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A Review: Development and validation of HPLC method

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ABSTRACT:

This review describes a strategy for the systematic development of High performance liquid chromatographic (HPLC) methods. HPLC is an analytical tool which is able to detect, separate and quantify the drug, its various impurities and drug related degradants that can be generate on synthesis or storage. Most of the drugs in multi component dosage forms can be analyzed by HPLC method because of the several advantages like rapidity, specificity, accuracy, precision and ease of automation in this method. HPLC methods development and validation play important roles in new discovery, development, manufacture of pharmaceutical drugs and various other studies related to humans and animals. It involves the understanding of chemistry of drug substance and facilitates the development of analytical method. A number of chromatographic parameters were evaluated in order to optimize the method. An appropriate mobile phase, column, column temperature, wavelength and gradient must be found that affords suitable compatibility and stability of drug as well as degradants and impurities. Force degradation studies are helpful in development and validation of stability-indicating methodology, determination of degradation pathways of drug substances and drug products, discernment of degradation products in formulations that are related to drug substances versus those that are related to non–drug substances (e.g. excipients). This review gives information regarding various stages involved in development and validation of HPLC method. Validation of HPLC method as per ICH Guidelines covers all the performance characteristics of validation, like Accuracy, precision, specificity, linearity, range and limit of detection, limit of quantification, robustness and system suitability testing.

KEY WORDS: HPLC, method development, validation, accuracy, specificity.

INTRODUCTION:

High Performance Liquid Chromatography (HPLC) was derived from the classical column chromatography and, is one of the most important tools of analytical chemistry today. The principle is that a solution of the sample is injected into a column of a porous material (stationary phase) and a liquid (mobile phase) is pumped at high pressure through the column. The separation of sample is based on the differences in the rates of migration through the column arising from different partition of the sample between the stationary and mobile phase. Depending upon the partition behavior of different components, elution at different time takes place. The technique, chromatography was originally developed by the Russian botanist M.S Tswett in 1903.[1] High Performance Liquid Chromatography is more versatile than gas chromatography since (a) it is not limited to volatile and thermally stable samples, and (b) the choice of mobile and stationary phases is wider. A schematic diagram of HPLC system is shown in Figure-1.

HPLC as compared with the classical LC technique is characterised by:

- High resolution.

- Small diameter (4.6 mm), stainless steel, glass or titanium columns.
- Column packing with very small (3, 5 and 10µm) particles.
- Relatively high inlet pressures and controlled flow of the mobile phase.
- Continuous flow detectors capable of handling small flow rates and detecting very small amounts.
- Rapid analysis [2]

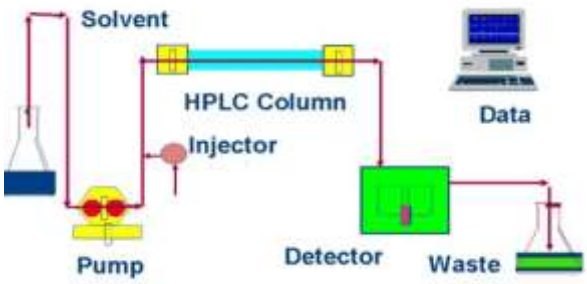

Figure-1: Flow Diagram of HPLC

Analytical method development

Analytical method development and validation play important roles in the discovery development and manufacture of pharmaceuticals. These methods used to ensure the identity, purity, potency, & performance of drug products. There are many factors to consider when developing methods. The initially collect the information about the analyte's physicochemical properties (pKa, log P, solubility) and determining which mode of detection would be suitable for analysis (i.e., suitable wavelength in case of UV detection).[3] The majority of the analytical development effort goes into validating a stability indicating HPLC–method. The goal of the HPLC-method is to separate quantify the main active drug, any reaction impurities, all available synthetic inter-mediates and any degradants.

Steps involve in method development are:

1. Qualified and calibrated instrument
2. Documented methods
3. Reliable reference standards
4. Qualified analysts
5. Sample selection and integrity
6. The analysis should take a minimal time and should be economical.
7. The accuracy of the analysis must accept the guidelines of Pharmacopoeia.
8. The chosen method should be precise and selective.
9. Set up HPLC conditions.
10. Preparation of sample solution for method development.
11. Method optimization.
12. Development and Validation of method (figure-2).[4]

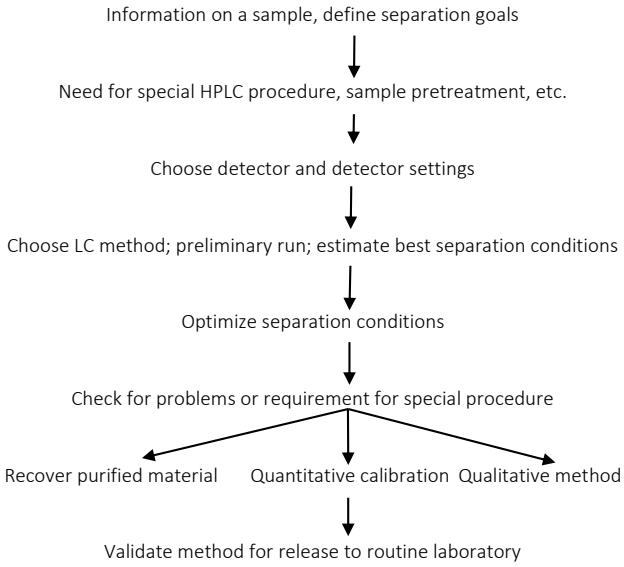

Figure -2: Steps involved in HPLC Method Development and Validation

Purpose of analytical method development

Drug analysis reveals the identification characterization & determination of the medication in mixtures like indefinite quantity forms & biological fluids. throughout producing method and drug development the most purpose of analytical ways is to produce info regarding efficiency (which is directly associated with the need of a notable dose), impurity (related to safety profile of the drug), bioavailability (includes key drug characteristics like crystal type, drug uniformity and drug release), stability (which indicates the degradation products), and impact of producing parameters to make sure that the assembly of drug merchandise is consistent. 5

The idea of internal control is meant to look at and establish a real and right product by series of measures designed to avoid and find eliminate errors at varied stages in production. to require a choice to unharness or discard a product relies on one or a lot of forms of management actions. Providing easy and analytical method for varied complicated formulations may be a subject material of utmost importance. fast increase in pharmaceutical industries and constant production of drug in varied components of the globe has brought a fast rise in demand for brand new analytical techniques within

the pharmaceutical industries as a consequence; analytical methodology development has become the essential activity of study during a internal control laboratory. 5

The reasons for the event of novel ways of drug analysis are:

a) once there's no official drug or drug combination out there within the pharmacopoeias.

b) once there's no decorous analytical method for the present drug within the literature thanks to patent laws.

c) once there aren't any analytical ways for the formulation of the drug thanks to the interference caused by the formulation excipients.

d) Analytical ways for the quantitation of the analyte in biological fluids square measure found to be unprocurable.

e) the present analytical procedures might have pricey reagents and solvents. it's going to additionally involve onerous extraction and separation procedure

Steps for the development of the method [6]

Development procedure follows with the proper documentation. All data relating to these studies must be recorded either in laboratory notebook or in an electronic database.

Analyte standard characterization

a) All known important information about the analyte and its structure that is to say physico-chemical properties like solubility, optical isomerism etc., is collected.

b) The standard analyte (≈100 % purity) is obtained. Necessary arrangement is to be made for the perfect storage (refrigerator, desiccators, and freezer).

c) In the sample matrix when multiple components are to be analyzed, the number of components is noted duly presenting the data and the accessibility of standards is estimated.

d) Methods like spectroscopic, HPLC, GC, MS etc., are considered when matched with the sample stability.

Method requirements

The requirements of the analytical method need to develop the analytical figures of merit such as linearity, selectivity, range, accuracy, precision, detection limits etc., shall be defined.

Literature search and prior methodology

All the information of literature connected with the drug is reviewed for physico-chemical properties, synthesis, solubility and appropriate analytical methods with reference to relevant books, journals, USP/NF, AOAC and ASTM publications and it is highly convenient to search Chemical Abstracts Service automated computerized literature.

Choosing a method

a) Duly utilizing the information available from theliterature, methodology is evolved since the methods are changed wherever required. Occasionally it is imperative to get additional instrumentation to develop, modify or reproduce and validate existing procedures for analytes and samples.

b) If there are no past suitable methods available to analyze the analyte to be examined.

Instrumental setup and initial studies

Installation, operational and performance qualification of instrumentation with reference to laboratory standard operating procedures is verified by setting up appropriate instrumentation.

Optimization

While performing optimization, one parameter is changed at a time and a set of conditions are isolated, before utilizing trial and error approach. The said work needs to be accomplished based on a systematic methodical plan duly observing all steps and documented with regard to dead ends.

Documentation of analytical figures of merit

The actual decided analytical figures of merit like Limit of quantitation, Limit of detection, linearity, time taken for analysis, cost, preparation of samples etc. are also documented.

Evaluation of development method with real samples

The sample solution should lead to unequivocal, total

identification of the peak interest of the drug apart from all other matrix components.

Estimation of percent recovery of real samples and demonstration of quantitative sample analysis

Percent recovery of spiked, genuine standard drug into a sample matrix which contains no analyte is estimated. Optimization to reproducibility of recovery (average ± standard deviation) from sample to sample has to be showed. It is not necessary to get 100% recovery so far as the results are reproducible to recognize with a high degree of certainty.

Understand the physicochemical properties of drug molecule [7, 8]

Physicochemical properties of a drug molecule play a very important role in methodology development. For methodology development one has got to study the physical properties like solubility, polarity, pKa and pH scale of the drug molecule.

Polarity could be a property of a compound. It helps associate degree analyst, to make your mind up the solvent and composition of the mobile part. during a nonionic bond, the electrons square measure shared equally between 2 atoms. A polar bond is one during which one atom incorporates a bigger attraction for the electrons than the opposite atom.

The solubility of molecules is often explained on the idea of the polarity of molecules. Polar, e.g. water, and nonionic, e.g. benzene, solvents don't combine. In general, like dissolves like i.e., materials with similar polarity square measure soluble in one another. choice of diluents is predicated on the solubility of analyte. The analyte should be soluble within the dilutants and should not react with any of the diluent elements. The dilutant ought to match to the beginning eluent composition of the assay to make sure that no peak distortion can occur, particularly for early eluting elements. pH scale associate degreed pKa plays a necessary role in HPLC methodology development. The pH scale worth is outlined because the negative of the exponent to base ten of the concentration of the cation.

$$pH = - \log10[H3O+]$$

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. Sharp, symmetrical peaks are necessary in quantitative analysis in order to achieve low detection limits, low relative standard deviations between injections, and reproducible retention times. The acidity of an aqueous solution is determined by the concentration of [H3O+] ions. Thus, the pH of a solution indicates the concentration of hydrogen ions in the solution. The concentration of hydrogen ions can be indicated as [H+] or its solvated form in as [H3O+] whose value normally lies between 0 and 14. The lower the pH, the more acidic is the solution. The pH of a solution can be changed simply by adding acid or base to the solution. The pKa is characteristic of a particular compound, and it tells how readily the compound gives up a proton. An acid dissociation constant is a particular example of equilibrium constant. For the specific equilibrium between a monoprotic acid, HA and its conjugate base A−,

$$HA + H2 \leftrightarrow A- + H3O+$$

The position of equilibrium is measured by the equilibrium constant, Keq.

$$Keq = \frac{[H3O+]\,[A-]}{[H2O]\,[HA]}$$

Now in dilute solutions of acid, [H2O] stays roughly constant. Therefore, define a new equilibrium constant- the acidity constant Ka.

$$Ka = \frac{[H3O+]\,[A-]}{[HA]}$$

This is also in logarithmic form are follows:

$$pKa = -\log10\ Ka.$$

It turns that the pKa of an acid is the pH at which it is exactly half dissociated. This can be shown by rearranging the expression for Ka:

$$pH = pKa - \log([AH]/[A-])$$

At half-neutralization [A−] / [HA] = 1; since log (1) = 0, the pH at half-neutralization is numerically equal to pKa. Conversely, when pH = pKa, the concentration of HA is equal to the concentration of A−.

The buffer region extends over the approximate range pKa ± 2, though buffering is weak outside the range pKa ± 1. At pKa ± 1, [A−]/[HA] = 10 or 1/10. If the pH is known, the ratio may be calculated. This ratio is independent of the analytical concentration of the acid.

When the pKa and analytical concentration of the acid are

known, the extent of dissociation and pH of a solution of a monoprotic acid can be easily calculated[7, 8].

Set up HPLC conditions

A buffer could be a partly neutralized acid that resists changes in pH scale. Salts like NaCl or Na- Lactate are usually accustomed partly neutralize the acid. Buffering capability is that the ability of the buffer to resist changes in pH scale (i) Buffering capability will increase because the molarity (molarity) of the buffer salt/acid answer will increase. (ii) The closer the buffered pH is to the pKa, the greater the Buffering Capacity. (iii) Buffering Capacity is expressed as the molarity of Sodium Hydroxide required to increase pH by 1.0.

Consideration of the effect of pH on analyte retention, type of buffer to use, and its concentration, solubility in the organic modifier and its effect on detection are important in reversed-phase chromatography (RPC) method development of ionic analytes. An improper choice of buffer, in terms of buffering species, ionic strength and pH, can result in poor or irreproducible retention and tailing in reverse-phase separation of polar and ionizable compounds[9, 10].

Buffer selection

Choice of buffer is often ruled by the required pH scale. The typical pH scale varies for reversed- part on silica-based packing is pH scale two to eight. It is vital that the buffer features a pKa on the point of the required pH scale since buffer controls pH scale best at their pKa. A rule is to choose a buffer with a pKa value < 2 units of the desired mobile phase pH see Table-1.

General considerations during buffer selection:

1. Phosphate is more soluble in methanol/water than in acetonitrile/water or THF/water.
2. Some salt buffers are hygroscopic. This may lead to changes in the chromatography (increased tailing of basic compounds, and possibly selectivity differences).
3. Ammonium salts are generally more soluble in organic/water mobile phases·
4. TFA can degrade with time, is volatile, absorbs at low UV wavelengths.
5. Microbial growth can quickly occur in buffered mobile phases that contain little or no organic modifier. This growth will accumulate on column inlets and can damage chromatographic performance.
6. At pH greater than 7, phosphate buffer accelerates the dissolution of silica and severely shortens the lifetime of silica-based HPLC columns.
7. If attainable, organic buffers should be used at pH greater than 7.
8. Ammonium bicarbonate buffers usually are prone to pH changes and are usually stable for only 24 to 48 hours.
9. The pH scale of this mobile part tends to become additional basic because of the discharge of dioxide.
10. After buffers are prepared, they should be filtered through a 0.2-µm filter.
11. Mobile phases should be degassed.

Buffer concentration

Generally, a buffer concentration of 10-50 metric linear unit is adequate for little molecules. Generally, not more than 50% organic solvent should be used with a buffer. This will rely on the particular buffer also as its concentration. Phosphoric acid and its sodium or potassium salts are the most common buffer systems for reverse - phase HPLC. Phosphonate buffers can be replaced with sulfonate buffers when analyzing organophosphate compounds[11, 12].

Table-1: HPLC Buffers, pKa Values and Useful pH Range

Buffer	pKa	Useful	UV cutoff
Ammonium acetate	4.8	3.8-5.8	
Ammonium formate	3.8	2.8-4.8	
Ammonium	9.2	8.2-	**205(10mM)**
KH_2PO_4/K_2PO_4	7.2	6.2-8.2	**<200nm**
KH_2PO_4/ phosphoric	2.1	1.1-3.1	**<200nm**
Potassium Acetate/	4.8	3.8-5.8	**210nm**
Potassium formate/	3.8	2.8-4.8	**210nm**
Trifluoroacetic acid	<2	1.5-2.5	**210nm**
Borate	9.2	8.2-	
Tri-K-	3.1	2.1-4.1	**230nm**
Tri-K-	4.7	3.7-5.7	**230nm**
Tri-K-	**5.4**	**4.4-6.4**	**230nm**

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 flexible, dual- wavelength absorbance detector for HPLC. This detector offers the high sensitivity needed for routine UV-based applications to low-level impurity identification and chemical analysis.

Photodiode Array (PDA) Detector offers advanced optical detection for Waters analytical HPLC, preparative HPLC, or LC/MS system solutions. Its integrated software package and optics innovations deliver high natural action and spectral sensitivity. Refractive Index (RI) Detector offers high sensitivity, stability and reproducibility, which make 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[13].

Column selection

The heart of a HPLC system is that the column. dynamical a column can have the best result on the resolution of analytes throughout methodology development. Generally, fashionable reverse part HPLC columns square measure created by packing the column housing with spherical colloid beads that square measure coated with the hydrophobic stationary part. The stationary part is introduced to the matrix by reacted a chlorosilane with the hydroxyl radical teams gift on the colloid surface. In general, the character of stationary part has the best result on capability issue, property, potency and extraction. There square measure many kinds of matrices for support of the stationary part, as well as silicon oxide, polymers, and aluminum oxide. silicon oxide is that the commonest matrix for HPLC columns. silicon oxide matrices square measure sturdy, simply derivatized, factory-made to consistent sphere size, and will not tend to compress underneath pressure. silicon oxide is with chemicals stable to most organic solvents and to low pH scale systems. One defect of a silicon oxide solid support is that it'll dissolve on top of pH scale seven. In recent years, silicon oxide supported columns are developed to be used at high pH scale.

The nature, form and particle size of the silicon oxide support effects separation. Smaller particle ends up in a bigger variety of theoretical plates or accumulated separation potency. However, the employment ofsmaller particles additionally results in accumulated backpressure throughout action and the column additional simply becomes obstructed.

In reverse part action the stationary part is non-polar and therefore the mobile part is polar, inflicting polar peaks to typically rinse previous non-polar peaks. to make a stationary part for reverse part action on silicon oxide support, the free silanols square measure reacted with a chlorosilane with hydrophobic practicality to introduce the non-polar surface. because of steric constraints, solely regarding 1/3 of the surface silanols square measure derivatized. The remaining free silanols will act with analytes, inflicting peak tailing. Typically, when the derivatization of a column with the desired stationary part, the column is additional reacted with chlorotrimethylsilane to finish cap the remaining free silanols and improve the column potency[14-16].

Mobile part

The mobile part effects resolution, property and potency. In reverse part action, the mobile part consists of associate degree binary compound buffer and a non-UV active water miscible organic solvent. The result of the organic and binary compound part and therefore the proportions during which they're mixed can have an effect on the analysis of the drug molecule. choice of the mobile-phase and gradient conditions depends on the ionogenic nature of the analyte and therefore the property of the analytes within the mixture severally. The binary compound buffer serves many functions. At low pH, the mobile part protonates free silanols on the column and reduces peak tailing. At sufficiently low pH scale basic analytes square measure protonated; once ionized the analyte can rinse additional quickly however with improved peak form. Acidic analytes in buffers of sufficiently low pH scale can stay dead, increasing retention. Conversely, at higher pH scale neutral basic compounds can be additional maintained, and ionized acidic compounds can rinse earlier. Peak cacophonous could also be ascertained if the pKa of a compound is analogous to the pKa of the buffer, and therefore the analyte elutes as each a charged and dead species. The pH scale of a buffer won't greatly have an effect on the retention of non-ionizable sample elements. usually a ten – fifty millimeter resolution of associate degree binary compound buffer is employed. the foremost normally used binary compound part is H3PO4 in water i.e. phosphate buffer. The pH scale of a phosphate buffer is definitely adjusted by mistreatment mono-, di-, or tribasic phosphate salts. However, once phosphate salts square measure used the answer ought to be filtered to get rid of insoluble particles with zero.22µm paper. alternative non-UV active acids and bases can also be accustomed result variations in peak form and retention[17].

Isocratic or gradient separations: Isocratic, constant eluent composition means equilibrium conditions in the column and the actual velocity of compounds moving through the column are constant; analyte-eluent and analyte-stationary- phase interactions are also constant throughout the whole run. This makes isocratic separations more predictable, although the separation

power (the number of compounds which could be resolved) is not very high. The peak capacity is low; and the longer the component is retained on the column, the wider is the resultant peak.

Gradient parting significantly upsurges the parting control of a arrangement mostly due to the dramatic increase of the apparent efficiency (decrease of the peak width). The condition where the tail of a chromatographic zone is always under the influence of a stronger eluent composition leads to the decrease of the peak width. Peak width varies depending on the rate of the eluent composition variation (gradient slope). [17]

Changing Gradient: 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. This leads to the general elution problem where no one set of conditions is effective in eluting all components from a column in a reasonable time period while still attaining resolution of each component. This necessitates the implementation of a gradient. Employing gradients shallow or steep permits for getting variations within the natural process property. This would be attributed to the various slopes of the retention versus organic composition for every analyte within the mixture. When a gradient method is used, the column must be allowed to equilibrate at the starting mobile-phase conditions prior to the next sample injection and the start of the next gradient run. [17]

Preparation of sample solutions for method development

The drug substance being analyzed ought to be stable in answer (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. The sample answer ought to be filtered; the employment of a zero.22 or 0.45 µm pore-size filter is generally recommended for removal of particulates. Filtration is a preventive maintenance tool for HPLC analyses. [18-21] Sample preparation is a critical step of method development that the analyst must investigate. The effectiveness of the syringe filters is largely determined by their ability to remove contaminants/insoluble components without leaching undesirable artifacts (i.e., extractables) into the filtrate. If any additional peaks are observed in the filtered samples, then the diluent must be filtered to determine if a leachable component is coming from the syringe filter housing/filter.

Method optimization

The experimental conditions ought to be optimized to urge desired separations and sensitivity once obtaining acceptable separations. Stability indicating assay experimental conditions will be achieved through planned/systemic examination on parameters including pH (if ionic), mobile phase components and ratio, gradient, flow rate, temperature, sample amounts, Injection volume and diluents solvent type.

Need of pharmaceutical validation [22]

Validation is AN integral a part of quality assurance; it involves the systematic study of systems, facilities and processes aimed at determining whether they perform their intended functions adequately and consistently as specified. A valid method is one that has been incontestible to supply a high degree of assurance that uniform batches are made that meet the desired specifications and has therefore been formally approved. Validation in itself does not improve processes but confirms that the processes have been properly developed and are under control.

Definitions [22-25]

European commission

1991 –Validation-"Act of proving, in accordance of GMPs that Any…" process actually leads to expected results.

2000 -"Documented evidence that the process, operated within established Parameters, can perform effectively and reproducibly to produce a Medicinal product meeting its predetermined specifications and quality attributes".

US FDA Definition

"Process validation is establishing documented evidence which provides a high degree of assurance that a specified process will consistently produce a product meeting its pre-determined specifications and quality characteristics."

ICH Definition

"Process Validation is the means of ensuring and providing documentary evidence that processes within their specified design parameters are capable of repeatedly and reliably producing a finished product of the required quality."

WHO Definition

"The documented act of proving that any procedure, process, equipment, material, activity or system actually leads to expected result."

Validation of method

Validation of an analytical procedure is the process by which it is established, by laboratory studies, that the performance characteristics of the procedure meet the requirements for its intended use. The methods validation process for analytical procedures begins with the planned

and systematic collection by the applicant of the validation data to support analytical procedures. [26] All analytical method that are intended to be used for analyzing any clinical samples will need to be validated. The validation of analytical methods is done as per ICH guidelines.

Components of method validation

The following are typical analytical performance characteristics which may be tested during methods validation: [27-28]

- Accuracy
- Precision
- Repeatability
- Intermediate precision
- Linearity
- Detection limit
- Quantitation limit
- Specificity
- Range
- Robustness
- System suitability determination
- Forced degradation studies
- Solution stability studies

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. It 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. [27]

The 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. [28] It consists of two components: repeatability and intermediate precision.

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. [28]

Intermediate precision is the variation within a laboratory such as different days, with different instruments, and by different analysts[24-25]. The precision is then expressed as the relative standard deviation[28].

$$\%RSD = \frac{\text{std dev.} * 100}{\text{mean}}$$

Accuracy and precision are not the same, as the diagram below indicates Figure-3. A method can have good precision and yet not be accurate.

Linearity is the ability of analytical procedure to obtain a response that is directly proportional to the concentration (amount) 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[28].

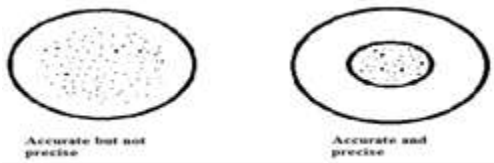

Figure 3: Difference between Accuracy and Precision

The detection limit (DL) or 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.(book) 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 [28].

The limit of Quantitation (LOQ) or Quantitation limit 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. 28

Specificity is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present such as impurities, degradation products, and excipients. Specificity measures only the desired component without interference from other

species that might be present, separation not necessarily required [28].

The range of an analytical method is the interval between the upper and lower levels that have been demonstrated to be determined with precision, accuracy and linearity using the method [28].

Robustness is defined as the measure of 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) 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 [28].

System Suitability tests are an integral part of liquid chromatographic methods. They are used to verify that the detection sensitivity, resolution and reproducibility of the chromatographic system are adequate for the analysis to be done. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. Factors, such as the peak resolution, number of theoretical plates, peak tailing and capacity have been measured to determine the suitability of the used method [28].

Forced Degradation 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. These studies may also help in the formulation development, manufacturing, and packaging to improve a drug product. Reasons for carrying out forced degradation studies include: development and validation of stability-indicating methodology, determination of degradation pathways of drug substances and drug products, discernment of degradation products in formulations that are related to drug substances versus those that are related to non–drug substances (e.g., excipients). 29

Solution Stability Studies

During validation the 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 [30].

CONCLUSION

In recent years development of the analytical methods for identification, purity evaluation and quantification of drugs has received a great deal of attention in the field of pharmaceutical analysis. This review describes HPLC method development and validation in general way. A general and very simple approach for the HPLC method development for the separation of compounds was discussed. Knowledge of the physiochemical properties of the primary compound is of utmost importance prior to the any HPLC method development. The selection of buffer and mobile phase composition (organic and pH) plays a dramatic role on the separation selectivity. Final optimization can be performed by changing the gradient slope, temperature and flow rate as well as the type and concentration of mobile-phase modifiers. Optimized method is validated with various parameters (e.g. specificity, precision, accuracy, detection limit, linearity, etc.) as per ICH guidelines.

ABBREVIATIONS

HPLC High Performance Liquid Chromatography
ICH International conference on Harmonization
Id Internal Diameter
LC Liquid Chromatography
LOD Limit of Detection
LOQ Limit of Quantitation
mm Mili meter
MS Mass Spectrometry
ODS Octyl decyl silane
RI Refractive index
THF Tetrahydrofuran
USP United states Pharmacopeia
µm Micron

AUTHORS CONTRIBUTION STATEMENT

Ms. Miral Patel and Ms. Dharti Patel conceptualized and

gathered the data with regard to this topic. Dr.Keyur Ahir and Dr.Sumer Singh reviewed these data and provided necessary inputs towards the designing of the manuscript. All authors were actively contributed towards the final manuscript.

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