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Review Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD IN MULTI-COMPONENT FORMULATION

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ABSTRACT:
The aim of the present work was focused on development of an analytical method for simultaneous estimation of drugs in multi-component dosage form by using RP-HPLC. Chromatographic separation techniques are one of the most widely used techniques for analysis of a multi-component formulation. HPLC technique allows for the separation as well as analysis of different drugs that are present in a combined formulation. The technique also allows for separation of the respective drugs from the excipients contained in the formulation and thus each drug in a multi-component formulation can be easily separated and hence analyzed. Analytical method was developed in order to carry out the assay of the respective drugs present in the tablet formulation. Validation studies were performed in order to assess the validation parameters for the analytical method developed in accordance to ICH Guidelines.
Keywords: Reverse Phase High Performance Liquid Chromatography, Multi-Component formulations, Analytical Method Development and Validation.

INTRODUCTION:

Analytical chemistry is primarily concerned about determining the qualitative and quantitative composition of material under study. Both these aspects are necessary to understand the sample material. Analytical chemistry is divided into two branches quantitative and qualitative. A qualitative analysis gives us the information about the nature of sample by knowing about the presence or absence of certain components. A quantitative analysis provides numerical information as to the relative amount of one or more of this component. For analyzing the drug samples in bulk, pharmaceutical formulations and biological fluids, different analytical methods are routinely being used.

In non-instrumental, the conventional and physicochemical property are use to analyze the sample. The instrumental methods of analysis are based upon the measurements of some physical property of substance using instrument to determine its chemical composition. The instrumental methods are simple, precise and reproducible as compared to classical methods. Therefore, analytical methods developed using sophisticated instruments such as spectrophotometer, HPLC, GC and HPTLC have wide applications in assuring the quality and quantity of raw materials and finished products.[1]

Quality assurance is a wide concept ranging and covering all matters that either individually or collectively influences the overall quality of the product. It is a total arrangement made with the object of ensuring that the pharmaceutical products are of the quality required for their intended use. So in order to above mentioned specifications, Quality assurance incorporates GMP and other factors.[2]

Chromatography

Chromatography is a technique employed for separation of the components of mixture by continuous distribution of the component between two phases. One phase moves (mobile phase) over the other phase (stationary phase) in a continuous manner. When the stationary phase is a solid support of adsorptive nature and mobile phase is liquid or gaseous phase it is called Adsorption Chromatography[1]

Chromatography according to USP can be defined as a procedure by which solute are separated by a differential migration process in a system consisting of two or more phases, one of which move continuously in a given direction.[4]

Theory of Chromatography[3]

Two theoretical approaches have been developed to describe the processes involved in the passage of solutes through a chromatographic system.

1. The Plate Theory[3]

According to Martin and Synge, a chromatographic system consists of discrete layers of theoretical plates. At each of these, equilibration of the solute between the mobile and stationary phases occurs. The movement of solute is considered as a series of stepwise transfers from plate to plate.

2. The Rate Theory[3]

This theory considers the dynamics of the solute particles as it passes through the void space between the stationary phase particles in the system as well its kinetic as it is transferred to and from the stationary phase.

Phases of Chromatography[1]

1. Normal Phase Mode:[1] 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 lower 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, therefore take more times to elute. Normal phase mode of separation is therefore, not generally used for pharmaceutical applications because most of the drug molecules are polar in nature and hence take longer time to elute.

2. Reversed Phase Mode:[1] It is the most popular mode for analytical and preparative separations of compound of interest in chemical, biological, pharmaceutical, food and biomedical sciences. In this mode, the stationary phase is non polar hydrophobic packing with octyl or octa decyl functional group bonded to silica gel and the mobile phase is polar solvent. The polar compound gets eluted first in this mode 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. The different columns used are Octa Decyl Silane (ODS) or C_{18}, C_8, C_4, (in the order of increasing polarity of the stationary phase). An aqueous mobile phase allows the use of secondary solute chemical equilibrium (such as ionization control, ion suppression, ion pairing and complexation) to control retention and selectivity.

3. Ion Exchange Chromatography:[1]
The stationary phase contains ionic groups like NR_3^+ or SO_3^-, which interact with the ionic groups of the sample molecules. This is suitable for the separation of charged molecules only. Changing the pH and salt concentration can modulate the retention.

4. Ion Pair Chromatography:[1] This technique is also referred to as Reversed Phase Ion Pair Chromatography or Soap Chromatography. It may be used for the separation of ionic compounds and this method can also substitute for Ion Exchange Chromatography. Strong acidic and basic compounds may be separated by reversed phase mode by forming ion pairs (coulumbic association species formed between two ions of opposite electric charge) with suitable counter ions.

5. Affinity Chromatography:[1] This technique uses highly specific biochemical interactions for separation. The stationary phase contains specific groups of molecules which can adsorb the sample if certain steric and charge related conditions are satisfied. This technique can be used to isolate proteins, enzymes as well as antibodies from complex mixtures.

6. Size Exclusion Chromatography:[1] It separates molecules according to their molecular mass. Largest molecules are eluted first and the smallest molecules last. This method is generally used when a mixture contains compounds with a molecular mass difference of at least 10%. This mode can be further subdivided into gel permeation chromatography (with organic solvents) and gel filtration chromatography (with aqueous solvents).

Separation Techniques[1]

1. **Isocratic Elution:** When the mobile–phase composition does not change throughout the course of the run, it is said to be isocratic. A mixed mobile phase can be delivered at a constant ratio by the pumps themselves or the solvent mixture can be prepared prior to analysis and pumped through a single reservoir. This is the simplest technique and should be the method of first choice when developing a separation.
2. **Gradient Elution:** HPLC can be performed with changes in composition of mobile phase over time (gradient elution). The elution strength of the eluent is increased during the gradient run by changing polarity, pH or ionic strength. Gradient elution can be a powerful tool to separate mixtures of compounds with widely different retention.

Quantitative Analysis in HPLC[1]
Three methods are generally used for quantitative analysis.

1. External Standard Method[1]
The External Standard Method is the simplest of the three methods. The accuracy of this method is dependent on the reproducibility of the injection of the sample volume. To perform this method, a standard solution of known concentration of the compound of interest is prepared. A fixed amount, which should be similar in concentration to the unknown, is injected. Peak height or area is plotted versus the concentration for each compound. The plot should be linear and pass through the origin. The concentration of the unknown is then determined based on Regression Equation.

2. **Internal Standard Method**[1]
The Internal Standard Method tends to yield the most accurate and precise results. In this method, an equal amount of an internal standard, a component that is not present in the sample, is added to both the sample and standard solutions. The internal standard selected should be chemically similar to the analyte, have a retention time close to that of the analyte and derivatise in a similar way to the analyte. For biological samples, the internal standard should extract similarly to the analyte without significant bias toward the internal standard or the analyte. Additionally, it is important to ensure that the internal standard is stable and that it does not interfere with any of the sample components. The internal standard should be added before any preparation of the sample so that extraction efficiency can be evaluated. Quantification is achieved by using ratios of peak height or area of the component to the internal standard.

3. Standard Addition Method[1]
In the standard addition method, a known amount of the standard compound is added to the sample solution to be estimated. This method is suitable if sufficient amount of the sample is available and is more realistic in the sense that it allows calibration in the presence of excipients or other components.

System Suitability Parameters[1]
System suitability parameters are used to determine the limits of the suitability of the chromatographic system. This approach facilitates the investigation of the worst case scenario, which reflects minimum performance standard used to ensure that the chromatography is not adversely affected.
SST parameters studied are as follows:

1. **Resolution (Rs):**[1] Resolution is the parameter describing the separation power of the complete chromatographic system relative to the particular components of the mixture. The resolution, R_s, of two neighboring peaks is defined as the ratio of the distance between two peak maxima. It is the difference between the retention times of two solutes divided by their average peak width. For baseline separation, the ideal value of R_s is 1.5.
2. **Capacity Factor (k'):**[1] Capacity factor is the ratio of the reduced retention volume to the dead volume. Capacity factor, k', is defined as the ratio of the number of molecules of solute in the stationary phase to the number of molecules of the same in the mobile phase. Capacity factor is a measure of how well the sample molecule is retained by a column during an isocratic separation. The ideal value of k' ranges from 2-10.
3. **Selectivity (a):**[1] The selectivity (or separation factor), a, is a measure of relative retention of two components in a mixture. Selectivity is the ratio of the capacity factors of both peaks, and the ratio of its adjusted retention times. Selectivity represents the separation power of particular adsorbent to the mixture of these particular components. This parameter is independent of the column efficiency; it only depends on the nature of the components, eluent type, and eluent composition, and adsorbent surface chemistry. In general, if the selectivity of two components is equal to 1, then there is no way to separate them by improving the column efficiency. The ideal value of a is 2.
4. **Column Efficiency:**[1] Efficiency, N, of a column is measured by the number of theoretical plates per meter. It is a measure of band spreading of a peak. Similar the band spread, higher is the number of theoretical plates, indicating good column and system performance. Columns with N ranging from 5,000 to 100,000 plates/meter are ideal for a good system.

5. **Peak asymmetry factor (Tf):**[1] Peak asymmetry factor, T_f, can be used as a criterion of column performance. The peak half width, b, of a peak at 10% of the peak height, divided by the corresponding front half width, a, gives the asymmetry factor. For a well packed column, an asymmetry factor of 0.9 to 1.1 should be achievable.

Strategy for Method Development in RP-HPLC[1]

'Best column, best mobile phase, best detection wavelength' efforts in their selection can make a world of difference while developing HPLC method for routine analysis. Determining ideal combination of this factor assures faster delivery of desired result -- a validated method for separation.

- On sample properties as much knowledge as possible should be collected.
- Chromatographic method is selected according to the sample property.
- The sample is chromatographed with HPLC condition where all compounds elute in a reasonable time.
- The HPLC method is optimized with regard to analysis time, resolution, selectivity and sensitivity.

Knowledge about Sample

- Origin (especially important with biological sample)
- History (sampling, storage, sample preparation)
- Components
- Amount
- Chemical and physical property
- Matrix

Important Chemical and Physical property of sample compounds

- Molecular weight
- Formula
- Acid-base property (pK value)
- Solubility (in organic or in water)
- UV Spectrum[1]

Validation of Analytical Method

Validation is an act of proving that any procedure, process, equipment, material, activity or system performs as expected under given set of conditions and also give the required accuracy, precision, sensitivity, ruggedness, etc.[2]

When extended to an analytical procedure, depending upon the application, it means that a method works reproducibly, when carried out by same or different persons, in same or different laboratories, using different reagents, different equipments, etc.[2]

In the US, there was no mention of the word validation in the cGMP's of 1971, and precision and accuracy were stated as laboratory controls. It was only in the cGMP guideline of March 1979 that the need for validation was implied. It was done in two sections: (1) Section 211.165, where the word 'validation' was used and (2) section 211.194, in which the proof of suitability, accuracy and reliability was made compulsory for regulatory submissions. Another guidance on validation of chromatographic methods was released by CDRE on 1st Nov. 1994.[4]

The WHO published guidelines under the title, 'Validation of analytical procedures used in the examination of pharmaceutical materials'. It appeared in the 32nd report of the WHO expert committee on 'specifications for pharmaceutical preparations' which was published in 1992.[7]

The International Conference on Harmonization (ICH), which has been on the forefront of developing the harmonized tripartite guidelines for adoption in the US, Japan and EC, has issued two guidelines under the titles-'Text on Validation of Analytical Procedures (Q_2A) and Validation of Analytical Procedure Methodology (Q_2B)'.[5-6]

Among the pharmacopoeias, USP XXII 1225 (1995) carries a section which describes requirements of validation of compendial methods. The British Pharmacopoeia includes the definition of method validation in 15 latest editions, but the term is completely missing from the Indian Pharmacopoeia. (1996).

Advantages of Analytical Method Validation[2]

- 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 proves to be inexpensive by eliminating frustrating repetitions and leads to better time management in the end.
- The method validation absorbs the shock of variations of analytical conditions and pays for more than invested on the process.

Key parameters of the Analytical Method Validation[5-6]

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:

a. Accuracy:[5-6]

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 be expressed 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.

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).

b. Precision:[5-6]

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

Validation of tests for assay and for quantitative determination of impurities includes an investigation of precision.

i. Repeatability

Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision.

Repeatability should be assessed using:

- A minimum of 9 determinations covering the specified range for the procedure (e.g., 3 concentrations/3 replicates each); or
- A minimum of 6 determinations at 100% of the test concentration.

ii. Intermediate Precision

Intermediate precision expresses within-laboratories variations: different days, different analysts, different equipment, etc. The extent to which intermediate precision should be established depends on the circumstances under which the procedure is intended to be used. The applicant should establish the effects of random events on the precision of the analytical procedure. Typical variations to be studied include days, analysts, equipment, etc. It is not considered necessary to study these effects individually. The use of an experimental design (matrix) is encouraged.

c. Limit of Detection[5-6]

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.

d. Limit of Quantification:[5-6]

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/or degradation products.

e. Selectivity and Specificity:[5-6]

The selectivity of an analytical method is its ability to measure accurately and specifically the analyte of interest in the presence of components that may be expected to be present in the sample matrix.

If an analytical procedure is able to separate and resolve the various components of a mixture and detect the analyte qualitatively the method is called selective. On the other hand, if the method determines or measures quantitatively the component of interest in the sample matrix without separation, it is said to be specific.

Hence one basic difference in the selectivity and specificity is that, while the former is restricted to qualitative detection of the components of a sample, the latter means quantitative measurement of one or more analyte.

f. Robustness:[5-6]

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 evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. It should show the reliability of an analysis with respect to deliberate variations in method parameters.

g. Ruggedness:[5-6]

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.

Analytical methods may be broadly classified as per WHO as follows:

- **Class A:** Tests designed to establish identity, whether of bulk drug substances or of a particular ingredient in a finished dosage form.
- **Class B:** Methods designed to detect and quantitative impurities in a bulk drug substance or finished dosage form.
- **Class C:** Methods used to determine quantitatively the concentration of a bulk drug substance or of a major ingredient in a finished dosage form.
- **Class D:** Methods used to assess the characteristic of finished dosage forms, such as dissolution profiles and content uniformity.[7]

As per USP analytical methods are broadly classified as:

- **Category I:** Analytical methods for quantitation of major components of bulk drug substances or active ingredients including preservatives in finished pharmaceutical products.
- **Category II:** Analytical methods for determination of impurities in bulk drugs or for determination of degradation compounds in finished pharmaceutical products.
- **Category III:** Identification tests for drugs.[4]

CONCLUSION

HPLC is one of the most widely used technique to carry out the analysis of pharmaceutical preparations. This technique is based on the separation of the drugs and excipients present in a formulation. Hence, analysis of drug which includes assay study, related substances study, dissolution study, can be carried out quite effectively by using HPLC. Multi-component formulations can also be analyzed using HPLC, as separation of two or more drugs in the formulation is achieved. As compared to other analytical methods, HPLC is more precise and accurate. Hence, HPLC can be used for analysis of drugs in bulk and in formulation.

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