# A Review on Novel Analytical Method Development and Validation by RP-HPLC Method

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## Abstract

A simple, precise, accurate, specific and RP-HPLC method was developed for determination of drug in pharmaceutical formulation. 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. The present study focuses on the various steps, parameters involved in HPLC condition. 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 main objective of this review is to elaborate the novel analytical techniques utilized in method development and validation of varied pharmaceuticals because it is extremely much significant for the steadiness, efficacy and quality of the drug product. Various validation parameters like accuracy, specificity, precision, linearity, LOD, LOQ, ruggedness, and robustness also are listed concerning ICH Guidelines. Validation is extremely much useful for the standard control and quality assurance of pharmaceuticals and therefore the safety of patients.

***Keywords:*** *RP-HPLC, Method Validation, Method development, ICH Guidelines*

## Introduction

Analytical chemistry is a branch of chemistry which deal with identification of components and determination of number of components of elements or samples or mixture [1]. Analytical method development and method validation is key role in drug discovery, method development and production of various pharmaceutical dosages form. These methods used to confirm the identity, transparency, effectiveness, and protection of drug products. The goal of the HPLC method is to separate, calculate the main active drug, any response impurities, all available synthetic inter-mediates and any degradants [2]. The chromatography derived from the word chromo means colour and graphs means to write i.e., colour groups are shaped in the process which are measured or analysed. These colour groups are shaped due to the separation of separate complexes [3]. Under these conditions, standards and analytical procedures for these drugs might not be available within the pharmacopoeias. Thus, it becomes necessary, to develop newer analytical methods for such drugs [4]. The object of the characterization is to generate a reliable, accurate and interpretable set of information describing the sample[5]. The analysis is vital in any product or service, and it is also important in drug

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because it involves life [6].

Chromatography is a process that is used for separate, identify, and quantify the compounds that are present in any sample that can be dissolved in a fluid. It is quantitative and qualitative analysis of drugs and the separated complexes can be identified by using any analytical method like UV-visible, Infrared, Mass spectroscopy, NMR etc. High Performance Liquid Chromatography (HPLC) is nowadays one of the most powerful tools in analytical chemistry [7]. HPTLC has become a routine analytical technique thanks to its advantages of reliability in quantitation of analytes at the micro and even in nanogram levels and price effectiveness [8].

The method of analysis is selected for highest purity of new chemical moieties, selected for reaction monitoring and evaluating new formulations. This is official test methods effect for the development are used to laboratories of quality control it is ensure the transparency, effectiveness, identity and presentation of drug products. In pharmaceutical industry useful to essential tool in the periods of development of drug discovery, growth, and manufacture [9]. The principle of separation followed is the adsorption of solute on stationary phase based on its attraction towards stationary phase. HPLC is the solution of sample is injected into a column of porous physical (stationary phase) and liquid phase (mobile phase) is pumped at higher pressure through the column [7, 9, 10]. The interaction of the solute with mobile and stationary Phases are often manipulated through different choices of both solvents and stationary phases has developed method for the determination of amiodarone hydrochloride in tablet and injectable formulations [11].

**Key features of HPLC:**

- High resolution
- Small diameter, Stainless steel, Glass column
- Rapid analysis
- Relatively higher mobile phase pressure
- Measured flow rate of mobile phase [12].

**Phases of chromatography:**

· **Normal phase chromatography**

In Normal Phase mode the stationary phase is polar and the mobile phase is non-polar in nature. In this method, non-polar complexes travel faster and are eluted first. This is because of the less attraction between the non-polar complexes and the stationary phase. Polar compounds are complexes, due to attraction with stationary phase take more times to elute. In the growth of separation in solute molecules in growths the adsorption volume is important to improved elution time. Chemically updated to silica (cyanopropyl, aminopropyl and diol) is used as a stationary phase in this chromatography [13]. For example. A typical column has an internal thickness of about 4.6 mm, and a length in the range of 150 to 250 mm. Polar compounds in the combination that are approved complete the column will stick longer to the polar silica than the non-polar complexes. Therefore, the non-polar ones will pass faster through the column [14].

· **Reversed phase chromatography (RP-HPLC):**

RP-HPLC is the most popular method for analytical and preparative separation of complex of attention in biological, food, chemical, pharmaceutical and biomedical sciences.

In this technique 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. As maximum of the drugs and pharmaceuticals are polar in nature, they are not retained for longer periods and hence elute faster [15].

RP-HPLC has a non-polar stationary phase and polar or moderately polar mobile phase. In based on the principle of hydrophobic interaction [16]. In a combination of components those analytes which are relatively less polar will be retained by the non-polar stationary phase longer than those which are relatively more polar. Therefore, the most polar section will elute first [14]. Uses water-organic as mobile phase, columns may be C18 (ODS), C8, phenyl, Trimethyl Silane (TMS), cyan as a stationary phase. It is first choice for most samples especially neutral or non-ionized compounds, that dissolve in water organic mixtures [17].

**Parameters used in HPLC**

- Retention time
- Retention volume
- Separation factor
- Resolution
- Theoretical plate
- HETP- Height Equivalent to the original Plate
- Efficiency (no of theoretical plates)
- Asymmetry factor- Fronting Tailing [18].

In every year the total amount of drugs growing in the market. These drugs also new objects and part of structural modification of standing one. The methods validation can be used to judge the quality, dependability and uniformity of analytical results, which is an essential role of any suitable analytical preparation. The need to analytical technique method development and method validation to formulated [19].

**Physicochemical properties of drug molecule:**

For the Technique of development, one has to study the physical properties like separation, dissociation, solubility, and pH of the drug particle. The essential role of physicochemical properties of drug molecule. The analyte is created on the solubility to collection of diluents. The mostly component determined by its pH value for acidity or basicity element [20].

The term of validation basically means assessment of validity or measures to demonstrate efficiency. Group of validation it is determination of including people from different regulations. The validation procedure is "creating documented evidence "that offers a high level of certainty that the product meets the requirements for the envisioned analytical applications [21].

The Pharmaceutical industry, new measurement technologies can be accepted if a complete systematic rationale for the submission has been established, confirmed, and defensible and the developed method has been agreed by internal company procedures [22].

**Method Development:**

The method development is known as definitive methods or techniques are present of original methods are being created for the evaluation of new or novel pharmaceutical product. The study of pharmacopeial or non-pharmacopeial produce original methods are established and decrease the rate also time for higher accuracy and power. These techniques are improved and authorised thorough primary routes [23].

Method development is the route of selecting a correct analyse method to control the arrangement of a preparation. Analytical techniques must be used within GMP and GLP environments and must be developed using the protocols and acceptance criteria set out in the ICH guidelines Q2(R1) [24].

These methodologies are optimized and valid through preliminary runs. Alternate ways are planned and place into practice to exchange this procedure within the comparative laboratory information with

all accessible merits and demerits [25].

**Determination of Analytical Method Development:**

Analytical method development is used in pharmaceutical industries, it is giving most essential data arranged the strength of the drug, it also checking the drug's stability, the drug's bioavailability and also its properties.in this method is traditional and determination of directing [26].

**Analyte standard characterization**:

· In this step, both the biological and chemical properties (such as solubility, optical isomerism, etc.) in addition to the physical properties of the analyte are collected.

· After that, the standard analyte is equal to 100% purity is obtained and stored according to its specific requirements (refrigerator, desiccators and freezer).

· When multiple components are to be analysed in the sample matrix, the number of components is noted, data is assembled and the availability of standards for each one is determined.

· Only those techniques (spectroscopic, MS, GC, HPLC etc.) that are compatible with sample stability are considered [27].

Basic criteria for new method development for drug analysis:

- 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 the quantitation of the drug in biological fluids may not be available.
- Analytical methods for a drug in combination with other drugs may not be available.
- The existing analytical procedures may require expensive reagents and solvents. It may also involve cumbersome extraction and separation procedures and these may not be reliable [28].

**The prerequisite for method development is as follows**.

· Qualified and calibrated instruments

· Documented methods

· Reliable reference standards

· Qualified analysts

· Sample selection and integrity

· Change control.

Problems in method development

· Stored samples are initially accurate but slowly become inaccurate with low bias.

· Absorption issue: A serially diluted curve is concave. The response factors drop with decreasing concentration. An increased exposure due to number of dilutions, surface area contact, and time may cause this problem

· Homogeneity: the sample to be analysed gets partitioned.

· These problems can be overcome by adding a surfactant to the sample under test [29].

**Method validation:**

Validation is an idea that has developed in the

U. S in 1978. The validation derived from the word Validus means strong and beneficial, and of a suitable or regular advises and proposes that something takes to verified to stand of correct [30]. Validation is procedure of method can be defined as the attesting that can specific and established analytical technique it is suitable for intended usage [31]. In the analytical method, must be important in the preparation of method validation. The understood in this method it is way to procedure is very important to analytical condition and it is the validating method that can take reflection to the show of competences it stable by submission [32]. The study of laboratory it is recognized by validation method [33].

The validation theory over the years to include an extensive collection of actions, it an analytical technique for quality control of drugs and pharmaceutical products to computer-aided organizations for clinical studies, category or process controller. Method validation of analytical technology requirements is determined almost regular in the field of pharmaceutical industry, since an exact method validation is important for approved approval application [34].

Method validation mean goal of analytical approaches is to illustrate that it is appropriate for its meant purpose. The dialogue of the validation of analytical approaches is directed to the 4 maxima not unusual place types.

- Identification tests.
- Quantitative tests for impurities content.
- Limit tests for the operate of impurities [35].

The proposed methods were validated as per ICH guidelines and successfully applied for the determination of investigated drugs in tablets [36]. High-performance liquid chromatography [37] and LC/ ESI-MS/MS [38].

**Validation guidelines**

1. ICH Q2A text on validation of analytical procedures: definitions and terminology (March 1995)

2. ICH Q2B validation of analytical procedures: methodology (June 1997)

3. FDA (Draft) guidance for industry: analytical methods validation and analytical procedures

4. Pharmacopoeias United states Pharmacopoeia (USP) and European Pharmacopoeia (EP).

Typical analytical parameters used in assay validation include

- Accuracy
- Precision
- Specificity
- Detection Limit
- Quantitation Limit
- Linearity
- Range
- Robustness

**Accuracy:** Accuracy is an amount of closeness among the measured and physical [39]. The proper of measured rate of accuracy is the nearness or recognized rate. Accuracy is decided through making use of the process of sample to which regarded quantities of analyte had been addition. It may also frequently be expressed because the healing through the assay of regarded, delivered quantities of analyte [40] .

**Precision:** The precision of an analytical method it is attachment of expresses compact connecting a sequence of measurements found from several samples the similar homogeneous sample under the

agreed repeatability conditions.

**Specificity:** The skill of Specificity degree of analyte of interest in the corporation of mechanisms that are estimated to be present. Classically, these may contain impurities, disintegrant, matrix and the like [41].

**Detection limit**: The limit of detection for a single analytical method is the lowest amount of analyte in a sample that can be detected but not necessarily quantified as an exact value. Can be determined visually, signal to noise. Standard deviation of the response and the slope [42].

**Quantitation Limit:** The Quantitation limit a single analytical technique is the bottom amount of analyte, quantitation limit is suitable preciseness in a sample is potency which quantitatively determined and accuracy. Quantitation limit sign to noise technique can handiest be implemented to analytical strategies which show off baseline noise [43].

**Linearity:** The linearity is analytical technique it is capability to achieve test result that are proportional to absorption of analytes in models within certain array or thrugh well-defined mathematical transformations [44].

ZZThe linearity should be assessed by graphic review of a signal plot as a role of the analyte content or concentration. It should be appropriate interpreted using arithmetical techniques it is linear connection for example, by calculating a line of deterioration using the smallest squares techniques, sometimes to control the linearity between model absorptions and tests, linearity is information test data essential to be exposed to a calculated alteration analysis [45].

**Range:** Range is defined as the interval between the upper and lower concentrations of analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity [46].

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 [47]. The range is normally expressed in the same units as the test results (e.g., percentage, parts per million) obtained by the analytical method [44].

**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 [48].

Methods need to be validation and revalidation

- Before their introduction into routine use.
- Whenever the condition change for which the method has been validated e.g., instrument with different characteristics.
- Whenever the method is changed and the changes are outside the original scope of the method.

We generally validate the method under following conditions:

- During method development
- Checking the system suitability
- Change of application, environment, and analyst
- While using after a prolonged period of time
- Checking reliability and consistency

The type of method and analytical technique used will determine the nature and extent of the validation studies required. The most common methods for validation are identification, assay and impurities

determination [49, 50].

**Important stages in validation:**

The action identifying with validation studies can be categorized mainly into three stages:

**Stage 1** This includes pre-validation qualification stage which covers all exercises identifying with product studies and improvement, formulation pilot batch testing, scale-up research, exchange of innovation to business scale groups, setting up stability conditions, and managing of in-process, finished pharmaceutical formulations, qualification of equipment, master documents, and process limit.

**Stage 2** This involves process validation phase. It is intended to check that every installed limit of the vital process parameter is substantial and that satisfactory products can be created even below the worst situations.

**Stage 3** It is also called as the validation maintenance stage, it requires constant review of all procedure related archives, including validation of the review reports, to guarantee that there have been no modifications, departure, failures, and alteration to the production procedure and that all standard operating procedures (SOPs), involving change control procedures observed. The approval team involving people representing all essential departments also guarantees that there have been no modifications/deviations that ought to have brought about requalification and revalidation [51, 52].

**Various steps involved in method development and validation are**:

- Method development plan.
- Background information gathering.
- Laboratory method development.
- Generation of test procedure.
- Methods validation protocol definition.
- Laboratory methods validation.
- Validated test method generation.
- Validation report. [31].

**Method Validation by Phase of Development**

Requirements for method validation are clear for new drug applications (NDA) and many other worldwide marketing applications. These requirements are specified in documents from the International Conference on Harmonization (ICH) [53, 54]. Regulatory agencies [55, 56]. And pharmacopeia. The validation guidelines applicable to early drug development phases, however, are not as specific [57, 58].

**Need of analytical method development and validation**

The need of validation of the analytical method development and validation emerged due to international competition, maintaining the standard of products in high commercial & market value and ethical reasons. Some of the famous organizations governing the quality standards are:

· United States Food and Drug Administration (US FDA)

· Current Good Manufacturing Practice (cGMP) regulations

· Good Laboratory Practice (GLP) regulations.

· The Pharmaceutical Inspection Cooperation Scheme's (PIC/S)

· Pharmaceutical Inspection Cooperation Scheme (PIC/S)

· The International Conference for Harmonization (ICH)

· Quality Manual ISO/IEC issued by International Organization for Standardization

· World Health Organization (WHO) [49, 50].

**Benefits and risks of phased method validation**

Although performing validation in phases has clear benefits, it also must be noted that potential risk is associated with this approach. The risk can be reduced significantly if the analytical scientist has a good understanding of the analytical methodology's limitations and a basic understanding of the chemistry or process used to produce the drug substance or product. With a strong technical base and the use of good method development practices [51].

**Ethical Clearance:** The study has been approved from institutional ethical committee.

**Conflict of Interest and Source of Funding:** Nill

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