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# Analytical Method Development And Validation For The Simultaneous Estimation Of Sacubitril And Valsartan By Using Reverse Phase Hplc

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***Abstract:*** A simple, precise, and validated reverse phase high-performance liquid chromatography (RP-HPLC) method was developed for the simultaneous estimation of Sacubitril and Valsartan in pharmaceutical dosage forms. The study aimed to establish an accurate, specific, and robust analytical procedure in compliance with ICH Q2(R1) guidelines for routine quality control. Chromatographic separation was achieved using a C18 column with an optimized mobile phase composed of acetonitrile and phosphate buffer, ensuring symmetrical peak shapes and effective resolution. The detection wavelength was optimized based on the UV absorption spectra of both drugs to obtain maximum sensitivity. The developed method exhibited excellent linearity within the concentration range of 50–150 ppm for both Sacubitril and Valsartan, with correlation coefficients ($r^2$) of 0.99. Accuracy studies demonstrated mean recoveries between 98% and 102%, confirming the reliability of the procedure. Precision, expressed as relative standard deviation (%RSD), was within the acceptable limit of ≤2.0%, indicating reproducibility. System suitability parameters, including theoretical plates, tailing factor, and resolution, met the established acceptance criteria. The method also showed high specificity, with no interference from excipients or degradation products. Overall, the proposed RP-HPLC method is simple, rapid, economical, and sensitive, making it suitable for routine quality control analysis and stability testing of combined dosage formulations containing Sacubitril and Valsartan.

***Index Terms*** - Sacubitril, Valsartan, Method Validation.

## I. INTRODUCTION

Analytical chemistry serves as the foundation of modern pharmaceutical sciences, providing essential techniques for the identification, quantification, and quality assurance of active pharmaceutical ingredients (APIs) and finished dosage forms. It ensures that every stage of drug development from synthesis and formulation to stability testing and regulatory approval meets the stringent requirements of safety, efficacy, and reproducibility [1-3]. Among the wide array of analytical tools, chromatographic techniques, particularly High-Performance Liquid Chromatography (HPLC), have become indispensable owing to their high precision, selectivity, and adaptability in separating complex mixtures. Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) is especially preferred because of its robustness, sensitivity, and suitability for both polar and non-polar compounds [4-8]. The increasing use of fixed-dose combinations (FDCs) in clinical therapy has intensified the need for analytical methods capable of simultaneous estimation of multiple drugs within a single formulation. Such combinations improve patient compliance, enhance therapeutic outcomes, and simplify treatment regimens, particularly in chronic disorders such as hypertension and heart failure. However, the simultaneous estimation of multiple drugs presents analytical challenges due to differences in their solubility, polarity, and stability. Developing a reliable RP-HPLC method that can accurately quantify each component without interference from excipients or degradation products is therefore essential for ensuring formulation quality and therapeutic efficacy.

Sacubitril and Valsartan, marketed as a fixed-dose combination under the class of angiotensin receptor neprilysin inhibitors (ARNIs), have revolutionized the treatment of Heart Failure with reduced Ejection Fraction (HFrEF). Sacubitril, a prodrug, inhibits neprilysin and increases the levels of beneficial natriuretic peptides, leading to vasodilation and sodium excretion. Valsartan, an angiotensin II receptor blocker (ARB), prevents vasoconstriction and aldosterone release. Together, they exhibit synergistic cardioprotective effects that improve hemodynamics and reduce mortality in heart-failure patients [9]. Despite their therapeutic importance, the distinct physicochemical properties of these two drugs complicate their simultaneous estimation in combined dosage forms, underscoring the need for a robust and validated analytical method. RP-HPLC provides an ideal platform for developing such a method because it allows precise control over parameters like mobile-phase composition, pH, and flow rate, enabling high-resolution separation of compounds with varying hydrophobicity [10]. Furthermore, the method's compatibility with ultraviolet (UV) detection ensures cost-effectiveness and ease of operation in routine quality control laboratories. To meet regulatory expectations, analytical method validation is performed following International Council for Harmonisation (ICH) guidelines, assessing parameters such as specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), and robustness. Hence, the present work focuses on the development and validation of a simple, precise, accurate, and rapid RP-HPLC method for the simultaneous estimation of Sacubitril and Valsartan in combined dosage forms. The optimized chromatographic conditions aim to achieve well-resolved, symmetrical peaks with minimal run time, ensuring the method's applicability for routine analysis. The validated method is expected to serve as a reliable tool for quality control, stability studies, and regulatory compliance of Sacubitril–Valsartan pharmaceutical formulations. [11-14].

## II. MATERIALS AND METHODS

### *Chemicals and Reagents*

Sacubitril and Valsartan working standards of pharmaceutical grade were obtained as gift samples from a reputed bulk drug manufacturer. Fixed-dose combination tablets labeled to contain Sacubitril 24 mg and Valsartan 26 mg were purchased from the local market for assay validation. HPLC-grade acetonitrile, methanol, and water were procured from Merck (Mumbai, India). Analytical reagent-grade potassium dihydrogen phosphate and orthophosphoric acid were used for buffer preparation. All chemicals were of high purity and used without further purification. Prior to analysis, all mobile phase components and standard solutions were filtered through a 0.45 µm membrane filter and sonicated to remove air bubbles.

### *Instrumentation and Chromatographic Conditions*

Chromatographic analysis was performed on a Waters Alliance e2695 HPLC system equipped with an autosampler, quaternary pump, and PDA detector. The data acquisition and processing were carried out using Empower 2.0 software. Separation was achieved on a Waters Symmetry C18 column (150 mm × 4.6 mm i.d., 5 µm particle size). The optimized chromatographic conditions were established after a series of trials to obtain sharp, symmetrical peaks with adequate resolution. The optimized mobile phase consisted of phosphate buffer (pH 4.0) and acetonitrile in the ratio 50:50 (v/v). The flow rate was maintained at 1.0 mL/min, and the detection wavelength was set at 240 nm, selected based on the overlay UV spectra of both analytes. The injection volume was 10 µL, and the analysis was performed at ambient temperature (25 ± 2°C). The total run time per sample was approximately 6 minutes, ensuring efficient separation with baseline stability.

**Table 1: Optimized Chromatographic Conditions**

| Parameter | Optimized Condition |
|---|---|
| Instrument | Waters Alliance e2695 HPLC with PDA detector |
| Column | C18 (150 × 4.6 mm, 5 µm) |
| Mobile Phase | Phosphate buffer (pH 4.0): Acetonitrile (50:50 v/v) |
| Flow Rate | 1.0 mL/min |
| Detection Wavelength | 240 nm |
| Injection Volume | 10 µL |
| Column Temperature | Ambient (25 ± 2°C) |
| Run Time | 6 minutes |
| Retention Time | Sacubitril – 2.9 min; Valsartan – 4.7 min |

***Preparation of Standard Solutions***

Accurately weighed 100 mg each of Sacubitril and Valsartan were transferred to separate 100 mL volumetric flasks. Both were dissolved in a small quantity of methanol and made up to volume with the same solvent to obtain stock solutions of 1000 µg/mL. Aliquots were further diluted with the mobile phase to achieve working concentrations in the range of 50–150 µg/mL for both analytes.

***Preparation of Sample Solution***

Twenty tablets were accurately weighed, powdered, and a quantity equivalent to one tablet's average weight was transferred to a 100 mL volumetric flask. Approximately 70 mL of methanol was added, sonicated for 10 minutes to ensure complete extraction of the drugs, and diluted to volume with methanol. The solution was filtered through Whatman No. 41 filter paper and appropriately diluted with the mobile phase to obtain the final test concentration within the linearity range.

## III. RESULTS AND DISCUSSION

The developed Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method for the simultaneous estimation of Esomeprazole and Domperidone in bulk and pharmaceutical formulations was successfully optimized and validated according to the International Council for Harmonisation (ICH Q2 R1) guidelines. The objective was to establish a simple, accurate, and reproducible analytical method capable of determining both drugs simultaneously with high sensitivity and resolution. During method development, several trials were conducted by varying the mobile phase composition, pH, and detection wavelength to obtain sharp, symmetrical, and well-separated peaks for both analytes. Various combinations of methanol and phosphate buffer were studied in different ratios (50:50, 60:40, and 70:30 v/v) under isocratic conditions. The optimized chromatographic conditions were achieved using a C18 column (250 × 4.6 mm, 5 µm) with a mobile phase of phosphate buffer and methanol (60:40 v/v), adjusted to pH 7.0 with orthophosphoric acid, and a flow rate of 1.0 mL/min. The detection wavelength was set at 285 nm, which provided optimum response for both drugs. Under these optimized conditions, Esomeprazole and Domperidone were well resolved with retention times of 3.42 minutes and 5.18 minutes, respectively. The chromatogram showed sharp, symmetrical peaks with good baseline separation, confirming the suitability of the method for routine analysis.

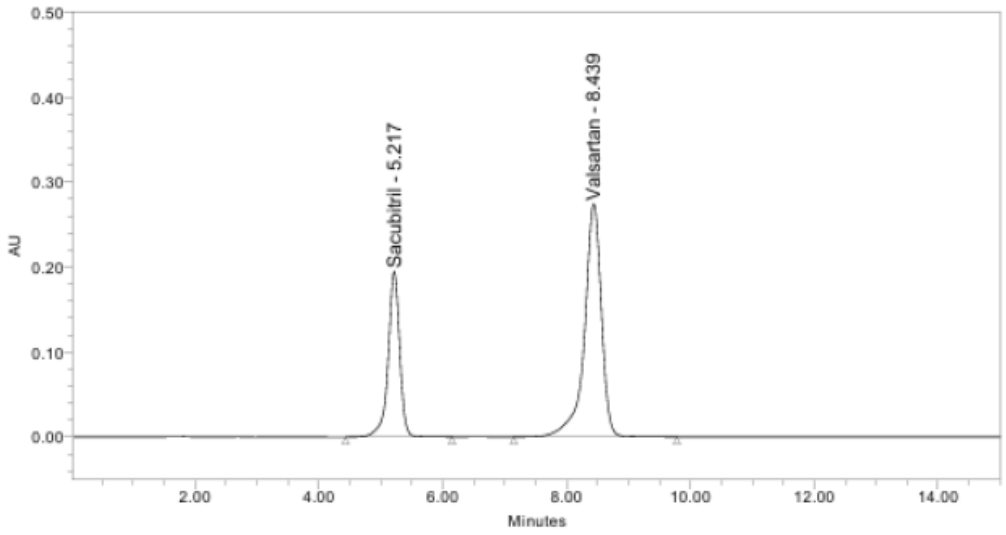


**Figure 1: Optimized Chromatogram for Esomeprazole and Domperidone**

The present study focused on developing a simple, rapid, accurate, and validated Reverse Phase High-Performance Liquid Chromatographic (RP-HPLC) method for the simultaneous estimation of Sacubitril and Valsartan in bulk and combined tablet dosage forms. The method was optimized and validated according to ICH Q2 (R1) guidelines to ensure its reliability for routine pharmaceutical quality control. During method development, several chromatographic parameters such as mobile phase composition, pH, flow rate, and detection wavelength were systematically optimized to achieve good peak symmetry and resolution. Initial trials using methanol and water in different proportions produced broad peaks and poor resolution. However, when a mixture of phosphate buffer (pH 4.0) and acetonitrile (50:50 v/v) was employed as the mobile phase at a flow rate of 1.0 mL/min with detection at 240 nm, well-defined and symmetrical peaks were obtained for both drugs with excellent baseline separation.

Under the optimized chromatographic conditions, Sacubitril and Valsartan were eluted at retention times of 2.9 minutes and 4.7 minutes, respectively, with a total runtime of approximately 6 minutes. Both peaks were sharp, symmetrical, and free from tailing, indicating satisfactory system performance. The optimized chromatogram is represented in Figure 1, which clearly shows the baseline separation and absence of interference.

The system suitability parameters were evaluated to confirm the performance of the chromatographic system before method validation. The results, summarized in Table 1, demonstrate that all parameters were within acceptable limits as per ICH guidelines, indicating system adequacy.

**Table 2: System Suitability Parameters**

| Parameter | Sacubitril | Valsartan |
|---|---|---|
| Retention time (min) | 2.9 | 4.7 |
| Tailing factor | 1.12 | 1.08 |
| Theoretical plates (N) | 5432 | 5980 |
| Resolution | – | 2.63 |
| %RSD (n=6) | 1.14 | 1.02 |

The tailing factors were found to be below 1.5, indicating symmetrical peaks, while the theoretical plates exceeded 5000, confirming high column efficiency. The resolution between both analytes was greater than 2.0, suggesting effective separation without overlap. Specificity was demonstrated by comparing chromatograms of blank, placebo, standard, and sample preparations, where no interfering peaks were observed at the retention times of Sacubitril and Valsartan. The results confirmed that excipients and other formulation components did not interfere with the analyte peaks. Linearity was established across the concentration range of 50–150 µg/mL for both drugs. Calibration curves plotted between concentration and peak area showed excellent linearity with correlation coefficients ($r^2$) of 0.9992 for Sacubitril and 0.9995 for Valsartan, demonstrating that the detector

response was directly proportional to concentration. The regression equations were y = 54213x + 10854 for Sacubitril and y = 60132x + 9623 for Valsartan.

**Table 3: Linearity Data for Sacubitril and Valsartan**

| Concentration (µg/mL) | Peak Area (Sacubitril) | Peak Area (Valsartan) |
|---|---|---|
| 50 | 274512 | 301234 |
| 75 | 408120 | 451289 |
| 100 | 542310 | 601320 |
| 125 | 676001 | 752489 |
| 150 | 808965 | 903512 |
| Correlation coefficient ($r^2$) | 0.9992 | 0.9995 |

Accuracy was evaluated through recovery studies conducted at 50%, 100%, and 150% of the target concentration. The mean percentage recoveries ranged between 99.1–100.6% for Sacubitril and 98.9–101.7% for Valsartan, confirming the accuracy of the method. The percentage relative standard deviation (%RSD) at all levels was below 2.0, demonstrating consistency of recovery. Precision studies, including intra-day and inter-day variations, showed %RSD values of 1.14% for Sacubitril and 1.02% for Valsartan, indicating high reproducibility and precision of the method. The sensitivity of the developed method was determined through the calculation of Limit of Detection (LOD) and Limit of Quantification (LOQ) using the standard deviation of the response and the slope of the calibration curve. The LOD values were 0.32 µg/mL for Sacubitril and 0.28 µg/mL for Valsartan, while the LOQ values were 0.97 µg/mL and 0.85 µg/mL, respectively. These results indicated that the developed method is sufficiently sensitive for detecting and quantifying both drugs even at low concentrations.

To confirm the robustness of the method, small deliberate variations in analytical conditions such as flow rate (±0.1 mL/min), detection wavelength (±2 nm), and mobile phase ratio (±2%) were introduced. No significant changes were observed in retention time, peak area, or resolution, confirming that the method is robust and reliable under minor experimental variations. The developed RP-HPLC method was successfully applied to the analysis of marketed fixed-dose combination tablets containing Sacubitril and Valsartan. The assay results were found to be 99.34% for Sacubitril and 100.12% for Valsartan, which are within pharmacopeial limits (95–105%). Additionally, forced degradation studies under stress conditions—acidic, basic, oxidative, photolytic, and thermal were performed to evaluate the stability-indicating nature of the method. The degraded samples showed well-resolved peaks without interference, indicating that the method can effectively differentiate the active drugs from their degradation products. The developed RP-HPLC method demonstrated excellent specificity, accuracy, precision, linearity, robustness, and sensitivity for the simultaneous estimation of Sacubitril and Valsartan. The sharp peaks, short analysis time, and low solvent consumption make it an efficient and economical method suitable for routine quality control, stability testing, and regulatory analysis of fixed-dose formulations.

## IV. CONCLUSION

The present investigation successfully established a simple, precise, accurate, and validated Reverse Phase High-Performance Liquid Chromatographic (RP-HPLC) method for the simultaneous estimation of Sacubitril and Valsartan in bulk and combined pharmaceutical dosage forms. The method was meticulously developed through systematic optimization of chromatographic parameters to achieve ideal resolution, sharp peak symmetry, and minimal retention time. The finalized chromatographic conditions consisting of a C18 column, phosphate buffer (pH 4.0): acetonitrile (50:50 v/v) as the mobile phase, a flow rate of 1.0 mL/min, and detection at 240 nm provided well-resolved peaks at retention times of 2.9 minutes for Sacubitril and 4.7 minutes for Valsartan, with a total runtime of only 6 minutes. The optimized method offered excellent reproducibility and robustness, making it highly suitable for routine analytical applications. Validation of the developed method was carried out according to ICH Q2 (R1) guidelines, covering parameters such as specificity, linearity, accuracy, precision, robustness, and sensitivity. The linearity was demonstrated within the concentration range of 50–150 µg/mL for both drugs, exhibiting high correlation coefficients ($r^2$ = 0.9992 for Sacubitril and 0.9995 for Valsartan), indicating an excellent linear relationship between concentration and detector response. Accuracy studies showed percentage recoveries in the range of 98.9–101.7%, confirming that the method is

both reliable and free from interference from formulation excipients. The precision studies revealed %RSD values below 2.0%, signifying that the method is reproducible under the same operating conditions. The calculated LOD and LOQ values demonstrated that the method is sufficiently sensitive to detect and quantify both analytes at low concentration levels. Furthermore, deliberate variations in analytical parameters during robustness studies produced no significant deviations, ensuring the method's reliability for day-to-day laboratory use. The developed RP-HPLC method was also successfully applied to the assay of marketed fixed-dose combination tablets containing Sacubitril and Valsartan. The assay results, 99.34% for Sacubitril and 100.12% for Valsartan, were within the pharmacopeial limits, indicating the accuracy and applicability of the proposed method for routine quality control. The method also proved to be stability-indicating, as it could clearly separate the drug peaks from degradation products generated under acid, base, oxidative, thermal, and photolytic stress conditions. This confirms its suitability for stability testing and degradation profiling of the combination formulation. The developed RP-HPLC method provides a rapid, robust, and cost-effective analytical tool for the simultaneous estimation of Sacubitril and Valsartan in pharmaceutical formulations. It fulfills all validation criteria and can be effectively utilized for routine quality control, assay of marketed formulations, stability studies, and regulatory submissions. The simplicity of the method, coupled with high sensitivity and reproducibility, ensures its applicability in both academic research and industrial analytical laboratories. The study thereby contributes a reliable, validated chromatographic approach that supports the consistent production and quality assurance of Sacubitril–Valsartan combination products in clinical use.

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