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A Review on High Performance Liquid Chromatography Analytical method development

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ABSTRACT:

High Performance Liquid Chromatographic Analytical method development and validation are the continuous and inter-dependent task associated with the research and development, quality control and quality assurance departments. Analytical procedures play a critical role in equivalence and risk assessment, management. It helps in establishment of product-specific acceptance criteria and stability of results .Validation should demonstrate that the analytical procedure is suitable for its intented purpose. Analyts should be comfortable to use it to characterize and optimize the analytical method. An effective analytical method development and its validation can provide significant improvements in precision and a reduction in bias errors. It can further help to avoid costly and time consuming exercises. The RP-HPLC strategy is precise, exact, particular, reproducible and Sensitive .The system has advantages, including fast investigation, a straightforward portable stage, basic specimen planning, and enhanced affectability. Method development involves the process of establishing an analytical method which is acceptable for measurement of the concentration of an API in a specific pharmaceutical dosage form and that using validated to verify or prove that an analytical procedure, accurately and consistently delivers a desirable specific guidelines to prove measurement of an active ingredient in pharmaceutical preparation during each analysis

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INTRODUCTION:

Instrumental Analytical Chemistry is the branch of Science that uses advance technologies in determining the composition by analytical method. We can achieve both qualitative as well as quantitative results [1].

The HPLC technique has its relative merits yet the greater part of them are done at increase temperatures, prolonged, utilize moderately costly reagents, include extraction, utilization of support framework [2] HPLC is a chromatographic system that can separate a mixture of mixes and is utilized as a part of natural chemistry and scientific science to distinguish measure and refine the individual segments of the mixture [3]. High liquid chromatography (HPLC; liquid chromatography), is a strategy in scientific science used to discrete the segments in a mixture, to distinguish every segment, and to measure every part. It depends on pumps to pass a pressurized liquid dissolvable containing the example mixture through a segment loaded with a strong adsorbent material [4].

Due to the small sample amount separated in analytical HPLC, typical column dimensions are 2.1–4.6 mm diameter, and 30–250 mm length. Also HPLC columns are made with smaller sorbent particles (2–50 micrometer in average particle size) [6].

This gives HPLC superior resolving power when separating mixtures, which is why it is a popular chromatographic technique [7]. HPLC routines investigated have the potential application to clinical examination of medication blend, multi-drug pharmacokinetics data and comparative studies [8-9].Reversed phase HPLC (RP-HPLC) has a non-polar stationary phase and a watery, tolerably polar portable phase [10]. One basic stationary phase is silica which has been surface-adjusted with RMe2SiCl, where R is a straight chain alkyl gathering, for example, C18H37 or C8H17. With such stationary phases, maintenance time is longer for atoms which are less polar, while polar particles elute all the more promptly (ahead of schedule in the examination) [11-13].

The RP-HPLC strategy is precise, exact, particular, reproducible and Sensitive . The system has advatanges, including fast investigation, a straightforward portable stage, basic specimen planning, and enhanced affectability . This makes the strategy suitable for routine examination in quality-control labs .Different investigative system (HPLC, UV-spectroscopy, HPTLC, Titration, Fluorescence spectroscopy) are utilized by Quality control research centers to guarantee the character, virtue, intensity and execution of medication items. The vast majority of the medications in multicomponent dose structures can be examined by HPLC system on account of the few favorable circumstances like speed, specificity, precision, exactness and simplicity of mechanization in this strategy [14].

The benefits of proposed analytical technique are its short investigation time and a basic methodology for testing sample [15]. RP-HPLC technique is helpful in routine lab investigation with a high level of exactness and accuracy and can be effectively sought the routine quantitative estimation [16]. Test strategy for any medication is exceptionally huge for pharmaceutical businesses and it is constantly alluring to choose and create basic, minimum time intensive, exact, precise and conservative system for the determination of medications in API pharmaceutical measurement structures and neurotic specimens like blood ,plasma and serum [17].Agreeability with good lab practice (GLPs) for conveying test examination of nonclinical (otherwise called preclinical) research facility studies and clinical studies is planned to guarantee the quality and honesty of the wellbeing information documented in backing of investigational new medication applications (INDs), [17,18] new medication applications (NDAs), abbreviated new medication applications (ANDAs), supplements in creating bioanalytical system tool approval data utilized as a part of human clinical pharmacology, bioavailability (BA), and bioequivalence (BE) studies obliging pharmacokinetic (PK) assessment [19].A definitive objective for any study test examination or system acceptance, paying little respect to whether GLP agreeability is implemented, is to guarantee the bioanalytical method strategies utilized are demonstrated powerful through advancement, approval (according to a convention, study arrangement or standard working method, and connected as written to powerfully evaluate the analyte in the vicinity of a particular network [19]. Analytical parameters consideration in method development and effects of change in parameters in method outcomes time can be enhanced by changing the pH that will prompt simple division of ionizable analytes from non-ionized structure [20]. By changing the versatile stage pH can likewise enhance segment effectiveness on the grounds that it adjusted both the ionization of the analyte and the lingering silanols and it additionally minimizes optional communications in the middle of analytes and the silica surface that will prompt poor crest shape [21,22]. To accomplish ideal determination, it has change in the pH of portable stage. Technique advancement can continues by examining parameters of chromatographic partitions first at low pH and afterward at higher pH until ideal results are attained and optimize [23].The use of silica-based packing is preferred in most of the present HPLC columns due to several physical characteristics. Totally porous silica particles with 5 µm diameter provide the desired characteristics for most HPLC separations [24].Separation of many samples can be enhanced by selecting the right column temperature. Higher column temperature reduces system backpressure by decreasing mobile phase viscosity, which in turn allows use of longer columns with higher separation efficiency. The acid dissociation constant (pKa) is the pH at which concentrations of ionized and unionized forms of drugs are equal. It is an essential parameter in drug discovery, particularly in physiological systems where ionization state will affect the rate at which the compound is able to diffuse across membranes including blood-brain barrier [25-26].

Need for Method development [27-28]

To developed an reliable method for drug or its combination that are not official in pharmacopoeias. the available method is not suitable for the drug metric for evaluation. New technology in instrumentation have evolved to provide improvement in analyte, identification and provide greater accuracy and precision. The existing developed method may not provide sensitivity and selectivity of analyte under investigation. When there is a need of conforming the analyte under legal or scientific way to provide valuble data. Required in identification and quantification of drug in blood fluid or human biological fluid. Analytical method for drug and it's new formulated combination which are recently approved by regulation authority.

Validation of Method as per ICH guidelines

Validation of Analytical Method [29-34]

It is the process of documenting or proving that the selected method provides analytical data for the intended use. As per the ICH guidelines, the objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose.

Validation is required for the following reasons

Assuring Quality., Achieving acceptance of products by the international agencies., Mandatory requirement for accreditation as per ISO 17025 guidelines., Mandatory requirement for registration of any pharmaceutical product or Pesticide formulation., Only validated methods are acceptable for undertaking proficiency testing., The biggest advantage of method validation is that it builds a degree of confidence, not only for the developer but also to the user.

Key parameters of the Analytical method validation

It is important for one to understand the parameters or characteristics involved in the validation process. The various Performance parameters, which are addressed in a validation exercise, are grouped as follows.

1. Selectivity (Specificity)

- Selectivity of a method refers to the extent to which it can determine particular analyte(s) in a complex mixture without any interference from other components in the mixture.
- The term specific generally refers to a method that produces a response for a single analyte only, while the term selective refers to a method that provides responses for a number of chemical entities that may or may not be distinguished from each other complex mixture.
- If the response is distinguished from all other responses, the method is said to be selective. Since very few analytical methods respond to only one analyte, the use of the term selectivity is more appropriate than specificity.

2. Linearity and range

- Linearity usually expressed in terms of the variance around the slope of regression line calculated according to an established mathematical relationship from test results obtained by the analysis of samples with varying concentrations of analyte.
- The linear range of detectability that obeys Beer's law is dependent on the compound analyzed and the detector used. The working sample concentration and samples tested for accuracy should be in the linear range. The claim that the method is linear is to be justified with additional mention of zero intercept by processing data by linear least square regression. Data is processed by linear least square regression declaring the regression co-efficient and b of the linear equation y= ax + b together with the correlation coefficient of determination r. For the method to be linear the r value should be close to 1.
- The range of an analytical method is the interval between the upper and lower levels of the analyte (including these levels) that have been demonstrated to be determined with method precision, accuracy and linearity using the method as written.

3. Accuracy

- The accuracy of an analytical method may be defined as the closeness of the test results obtained by the method to the true value. It is the measure of the exactness of the analytical method developed. Accuracy may often express as percent recovery by the assay of a known amount of analyte added.
- Accuracy may be determined by applying the method to samples or mixtures of excipients to which known amount of analyte have been added both above and below the normal levels expected in the samples. Accuracy is then calculated from the test results as the percentage of the analyte recovered by the assay. Dosage form assays commonly provide accuracy within 3-5% of the true value.

% Recovery calculated by formula;

$$\% \text{ Recovery} = \frac{N(\sum xy) - (\sum x)(\sum y)}{N(\sum x^2) - (\sum x)^2} \times 100$$

N = Number of observations

Y = Amount of drug found.

X = Amount of standard drug added.

- The ICH documents recommend that accuracy should be assessed using a minimum of nine determinations over a minimum of three concentration levels, covering the specified range (i.e. three concentrations and three replicated of each concentration).

4. Precision

- The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of homogenous samples. This is usually expressed as the standard deviation or the relative standard deviation (coefficient of variation). Precision is a measure of the degree of reproducibility or of the repeatability of the analytical method under normal operating circumstances.
- Repeatability involves analysis of replicates by the analyst using the same equipment and method and conducting the precision study over short period of time while reproducibility involves precision study at
- Different Occasions,
- Different Laboratories,
- Different Batch of Reagent,
- Different Analysts,
- Different Equipments.
- The standard deviation (SD) is calculated from the following formula;

$SD = \sum (Xi - X)^2 / N-1$

Xi = individual measurement in a set

X = arithmetic mean of the set and

N = total number of replicated measurements taken in the set.

Precision between different samples can be compared with relative standard deviation (RSD) as follows.

$$RSD = S/X$$

% RSD or coefficient of variance (CV) = $(S/X) \times 100$

5. Limit of Detection (LOD)

- The detection limit of an analytical procedure is the lowest amount of an analyte in a sample that can be detected, but not necessarily quantitated as an exact value. The LOD may be determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level (lowest calibration standard) at which the analyte can be reliably detected. The lowest calibration standard which produces a peak response corresponding to the analyte should be measured n times (normally 6-10).

$$\mathbf{LOD} = \frac{\mathbf{3.3}\,\sigma}{\mathbf{S}}$$

Where, σ = the standard deviation of the response

S = the slope of the calibration curve

6. Limit of quantitation(LOQ)

- The limit of quantification is the lowest amount of the analyte in the sample that can be quantitatively determined with defined precision under the stated experimental conditions.
- The limit of quantitation is a parameter of quantitative assays for low levels of compounds in sample matrices and is used particularly for the determination of impurities and/or degradation products or low levels of active constituent in a product.
- The solution should be injected and analyzed n times. The average response and the standard deviation should be calculated and the SD should be less than 20%. If the SD exceeds 20%, a new standard solution of higher concentration should be prepared and the above procedure repeated. LOQ can be calculated by formula;

$$\mathbf{LOQ} = \frac{\mathbf{3.3}\,\sigma}{\mathbf{S}}$$

Where, σ = the standard deviation of the response

S = the slope of the calibration curve

7. Robustness

- The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variation in method parameters and provides an indication of its reliability during normal usage. The determination of robustness requires that methods characteristic are assessed when one or more operating parameter varied.

8. Ruggedness

- The ruggedness of an analytical method is the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of normal test conditions such as different laboratories, different analysts, using operational and environmental conditions that may differ but are still within the specified parameters of the assay. 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.

9. System suitability tests

- System suitability testing is an integral part of many analytical procedures. 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. System suitability test parameters to be established for a particular procedure depend on the type of procedure being validated.

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