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Review Article

REVIEW ON METHOD DEVELOPMENT AND VALIDATION

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Abstract

High performance liquid chromatography is most accurate methods widely used for the qualitative and quantitative analysis of drug product. Analytical method development and validation play important roles in the Drug discovery, Drug development and Manufacture of pharmaceuticals. It involves detection of the purity and toxicity of a drug substance. A number of chromatographic parameters have been evaluated in order to optimize the methods in the analysis of method development in HPLC. An appropr- iate mobile phase, column, column tempe- rature, wavelength and gradient are develop- ped. Force degradation studies are helpful in development and validation of stability-indicating studies, determination of degrad- ation pathways of drug substances and drug products. Validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. The parameters described here are according to ICH guidelines and include accuracy, precision, specificity, limit of detection, limit of quantitation, linearity, range, ruggedness and robustness. The objective of this paper is to review the method development, optimize method parameters and validation of method for drug product from developm- ental stage of formulation to commercial batch of product.

Keywords: Qualitative, Quantitative, Force degradation, Method development, Stability, ICH.

Introduction:

Chromatography is a procedure that is used for separating a complex mixture into its individual particular fractions or compon- ents. It is a separation technique and the separated compounds can be identified by using any analytical technique like UV-visible, Infrared, Mass spectroscopy, NMR etc. "Chromato" "graphy" derives its name from two words as chromo means colour and graphy means to write i.e. colour bands are formed in the procedure which are measured or analyzed. These colour bands are formed due to the separation of individual compounds.[1] Analytical chemi- stry deals with methods for identification, separation, and quantification of the chemical components of natural and artificial materials. [2][3][4] HPLC is the method of choice for checking peak purity of new chemical moieties, reaction monitoring and evaluating new formulations. The official test methods that result from these processes are used by quality control laboratories to ensure the identity, purity, potency, and performance of drug products. In the modern pharmaceutical industry, high-performance liquid chromato- graphy (HPLC) is the major and integral analytical tool applied in all stages of drug discovery, development, and production.[5] The Goal of HPLC method is to try & separate, quantify the main drug, any reaction impurities, synthetic intermediates and any degradation products. HPLC 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.[6][7][8]

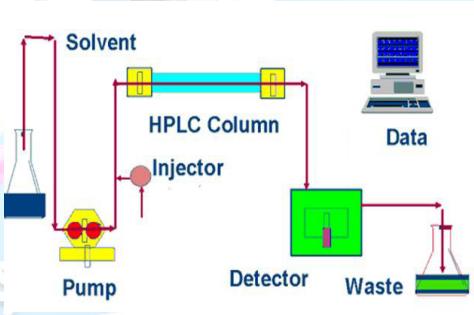

Figure 1. Flow diagram of HPLC

Main features of HPLC:[7]

High resolution

Small diameter, Stainless steel, Glass column

Rapid analysis

Relatively higher mobile phase pressure

Controlled flow rate of mobile phase

Phases of chromatography:[4]

Normal phase chromatography:

In Normal Phase mode the stationary phase is polar and the mobile phase is non-polar in nature. In this technique, non-polar compounds travel faster and are eluted first. This is because of the less affinity between the non-polar compounds and the stationary phase. Polar compounds are retained for longer times because of their higher affinity with the stationary phase. These compounds, due to affinity with stationary phase take more times to elute.[9]

Reversed phase chromatography:

It is the popular mode for analytical and preparative separations of compound of interest in chemical, biological, pharma- ceutical, food and biomedical sciences. In this mode the stationary phase is non polar hydrophobic packing with octal or octadecyl functional group bonded to silica gel and the mobile phase is polar solvent. The polar compound gets eluted first because of less affinity for stationary phase and non-polar compounds are retained for longer time. As most of the drugs and pharmaceuticals are polar in nature, they are not retained for longer times and hence elute faster.[10]

Analytical Method Development:

Analytical method development and validation play important roles in the discovery, development and manufacture of pharma- ceuticals. These methods used to ensure the identity, purity, potency, and safety of drug products. 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.[11][12]

Need for developing a method:[13]

Available method may be too expensive, time consuming or energy intensive, or that may not be easily automated.

Existing method may be too much error, contamination prone or they may be unreliable.

There may be need for an alternative method to confirm, for legal or scientific reasons, analytical data originally obtained by existing methods.

There may not be a suitable method for a particular analyte in the specific sample matrix.

Existing method may not provide adequate sensitivity

For regulatory requirements it is required.

Newer instrumentation and techniques may have evolved that provide opportunities for improved methods, including improved analyte identification or limit of detection, greater accuracy or better return on investment.

Requirements for new Method Development:

- The drug or drug combination may not be official in any pharmacopoeias.
- A proper analytical procedure for the drug may not be available in the literature due to patent regulations.
- Analytical methods may not be available for the drug in the form of a formulation due to the interference caused by the formulation excipients.
- Analytical methods for a drug in combination with other drugs may not be available.
- The existing analytical procedures may require costly reagents and solvents. It may also involve cumbersome extraction and separation procedures and these may not be reliable.

Steps in method development:[2][14]

Physicochemical properties of drug molecule

Selection of chromatographic conditions

Developing the approach of analysis

Preparation sample

Method optimization

Method validation

Physicochemical properties of drug molecule:

For Method development one has to study the physical properties like solubility, polarity, dissociation constant and pH of the drug molecule. Physicochemical properties of drug molecule are very

important tool. It helps an analyst, to decide the solvent and composition of the mobile phase. Selection of diluents is based on the solubility of analyte. The acidity or basicity of a substance is mainly determined by its pH value. Selecting a proper pH for ionizable analytes often leads to symmetrical and sharp peaks in HPLC.[15][16]

Selection of chromatographic conditions:

During initial method development, a set of initial conditions (column, mobile phase and detector) is selected. In most cases, these are based on reversed-phase separations on a C_{18} column with UV detection. A decision on developing either an isocratic or a gradient method should be made at this point.

It includes:

1. Selection of column:[17][18][19][20]

The principle part of a HPLC system is the column. Changing a column will have the greatest effect on the resolution of analytes during method development. An appropri- ately selected column can produce a good chromatographic separation and it provides accurate and reliable analysis. An improperly used column can often generate confusion, difficulties, and poor separations which can lead to results that are invalid or complex to interpret. There are several types of matrices for support of the stationary phase, including silica, polymers, alumina, and zirconium. Silica is the most commonly used matrix for HPLC columns. Silica matrices are robust, easily derivatized, manufactured to consistent sphere size.[21]

2. Chromatographic conditions:

Next step in the method development is to select appropriate chromatographic condi- tions. It includes selection of composition of mobile phase, selection of temperature, pH of mobile phase and Flow rate etc. A decision on developing either an isocratic or a gradient method should be made at this point. In most cases, these are based on reversed-phase separations on a C_{18} column with UV detection.[22]

3. Optimization of mobile phase:

The most useful solvents are Acetonitrile, Methanol and Tetrahydofuron. The selection of organic modifier is depending on the elution of the molecules and peak shapes and buffers present in mobile phase. Choosing a proper mobile phase for the given analyte is the most important stage in developing a method for HPLC. A mobile phase which has the capability of pulling the analyte from the column is chosen. When dealing with weak acids and bases, we have to adjust the pH also as it affects the retention.

Role of buffer in mobile phase selection:

Buffer and its strength play a major role in deciding the peak symmetries and separations. The retention time depends on molar strength of buffer. Molar strength is proportional to retention time.

pH of the Buffer:

It is important to maintain the pH of mobile phase in the range of 2.0-8.0 as most of the columns does not withstand out of this range. e.g. As siloxane linkages cleaved below pH 2 and at above pH 8 silica dissolves.[23]

Buffer	pKa	Useful pH range
Ammonium acetate	4.8,9.2	3.8-5.8, 8.2-10.2
Phosphoric acid	2.1	1.1-3.1
K_2PO_4	7.2	6.2-8.2
Acetic acid	4.8	3.8-5.8
Borate	9.2	8.2-10.2
Ammonium hydroxide	9.2	8.2-10.2
Trifluroacetic acid	<2	1.5-2.5
Formic acid	3.8	2.8-4.8

Table. Buffers and its pH ranges

4. Selection of wavelength and detector:[24][25]

All listed molecules UV/Visible and FT-IR 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. After the chromatographic separation, the analyte of interest is detected by using suitable detectors. The detectors are designed to have certain properties like:

They should be inert (non-reactive) to the samples injected and the mobile phases passing through it.

They should be preferably non-destructive to the sample.

Should be able to produce quick and quantitative response.

Reliable, uniform and reproducible detection and analytic data.

Compatible with all types of compounds under testing.

Should have good sensitivity (Ability to detect compounds at very low concentration in the ranges below μg, ng, etc.)

Types of HPLC detectors:

UV detectors: UV wavelength range of absorption is specific for sample.

Fluorescent detectors: In this detector the fluorescence rays emitted by sample after absorbing incident light is measured as a function of quality and quantity of the sample.

Electrochemical detectors: The principle is that when compound is either oxidized or reduced, the chemical reaction produces electron flow. This flow is measured as current which is the function of type and quantity of compound.

Photo diode-array detectors (PDA): It measures the entire absorption range i.e. it gives the scan of the entire spectrum.

Refractive index detectors: These are detectors which measure the change of refractive index of the eluant from the column with respect to pure mobile phase.

Developing the approach for analysis:

The selections of various chromatographic parameters like selection of mobile phase, selection of column, selection of flow rate of mobile phase, selection of pH of mobile phase. All of these 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 %.[16]

Sample preparation:

The prepared sample should be an aliquot relatively free of interferences that is suitable with the HPLC method and that will not damage the column.[26][27]

Method optimization:

The mobile 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 decrease 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.[19][21]

Method validation:

The methods were validated according to International Conference on Harmonization (ICH) guidelines for validation of analytical procedures. Validation is required for any new drug or amended method to ensure that it is capable of giving reproducible, precise and reliable results.[14]

Method validation:

"Validation of an analytical method is the process by which it is established by laboratory studies, that the performance characteristics of the method meet the requirements for the intended analytical application.[16][28][29] "Guidelines from the USP, ICH, FDA etc., can provide a framework for validations of pharmaceutical methods. The methods were validated according to International Conference on Harmonization (ICH) guidelines for validation of analytical procedures. Validation is required for any new drug or method to ensure that it is capable of giving reproducible and reliable results, when used by different operators employing the same equipment in the same or different laboratories.[14]

Why is analytical method validation required?[30][31]

For achieving the approval of the products by the international agencies.

For assuring the quality and safety of the product.

It is a mandatory requirement for accreditation as per ISO 17025 guidelines.

Requirement for registration of any pharmaceutical product.

Validation not only improves the processes, but also confirms that the process is properly developed.

Importance for the manufacturer:

It decreases the risk of preventing problems.

It decreases the risk of defect costs.

It decreases the risk of regulatory failure.

A fully validated process may require less in-process controls and final product testing.

Advantages:

The biggest advantage of method validation is that it builds a degree of confidence, not only for the developer but also to the user.

Although the validation exercise may appear costly and time consuming, it results inexpensive, eliminates frustra- ting repetitions and leads to better time management in the end.

Minor changes in the conditions such as reagent supplier or grade, analytical setup are unavoidable due to obvious reasons but the method validation absorbs the shock of such conditions and pays for more than invested on the process.

It builds confidence for not only the developer but also the user.

Types of analytical procedures:[32]

Discussion of the validation of analytical procedures is directed to the four most common types of analytical procedures:

Identification tests

Quantitative tests for impurities content

Limit tests for the control of impurities

Quantitative tests of the active moiety in samples of a drug substance.

Types of analytical parameters:

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

Accuracy:

It is sometimes termed as trueness. Accuracy is a measurement of closeness between the measured and real value. Comparison to reference standard. Recovery of analyte spiked into blank matrix standard addition of the analyte. The reported limits for accuracy for drug substances and products are 98.0 – 102.0 % and 97.0 – 103.0 % respectively. For the impurity determination, range from 50 - 150 % of average recovery may be accepted.[33]

Precision:[22]

Precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions of Repeatability, Intermediate precision, Reproducibility.[34]

To ensure precision of method for major analytes, RSD should be 2 %. For low level impurities, RSD of 5-10 % is usually acceptable.

Repeatability:

Repeatability means the methods performs in one lab and on one instrument, within a given day. It is useful to calculate the 'Repeatability limit'. Performed by prepa- ring a minimum of 6 determinations at 100% of the test concentration.

Reproducibility:

Reproducibility refers to how that method performs from lab to lab, day to day, analyst to analyst and from instrument to instrument again in both qualitative and quantitative terms. It is useful to calculate the 'reproducibility limit', *R*.

Intermediate precision:

Intermediate precision refers to how the method performs, both qualitatively and quantitatively, within one lab, but now from instrument to instrument and day to day and calculated the % RSD of assays.

Linearity:

The linearity of an analytical procedure is its ability to obtain test results which are directly proportional to the concentration of analyte in the sample. Test results should be evaluated by appropriate statistical methods. For example, by calculation of a regression line by the method of least squares. [27]

Level	Acceptance criteria
Assay (5 levels, 50-150% of label claim)	Correlation coefficient, $R^2 = 0.999$
Dissolution (5-8 levels, 10-150% of label claim)	% y intercept NMT 2.0%, $R^2 = 0.99$
Related substances (5 levels, LOQ to acceptance)	% y intercept NMT 5.0%, $R^2 = 0.99$

Table: Linearity ranges and Acceptance criteria for various pharmaceutical methods

Detection limit: [34][35]

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

The formula for calculating LOD is,

$LOD = 3.3 \times \delta/S$

Where,

δ = standard deviation of intercepts of calibration curves.

S = the slope of linearity plot.

Detection limit can be determined visually

Signal to Noise ratio

Standard Deviation of the Response and the Slope

Quantitation limit:[35]

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and degradation products. It can be determined by following formula,

$LOQ = 10 \times \delta/S$

Where,

δ = standard deviation of response.

S = Mean of slopes of the calibration curves.

Specificity:

Specificity is the ability to measure the desired analyte in the presence of components which may be expected to be present. Typically it may include impurities, degradants, matrix, etc. The peak purity value must be more than 0.999 (for Agilent system) or purity angle is less than threshold (for Waters system) in every case.[35]

Range:

The range of an analytical procedure is the interval between the upper and lower concentration of analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.[36]

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. The variable method parameters in HPLC technique may include flow rate, column temperature, sample temperature, pH and mobile phase composition.[37][17]

System suitability:

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 ca-

pacity have been measured to determine the suitability of the used method.

Parameter	Limit
Resolution (Rs)	>2.0
Repeatability (RSD)	<1.0% for five replicates
Plate count (N)	>2000
Tailing factor (Tf)	2.0
Separation factor	>1.0

Table. System suitability parameters and its limits

Parameters used in the system suitability tests (SST):

Number of theoretical plates or Efficiency (N)

Capacity factor (K)

Separation or Relative retention (α)

Resolution (Rs)

Tailing factor (T)

Relative Standard Deviation (RSD)

Table: Limits for system suitability tests

Force degradation:

Force degradation of the drug substance can help identify the likely degradation products, which can in turn help establish the degradation pathways and the intrinsic stability of the molecule.[38]

Solution state stability:

Acidic hydrolysis

Alkaline hydrolysis

Hydrolytic

Oxidative degradation

Solid state stability:

Thermal degradation

Photolytic degradation

Conclusion:

Method development and validation play a major role in the pharmaceutical industry. Recent development in pharmaceutical and biotechnological field generates demand for analytical methods. Rapid and accurate qua- ntification of the drug and its product is important in the process development. Improvements in analytical instrumentation leads to development of new techniques like isocratic and gradient RP-HPLC, which evolved as the primary techniques for the analysis of APIs and impurities. These analytical methods are critical elements of pharmaceutical development so it is very important to develop efficient and accurately validated analytical methods to develop safe and effective drugs.

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