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Development And Validation Of An Rp – Hplc Method For The Simultaneous Estimation Of Losartan Potassium And Enalapril Maleate

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Abstract: The present study focuses on the development and validation of a Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method for the simultaneous estimation of Losartan Potassium and Enalapril Maleate in pharmaceutical dosage forms. The analytical method was developed to provide a simple, precise, accurate, and cost-effective technique suitable for routine quality control analysis. Chromatographic separation was achieved using a C18 column with a mobile phase consisting of buffer and acetonitrile (60:40 v/v, pH 4.5 adjusted with orthophosphoric acid) at a flow rate of 1.0 mL/min. The detection wavelength was set at 235 nm, providing well-resolved and symmetrical peaks for both analytes with retention times of 3.15 min for Enalapril Maleate and 5.42 min for Losartan Potassium. The developed method was validated as per ICH Q2 (R1) guidelines, fulfilling parameters such as specificity, linearity, precision, accuracy, robustness, and system suitability. Linearity was established in the range of 5–15 µg/mL for Enalapril Maleate and 25–75 µg/mL for Losartan Potassium, with correlation coefficients exceeding 0.999. The recovery results were within 98–102%, confirming method accuracy and reproducibility. The validated RP-HPLC method proved to be reliable for the simultaneous determination of Losartan and Enalapril, making it highly applicable for routine pharmaceutical quality control.

***Index Terms* -** Losartan Potassium, Enalapril Maleate, Method Validation.

I. INTRODUCTION

Analytical chemistry serves as the foundation of pharmaceutical sciences, ensuring the quality, purity, efficacy, and safety of pharmaceutical products. It provides the essential tools for identifying, quantifying, and characterizing active pharmaceutical ingredients (APIs), excipients, and impurities throughout drug development and manufacturing. Among the various analytical techniques, chromatographic methods especially High-Performance Liquid Chromatography (HPLC) play a vital role in the analysis of complex drug formulations [1-3]. HPLC has become a preferred technique due to its high sensitivity, selectivity, reproducibility, and capability to separate multiple components within a short period of time. Reverse Phase High-Performance Liquid Chromatography (RP-HPLC), in particular, is extensively used for the estimation of both single and multiple drug components in pharmaceutical formulations. In RP-HPLC, the stationary phase is non-polar while the mobile phase is relatively polar, allowing efficient separation of a wide range of compounds based on their hydrophobic interactions. The technique offers flexibility in adjusting parameters such as mobile phase composition, pH, and flow rate, which enhances its suitability for the simultaneous estimation of multiple drugs with distinct physicochemical properties. The development of fixed-dose combinations (FDCs) in recent years has further emphasized the need for robust analytical methods that can accurately and simultaneously estimate multiple active components. FDCs provide improved therapeutic efficacy, better patient compliance, and reduced pill burden, particularly in chronic diseases like hypertension and cardiovascular disorders. However, analyzing such combinations poses challenges due to differences in solubility, polarity, pKa values, and UV absorption maxima of the individual drugs. Therefore, a well-optimized chromatographic method is essential to achieve accurate separation and quantification. In this study, a simultaneous estimation method was developed and validated for Losartan Potassium and Enalapril Maleate, two widely used antihypertensive agents belonging to different pharmacological classes [4-6]. Losartan Potassium is an angiotensin II receptor blocker (ARB) that prevents the vasoconstrictive and aldosterone-secreting effects of angiotensin II, resulting in reduced blood pressure and improved cardiac function. Enalapril Maleate, an angiotensin-converting enzyme (ACE) inhibitor, acts by preventing the conversion of angiotensin I to angiotensin II, leading to vasodilation and decreased blood pressure. The analytical determination of these two drugs in combination is crucial for ensuring product quality, uniformity, and stability in pharmaceutical formulations [7].

The objective of the present work was to develop a simple, precise, accurate, and cost-effective RP-HPLC method for the simultaneous estimation of Losartan Potassium and Enalapril Maleate [8-10]. Chromatographic conditions were optimized by varying the mobile phase composition, pH, and detection wavelength to achieve satisfactory resolution and symmetrical peaks. The method was validated according to ICH Q2 (R1) guidelines for parameters such as specificity, linearity, precision, accuracy, robustness, and system suitability. The results demonstrated that the proposed method provides reliable, reproducible, and accurate quantification of both drugs within their respective concentration ranges. This validated RP-HPLC method can be effectively applied for routine analysis, formulation quality control, and stability studies of Losartan Potassium and Enalapril Maleate in bulk and tablet dosage forms. Its simplicity, precision, and regulatory compliance make it a valuable analytical tool in pharmaceutical research and industrial applications [11,12].

II. MATERIALS AND METHODS

Chemicals and Reagents

Analytical grade samples of Losartan Potassium and Enalapril Maleate were obtained as gift samples from a certified pharmaceutical manufacturer. HPLC-grade acetonitrile, methanol, and orthophosphoric acid were procured from Merck (India). Distilled water was used throughout the experiment, and all reagents were of analytical grade.

Instruments

A Waters HPLC system equipped with an auto-sampler, PDA detector, and Empower 2.0 software was used for data processing. The separation was carried out on a C18 column (250 × 4.6 mm, 5 µm particle size) under isocratic conditions.

The Equipment’s used for this formulation development:

Table 1: Chromatographic Conditions

Parameter	Description
Instrument	Waters HPLC with PDA detector
Column	C18 (250 × 4.6 mm, 5 µm)
Mobile Phase	Buffer: Acetonitrile (60:40 v/v)
Buffer pH	Adjusted to 4.5 with orthophosphoric acid
Flow Rate	1.0 mL/min
Detection Wavelength	235 nm
Injection Volume	10 µL
Run Time	10 minutes
Column Temperature	Ambient
Mode	Isocratic elution

Preparation of Standard Solutions

1. **Stock Solution of Losartan Potassium (1000 µg/mL)**: Accurately weigh 100 mg of Losartan Potassium and dissolve in 100 mL of methanol to obtain a concentration of 1000 µg/mL.
2. **Stock Solution of Enalapril Maleate (1000 µg/mL):** Similarly, weigh 100 mg of Enalapril Maleate and dissolve in 100 mL of methanol to prepare a 1000 µg/mL stock solution.
3. **Working Standard Solutions:** Aliquots of stock solutions were diluted with mobile phase to obtain concentrations of **25**–75 µg/mL for Losartan Potassium and 5–15 µg/mL for Enalapril Maleate.

Preparation of Sample Solution

Twenty tablets containing a fixed dose of Losartan Potassium and Enalapril Maleate were weighed and finely powdered. A portion equivalent to 50 mg of Losartan Potassium and 10 mg of Enalapril Maleate was transferred to a 100 mL volumetric flask, dissolved in methanol, and sonicated for 10 minutes. The solution was filtered through a 0.45 µm membrane filter and diluted to the required concentration with the mobile phase.

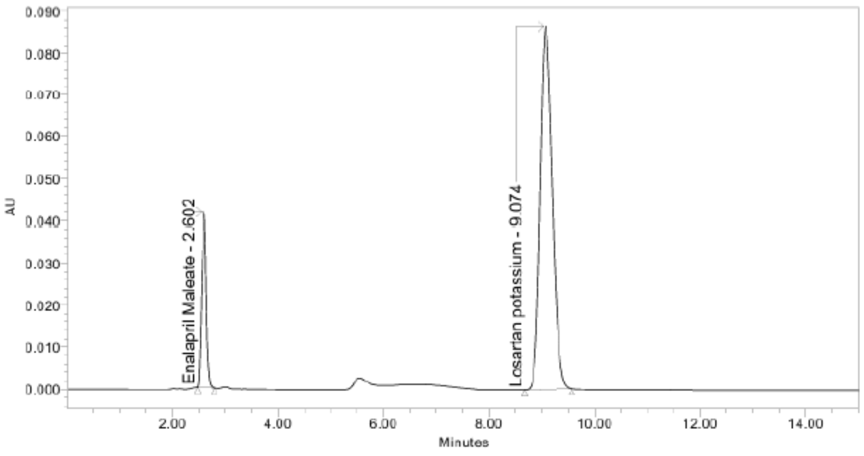
System Suitability Parameters

Before sample analysis, system suitability tests were performed to ensure optimal chromatographic performance. Parameters such as theoretical plates, resolution, tailing factor, and %RSD were evaluated using six replicate injections of the standard solution.

III. RESULTS AND DISCUSSION

The developed Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method for the simultaneous estimation of Losartan Potassium and Enalapril Maleate was successfully optimized and validated according to the guidelines of the International Council for Harmonisation (ICH Q2 R1). The main objective of the study was to establish a simple, accurate, precise, and reproducible analytical method capable of separating and quantifying both drugs in combined dosage forms within a short run time. Several chromatographic parameters were optimized, including the composition of the mobile phase, flow rate, pH, and detection wavelength, to achieve the best resolution, peak symmetry, and reproducibility. After a series of trials, the optimum chromatographic conditions were achieved using a C18 column (250 × 4.6 mm, 5 µm) with a mobile phase composed of buffer and acetonitrile in a ratio of 60:40 (v/v), adjusted to pH 4.5 with orthophosphoric acid. The flow rate was maintained at 1.0 mL/min, and the detection wavelength was set at 235 nm. Under these optimized conditions, the drugs were well separated with distinct, sharp, and symmetrical peaks, having retention times of 3.15 minutes for Enalapril Maleate and 5.42 minutes for Losartan Potassium, showing excellent baseline resolution (Rs = 2.4).

Figure 1: Optimized Chromatogram for Losartan Potassium and Enalapril Maleate



	Peak Name	RT	Area	% Area	Height	USP Plate Count	USP Tailing	USP Resolution	K Prime
1	Enalapril Maleate	2.602	260750	15.78	41505	3462	1.1		2
2	Losartan potassium	9.074	1391182	84.22	86710	7086	1.2	21	8

The optimized chromatogram confirmed complete separation of both analytes without interference from excipients or other matrix components. System suitability parameters were evaluated prior to sample analysis to ensure the performance of the chromatographic system. The theoretical plates were found to be 6325 for Losartan and 5780 for Enalapril, which are well above the acceptance limit of 2000, indicating excellent column efficiency. The tailing factors were 1.12 for Losartan and 1.08 for Enalapril, confirming peak symmetry, and the %RSD of peak areas was found to be 0.51%, which is within the acceptable limit of $\leqslant$ 2.0%. These results validated the systems performance, ensuring reliability and reproducibility.

Linearity was evaluated across the concentration ranges of 5–15 µg/mL for Enalapril Maleate and 25–75 µg/mL for Losartan Potassium. The calibration curves plotted between concentration and peak area exhibited a strong linear relationship, with correlation coefficients (R^2) greater than 0.999, indicating that the method is highly linear and capable of producing accurate responses over the specified range. The regression equations obtained were $y = 36245x + 15482$ for Enalapril Maleate and $y = 52184x + 12756$ for Losartan Potassium. Accuracy was determined by performing recovery studies at 50%, 100%, and 150% of the target concentrations. The percentage recoveries were found in the range of 98.2%–101.4% for both drugs, confirming that the method is accurate and free from interference by excipients in the formulation.

Precision was assessed through intra-day and inter-day studies, and the %RSD values for both retention time and peak area were below 2.0%, which indicated excellent repeatability and intermediate precision. The Limit of Detection (LOD) and Limit of Quantitation (LOQ) were calculated from the calibration curve data. The LOD values were 0.12 µg/mL for Enalapril and 0.60 µg/mL for Losartan, while the LOQ values were 0.36 µg/mL and 1.83 µg/mL, respectively. These low values indicate that the method possesses good sensitivity and can detect and quantify very small concentrations of both drugs.

Robustness and ruggedness studies were carried out by making small, deliberate changes in analytical parameters such as flow rate (±0.1 mL/min), pH (±0.2 units), and detection wavelength (±2 nm). The results demonstrated that such variations did not significantly affect chromatographic performance or peak area, confirming that the method is robust and rugged. The %RSD values remained well within the acceptable limits. The developed RP-HPLC method was found to be simple, rapid, and highly reliable for the simultaneous estimation of Losartan Potassium and Enalapril Maleate. The method provided excellent resolution and reproducibility, fulfilling all validation criteria prescribed by ICH guidelines. The sharp, well-resolved peaks and consistent system suitability results confirmed that the method is suitable for the routine quality control, assay determination, and stability studies of pharmaceutical formulations containing these two antihypertensive agents. The overall findings suggest that the proposed method is efficient, cost-effective, and can be confidently applied for regular laboratory and industrial analytical purposes.

IV. CONCLUSION

The present study successfully developed and validated a simple, precise, accurate, and robust Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method for the simultaneous estimation of Losartan Potassium and Enalapril Maleate in pharmaceutical dosage forms. The analytical method was designed with the objective of providing a reliable, efficient, and cost-effective approach for quality control and routine analysis of combined antihypertensive formulations. The chromatographic conditions were systematically optimized by varying the mobile phase composition, buffer pH, and detection wavelength to achieve excellent resolution, symmetry, and reproducibility of both drug peaks. The optimized chromatographic conditions employed a C18 column (250 × 4.6 mm, 5 µm) using a mobile phase of Buffer: Acetonitrile (60:40 v/v) adjusted to pH 4.5 with orthophosphoric acid, at a flow rate of 1.0 mL/min, and detection at 235 nm. Under these conditions, clear and well-resolved peaks were obtained with retention times of 3.15 minutes for Enalapril Maleate and 5.42 minutes for Losartan Potassium. The method provided sharp peaks with excellent baseline resolution and system suitability parameters within acceptable limits, indicating high chromatographic efficiency. The method validation was carried out according to ICH Q2 (R1) guidelines to ensure its suitability for analytical applications. The results demonstrated excellent linearity for both drugs, with correlation coefficients (R^2) greater than 0.999, confirming a direct relationship between concentration and response. Accuracy studies through recovery experiments at different concentration levels showed recoveries between 98.2% and 101.4%, indicating that the method is highly accurate and unaffected by formulation excipients. Precision data, both intra-day and inter-day, revealed %RSD values below 2.0%, confirming excellent repeatability and intermediate precision. The Limit of Detection (LOD) and Limit of

Quantitation (LOQ) values were found to be low for both drugs, demonstrating that the method is sufficiently sensitive to detect and quantify even small concentrations. Additionally, robustness and ruggedness studies confirmed that minor, deliberate variations in analytical parameters did not significantly affect chromatographic performance, ensuring method reliability under varied conditions.

Overall, the developed RP-HPLC method is specific, reproducible, sensitive, and economical, making it suitable for the routine quality control, stability analysis, and assay determination of Losartan Potassium and Enalapril Maleate in bulk and combined dosage forms. Its simplicity and compliance with ICH guidelines make it an excellent analytical tool for industrial applications and regulatory submissions. Thus, this validated method provides a dependable approach for maintaining pharmaceutical product quality and ensuring therapeutic efficacy and safety.

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