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A REVIEW: METHOD DEVELOPMENT AND VALIDATION

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ABSTRACT

Now days, there are various strategies of high pressure liquid chromatography method development and validation. This review describes method, optimization and validation of high pressure liquid chromatography. Method development depends on chemical structure of the molecules, synthetic route, solubility, polarity, pH and pKa values, and functional group activity. Validation of high pressure liquid chromatography method as per ICH guidelines coves all the performance characteristics of validation like accuracy, precision, specificity, linearity, range and limit of detection, limit of quantification, robustness, and system suitability testing. Most of the drugs in multi component dosage forms can be analyzed by high pressure liquid chromatography method because of the several merits that is rapidity, specificity, accuracy, and ease of automation in this method.

KEYWORDS: Method Development, Validation, linearity, precession, column, mobile phase, stationary phase.

INTRODUCTION

Analysis is essential in any administration, and it is likewise significant in drug since it includes life. Analytical chemistry is the analysis of separation, quantification and chemical additives distinguishing proof of herbal and synthetic materials established with at least one factors.[1] High Performance Liquid Chromatography (HPLC) was gotten from the old style column chromatography and, is one of the main instruments of analytical chemistry today.[2] HPLC is the strategy for checking peak purity of chemical entities, monitoring reaction changes is in synthetic procedures or scale up, evaluating new formulations and carrying out quality control / assurance of the final drug products.[3] The principle is that an solution of the sample is infused into a column of a permeable material (fixed phase) and a fluid (portable phase) is siphoned at high pressure through the column. The separation of sample depends on the differences in the rates of migration through the column emerging from various partition of the sample between the fixed and portable phase. Depending upon the partition behaviour of various segments, elution at different time happens. The sample compound with the more affinity to the fixed phase will travel increasingly slow a more limited separation in contrast with compounds with less affinity which travel quicker and for a more extended distance.[4]

HPLC has numerous advantages like[5]

1. Simultaneous Analysis
2. High Resolution
3. High Sensitivity
4. Good repeatability
5. Small sample size
6. Moderate analysis condition
7. Easy to fractionate the sample and purify

HPLC has numerous limitations like[51]

1. HPLC can be costly.
2. Requiring large quantities of expensive organics.
3. HPLC does have low sensitivity for certain compounds, and some cannot be detected as they are irreversibly adsorbed.
4. Volatile substances are better separated by gas chromatography.

Classification of HPLC can be done as[6]

1. Normal phase chromatography
2. Reverse phase chromatography (based on modes of operation)

1 Normal phase chromatography:- In normal phase chromatography, mobile phase is non-polar and stationary phase is polar. Subsequently, the stationary phase holds the polar analyt. An expansion in polarity of solute particles builds the adsorption limit prompting an expanded elution time. Synthetically modified silica (cyanopropyl, aminopropyl and diol) is utilized as a stationary phase in this chromatography.[7] For example, An ordinary column has an internal diameter of around 4.6 mm, and a length in the range of 150 to 250 mm. Polar compounds in the mixture that are gone through the column will adhere longer to the polar silica than the non-polar compounds. Hence, the non-polar ones will go all the more rapidly through the column.[8]

2 RP-HPLC (Reversed phase HPLC):- RP-HPLC has a non-polar stationary phase and polar or moderately polar mobile phase.[9] RP-HPLC depends on the rule of hydrophobic interaction. In a combination of components those analytes which are moderately less polar will be held by the non-polar stationary phase longer than those which are generally more polar. In this way the most polar part will elute first.[10]

Method Development and Validation

Analytical method development and validation are non-stop and interconnected activities conducted through the drug development process.The practice of validation that a given technique quantifies a parameter as proposed and sets up the performance limits of the estimation.

Analytical (HPLC) Method Development:- Methods are developed for new products when no official methods are available. Alternate method for existing (Non-Pharmacopoeial) products is to reduce the cost and time for better precision and ruggedness. When alternate method proposed is intended to replace the existing procedure comparative laboratory data including merit/demerits are made available. 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.[11]

1. Understand the physicochemical properties of drug molecule.
2. Set up HPLC conditions.
3. Preparation of sample solution for method development.
4. Method optimization.
5. Validation of method

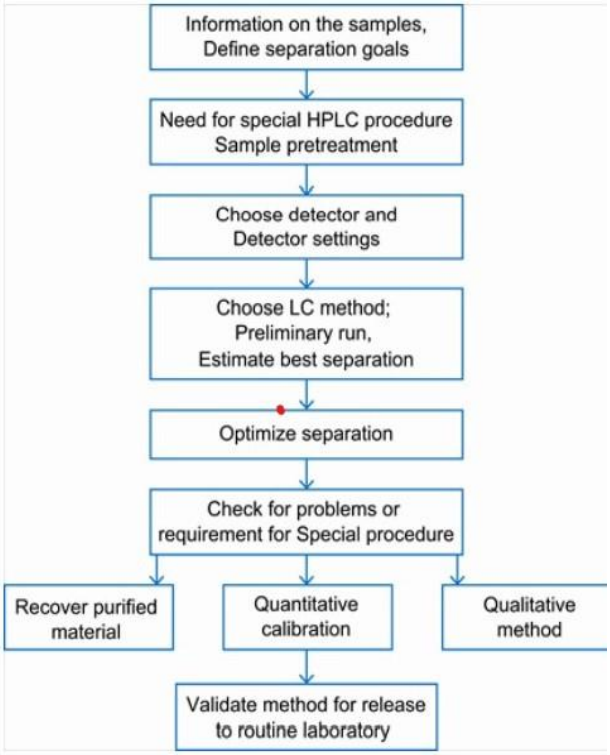

Figure 1: Steps of Method Development.

1:- PHYSICOCHEMICAL PROPERTIES OF DRUG MOLECULE

a) Chemical properties:- Chemical structure of the known and expected product chemical structures is acceptable contributions for starting the method development and it will give scientific approach for the method development. Draw the relative difference between impurities, beginning materials, by-Products and degradation products with final outcomes. Solubility learns at various pH esteems data for all focused on molecules is best contributions for choosing the regular diluent for all particles. Choosing the polar/non polar HPLC column the data of polarity of atoms is significant.[12]

b) pH and pKa value of compound:- The nature and polarity of the compound is accepted dependent on pH and pKa values. The compound is half ionized, when pH is comparable to pKa. Practically all the pH related change happens inside the ± 1.5 units of the pKa values. Outside the range the compound is either ionized or non-ionized, and its retention doesn't change much with pH.[12]

2:- SELECTION OF CHROMATOGRAPHIC CONDITIONS:- During beginning method development, a bunch of starting conditions (column, mobile phase and detectors) is chosen. By and large, these depend on reversed phase separations on a C18 column with UV detection. A choice on developing either an isocratic or a gradient method ought to be made now.[13]

a) Selection of column:- A column is of course, the starting and valuable piece of a chromatograph. A correctly decided on column can produce a very good chromatographic separation which offers a correct and reliable analysis. An improperly used column can frequently generate confusion, insufficient, and bad separations which can lead to results which can be invalid or complicated to interpret. The heart of a HPLC machine is the column. Changing a column will have the greatest impact at the resolution of analytes all during method development. There are several types of matrices for support of the stationary phase, along with silica, polymers, alumina, and zirconium. The nature of the stationary phase will determine whether a column can be used for normal phase or reverse phase chromatography. Commonly used reverse phase columns and their uses are listed below. Propyl (C3), Butyl (C4), and Pentyl (C5) phases are useful for ion-pairing chromatography (C4) and peptides with hydrophobic residues, and other large molecules. C3–C5 columns generally retain non-polar solutes more poorly when compared to C8 or C18 phases. 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.[14]

b) Chromatographic conditions:- Selection of suitable chromatographic conditions is the next step within the method development. This includes choice of temperature, selection of composition of mobile phase, pH of mobile phase and flow rate etc. A decision of developing either an isocratic or a gradient approach need to be made at this point. In most instances, those are based on reversed phase separations on a C18 column with UV detection.[15]

c) Optimization of mobile phase:- When samples contain ionizable compounds, the mobile phase pH can be one of the main factors in the control of maintenance in a reversed-phase HPLC (RP-HPLC) separation. However, if it is not controlled properly, pH can be a source of many problems. Since most compounds analyzed by RP-HPLC contain one or more acidic or basic functional groups, most mobile phases require pH control. For this reason, buffers are widely used. In RP chromatography, water and support are feeble dissolvable and CAN, methanol, THF is strong solvents and this are most generally use solvents.[16] Different considerations while choosing solvents: Methanol – High thickness may restrict utilization of more modest molecule size or longer columns at elevated flow rates. Acetonitrile – Relatively significant expense. THF – UV absorbance at low frequencies; high consistency.[17]

d) Column temperature:- The use of temperature in HPLC method improvement provides a mission because it can have unpredictable effects on selectivity. The use of elevated temperatures will Reduce mobile phase viscosity and back-pressure. This can assist you to perform at better flow rates, or to apply longer columns smaller particle sizes. Reduce elution time. Improve method reproducibility (instead of operating at room temperature). However, it's far not possible to decide if the usage of increased temperatures will assist or hinder a specific separation. For complicated separations, enhancements in one portion of the chromatogram are nearly always followed with the decreases in some other part of the same chromatogram.[18]

e) Selection of wavelength and detector:- All listed molecules UV/Visible and FT-1R spectrums are required to select the UV detector nm for all molecules. FTIR spectral data is the main source for understanding the functional groups activity.[19]

Table 1:- Types of detectors.

Detector	Type of compound can be detected
UV-Visible &Photodiode array	Compounds with chromophores, such as aromatic rings or multiple alternating double bonds.
Fluorescence detector	Fluorescent compounds, usually with fused rings or highly conjugated planer system.
Conductivity detector	Charged compounds, such as inorganic ions and organic acid.
Electrochemical detector	For easily oxidized compounds like quinines or amines
Refractive Index detector & Evaporative light scattering detector	Compounds that do not show characteristics usable by the other detectors, eg.polymers, saccharides.

3:- SAMPLE PREPARATION:- The drug substance being examined should be stable in solution (diluent). During starting method development, preparation of the solution in golden jars ought to be performed until it is resolved that the active part is stable at room temperature and doesn't degrade under ordinary research laboratory conditions. The sample solution ought to be separated; the utilization of a 0.22 or 0.45 µm pore-size channel is by and large suggested for evacuation of particulates. Filtration is a preventive upkeep tool for HPLC analysis.

Sample preparation is a critical step of method development that the examiner must explore. The viability of the needle channels is to a great extent controlled by their capacity to eliminate foreign substances/insoluble components without leaching undesirable artifacts (i.e., extractables) into the filtrate. On the off chance that any extra peaks are seen in the separated samples, at that point the diluent must be separated to decide whether a leachable component is coming from the syringe filter housing/filter.[20,21,22,23]

4:- METHOD OPTIMIZATION:- Most of the optimization of HPLC method development has been focused on the optimization of HPLC conditions. The mobile phase and stationary phase compositions need to be taken into account. Optimization of mobile phase parameters is always considered first as this is much easier and convenient than stationary phase optimization. To minimize the number of trial chromatograms involved, only the parameters that are likely to have a significant effect on selectivity in the optimization must

be examined. Primary control variables in the optimization of liquid chromatography (LC) methods are the different components of the mobile phase determining acidity, solvent, gradient, flow rate, temperature, sample amounts, injection volume, and diluents solvent type. This is used to find the desired balance between resolution and analysis time after satisfactory selectivity has been achieved. The parameters involved include column dimensions, column-packing particle size and flow rate. These parameters may be changed without affecting capacity factor or selectivity.[24]

5:-METHOD VALIDATION:- Analytical method validation is "A Documented proof, which gives a serious level of confirmation that a specific process will consistently produce, an item meeting its predetermined details and quality attributes".

Validation parameters

1) Accuracy
2) Precision
   - Repeatability
   - Intermediate Precision
   - Reproducibility
3) Specificity
4) Detection Limit
5) Quantitation limit
6) Linearity
7) Range
8) Stability
9) Robustness
10) Ruggedness
11) System Suitability

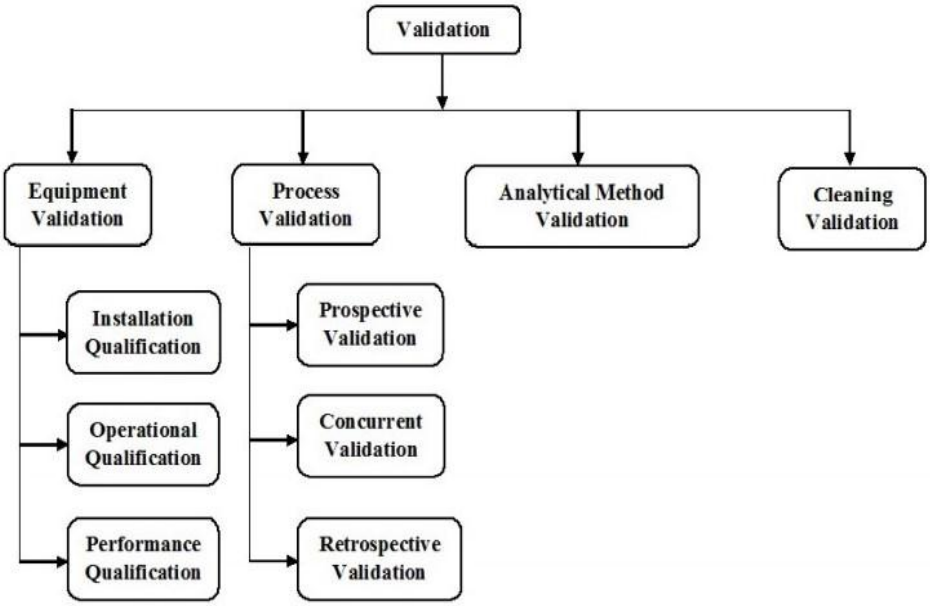

Figure 2: Types of Validation.

1) Accuracy:- Accuracy is communicated as the proximity of arrangement between the qualities found and qualities that are already available. It can also be characterized as the closeness between the true value and the observed value. It is sometime called as trueness and determinations over a minimum of 3 concentrations it could be determined by using at least 9 predefined range.[25]

2) Precision:- Precision communicates closeness of agreement (degree of scatter) between a progression of estimations acquired from numerous sampling of same homogeneous sample under the recommended conditions.[26]

Precision might be considered at three levels

- Repeatability
- Intermediate precision
- Reproducibility

Repeatability:- Repeatability is also referred to as intra-assay precision. It is a proportion of precision of precision in one research facility by one administrator utilizing piece of equipment over a moderately short timeframe range. It is level of arrangement of results when experimental conditions are kept up as consistent as could be expected under the circumstances, and communicated as RSD of reproduce values.[27]

Intermediate precision:- ICH characterize moderate precision as long term variability of the estimation process and is controlled by looking at the aftereffects of a method run inside a solitary research facility over various weeks. It is additionally called as intraday precision.[28]

Reproducibility:- Reproducibility communicates precision of analysis of similar sample by various different in various research facilities utilizing

operational and environmental conditions that may differ yet are still inside the predetermined boundaries of the method.[27]

3) SPECIFICITY:- For each phase of advancement, the analytical technique ought to show specificity. The technique was should have the power to unequivocally survey the analyte of interest inside the presence of every expected parts, which can incorporate degradants, excipients/ sample matrix, and sample blank peaks.

Specificity was performed to decide the retention time of each drug in a combination and in the sample. The retention time of standard drugs individually was resolved, and it was found to be 3.750 min and 1.533 min for nitazoxanide and ofloxacin and retention time of the two medications in the standard blend was discovered to be 3.760 min for nitazoxanide and 1.542 min for ofloxacin individually.[29]

4) LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTITATION (LOQ):- The limit of detection (LOD) is characterized as the lowest concentration of an analyte in a sample that can be detected, not quantified. LOD is communicated as a fixation at a predefined signal: noise proportion, generally 3:1. The limit of quantitation (LOQ) is characterized as the most reduced grouping of an analyte in a sample that can be resolved with acceptable precision and accuracy under the expressed operational states of the technique. For LOQ, ICH has suggested a signal: noise proportion 10:1. LOD and LOQ may likewise be determined dependent on the standard deviation of th e reaction (SD) and the slope of the calibration curve(s) at levels approximating the LOD as per the given beneath formulae.[30,31]

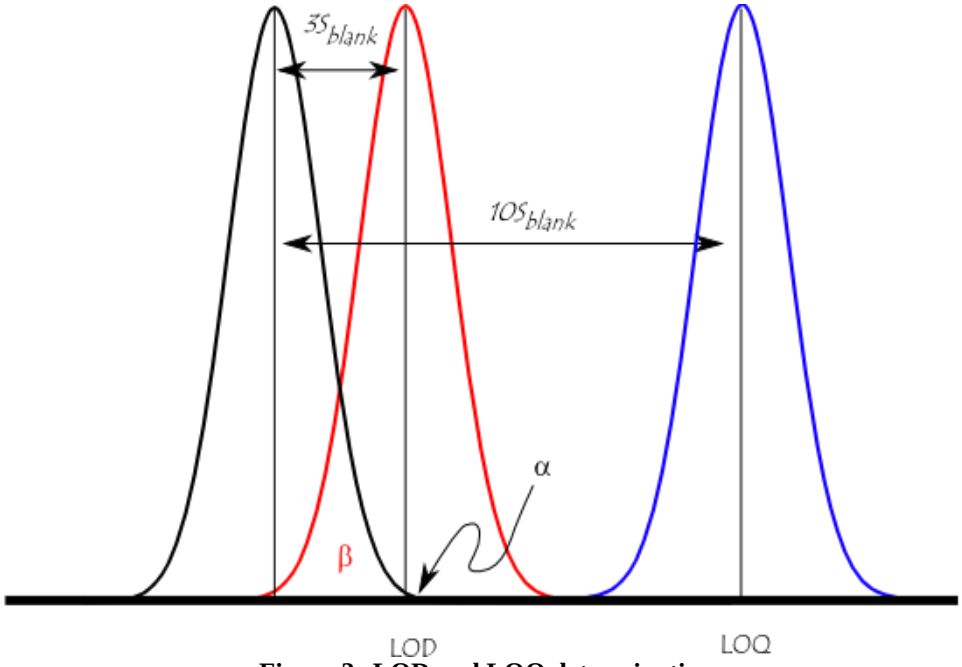

Figure 3:-LOD and LOQ determination.

5) LINEARITY:- The linearity of a systematic technique is its capacity (inside an offered range) to acquire test results which are directly proportional to the concentration (amount) of analyte in the sample (32). Linearity is controlled by a progression of five to six infusions of five standard whose concentration span 80–120 percent expected concentration range. The reaction ought to be directly proportional to the groupings of the analytes or relative by methods for a very much characterized calculation. A linear regression equation applied to the outcomes ought to intercept not significantly different from zero. If a significant non-zero intercept is acquired, it should be shown that this has no impact on the accuracy of the strategy.[33]

6) RANGE:-The range of the method is the interval between the upper and lower levels of an analyte that have been determined with acceptable precision, accuracy and linearity. It is determined on either a linear or nonlinear response curve (i.e. where more than one range is involved, as shown below) and is normally expressed in the same units as the test results.[34]

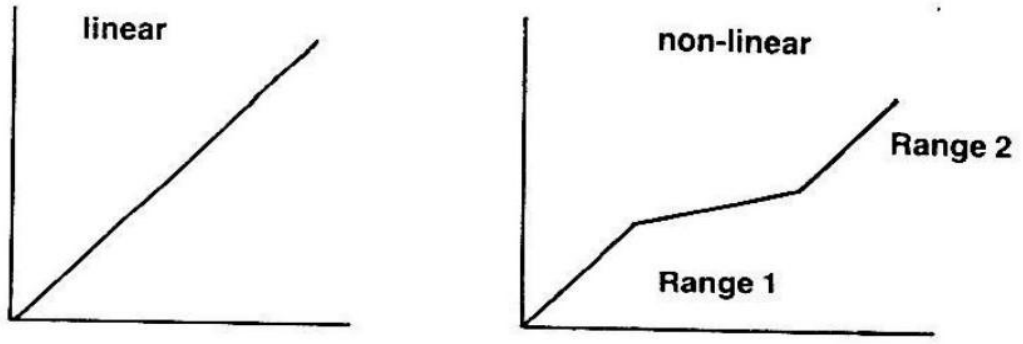

Figure 4:- Determination of Range.

7) STABILITY:- Solution stability is soundness of standard and extracted sample solution (prepared to infuse) from the sample or matrix and analysed according to indicated method, and it ought to be put away appropriately in room temperature and refrigerated condition depending on the strength of the sample and standard solution. The stability of standard and test solution ought to be set up in room temperature and refrigerated, whenever refrigerated before investigating it should place to room temperature[50]. A minimum two preparation of standard and test solution ought to be arranged and broke down according to determined method. The analysed solutions put away in necessary condition and the stability can be set up for two days or solution stability can be set up by an hour depending upon the nature of the product.[35]

8) Robustness:-The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.[36]

In the case of liquid chromatography, examples of typical variations are[36]

- Influence of variations of pH in a mobile phase
- Influence of variations in mobile phase composition
- Different columns (different lots and/or suppliers)
- Temperature
- Flow rate

9) Ruggedness:- Ruggedness is the degree or measure of reproducibility under different situations such as in different laboratories, different analyst, different machines, environmental conditions, operators etc. The testing of ruggedness is normally suggested when the method is to be used in more than one laboratory. Ruggedness is normally expressed as the lack of the influence on the test results of operational and environmental variables of the analytical method.[37]

10) SYSTEM SUITABILITY:- According to the USP, system suitability tests are an integral part of chromatographic methods. These tests are used to verify that the resolution and reproducibility of the system are adequate for the analysis to be performed. System suitability tests are based on the concept that the equipment, electronics, analytical operations, and samples constitute an integral system that can be evaluated as a whole.[44]

System suitability parameters[39,40,41]

- Tailing factor (T)
- Capacity factor (K)
- Retention time
- Resolution (R)

CONCLUSION

This article gives a thought that what is validation, its sorts, why it is vital, how to develop a method and how to do the validation procedure to exhibit that the procedure is capable for its proposed reason. All validation parameters, for example, linearity, LOQ, LOD, Range, specificity, robustness, ruggedness and system suitability are characterized well with instances of specific medications. Validation is a necessary method in the pharmacy department, and it is utilized to guarantee that the quality is worked into the techniques supporting the advancement of drug and production.

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