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"Development of an analytical method at the level of its validation employing liquid phase chromatography using HPLC"

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Abstract

HPLC method development and validation plays an important role in the discovery, manufacturing, and development of pharmaceutical products. HPLC methods are able to separate, detect and quantify various drugs their related substances, degraded products and impurities that may be introduced during the synthesis of drug substance. Method validation establishes the performance characteristics and limitations of the developed method. Optimization of chromatographic conditions includes fixation of parameters like mobile phase, stationary phase, detection wavelength, elution mode that must affords to system suitability as well as stability of drugs, degradants, impurities. Force degradation studies are helpful in the development and validation of stability indicating assay. They also demonstrate the specificity while developing stability indicating assay.

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1. Introduction

Analytical chemistry is an art and science of recognizing the different substances in a particular matrix and determining their constituents. The instrumental methods of analysis are based upon measurement of physical properties of material using some instrument to determine its composition. Analytical method development and validation are continuous and interconnected activities throughout the drug development process. It is very much trial and error basis approach. Here in this paper the method development on RP-HPLC has been described and its further validation. The techniques of high-performance liquid chromatography is so called because of its improved performance over classical column chromatography, and it is the most important tools of analytical chemistry today. Principle is the solution of sample is injected into a column of porous material (stationary phase) and liquid phase (mobile phase) is pumped at higher pressure through the column. The principle of separation followed is the adsorption of solute on stationary phase based on its affinity towards stationary phase.

Method development on HPLC: Steps involved in method development of HPLC is as follows

a) Understanding the physicochemical properties of drug molecule:

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 helps an analyst, to decide the solvent and composition of the mobile phase. The solubility of molecules can be explained on the basis of the polarity of molecules. The selection of mobile phase or diluents is based on the solubility of analyte. The analyte must be soluble in diluents and must not react with any of its components. pH and pKa plays an important role in HPLC method development.

b) Selection of chromatographic conditions:

Selection of column andcolumn oven temperature:

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Selection of the stationary phase/column is the first and the most important step in method development. To avoid problems from irreproducible sample retention during method development, it is important that columns be stable and reproducible. A C8 or C18 column made from specially purified, less acidic silica and designed specifically for the separation of basic compounds is generally suitable for all samples andis strongly recommended. Column dimensions, silica substrate properties and bonded stationary phase characteristics are the main ones. The use of silica-based packing is favored in most of the present HPLC columns due to several physical characteristics. Silica substrates are available in spherical or irregular shapes and can be prepared with different surface areas, pore sizes and particle sizes, which make them suitable for most HPLC applications.

Separation of many samples can be enhanced by selecting the right column temperature. Higher column temperature reduces system backpressure by decreasing mobile phase viscosity.

Selection of Mobile Phase:

The mobile phase effects resolution, selectivity and efficiency. Mobile phase composition (or solvent strength) plays an important role in RP-HPLC separation. Acetonitrile, Methanoland Tetrahydrofuranare commonly used solvents in RP-HPLC having low UV cut-off of 190, 205 and 212nm respectively. These solvents are miscible with water. Mixture of acetonitrile and water is the best initial choice for the mobile phase during method development. Selection of the mobile-phase and gradient conditions is dependent on the ionogenic nature of the analyte and the hydrophobicity of the analytes in the mixture respectively. The aqueous buffer serves several purposes. At low pH, the mobile phase protonates free silanols on the column and reduces peak tailing. At sufficiently low pH basic analytes are protonated; when ionized the analyte will elute more quickly but with an improved peak shape. Acidic analytes in buffers of sufficiently low pH will remain uncharged, increasing retention. Conversely, at higher pH neutral basic compounds will be more retained, and ionized acidic compounds will elute earlier. Peak splitting may be observed if the pKa of a compound is similar to the pKa of the buffer, and the analyte elutes as both a charged and uncharged species. The pH of a buffer will not greatly affect the retention of non-ionizable sample components.

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Selection of elution mode:

There are basically 2 modes of elution are employed Isocratic and Gradient. Isocratic, constant eluent composition means equilibrium conditions in the column and the actual velocity of compounds moving through the column are constant; This makes isocratic separations more predictable, although the separation power is not very high. Gradient separation significantly increases the separation power of a system mainly because of the dramatic increase of the apparent efficiency. Gradient elution is employed for complex multicomponent samples since it may not be possible to get all components eluted between k (retention factor) 1 and 10 using a single solvent strength under isocratic conditions.

Selection of detector:

Detector is a very important part of HPLC. Selection of detector depends on the chemical nature of analytes, potential interference, limit of detection required, availability and/or cost of detector. UV-Visible detector is versatile, dual-wavelength absorbance detector for HPLC. This detector offers the high sensitivity required for routine UV-based applications to low-level impurity identification and quantitative analysis. Photodiode Array Detector offers advanced optical detection for Waters analytical HPLC. Refractive Index Detector offers high sensitivity, stability and reproducibility, which makes this detector the ideal solution for analysis of components with limited or no UV absorption. Multi-Wavelength Fluorescence Detector offers high sensitivity and selectivity fluorescence detection for quantitating low concentrations of target compounds.

c) Developing the approach for analysis:

All the above parameters are selected on the basis of trials and followed by considering the system suitability parameters. Typical parameters of system suitability are e.g., retention time should be more than 5 min, the theoretical plates should be more than 2000, the tailing factor should be less than 2, resolution between 2 peaks should be more than 5, % R.S.D. of the area of analyte peaks in standard chromatograms should not be more than 2.0 %. like others.

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d) Sample preparation:

The drug substance being analyzed should be stable and ideally dissolved in solution (diluent). During initial method development, preparations of the solutions in amber flasks should be performed until it is determined that the active component is stable at room temperature and does not degrade under normal laboratory conditions. Sample preparation is a critical step of method development that the analyst must investigate. If it is not dissolved due to stability or solubility problems, formic acid, acetic acid or salt can be added to the sample to increase solubility. These additives do not usually effect the separation so long as the volume of the sample loaded is small compared to the column volume.

e) Method optimization:

The experimental conditions should be optimized to get desired separations and sensitivity after getting appropriate separations. This will be achieved through planned/systemic examination on parameters including pH, mobile phase components and ratio, gradient, flow rate, temperature, sample amounts, Injection volume and diluents solvent type.

f) Method validation:

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

Accuracy:

Accuracy is the nearness of a measured value to the true or accepted value. Accuracy indicates the deviation between the mean value found and the true value. lt is determined by applying the method to samples to which known amounts of analyte have been added. These should be analysed against standard and blank solutions to ensure that no interference exists. The accuracy is then calculated from the test results as a percentage of the analyte recovered by the assay. It may often be expressed as the recovery by the assay of known, added amounts of analyte

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Precision:

Precision of an analytical method is the degree of agreement among individual test results obtained when the method is applied to multiple sampling of a homogenous sample. Precision is a measure of the reproducibility of the whole analytical method. It consists of two components: repeatability and intermediate precision.

Repeatability:

Repeatability is the variation experienced by a single analyst on a single instrument. It does not distinguish between variation from the instrument or system alone and from the sample preparation process. During validation, repeatability is performed by analyzing multiple replicates of an assay composite sample by using the analytical method. The recovery value is calculated.

Intermediate precision:

Intermediate precision is the variation within a laboratory such as different days, with different instruments, and by different analysts. The precision is then expressed as the relative standard deviation.

Linearity:

Linearity is the ability of analytical procedure to obtain%RSD a response that is directly proportional to the concentration of analyte in the sample. If the method is linear, the test results are directly or by well-defined mathematical transformation proportional to concentration of analyte in samples within a given range. Linearity is usually expressed as the confidence limit around the slope of the regression line.

Limit of detection (LOD):

Limit of detection (LOD)of an individual procedure is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated as an exact value. 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. h = largest noise fluctuation from the baseline.

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Limit of Quantitation (LOQ):

Limit of Quantitation (LOQ)of an individual analytical procedure is the lowest amount of analyte in a sample that can be quantitatively determined with suitable precision and accuracy. For analytical procedures such as HPLC that exhibit baseline noise, the LOQ is generally estimated from a determination of S/N ratio (10:1) and is usually confirmed by injecting standards which give this S/N ratio and have an acceptable percent relative standard deviation as well.

Specificity:

Specificitybe present such as impurities, degradation products, and excipients. Specificity measures only the desired component without interference from other species that might be present; separation is not necessarily required.

Range:

Rangeis defined as the interval between the upper and lower concentrations of analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy, and linearity.

Robustness:

Robustness is defined as the measure of the ability of an analytical method to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Determination of robustness is a systematic process of varying a parameter and measuring the effect on the method by monitoring system suitability and/or the analysis of samples.

System Suitability Determination:

System Suitability Determinationis the evaluation of the components of an analytical system to show that the performance of a system meets the standards required by a method. These parameters can be calculated experimentally to provide a quantitative system suitability test report: number of theoretical plates, capacity factor, separation, resolution, tailing factor, relative standard deviation. These are measured on a peak or peaks of known retention time and peak width.

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Forced degradation or stress studies:

Forced degradation or stress studies are undertaken to deliberately degrade the sample. These studies are used to evaluate an analytical method's ability to measure an active ingredient and its degradation products, without interference, by generating potential degradation products. During validation of the method, drug substance are exposed to acid, base, heat, light and oxidizing agent to produce approximately 10% to 30% degradation of active substance. The studies can also provide information about the degradation pathways and degradation products that could form during storage.

Solution Stability Studies:

stability of standards and samples is established under normal conditions, normal storage conditions, and sometimes in the instrument to determine if special storage conditions are necessary, for instance, refrigeration or protection from light.

2. Conclusion

Analytical method development and validation are interconnected activities throughout the drug development process. This paper describes the general technique of HPLC method development and validation of optimized method. The analytical validation verifies that a given method measures a parameter as intended and establishes the performance limits of the measurement. Reproducible quality HPLC results can only be obtained if proper attention has been paid to the method development, validation and system's suitability to carry out the analysis. While developing the analytical methods for pharmaceuticals by RP-HPLC, must have good practical understanding of chromatographic separation to know how it varies with the sample and with varying experimental conditions in order to achieve optimum separation.

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