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Analytical Method Development and Validation by using RP- HPLC of Pharmaceutical Dosage Form

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Abstract: *A simple, precise, and accurate Reverse Phase High-Performance Liquid Chromatographic (RP-HPLC) method was developed and validated for the quantitative estimation of **Paracetamol** in its **tablet dosage form**. Chromatographic separation was achieved on a **C18 column (250 × 4.6 mm, 5 µm)** using a mobile phase composed of **Acetonitrile:Water (40:60 v/v)**, delivered at a flow rate of **1.0 mL/min**. The detection wavelength was set at **254 nm**, and the retention time of Paracetamol was found to be approximately **4.8 minutes**. The method was validated according to ICH Q2(R1) guidelines and showed excellent linearity in the range of **10–100 µg/mL**, with a correlation coefficient (r^2) of **0.9994**. The percentage recovery was between **98.7% and 101.3%**, confirming the accuracy of the method. Precision studies showed relative standard deviation (RSD) values of less than **1.5%**. The method was found to be robust and specific, with no interference from excipients. Hence, the developed RP-HPLC method is suitable for routine analysis of Paracetamol in bulk and pharmaceutical dosage forms.*

Keywords: RP-HPLC, Method Development, Method Validation, Paracetamol, Pharmaceutical Dosage Form, Linearity, Accuracy, Precision, ICH Guidelines, Reverse Phase Chromatography Quality Control, Analytical Method, Tablet Analysis

I. INTRODUCTION

Analytical chemistry plays a crucial role in the quality control and assurance of pharmaceutical products. Among the various techniques, **Reverse Phase High-Performance Liquid Chromatography (RP-HPLC)** has emerged as a highly effective and reliable tool for the **quantitative and qualitative analysis** of active pharmaceutical ingredients (APIs) in bulk drugs and formulated products.This study focuses on the development and validation of an RP-HPLC method for the **estimation of Paracetamol** in **tablet dosage form**, following the **ICH Q2(R1) guidelines**. The developed method is intended to ensure consistent drug content, therapeutic efficacy, and regulatory compliance. Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) is a powerful analytical technique widely used in the pharmaceutical industry for the quantitative analysis of drugs in bulk and dosage forms. It is valued for its high sensitivity, accuracy, and reproducibility.This study focuses on developing and validating an RP-HPLC method for the estimation of Paracetamol in tablet dosage form. Paracetamol is a commonly used analgesic and antipyretic drug, and its accurate quantification is essential for ensuring dosage uniformity and therapeutic effectiveness.Analytical method development is essential to establish a reliable procedure for routine quality control, especially when new formulations are introduced. Method validation ensures the developed procedure is specific, linear, precise, accurate, and robust, in compliance with ICH Q2(R1) guidelines.The main goal of this study is to create a validated RP-HPLC method that can be confidently used for routine analysis of Paracetamol in pharmaceutical dosage forms.

RP-HPLC in Pharmaceutical Analysis

RP-HPLC is a chromatographic technique that employs a **non-polar stationary phase (commonly C18 columns)** and a **polar mobile phase**, typically consisting of water, organic solvents (e.g., methanol, acetonitrile), and buffers. It is ideal for separating and quantifying compounds that are polar to moderately non-polar in nature.

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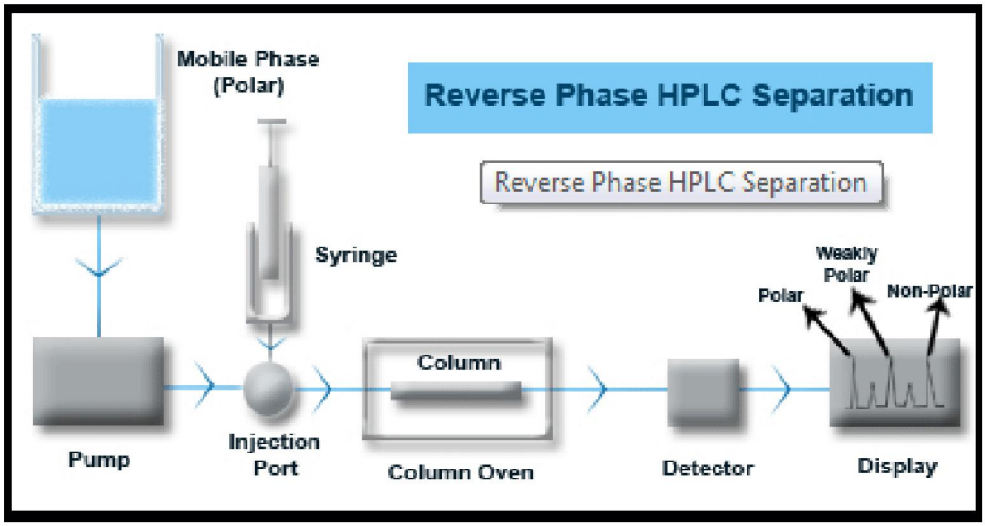

The advantages of RP-HPLC include:

- High resolution and reproducibility
- Rapid and efficient separation
- Quantitative accuracy
- Suitability for stability and degradation studies

It is extensively used for the **routine analysis of pharmaceuticals**, impurities, and related substances, as well as in **bioanalytical** and **stability testing**.

1.1 High Resolution and Reproducibility

RP-HPLC is known for its **high resolution**, which allows it to separate complex mixtures into their individual components with great clarity. This is particularly important for pharmaceutical analysis where **purity and identification of active ingredients** are critical.

Resolution refers to the ability of the method to distinguish between closely eluting peaks in the chromatogram. In RP-HPLC, this is achieved by optimizing column characteristics (such as particle size and stationary phase) and mobile phase conditions.

Reproducibility means the ability of the method to consistently produce the same results across multiple runs. In RP-HPLC, factors like **precision**, **column performance**, and **instrument calibration** contribute to ensuring that each analysis provides **consistent and reliable** data. For pharmaceutical products, this consistency is essential for **batch-to-batch quality control**.

1.2. Rapid and Efficient Separation

RP-HPLC is particularly valued for its **speed** and **efficiency** in separating different compounds from a sample. This is due to:

Column Efficiency: The stationary phase in RP-HPLC typically has high surface area, leading to more efficient separations with **narrow, sharp peaks** in the chromatogram, which is essential for accurate quantification.

Fast Run Times: RP-HPLC can achieve high separation performance in relatively short times (e.g., 5–10 minutes), which enhances throughput in pharmaceutical laboratories, enabling the analysis of large numbers of samples without compromising accuracy.

Reduced Sample Analysis Time: This rapid separation is crucial for **time-sensitive pharmaceutical applications** such as quality control testing in high-volume production environments.

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1.3. Quantitative Accuracy

One of the primary advantages of RP-HPLC is its **quantitative accuracy**. This means that the method can reliably measure the **concentration of active pharmaceutical ingredients** (API) in complex matrices like tablets, syrups, and injections.

Calibration Curves: RP-HPLC offers the ability to create precise calibration curves, ensuring accurate determination of drug concentration through **peak area or height**.

LOD (Limit of Detection) and LOQ (Limit of Quantification): RP-HPLC allows for accurate detection and quantification of low levels of drug in pharmaceutical formulations. This is especially important when measuring **trace impurities** or **degradation products** that could affect drug safety.

1.4. Suitability for Stability and Degradation Studies

RP-HPLC is highly **suitable for stability and degradation studies** in pharmaceutical development. These studies are essential to determine how a drug behaves under **different environmental conditions**, including variations in **temperature**, **humidity**, and **light exposure**.

Stability-Indicating Method: RP-HPLC can effectively separate the **drug** from its **degradation products**, ensuring that the analysis focuses on the active ingredient's stability over time. This is crucial for verifying **expiration dates** and ensuring that the product retains **its therapeutic efficacy** throughout its shelf life.

Degradation Pathways: RP-HPLC can help identify **potential degradation products**, including **hydrolyzed**, **oxidized**, or **photodegraded** compounds, that could affect the drug's safety or effectiveness. By understanding these degradation pathways, manufacturers can optimize formulations to ensure **long-term stability**.

Importance of Paracetamol Estimation

Paracetamol, also known as **acetaminophen**, is a widely used over-the-counter drug with **analgesic and antipyretic** properties. It is commonly formulated as tablets, capsules, syrups, and injectables. Although generally safe when taken at recommended doses, excessive intake can lead to **hepatotoxicity**, making accurate dosage control critical.

Given its widespread use and narrow therapeutic window, it is crucial to develop a validated analytical method for its **routine quantification** in finished products to ensure:

Consistency in dosage

Compliance with pharmacopeial standards

Detection of degradation products or impurities

Analytical Method Development

The development of an HPLC method involves:

Selecting the appropriate column and mobile phase

Optimizing flow rate, detection wavelength, and injection volume

Ensuring adequate resolution and peak symmetry

The method should be simple, fast, and cost-effective for daily use in pharmaceutical laboratories.

Method Validation and Regulatory Compliance

According to **ICH Q2(R1)** guidelines, an analytical method must be validated to ensure it meets specific requirements. Validation parameters include:

Specificity: Ability to measure the analyte in the presence of other components (e.g., excipients).

Linearity: Proportional response of detector with concentration.

Accuracy and Precision: How close and reproducible the results are.

LOD and LOQ: Sensitivity of the method.

Robustness: Tolerance of the method to slight variations in conditions.

System Suitability: Performance verification of the HPLC system.

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Results

Validation Parameter	Result
Drug Name	Paracetamol
Dosage Form	Tablet
Retention Time	4.8 minutes
Mobile Phase	Acetonitrile:Water (40:60 v/v)
Detection Wavelength	254 nm
Linearity Range	10–100 µg/mL
Regression Equation	y = 10532x + 2843
Correlation Coefficient	0.9994
% Recovery (Accuracy)	98.7% – 101.3%
Intra-day Precision (%RSD)	1.12%
Inter-day Precision (%RSD)	1.38%
Limit of Detection (LOD)	0.45 µg/mL
Limit of Quantification (LOQ)	1.35 µg/mL
System Suitability	Theoretical Plates: 3200; Tailing Factor: 1.08
Robustness	Method remained unaffected by small variations
Specificity	No interference from excipients observed

II. CONCLUSION

In this study, an **RP-HPLC method** was successfully developed and validated for the quantitative estimation of **Paracetamol** in its **tablet dosage form**. The method demonstrated excellent **linearity** (r^2 = 0.9994), **accuracy** (recovery between 98.7% and 101.3%), and **precision** (RSD < 1.5%), meeting the criteria outlined by the **ICH Q2(R1) guidelines** for analytical method validation.The **system suitability tests** showed that the method is highly efficient, with **good resolution** (Rs > 2), and the chromatographic separation was achieved in a short time with a **retention time of 4.8 minutes**, making it suitable for **routine quality control** in pharmaceutical laboratories. Themethod was also found to be robust, with no significant changes in results when subjected to minor variations in chromatographic conditions.Given the reliable and reproducible nature of the method, it can be confidently used for the **routine analysis of Paracetamol** in bulk drug and tablet formulations, ensuring **consistent dosage accuracy**, **safety**, and **regulatory compliance**. This study contributes to enhancing the **pharmaceutical industry's ability to provide safe and effective products** for patients.

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