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A Review on Development and validation of HPLC method

Kalyani P.Patil*,Damini M.Patil, Sunila A.Patil, Sunil.P.Pawar.
Department of Quality Assurance P.S.G.V.P.M's College of Pharmacy, Shahada

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Corresponding Author:
Kalyani P.Patil

Email ID:
patilkalyani355@gmail.com

Abstract:
This review describes a strategy for the systematic development of HPLC. High Performance Liquid Chromatography has evolved into one of the most potent analytical chemistry tools available. It is capable of separating, identifying, and quantifying the compounds present in any liquid-soluble sample. To optimize the method, several chromatographic parameters were investigated, including sample pretreatment, mobile phase selection, column selection, and detector selection. The purpose of this article is to go over the method development, optimization, and validation processes.HPLC method development depends on chemical structure of the molecules, synthetic route, solubility, polarity, pH and pKa values, and functional groups activity et.. This article was prepared with the aim to review the method development and validation of HPLC.

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

INTRODUCTION –

High Performance Liquid Chromatography has evolved into one of the most potent analytical chemistry tools available. It is capable of separating, identifying, and quantifying the compounds present in any liquid-soluble sample. HPLC stands for high performance liquid chromatography.The most precise analytical methods widely used for quantitative and qualitative analysis of drug product.[1] The sample is separated based on differences in migration rates through the column caused by different partitions of the sample Between the stationary and mobile phases. Depending on the partitioning behaviour of various components Elution occurs at various times. The procedure the Russians were the first to develop chromatography M.S Tswett, a botanist, invented High Performance Liquid in 1903.1Chromatography is more adaptable than gas chromatography. because (a) it is not limited to volatile and non-volatile thermally stable samples, as well as (b) the selection

of mobile and The range of stationary phases is broader. Figure-1 depicts a schematic diagram of an HPLC system HPLC is more efficient than traditional LC techniques distinguished by:

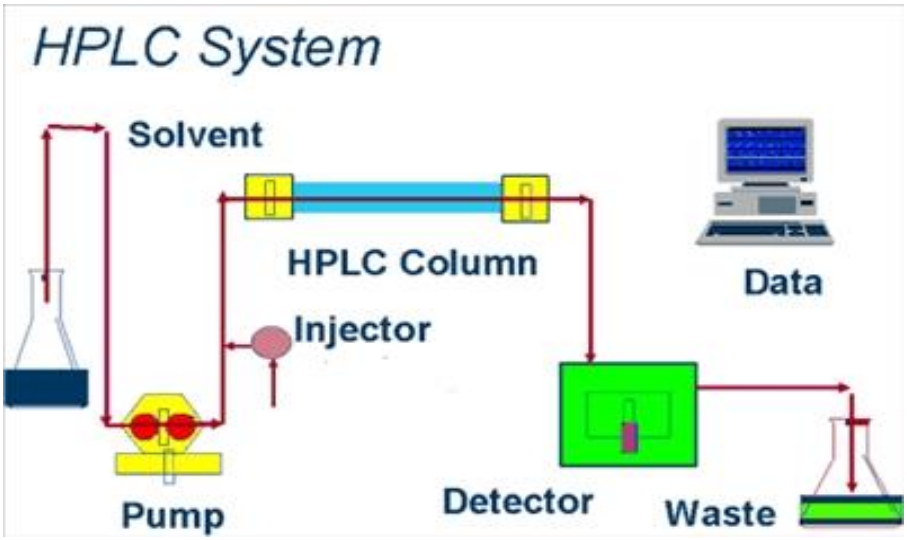
• Excellent resolution. Glass, stainless steel, and a small diameter (4.6 mm).or columns made of titanium.

• Column packing with small (3, 5, and 6) column Particles with a diameter of 10m.

• Relatively high inlet pressures and precise The movement of the mobile phase.

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

• Quick analysis.[2]



•HPLC Method Development:

When there are no official methods for a new product, methods are developed. Alternative methods for existing (non-pharmacopoeial) products are to reduce cost and time in order to improve precision and ruggedness. When an alternate method is proposed to replace an existing procedure, comparative laboratory data, including merits and demerits, is made available. The HPLC-method attempts to separate and quantify the main active drug, any reaction impurities, all available synthetic intermediates, and any degradants.

Method development steps are as follows.

•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:

A drug molecule's physicochemical properties are important in method development. To develop a method, one must first investigate the physical properties of the drug molecule, such as its solubility, polarity, pKa, and pH. Polarity is a compound's physical property. It aids an analyst in determining the solvent and mobile

phase composition. 6 The polarity of molecules can explain molecule solubility. Solvents that are polar, such as water, and nonpolar, such as benzene, do not mix. Like dissolves like, which means that materials with similar polarities dissolve in each other. The analyte solubility is used to select diluents. The pH value is commonly used to define a substance's acidity or basicity.[3]

Choosing chromatographic conditions

During initial method development, a set of initial conditions (detector, column, mobile phase) is chosen to generate the sample's first "scouting" chromatograms. These are typically based on reversed-phase separations on a C18 column with UV detection. At this point, a decision should be made whether to develop an isocratic or a gradient method.[3]

Column selection

The column is at the heart of an HPLC system. Throughout methodology development, dynamical a column can have the best result on analyte resolution. Generally, square measure fashionable reverse part HPLC columns created by packing the column housing with spherical square-measuring colloid beads coated with The stationary part is hydrophobic. The stationary portion is introduced to the matrix by reacting a chlorosilane with the On the colloid surface, hydroxyl radical teams present. In general the character of the stationary part has the best effect on Property, potency, and extraction are all issues of capability. There Many different types of matrices are square measured for support of the stationary component, as well as silicon oxide, polymers, and aluminium oxide is a type of oxide. The most commonly used matrix is silicon oxide For HPLC columns.The silicon oxide's nature, form, and particle size aid in the separation of effects. Smaller particles accumulate in a greater number of theoretical plates Separation potency. However, the use of smaller Additionally, particles result in accumulated backpressure. During action and the column more simply Becomes obstructed. The stationary part in reverse part action is non-polar.As a result, the mobile part is polar, resulting in polar peaks Typically, previous non-polar peaks are rinsed. To create a On silicon oxide, a stationary part is used for reverse part action Support, the square measure of free silanols reacted with a To introduce the chlorosilane with hydrophobic practicality Surface that is not polar. Solely due to steric constraints Approximately one-third of the surface silanols Square measure Derivatized. The remaining free silanols will interact with each other Peak tailing is caused by analytes. Normally, when the Column derivatization with the desired stationary part, The column is then further reacted with chlorotrimethylsilane.To complete the capping of the remaining free silanols and improve the Column effectiveness[4]

Buffer selection

The desired pH typically governs the buffer selection. The typical pH range for reversed-phase on silica-based packing is 2 to 8. It is critical that the buffer has a pKa close to the desired pH because buffers control pH best at their pKa. A general rule is to select a buffer with a pKa value of 2 units of the desired mobile phase Ph.[5]

Concentration of the Buffer

For small molecules, a buffer concentration of 10-50 mM is usually adequate. In general, no more With a buffer, less than 50% organic should be used. This depends on the particular buffer as well as its concentration. Phosphoric acid and its sodium or potassium salt The most widely used buffer systems are potassium salts. For reversed-phase HPLC. Sulfonate buffers can be useful. When analysing, replace phosphonate buffers Compounds containing organophosphate.

Mobile Phase Selection: The mobile phase has an effect on resolution, selectivity, and efficiency. The composition of the mobile phase (or the strength of the solvent) is critical. In RP-HPLC separation, this plays a role. Acetonitrile (ACN) (ACN),Methanol (MeOH) and tetrahydrofuran (THF) are examples of solvents-blocking solvents are commonly used in RP-HPLC.The cut-off wavelengths are 190, 205, and 212nm, respectively. These Water is miscible with solvents. Combination of The best first choice is acetonitrile and water.During the method development phase, you will be mobile.[6]

Selection of detector

The detector is an essential component of HPLC. The chemical nature of the analysts, potential interference, detection limit required, availability and/or cost of the detector all influence detector selection. The UV-Visible detector is versatile and dual-wavelength.For HPLC, an absorbance detector is used. This detector provides the high sensitivity required for routine UV-based applications to low-level Identification and quantification of impurities. The Photodiode Array (PDA) Detector provides advanced optical detection for Waters. Solutions for analytical HPLC, preparative HPLC, and LC/MS systems. Its integrated software and optics innovations deliver high performance. Sensitivity to chromatography and spectroscopy. The Refractive Index (RI) Detector has a high sensitivity, stability, and reproducibility, making it an ideal solution for analysing components with little or no UV absorption. The Multi-Wavelength Fluorescence Detector provides high sensitivity and selectivity in fluorescence detection for quantifying low concentrations of target compounds.[7]

Collection and preparation of the sample:

Ideally, the sample should be dissolved in the initial mobile phase. If this is not possible due to issues with stability or solubility,It is possible to add formic acid, acetic acid, or salt to the Solubility can be increased by adding a sample. These additives are not harmful. As long as the volume of The loaded sample is small in comparison to the column.Volume. When large sample volumes are used, the only effect Are used, there could be an additional peak or two eluting in the After sample injection, the void volume is calculated.

Sample preparation is an essential part of HPLC analysis, with the goal of producing a repeatable and homogeneous solution suitable for injection onto the column. The column. The purpose of sample preparation is to create a sample aliquot that,

•Is relatively free of interferences,

•Will not harm the column and

• Is it compatible with the intended HPLC method that is, the sample solvent will dissolve in the mobile phase without affecting sample retention or resolution[8]

Method optimization

Using experimental design, identify the method's "weaknesses" and optimise the method. Learn how the Approach performs in a variety of settings, with varying degrees of success Different instrument setups and samples are used. The vast majority of HPLC technique development optimization has been completed. The focus has been on optimising HPLC conditions. The Mobile and stationary phase compositions The mobile phase has been optimised. The optimization of parameters is always prioritised over the optimization of parameters because it is much easier And more relaxed[9]

Method validation

The process of validating an analytical method is the process of determining that the method's performance characteristics meet the requirements for the intended analytical application through laboratory studies. Any new or altered method must be validated to ensure its effectiveness. That it can produce reproducible and dependable results when used by different people Operators using the same or different laboratory equipment. the kind of the validation programme that is needed is entirely dependent on the specific method and its proposed implementation.

The results of method validation can be used to evaluate the quality, reliability, and consistency of analytical results; it is a necessary component of any good analytical practise. The use of equipment that is within specification, operational, and calibrated properly is essential to the method validation procedure. Analytical methods must be validated or revalidated Required.[10]

Components of method validation

The following are typical analytical performance characteristics which may be tested during methods validation: [11]

Accuracy:

The closeness of a measured value to the true or accepted value is referred to as accuracy. Accuracy indicates the difference in mean value found and the true value. It is decided by using the method on samples that are know Analyte in various concentrations has been added. These ought to be compared to standard and blank solutions to Make certain that there is no interference. The precision is then calculated as a percentage from the test results of the analyte recovered by the assay. It may occur frequently be expressed as the assay recovery of known,additional analyte amounts.[12]

Precision

The precision of an analytical procedure expresses the degree of agreement between a series of measurements obtained from multiple samples of the same homogeneous sample.Under the specified conditions, collect a sample. Precision can be divided into three categories: Repeatability, intermediate precision, and

reproducibility are all important considerations. Precision should be researched. Using homogeneous, authentic samples. However, if you are unable to obtain a It may be investigated using artificially prepared samples or a sample that is homogeneous. Solution The variance is commonly used to express the precision of an analytical procedure. A series of measurements' standard deviation or coefficient of variation.[13]

(A)Repeatability

(1) A minimum of 9 determinations covering the procedure's specified range should be used to assess repeatability. (For example, three concentrations with three replicates each); or

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

(B)Intermediate Precision

The degree to which intermediate precision should be established is determined by the circumstances in which the procedure is to be used. The applicant must Determine the impact of random events on the analytical procedure's precision. Typical Days, analysts, and equipment are among the variables to be investigated. It is not required to study. These effects on their own. It is encouraged to use an experimental design (matrix).

(C) Reproducibility

An interlaboratory trial is used to assess reproducibility. When standardising an analytical procedure, for example, reproducibility should be taken into account. Inclusion of procedures in pharmacopoeias. These are not marketing data. Authorization dossier.

(D)Recommended Data (5.

The standard deviation, relative standard deviation (coefficient of variation), and confidence interval should be reported for each type of precision investigated.[14]

Linearity:

A method's linearity refers to its ability to produce test results that are directly proportional to the sample concentration over a given range. The linear relationship between detector response (peak area and height) and sample concentration is determined for HPLC methods. By dilution of standard stock or separate weighing of drug substance, the relationship can be demonstrated directly on drug substance. Using the proposed procedures, prepare the sample components.

Linearity should be assessed visually by inspecting a plot of signals as a function of analyte concentration or content. If there is a linear relationship, test results should be evaluated using appropriate statistical methods, such as regression analysis. Data from the regression line It is useful to provide mathematical estimates of the degree of linearity. It is typically expressed in Terms of variance around the slope of regression line. In some cases, the analytical responses Should be described by the appropriate function of analyte concentration. The widely used Linearity ranges and acceptance criteria for various pharmaceutical methods.[15]

Limit of detection:

The LOQ is determined by analysing samples with known analyte concentrations and determining the minimum level at which the analyte can be reliably detected, but not necessarily quantitated as a precise value, under the specified experimental conditions. The detection limit is commonly expressed in terms of Analyte concentration in the sample (ppm).The ICH recommends a number of approaches for determining sample detection limits.Depending on the instrument used for analysis, the nature of the analyte, and the method's suitability. The suitable Strategies are

Visual assessment.

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 smallest amount of analyte in a sample that can be quantified Determined with appropriate precision and Accuracy.in the case of analytical procedures such as HPLC,Show baseline noise, and the LOQ is generally Estimated from a S/N ratio determination (10:1) and is typically confirmed by injection Standards that provide this S/N ratio while maintaining an acceptable percent relative standard As well as deviation[17]

ROBUSTNESS

The robustness of an analytical procedure is a measure of its ability to remain unaffected by minor but deliberate changes in method parameters, and it indicates its dependability.During normal operation.[18]

Specificity:

The ability to clearly evaluate the substance in the presence of components that might be anticipated to be present is known as specificity. Comparing the test findings with samples of placebo particles obtained through sample analysis, upgrade products, or sample analysis, without impurity, placebo components, allows for the specification of the test method. Resolution between the analyte peak and the other closely eluting peak is the greatest indicator of specificity.[19]

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