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HPLC METHOD DEVELOPMENT VALIDATION AND STABILITY INDICATING FOR DETERMINATION OF ESOMEPRAZOLE IN CAPSULE

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ABSTRACT

HPLC produces highly efficient separations and, in most cases, high detection sensitivity, it is the most widely used separation technique in modern pharmaceutical and biomedical research. Because of its advantages such as rapidity, specificity, accuracy, precision, and ease of automation, the HPLC approach can be used to test the majority of drugs in multi-component dosage types. An analytical technique is created to compare a given characteristic of a drug substance or drug product to accepted acceptance criteria. This article discusses the different stages involved in the creation and validation of HPLC methods. Accuracy, precision, accuracy, linearity, range and limit of detection, limit of quantification, robustness, and device suitability testing are all covered by validation of HPLC methods according to ICH Guidelines.

KEYWORDS: HPLC, Instrumentation, Method and Validation.

1. INTRODUCTION

HPLC (High Performance Liquid Chromatography) evolved from traditional column chromatography and is now one of the most important analytical chemistry tools available.[1] High-performance liquid chromatography (HPLC) is a critical analytical method used in all stages of drug discovery, growth, and production in today's pharmaceutical industry.[2] HPLC is the perfect tool for determining peak purity of new chemical substances, tracking reaction changes during synthetic procedures or scale-up, testing new formulations, and performing quality control / assurance on finished drug products.[3] The aim of the HPLC method is to isolate and quantify the active ingredient, any reaction impurities, all available synthetic intermediates, and any degradants.[4] High-Performance Liquid Chromatography is now one of analytical chemistry's most efficient instruments. It is capable of separating, identifying, and quantifying the compounds found in any sample that can be dissolved in a liquid. HPLC is one of the most precise analytical methods for quantitative and qualitative drug product analysis, as well as for determining drug product stability.[5] The theory of HPLC is that a sample solution is inserted into a porous material column (stationary phase), and the liquid phase (mobile phase) is pumped through the column at a higher pressure. The adsorption of solute on stationary phase based on its preference for stationary phase is the separation theory used. The HPLC technique has the following characteristics.[6]

- High resolution
- Small diameter, Stainless steel, Glass column
- Rapid analysis
- Relatively higher mobile phase pressure
- Controlled flow rate of mobile phase

1.1 INSTRUMENTATION

The HPLC instrumentation involves pump, injector, column, detector, integrator and display system. In the column the separation occurs. The parts include.

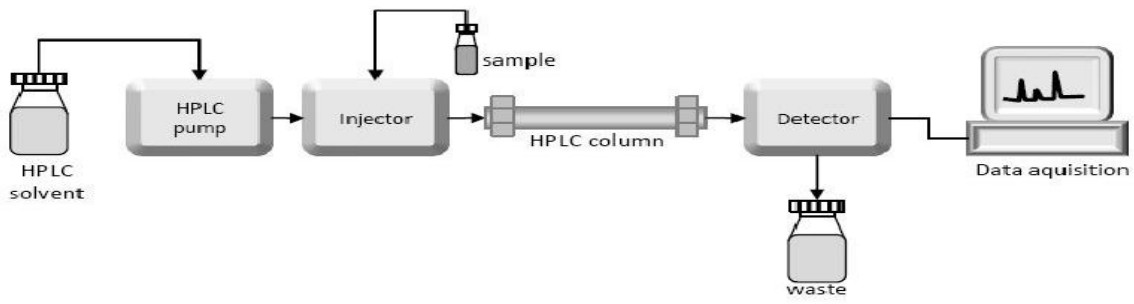

Fig.1: Instrumentation of HPLC.

• **Solvent Reservoir:** In the glass jar, the contents of the mobile process are present. The mobile phase, also known as the solvent, in HPLC is a mixture of polar and non-polar liquid components. The polar and non-polar solvents can be modified depending on the sample composition.

• **Pump:** The pump draws the mobile phase from the solvent tank, drives it into the column, and then sends it to the detector. The working pressure of the pump is 42000 KPa. The operating pressure is determined by the column lengths, particle size, flow rate, and mobile phase composition.

• **Sample Injector:** A single infusion or a computerised infusion framework may be used as the injector. An injector for an HPLC system can provide fluid specimen infusions with high reproducibility and pressure in the range of 0.1 mL to 100 mL of volume (up to 4000 psi).

• **Columns:** Columns are usually made of cleaned stainless steel, measure between 50 and 300 millimetres in length, and have a 2 to 5 millimetre inward diameter. They are usually filled with a stationary phase with molecules ranging in size from 3 to 10 micrometres. Microbore segments are described as columns with an inner diameter of less than 2 mm. During the investigation, the temperature of the mobile process and the column should ideally be kept constant.

• **Detector:** As the analytes elute from the chromatographic column, the HPLC detector near the end of the column separates them. UV spectroscopy, fluorescence, mass spectrometry, and electrochemical identifiers are all commonly used detectors.

• **Data Collection Devices or Integrator:** Graph recorders or electronic integrators may collect signals from the detector, which vary in many-sided quality and capacity to process, store, and reprocess chromatographic data. The PC coordinates the indicator's reaction to each component and inputs it into a chromatograph that is simple to understand.[21]

2. HPLC METHOD DEVELOPMENT

When there are no official methods for a new product, methods are created for Reduction the cost and time for greater accuracy and ruggedness for current (non-pharmacopoeial) products. Comparative laboratory results, including merits and demerits, are made available when an alternative approach is proposed to replace an existing technique. The goal of the HPLC-method is to try & separate, quantify the main active drug, any reaction impurities, all available synthetic inter-mediates and any degradants.[7]

Steps involved in Method development are.[6,7]

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

2.1 Understanding the physicochemical properties of drug molecules

Physicochemical properties of a drug molecule play an important role in method development. For Method development one has to study the physical properties like solubility, polarity, pKa and pH of the drug molecule. Polarity is a physical property of a compound. It assists an analyst in evaluating the solvent and mobile phase composition.[6] The polarity of molecules can be used to describe the solubility of molecules. Solvents that are polar, such as water, and nonpolar, such as benzene, do not combine. Like dissolves like, which means that materials with identical polarities dissolve in each other. Selection of diluents is based on the solubility of analyte. The acidity or basicity of a substance is defined most typically by the pH value. Selecting a proper pH for ionizable analytes often leads to symmetrical and sharp peaks in HPLC.[7]

2.2 Selection of chromatographic conditions

It assists an analyst in determining the composition of the solvent and mobile phase.[6] The solubility of molecules can be defined using the polarity of molecules. Solvents that are both polar and nonpolar, such as water and benzene, do not mix. Materials with equal polarities dissolve in each other, which is known as "like dissolves like."

2.2.1 Selection of Column

A chromatograph's beginning and central piece is, of course, a column. A successful chromatographic separation can be achieved with the right column, resulting in an accurate and reliable analysis. When a column is used incorrectly, it may cause confusion, insufficient separations, and invalid or difficult-to-understand results.[9] The column is the nucleus of an HPLC scheme. During method growth, changing a column will have the greatest impact on analyte resolution. The stationary phase chemistry, retention power, particle size, and column dimensions must all be considered when selecting the best column for your application. The hardware, matrix, and stationary phase are the three main components of an HPLC column. The stationary phase is assisted by a variety of matrices, including silica, polymers, alumina, and zirconium. The most popular matrix for HPLC columns is silica. Silica matrices are tough, easy to derivatize, and can be produced to a consistent sphere scale. They also don't compress under pressure. Many organic solvents and low pH systems are chemically stable for silica. One disadvantage of a silica solid help is that it dissolves at pH levels higher than 7. In recent years, silica-supported columns for use at high pH have been created. Separation is supported by the design, form, and particle size of silica. The number of theoretical plates increases

as the particle size decreases. Whether a column may be used for normal phase or reverse phase chromatography depends on the type of the stationary phase. A polar stationary phase and a non-polar mobile phase are used in normal phase chromatography. Polar compounds elute later than non-polar compounds in general. The most common reverse phase columns and their applications are described below. Ion-pairing chromatography (C4), peptides with hydrophobic residues, and other large molecules gain from the propyl (C3), butyl (C4), and pentyl (C5) phases. As compared to C8 or C18 phases, C3–C5 phases hold non-polar solutes relatively poorly. Examples include Zorbax SB-C3, YMC-Pack C4, and Luna C5. These columns are generally less stable to hydrolysis than columns with longer alkyl chains. Octyl (C8, MOS) phases have wide applicability. This phase is less retentive than the C18 phases, but is still quite useful for pharmaceuticals, nucleosides, and steroids.[10] Selection of the stationary phase/column is the first and the most important step in method development. The development of a rugged and reproducible method is impossible without the availability of a stable, high performance column. It's important for columns to be stable and repeatable during method creation to prevent issues with irreproducible sample retention. Separation selectivity for specific components varies between manufacturers' columns as well as between output batches of the same manufacturer's columns. The key ones are column lengths, silica substrate properties, and bonded stationary phase characteristics. Because of certain physical characteristics, silica-based packing is preferred in most current HPLC columns.[6]

2.2.2 Selection of Chromatographic mode

chromatographic modes dependent on the molecular weight and polarity of the analyte The most popular mode for small organic molecules is reversed-phase chromatography (RPC), which will be the subject of all case studies. RPC is commonly used to isolate ionizable compounds (acids and bases) using buffered mobile phases (to hold the analytes non-ionized) or ion-pairing reagents.[8]

2.2.3 Optimization of Mobile phase

Buffer Selection: Different buffers such as potassium phosphate, sodium phosphate and acetate were evaluated for system suitability parameters and overall chromatographic performance.

Effect of pH.: If analytes are ionisable, the appropriate mobile-phase pH must be selected based on the analyte pKa, ensuring that the target analyte is in one of two ionisation states: ionised or neutral. One of the most powerful tools in the "chromatographer's toolbox" is changing the mobile-phase pH, which allows for simultaneous changes in retention and selectivity between essential pairs of components.[12]

Effect of organic modifier: In reverse phase HPLC, choosing an organic modifier is relatively easy. The most common options are acetonitrile and methanol (rarely THF). Gradient elution is widely used for complex multicomponent samples because all components cannot be eluted between k (retention factor) 1 and 10 under isocratic conditions using a single solvent strength.[12]

2.2.4 Selection of detector and wavelength

The analyte of interest is detected using appropriate detectors after chromatographic separation. UV detectors, fluorescence detectors, electrochemical detectors, refractive index (RI) detectors, and mass spectrometry (MS) detectors are some of the industrial detectors used in LC. The detector to use is determined by the sample and the analysis' goal. In the case of multicomponent analysis, the absorption spectra of the parent compound could have been moved to longer or shorter wavelengths. As a result, the UV spectra of the target analyte and impurities must be taken and overlaid, and the spectra must be standardised due to the varying quantities of each present in the mixture. A wavelength must be chosen such that adequate response is for most of the analytes can be obtained.[12,13]

2.3. Developing the approach for analysis

The selection of various chromatographic parameters, such as mobile phase, column, flow rate of mobile phase, and pH of mobile phase, is the first step in developing an analytical method on RP-HPLC. All of these parameters are chosen based on experiments, and then the device suitability parameters are taken into account. For example, the retention time should be greater than 5 minutes, the theoretical plates should be greater than 2000, the tailing factor should be less than 2, the resolution between two peaks should be greater than 5, and the percent R.S.D. of the area of analyte peaks in standard chromatograms should not exceed 2.0 percent similar to other. Detection wavelength is usually isobestic point in the case of simultaneous estimation of 2 components.[6]

2.4 Sample preparation

The analyst must examine sample preparation as a critical phase in method production. For example, if there are insoluble components in the sample, the analyst should investigate whether centrifugation (determining the optimal rpm and time), shaking, and/or filtration of the sample is required. The purpose is to evaluate that sample filtration has no effect on the analytical result due to leachable adsorption and/or extraction. The capacity of syringe filters to eliminate contaminants/insoluble materials without leaching undesirable objects (i.e., extractable) into the filtrate determines their effectiveness. The sample preparation technique for subsequent HPLC analysis should be adequately defined in the respective analytical method that is applied to a real in-process sample or a dosage type. The maker, type of filter, and pore size of the filter media must all be specified in the analytical procedure.[12] The aim of sample preparation is to produce a processed sample that yields better analytical results than the raw sample. The

prepared sample should be a reasonably interference-free aliquot that is consistent with the HPLC method and won't harm the column.[13,15]

2.5 Method Optimization

The majority of HPLC method growth optimization has been based on improving HPLC conditions.[14] The compositions of the mobile and stationary phases must be considered. The optimization of mobile phase parameters is often prioritised over stationary phase optimization because it is much simpler and more convenient. Only the parameters that are likely to have a major impact on selectivity in the optimization should be investigated to reduce the number of trial chromatograms. The different components of the mobile process that determine acidity, solvent, gradient, flow rate, temperature, sample quantities, and injection volume, diluents and solvent type are primary control variables in the optimization of liquid chromatography (LC) methods. After satisfactory selectivity has been achieved, this is used to find the optimal balance between resolution and study time. Column lengths, column-packing particle size, and flow rate are all factors to consider. Changes to these parameters have no impact on capacity factor or selectivity.[10]

2.6 Method Validation

The process of validating an analytical method is establishing by laboratory studies that the method's output characteristics meet the requirements for the intended analytical use. Every new or modified approach must be validated to ensure that it can produce repeatable and accurate results when used by different operators using the same equipment in the same or different laboratories. The type of validation programme needed is entirely dependent on the method in question and its intended applications.[13] Method validation results can be used to assess the efficiency, accuracy, and reliability of analytical results; it is an essential component of any successful analytical practise. The use of equipment that is within specification, operationally sound, and properly calibrated is critical to the method validation process.

Analytical methods need to be validated or revalidated.[16]

• Before their introduction into routine use;
• Whenever the conditions change for which the method has been validated
• Whenever the method is changed

Typical parameters recommended by FDA, USP, and ICH are as follow.[16,18]

1. Specificity
2. Linearity & Range
3. Precision
   - Method precision (Repeatability)
   - Intermediate precision (Reproducibility)
4. Accuracy (Recovery)
5. Solution stability
6. Limit of Detection (LOD)
7. Limit of Quantification (LOQ)
8. Robustness
9. System suitability

Specificity: The ability of an analytical method to accurately calculate an analyte in the presence of interference, such as synthetic precursors, excipients, enantiomers, and established (or likely) degradation products that may be present in the sample matrix, is referred to as selectivity.[17]

Linearity and range: The ability of an analytical technique to produce test results that are directly proportional to the concentration of analyte in the sample (within a given range) is known as linearity. The range of the analytical method should be used to determine a linear relationship. It is seen directly on the drug substance by diluting a normal stock solution of the drug product components with using the proposed procedure. The trust limit along the slope of the regression line is often used to express linearity. 16-18 years old The ICH guideline recommends a minimum of five concentrations for establishing linearity.[19] The distance between the upper and lower levels that have been shown to be calculated with precision and accuracy is the range of an analytical and linearity using the method.[17]

Precision: The degree of scatter (closeness of agreement) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the specified conditions is expressed by the precision of an analytical method. There are three degrees of precision: repeatability, intermediate precision, and reproducibility.[19] The standard deviation or relative standard deviation of a set of measurements is widely used to express the accuracy of an analytical method. Precision may refer to the reproducibility or repeatability of an analytical procedure under normal circumstances. Within a laboratory, intermediate precision (also known as ruggedness) communicates variations, such as on different days or with different analysts or equipment. An analytical procedure's precision is calculated by testing a large enough number of aliquots of a homogeneous sample to measure statistically accurate standard deviation or relative standard deviation estimates.[20]

Accuracy (Recovery): The closeness of agreement between the value accepted as a traditional true value or an accepted reference value and the value found is expressed by the accuracy of an analytical method. lt is measured by applying the method to samples containing known concentrations of analyte. To ensure that there is no intrusion, these should be compared to normal and blank solutions. The accuracy is then measured as a percentage of the analyte recovered by the assay using the test results. It is frequently expressed as the recovery of identified, added amounts of analyte by assay.[18,19]

Solution stability: The stability of standards and samples is defined during validation under normal conditions, normal storage conditions, and sometimes in the instrument to decide whether special storage conditions, such as refrigeration or light protection, are needed**.**[18]

Limit of Detection (LOD): The lowest quantity of analyte in a sample that can be detected but not actually quantified as an exact value is the limit of detection (LOD) of an individual procedure**.** In analytical procedures that exhibit baseline noise, the LOD can be based on a signal-to-noise (S/N) ratio (3:1), which is usually expressed as the concentration of analyte in the sample. The signal-to-noise ratio is determined by: s = H/h Where H = height of the peak corresponding to the component. h = absolute value of the largest noise fluctuation from the baseline of the chromatogram of a blank solution.[18-20]

Limit of Quantification (LOQ): The lowest quantity of analyte in a sample that can be quantitatively measured with sufficient precision and accuracy is known as the limit of quantitation (LOQ) or quantitation limit of an individual analytical technique**.** The LOQ is usually calculated from a determination of S/N ratio (10:1) and is usually verified by injecting standards that give this S/N ratio and have a reasonable percent relative standard deviation. For analytical procedures such as HPLC that exhibit baseline noise, the LOQ is usually estimated from a determination of S/N ratio (10:1) and is usually confirmed by injecting standards that give this S/N ratio and have an acceptable percent relative standard deviation as well.[19,20]

Robustness: The ability of an analytical method to remain unaffected by small but deliberate variations in method parameters (e.g. pH, mobile phase composition, temperature, and instrumental settings) is characterised as robustness, which indicates its reliability during normal use. The process of varying a parameter and calculating the impact on the procedure by testing device suitability and/or sample analysis is known as robustness determination.[18,19]

System Suitability: Liquid chromatographic approaches include device suitability checks as a normal protocol. They are used to ensure that the chromatographic system's detection sensitivity, resolution, and reproducibility are sufficient for the study. The experiments are based on the premise that the equipment, circuitry, analytical operations, and samples to be tested are all part of a broader framework that can be evaluated. To evaluate the suitability of the used system, factors such as peak resolution, number of theoretical plates, peak tailing, and capability were calculated**.**[16-20]

3. CONCLUSION

In the field of pharmaceutical science, the advancement of analytical methods for drug detection, purity assessment, and quantification has gained a lot of attention in recent years. In general, this analysis discusses HPLC method development and validation. The development of HPLC methods for the separation of compounds was addressed using a general and very simple approach. Prior to the production of any HPLC process, it is critical to have a thorough understanding of the primary compound's physiochemical properties. The composition of the buffer and mobile phase (organic and pH) has a significant impact on separation selectivity. The gradient slope, temperature, and flow rate, as well as the type and concentration of mobile-phase modifier, can all be changed for final optimization. As per ICH guidelines, the optimised approach is validated using various parameters (e.g., specificity, precision, accuracy, detection limit, linearity, and so on).

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