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Development and Validation of RP-HPLC Method: An Overview.

M Sangeetha[1]*, C Rubina Reichal[2], VN Indulatha[1], and N Thirumoorthy[2].

[1]Department of Pharmaceutical Chemistry, Cherraan's College of Pharmacy, Coimbatore, Tamil Nadu, India.
[2]Department of Pharmaceutics, Cherraan's College of Pharmacy, Coimbatore, Tamil Nadu, India.

Review Article

ABSTRACT

The present study focuses on the various steps, parameters involved in HPLC condition. Various applications of this system also discussed. HPLC process development is important in case of drug discovery, drug development and pharmaceutical products. It can be adopted apparently for routine quality control study of research and formulation tests. This article mainly focuses on the optimization of HPLC conditions and other important aspects during method of process development and validation of drug substances. The steps involved in the method development and analytical validation was discussed, an advantage over the method was explained elaborately.

INTRODUCTION

Analytical chemistry may be defined as the science and the art of determining the composition of materials in terms of the elements of composition contained. In this it is of prime importance to gain the qualitative and quantitative information of the substance and chemical species. i.e., it find out what a substance is composed of and exactly how much.[1]

Analytical techniques hold the key to the design, development, standardization and quality control of medical products. They are equally important in pharmacokinetics and in drug metabolism studies. Both of which are fundamental to the assesment of bioavailability and the duration of clinical response. Analytical instrumentation place an important role in the production and evaluation of new products, and in the production of consumers and the environment.

Chemical analysis is generally applied in two areas.

    (i)   Quantitative Analysis
    (ii)   Qualitative Analysis

Highly specific sensitive analytical techniques hold the key to the design, development, standardization and quality control of medicinal products. Modern physical methods of analysis are extremely sensitive, providing precise and accurate information about the standards of chemicals (or) drugs up to a nanogram level.

HPLC is one among must useful tools available for quantitative analysis. Reverse Phase Chromatography refers to the use of a polar mobile phase with non-polar stationary phase in contract to normal phase being employed with a non-polar mobile phase HPLC is always used in injection with another analytical tool for quantitative and qualitative analysis. The mode of operation of the system is isocratic i.e. one particular solvent or mixture is pumped throughout the analysis for some determination. The solvent composition may be attended gradually to give gradient elution. The rate of distribution

between stationary and mobile phase is controlled by diffusion process. I diffusion is minimized a faster and effective separation can be achieved.[2,3,4,5]

Mode of Separation[6]

In normal phase mode, the stationary phase eg. Silica Gel is polar in nature and the mobile phase is non-polar. In this technique non-polar compounds travel faster and are eluted first. This is because less affinity between solute and stationary phase polar compounds are retained for longer time in the column because more affinity towards stationary phase and takes more time to be eluted from the column.

Importance of polarity in HPLC

Polarity is a term i.e used in chromatography as an index of the ability of compounds to interact with one another. It is applied very freely to solutes. Stationary and mobile phase HPLC the eluting power or solvent strength by its polarity. If the polarity of stationary phase and the non-polar phase are similar it is likely that the interaction of solute with each phase may also be similar resulting in poor separation. Retention of solutes is usually altered by changing the polarity of the mobile phase.

Method validation [7,8]

Validation means assessment of validity or action of proving effectiveness. Method validation is the process of proving that an analytical method is acceptable for its intended purpose. For pharmaceutical methods guidelines from the United States of Pharmacopeia (USP), International Conference on Harmonization (ICH) and the Food and Drug Administration (FDA) provide a frame work for performing such validations.

Parameters used for Assay Validation

The validation of the assay procedure was carried out as per ICH guidelines using the following parameters.

Specificity

Specificity is the ability to access unequivocally the analyte in the presence of the components which may be expected to be present lack of specificity of an individual analytical procedure may be compensated by other analytical procedures. For the chromatographic methods developing a separation involves demonstrating specificity, which is the ability of the method to accurately measure the analyte response in the presence of all potential sample components. The response of the analyte in test mixtures containing the analyte and all potential sample components (placebo formulation, process impurities etc) is compared with the response of the solution containing only the analyte. A specificity criterion for an assay method is that the analyte peak will have baseline chromatographic resolution of at least 1.5 from all other sample components. If this cannot be achieved the unresolved components at their maximum expected level will not affect the final assay result by more than 0.5%.

Linearity

Linearity of an analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to the concentration (amount) of the analyte in the sample. For assay methods, this study is generally performed by preparing standard solutions at five concentration levels. Five levels are required to allow detection of curvature in the plotted data acceptability of linearity data is often judged by examining the correlation and y-intercept of the linear regression line for the response verses concentration plot. A correlation coefficient of >0.999 is generally considered as evidence of the data to the regression line. The y-intercept should be less than a few percent responses obtained for the analyte at the target level.

Accuracy

The accuracy of an analytical procedure expresses the closeness of the agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. The accuracy of the method is the closeness of the measured value to the true value for the sample. Accuracy is usually determined in one of four ways.

- Accuracy can be assessed by analyzing a sample of known concentration and comparing the measured value to the true value.
- To compare test results from the new method with results from an existing alternate method that is known to be accurate.
- The most widely used recovery study, is performed by spiking analyte in blank matrices. For assay methods, spiked samples are prepared in triplicate at three levels over the range of 50-150% of the target concentration.
- The technique of the standard additions, which can also be used to determine recovery of spiked analyte. This approach is used if it is not possible to prepare a blank sample matrix without the presence of the analyte.

Accuracy criteria for an assay method is that the mean recovery will be 100+2% at each concentration over the range of 80-120% of the target concentration.

Range

Range of analytical procedure is the interval between the upper and lower concentration amounts of analyte in the sample including these concentrations for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity. The range is determined using data from the linearity and accuracy studies. Range criteria for an assay method is the at the acceptable range will be defined as the concentration interval over which linearity and accuracy are obtained per previously discussed criteria and that yields a precision of 3% RSD.

Precision

The precision of an analytical procedure express the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions. Precision of an analytical procedure is usually expressed the variance, standard deviation or coefficient of variance of a series of measurements. The first type of precision study is instrument precision or injection repeatability. A minimum of 10 injections of one sample solution is made to test the performance of the chromatographic instrument. The second type is repeatability or intra-assay precision. Intra-assay precision data are obtained on one day. Aliquots of a homogenous sample, each of which has been independently prepared according to the method procedure. From these precision studies, the samples preparation procedure the number of replicate samples to be prepared and the number of injections required for each sample in the final method procedure will be set. An example of precision criteria for an assay method is that the instrument precision (RSD) will be 1% and the intra assay precision will be 2%.

Detection limit

The detection limit of an individual analyte procedure is the lowest amount of analyte in a sample which can be detected but not necessarily qualitated as an exact value. Detection limit based on the standard derivation of the response and the slope.

Detection limit (or) limit of detection may be expressed as,

$$DL= [3.3\sigma/S]$$

Where,
σ =standard deviation of the response
S =slope of the calibration curve (of the analyte)

Figure 1: Components of HPLC System
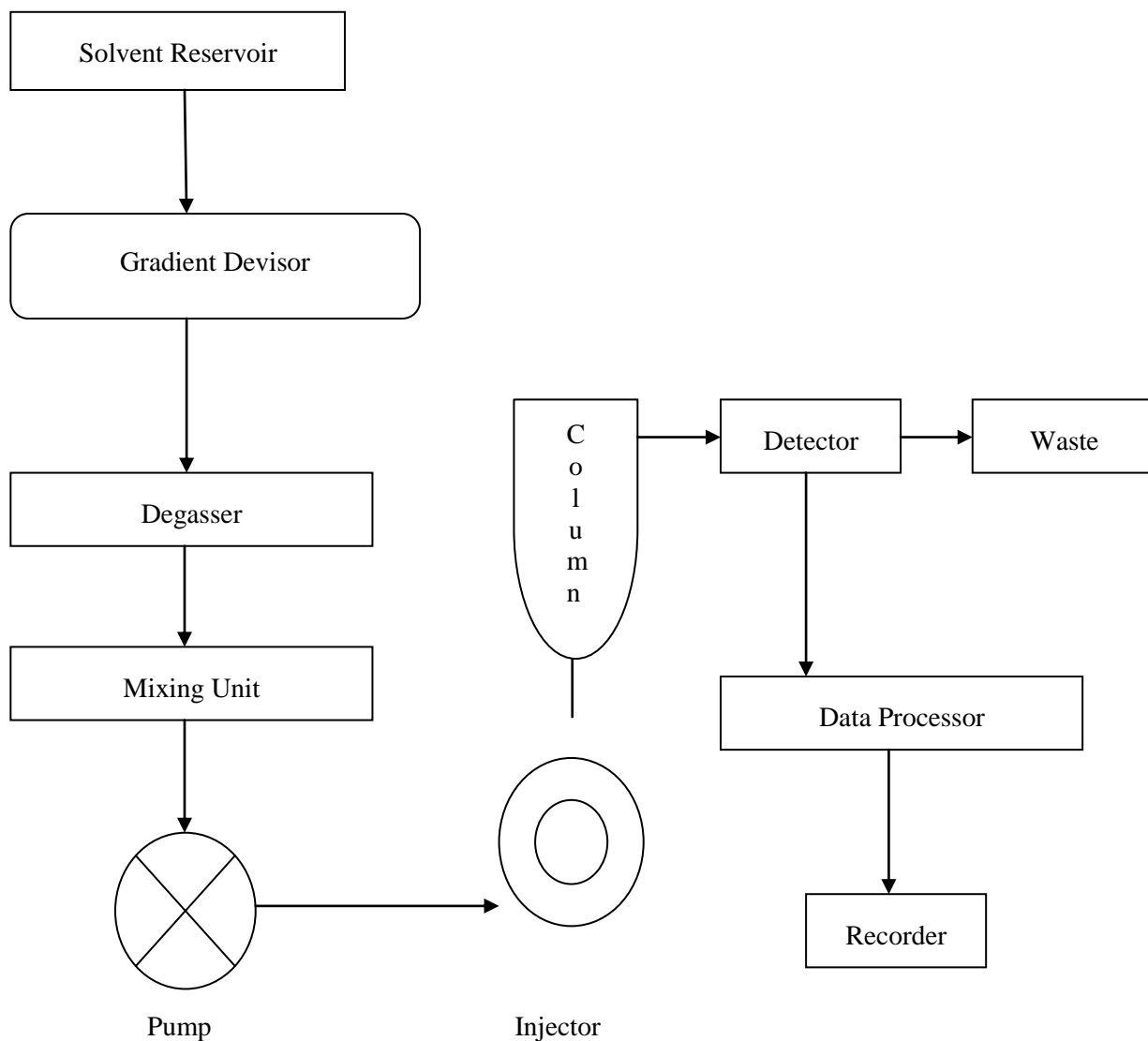
Quantitation limit

The quantitation of an analytical procedure is the lowest amount of analyte in a sample, which can be quantitatively determined with suitable precision and accuracy.

Quantitation limit based on the standard deviation of the response and the slope. It can be expressed as,

$$QL = [10\sigma/S]$$

σ = standard deviation of the response
S = slope of the calibration curve (of the analyte)

Ruggedness

The ruggedness of an analytical method is the degree of reproducibility of test result obtained, the analysis of conditions such as different laboratories, different analysis using different instrument, on different days. Different source of reagent, elapsed assay, times, assay temperature conditions. Ruggedness is the measure of reproducibility of test result under the variation in conditions normally expected from analyst to analyst. The criteria of the ruggedness is the RSD should be not more than 2%.

Figure 2: Various steps for RP-HPLC Method Development[7]

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│               Drug Development                │
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│    Drug approval process by regulatory agencies │
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│          Analytical procedure development      │
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│    System suitability of RP-HPLC method and process │
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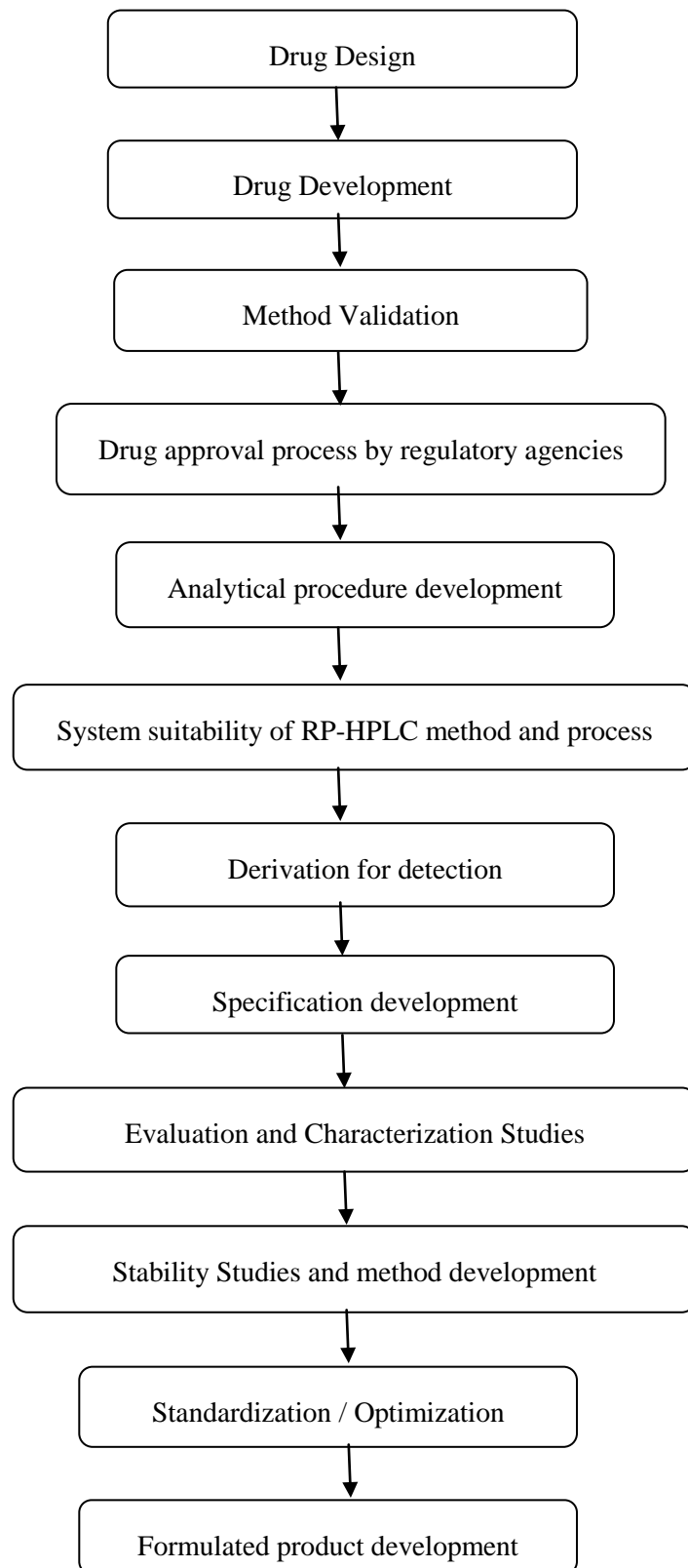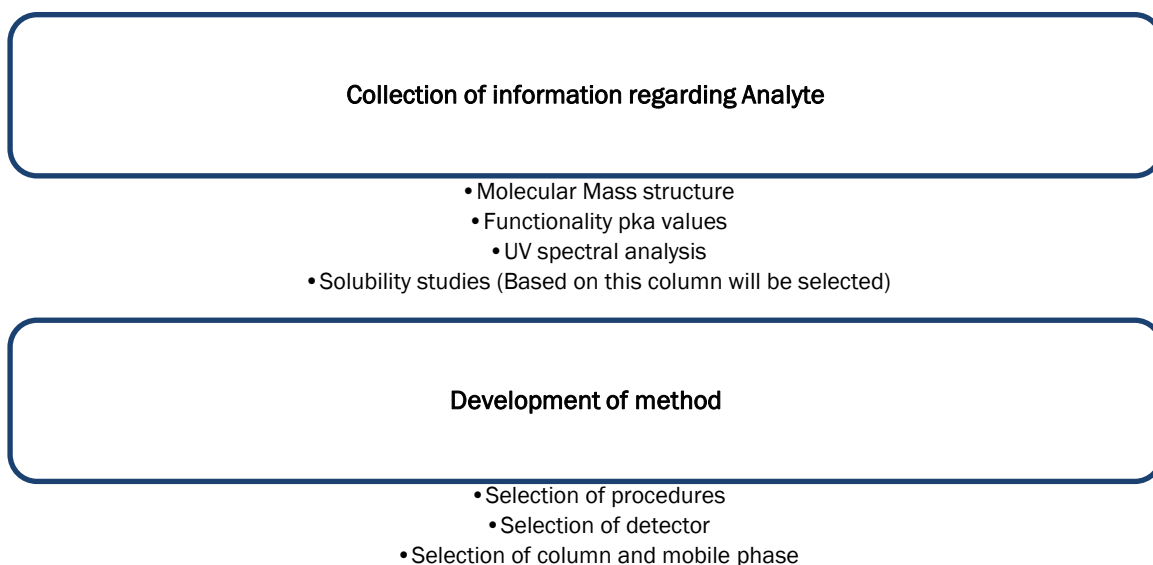
Figure 3: Parameters involved in RP-HPLC Method

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Collection of information regarding Analyte

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- Molecular Mass structure
- Functionality pka values
- UV spectral analysis
- Solubility studies (Based on this column will be selected)

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Development of method

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- Selection of procedures
- Selection of detector
- Selection of column and mobile phase

Robustness

The robustness of a method is its ability to remain unaffected by small changes in parameter such as percent organic content, p^H of the mobile phase, buffer concentration, temperature and injection volume. The criteria for robustness are the RSD should be not more than 2%.

System suitability testing

System suitability testing is an integral part of many analytical procedures the tests are based on the concept that the equipment, electronics, analytical operation and samples to be analyzed constitute an integral system that can be evaluated as such. Typically the process involves making five injections of a standard solution and evaluating several chromatographic parameters such as resolution, area % reproducibility, number of theoretical plates and tailing factor.

Applications of HPLC method [10]

- The various applicability, speed, sensitivity of HPLC is the most popular chromatography technique used for purification and all types of biological molecules.
- The system is widely used in clinical and pharmaceutical work as it possible to apply biological fluids such as serum and urine directly to the column.
- RP-HPLC has biggest impact on the separation of oligo peptides and proteins.
- Wide range of applications in organic chemistry.
- Chromatography separation of anions can be carried out by using ion exchange ion pair chromatography and ion exclusion chromatography.
- Chromatography separation of cation superficially sulphonated inert polymer resins have been used.
- Most widely used in Agri chemicals i.e. analysis of pesticides in cleaning water.
- Mainly applied in food analysis
- Widely applied in forensic science for the separation of morphine and metabolites extracted from blood plasma.
- Modern applications are mainly in pharmaceutical field.

CONCLUSION

The method development and validation of HPLC was found to be accurate, precise and reliable. The method was proposed for the quality control studies of various pharmaceutical dosage forms and to find out the efficacy or therapeutic activity. It could be effectively separate the drugs and further studies

should be preferred to evaluate the stability of Pharmaceutical formulation. The advantages of HPLC were high selectivity, sensitivity, economic, less time consuming and low limit of detection.

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