obtain a homogeneous sample or one that has been intentionally manufactured, it may be investigated. The answer The precision of an analytical process is frequently expressed using the variance. The standard deviation or coefficient of variation of a set of measurements.[13]

(A) Replicability :

(1) To evaluate repeatability, at least nine conclusions throughout the procedure's designated range should be employed.

(2 2) At least six determinations at 100% of the test concentration

(3) three concentrations with three duplicates each.

(B) Moderate Accuracy :

The conditions under which the process is to be employed dictate the level of intermediate precision that should be established. The applicant must ascertain how random events affect the accuracy of the analytical process. Typical Among the variables to be examined are days, analysts, and equipment. Studying is not necessary. these impacts independently. Using an experimental design (matrix) is advised.

(C) The ability to reproduce :

considered when standardizing an The repeatability is evaluated using an interlaboratory trial. For instance, repeatability should be analytical technique. Procedures are added to pharmacopoeias. This isn't marketing information. dossier of authorization.

(D) Suggestions for Information :

For every accuracy type under investigation, the standard deviation, confidence interval, and relative standard deviation (coefficient of variation) should be supplied.[14]

Linearity:

The capacity of a procedure to yield test findings that are exactly proportionate to the concentration of the sample over a specified range is known as linearity. For HPLC procedures, the linear relationship between sample concentration and detector response (peak area and height) is established. The relationship can be directly shown on drug substance by dilution of standard stock or independent weighing of drug substance. Prepare the sample components according to the suggested methods.

Examining a plot of signals as a function of analyte content or concentration is the best way to visually evaluate linearity. Regression analysis and other suitable statistical techniques should be used to assess test findings if there is a linear relationship. Regression line data The degree of linearity can be usefully estimated mathematically. Usually, it is stated in terms of variance around the regression line's slope. In certain situations, the proper function of analyte concentration should be used to characterize the analytical reactions. The commonly used acceptability criteria and linearity ranges for different pharmaceutical techniques.[15]

Limit of detection:

Analyzing samples with known analyte concentrations allows one to calculate the limit of quantification (LOQ), which is the lowest level at which the analyte can be consistently identified under the given experimental conditions, without necessarily being quantified as a precise value. The analyte concentration in the sample (ppm) is a typical way to indicate the detection limit.The ICH suggests several methods for figuring out sample detection limits.based on the kind of analyte, the analytical tool, and the applicability of the technique.

Visual assessment is one of the appropriate strategies.

Signal-to-noise ratio (SNR).

The response's standard deviation

The standard deviation of the linearity plot's slope.

The LOD calculation formula is LOD = 3.3 δ/S

Where δ = standard deviation of calibration curve intercepts.

S =denotes the slope of linearity plot [16]

Limit of Quantification (LOQ) –

The lowest concentration of analyte in a sample that can be accurately and precisely quantified.The LOQ is usually estimated from a S/N ratio determination (10:1) in the case of analytical procedures like HPLC, which exhibit baseline noise. This S/N ratio is usually provided by injection standards that maintain an acceptable percent relative standard and deviation [17].

ROBUSTNESS :

An analytical procedure's robustness is a measure of its reliability and capacity to withstand small but intentional changes in technique parameters.while things are running normally.[18]

Specificity:

Specificity refers to the ability to clearly assess the substance in the presence of potentially expected components. The test technique can be specified by comparing the test results with samples of placebo particles that were obtained through sample analysis, upgrade products, or sample analysis without impurity, placebo

components. The best measure of specificity is the resolution between the analyte peak and the other closely eluting peak.[19]

REFERENCE :

1.Yadav Vidushi, Bharkatiya Meenakshi A REVIEW ON HPLC METHOD DEVELOPMENT AND VALIDATION Peer review under responsibility of Life Science Informatics Publications 2017 March- April RJLBPCS 2(6) Page No.166

2.Miral Patel1, Dharti Patel, Keyur Ahir3, Sumer Singh4A Review: Development and validation of HPLC method, J Pharm Sci Bioscientific Res. 2019. 9 (3):173-182

3 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 2015; 5(4): 76-81

4.Miral Patel1, Dharti Patel2, Keyur Ahir3, Sumer Singh4A Review: Development and validation of HPLC method ,J Pharm Sci Bioscientific Res. 2019. 9 (3):173-182

5.Ms Pallavi Nemgonda Patil HPLC METHOD DEVELOPMENT – A REVIEW,SGVU Journal of Pharmaceutical Research & Education, 2017, 2(1), 243-26**0**

6.Manjiri Shinde, Dr. Satish Kumar,Dr.Arunabha Mallik,Dr. N Jyothi A REVIEW ON HPLC METHOD DEVELOPMENT AND VALIDATION EPRA International Journal of Research and Development (IJRD) October 2021

7 D. Satheeshkumar,M. Gokul,S. Shalini,M. Ukeshraj, M. Mullaivendan ,HPLC method development and validation – A review,International Journal of Advance Research, Ideas and Innovations in Technology ISSN: 2454-132X Impact Factor: 6.078 (Volume 7, Issue 3 - V7I3-1429)

8.Sanjay Kumar D and D.R. Harish Kumar IMPORTANCE OF RP-HPLC IN ANALYTICAL METHOD DEVELOPMENT: A REVIEW IJPSR, 2012; Vol. 3(12): 4626-4633

9.Priya Sadapha, Kavita Dhamak Review Article on High-Performance Liquid Chromatography (HPLC) Method Development and Validation,Int. J. Pharm. Sci. Rev. Res., 74(2), May - June 2022; Article No. 03, Pages: 23-29

10.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.

11.Vikram Kumar, Rabijit Bharadwaj, Gaurav Gupta, Shailesh Kumar,An Overview on HPLC Method Development, Optimization and Validation process for drug analysis, The Pharmaceutical and Chemical Journal, 2015, 2(2):30-40

12.Murugan S, Elayaraja A, Niranjan Babu M, Chandrakala K, Prathap Naik K, Ramaiah P and Chathurya .Vulchi,A REVIEW ON METHOD DEVELOPMENT AND VALIDATION BY USING HPLC ,INTERNATIONAL JOURNAL OF NOVEL TRENDS IN PHARMACEUTICAL SCIENCES,VOLUME 3 | NUMBER 3 | OCT | 2013

13.Pawan Tiwari and B. K. Singh HPLC: A MODERN APPROACH OF DEVELOPMENT AND VALIDATION, World Journal of Pharmaceutical Research,Volume 5, Issue 9, 1616-1631.

14.Validation of Analytical Procedures: Methodology.ICH-Guidelines Q2B, Geneva. 1996; 11. (CPMP/ICH/281/95)

15.Harshad V. Paithankar ,HPLC METHOD VALIDATION FOR PHARMACEUTICALS: A REVIEW,International Journal of Universal Pharmacy and Bio Sciences 2(4): July-August 2013.

16.Panchumarthy Ravisankar Ch. Naga Navya1, D. Pravallika D. Navya Sri,A Review on Step-by-Step Analytical Method Validation, .iosrphr.org Volume 5, Issue 10 (October 2015), PP. 07-19

17.Kusum Mishra, B. K. Sing,A Review on Method Development and Validation by Using HPLC International Journal for Pharmaceutical Research Scholars (IJPRS),V-5, I-3, 2016

18.Ms Pallavi Nemgonda Patil ,HPLC METHOD DEVELOPMENT - A REVIEW,Journal of Pharmaceutical Research & Education, 2017, 2(1), 243-260

19.Punam S. Desai, Rajeshwar V. Chavan, Nikita B. Amane, Sanmati D. Shete, Archana R. Dhole, High Performance Liquid Chromatography – A Validation View,ASian J. Pharm. Ana. 2019; 9(4):232-236.