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Stability-Indicating RP-HPLC Method for the Quantitative Determination of Amlodipine Besylate and Lisinopril in Combined Antihypertensive Formulations

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Abstract
A simple, accurate, and precise reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Amlodipine Besylate and Lisinopril in fixed-dose combination formulations. Chromatographic separation was achieved using a C18 column (250 × 4.6 mm, 5 µm) with a mobile phase consisting of acetonitrile and phosphate buffer (pH 3.5) in the ratio of 60:40 v/v, at a flow rate of 1.0 mL/min. The detection was carried out at 210 nm using a UV detector. The retention times for Amlodipine Besylate and Lisinopril were found to be approximately 5.8 and 3.2 minutes, respectively. The method was validated according to ICH Q2(R1) guidelines for parameters including linearity, accuracy, precision, specificity, robustness, LOD, and LOQ. The linearity range was established between 2–12 µg/mL for Amlodipine and 5–30 µg/mL for Lisinopril, with correlation coefficients (r^2) exceeding 0.999. Recovery studies demonstrated accuracy within 98–102% for both drugs. The method proved to be robust and suitable for routine quality control of the fixed-dose combination tablet containing Amlodipine and Lisinopril.
Keywords: *RP-HPLC, Amlodipine Besylate, Lisinopril, Method Validation, Fixed-Dose Combination, ICH Guidelines*

1. INTRODUCTION

Hypertension is a major risk factor for cardiovascular diseases and is frequently managed using combination drug therapy to enhance patient compliance and therapeutic outcomes. Amlodipine Besylate, a calcium channel blocker, and Lisinopril, an angiotensin-converting enzyme (ACE) inhibitor, are often prescribed together in fixed-dose combination (FDC) formulations for the effective management of high blood pressure (Cryer et al., 2016; Meissner, 2016; Yildiz et al., 2020). The synergistic action of these two agents reduces the risk of adverse cardiovascular events while minimizing the dose-related side effects of monotherapy. To ensure the safety, efficacy, and quality of these FDC products, it is essential to develop a robust and validated analytical method capable of simultaneously quantifying both active pharmaceutical ingredients (APIs) (Burnier & Egan, 2019; Landsberg et al., 2013; Messerli et al., 2007; Yildiz et al., 2020).

High-performance liquid chromatography (HPLC) has emerged as one of the most powerful tools in pharmaceutical analysis, offering high precision, reproducibility, and selectivity. Reverse-phase HPLC (RP-HPLC) is particularly well-suited for the analysis of polar and non-polar compounds, making it a suitable choice for simultaneous estimation of Amlodipine and Lisinopril. Despite the availability of individual methods for the estimation of these drugs, there exists a need for a single, simple, and validated RP-HPLC method that can effectively quantify both drugs in combined dosage forms (Dalawai et al., 2021; Jiménez-Nava et al., 2023; Nag et al., 2020; Patel et al., 2024).

Previous studies have explored various chromatographic methods for either Amlodipine or Lisinopril individually; however, simultaneous estimation in a single run is limited in literature, especially with improved sensitivity and system suitability. Therefore, the aim of this study was to develop and validate an accurate, precise, and robust RP-HPLC method for the simultaneous estimation of Amlodipine Besylate and Lisinopril in fixed-dose combination tablets in accordance with the International Council for Harmonisation (ICH) Q2(R1) guidelines (Baj et al., 2022; Dalawai et al., 2021; Flieger, 2014; Josic & Kovac, 2010). This paper details the method development process, selection of chromatographic conditions, optimization strategies, and method validation studies. Emphasis was placed on achieving good resolution between the two drugs with acceptable peak symmetry, system suitability, and run time, enabling its application in routine quality control and stability testing of pharmaceutical formulations.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Amlodipine Besylate and Lisinopril reference standards were obtained as gift samples from a reputed pharmaceutical manufacturer named Zoripan Pharma, Baddi, India. Fixed-dose combination tablets containing 5 mg of Amlodipine Besylate and 10 mg of Lisinopril were procured from a local pharmacy. HPLC-grade acetonitrile and methanol were purchased from Merck (India), while analytical-grade potassium dihydrogen phosphate and orthophosphoric acid were used for buffer preparation. Double-distilled water was used throughout the study and filtered using a 0.45 µm membrane filter prior to use.

2.2 Instrumentation

The chromatographic analysis was performed on a Shimadzu LC-20AT HPLC system equipped with a UV-Visible detector (SPD-20A) and a manual injector with a 20 µL loop. The system was controlled through LC Solutions software. Separation was achieved using a Phenomenex C18 column (250 mm × 4.6 mm, 5 µm particle size), maintained at ambient temperature (Jiménez-Nava et al., 2023; Le et al., 2024; Patel et al., 2024).

2.3 Chromatographic Conditions

The mobile phase consisted of a mixture of acetonitrile and phosphate buffer (pH 3.5 adjusted with orthophosphoric acid) in the ratio of 60:40 v/v. The flow rate was set at 1.0 mL/min, and the detection was carried out at 210 nm. The injection volume was 20 µL, and the total run time for each sample was approximately 8 minutes (Baj et al., 2022; Gondhale-Karpe & Manwatkar, 2023; Illendula & Sharma, 2024; Itigimatha et al., 2022).

2.4 Preparation of Standard Solutions

Amlodipine Besylate Standard: An accurately weighed amount of Amlodipine Besylate equivalent to 10 mg was transferred to a 100 mL volumetric flask and dissolved in the mobile phase. The solution was sonicated and diluted to volume to obtain a stock solution of 100 µg/mL.

Lisinopril Standard: Similarly, 10 mg of Lisinopril was weighed, transferred to a 100 mL volumetric flask, dissolved in mobile phase, sonicated, and diluted to obtain a stock solution of 100 µg/mL.

Working standards were prepared by suitable dilution of the stock solutions with the mobile phase to obtain final concentrations in the range of 2–12 µg/mL for Amlodipine and 5–30 µg/mL for Lisinopril (Dalawai et al., 2021; Nag et al., 2020; Salunkhe et al., 2019).

2.5 Preparation of Sample Solution

Twenty tablets were weighed and powdered. A quantity of powder equivalent to 5 mg of Amlodipine and 10 mg of Lisinopril was transferred to a 100 mL volumetric flask, and about 70 mL of mobile phase was added. The contents were sonicated for 20 minutes, filtered through Whatman filter paper No. 41, and made up to volume with the mobile phase. This solution was further diluted to obtain final concentrations within the linear range of the calibration curve (Gondhale-Karpe & Manwatkar, 2023; Illendula & Sharma, 2024; Itigimatha et al., 2022; Nouwade et al., 2021).

2.6 System Suitability Parameters

Prior to analysis, system suitability tests were conducted by injecting six replicate injections of standard solution to assess parameters such as retention time, theoretical plates, tailing factor, and resolution (Gondhale-Karpe & Manwatkar, 2023; Illendula & Sharma, 2024; Itigimatha et al., 2022; Nouwade et al., 2021).

2.7 Method Validation

The developed RP-HPLC method for the simultaneous estimation of Amlodipine Besylate and Lisinopril was validated in accordance with the International Council for Harmonisation (ICH) Q2(R1) guidelines. The validation process involved the assessment of the following parameters to ensure the method's suitability, reliability, and reproducibility for routine pharmaceutical analysis (Gondhale-Karpe & Manwatkar, 2023; Illendula & Sharma, 2024; Itigimatha et al., 2022; Nouwade et al., 2021):

Linearity and Range: Linearity was evaluated by analyzing a series of standard solutions at six concentration levels ranging from 2 to 12 µg/mL for Amlodipine Besylate and 5 to 30 µg/mL for Lisinopril. Calibration curves were constructed by plotting peak area versus drug concentration. The correlation coefficient (r^2), slope, and intercept of the regression line were calculated to assess the linear relationship.

Precision (Repeatability and Intermediate Precision): Repeatability (intra-day precision) was determined by injecting six replicates of a standard solution at a specified concentration and analyzing them under the same conditions within a single day. Intermediate precision (inter-day precision) was evaluated by performing the same procedure on three consecutive days by different analysts. The precision was expressed as percent relative standard deviation (%RSD) of peak areas.

Accuracy (Recovery Studies): The accuracy of the method was determined using recovery studies by spiking known amounts of standard drug into pre-analyzed samples at three levels—80%, 100%, and 120% of the target concentration. Each level was analyzed in triplicate, and percent recovery was calculated to assess the method's accuracy and absence of matrix interference.

Limit of Detection (LOD) and Limit of Quantitation (LOQ): LOD and LOQ were calculated using the standard deviation of the response (σ) and the slope (S) of the calibration curve, based on the formulae:

- LOD = 3.3 × (σ/S)
- LOQ = 10 × (σ/S) These values indicate the method's sensitivity in detecting and quantifying low concentrations of both analytes.

Robustness: Robustness was assessed by deliberately varying method parameters such as flow rate (±0.1 mL/min), mobile phase composition (±2%), and detection wavelength (±2 nm). The impact of these small changes on retention time, peak area, and system suitability parameters was evaluated to determine the method's reliability under varied conditions.

Specificity: Specificity was established by analyzing the chromatograms of blank, placebo, standard, and sample solutions to confirm the absence of interference at the retention times of Amlodipine and Lisinopril. Peak purity was verified to ensure that the method could clearly distinguish the target analytes from other components present in the formulation.

This comprehensive validation confirmed that the developed method is linear, accurate, precise, specific, robust, and sensitive for the simultaneous determination of Amlodipine Besylate and Lisinopril in fixed-dose combination tablets.

3. RESULTS AND DISCUSSION

The RP-HPLC method developed for the simultaneous estimation of Amlodipine Besylate and Lisinopril in fixed-dose combination tablets was successfully optimized and validated in accordance with ICH Q2(R1) guidelines. The selection of chromatographic conditions—including a C18 column, mobile phase of acetonitrile and phosphate buffer (pH 3.5) in a 60:40 v/v ratio, flow rate of 1.0 mL/min, and detection at 210 nm—yielded well-resolved and sharp peaks for both drugs. The retention times were found to be approximately 3.20 minutes for Lisinopril and 5.80 minutes for Amlodipine, ensuring complete separation within a short runtime of under 8 minutes. The method exhibited excellent linearity, precision, accuracy, and robustness across all validation parameters, demonstrating its suitability for routine quality control and stability studies of fixed-dose formulations containing both antihypertensive agents.

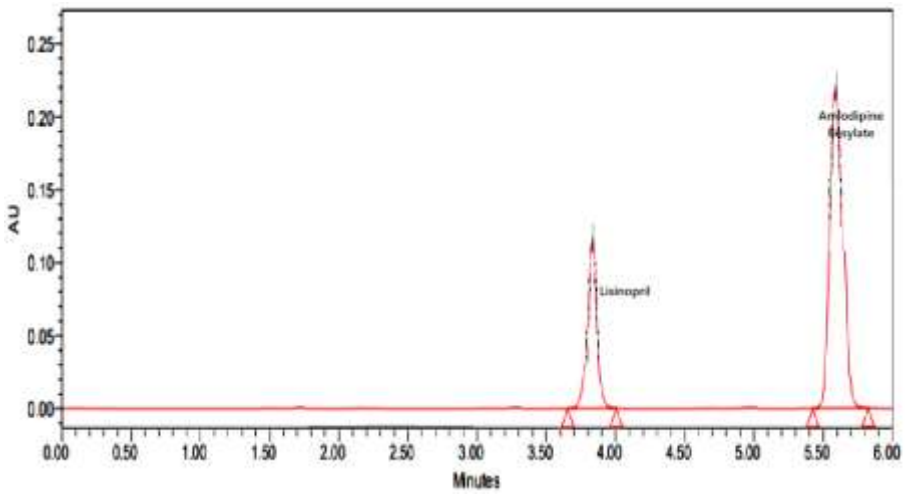

Figure 1. Optimized chromatogram

3.1 Method validation

3.1.1 Linearity

The linearity data presented in Table 1 illustrates a strong and consistent relationship between drug concentration and corresponding peak area for both Amlodipine Besylate and Lisinopril. For Amlodipine, as the concentration increased from 2 to 12 µg/mL, the peak area increased proportionally from 10,500 to 63,000. Similarly, for Lisinopril, a linear rise in peak area from 9,000 to 54,000 was observed with increasing concentrations from 5 to 30 µg/mL. This indicates that the detector response is directly proportional to the concentration of analyte over the tested range. The regression analysis further confirmed this linearity with correlation coefficients (R^2) of 0.9999 for Amlodipine and 1.0000 for Lisinopril, both of which are within the acceptable criteria ($R^2 > 0.999$) for analytical method validation as per ICH Q2(R1) guidelines. The slope and intercept values also demonstrate the sensitivity of the method, particularly for Amlodipine with a higher slope, reflecting its greater response factor under the given chromatographic conditions. These results validate the method's capability to produce accurate and reliable responses across a wide concentration range, ensuring its applicability for quality control testing of both low- and high-dose formulations. The excellent linearity also ensures that the method can be used confidently for determining drug content, conducting dissolution studies, and assessing stability during formulation development and production.

Table 1. Linearity Data for Amlodipine Besylate and Lisinopril

Drug	**Concentration (µg/mL)**	**Peak Area**
Amlodipine	2	10,500
Amlodipine	4	21,000
Amlodipine	6	31,000
Amlodipine	8	42,000
Amlodipine	10	52,000
Amlodipine	12	63,000
Lisinopril	5	9,000
Lisinopril	10	18,000
Lisinopril	15	27,000
Lisinopril	20	36,000
Lisinopril	25	45,000
Lisinopril	30	54,000

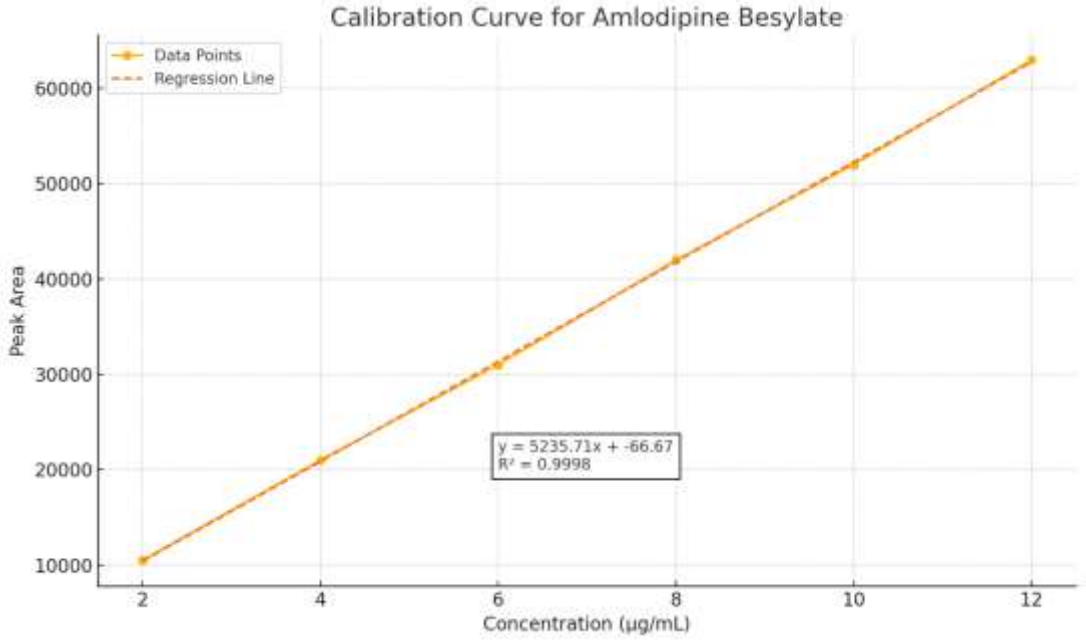

Figure 2. Calibration Curve for Amlodipine Besylat

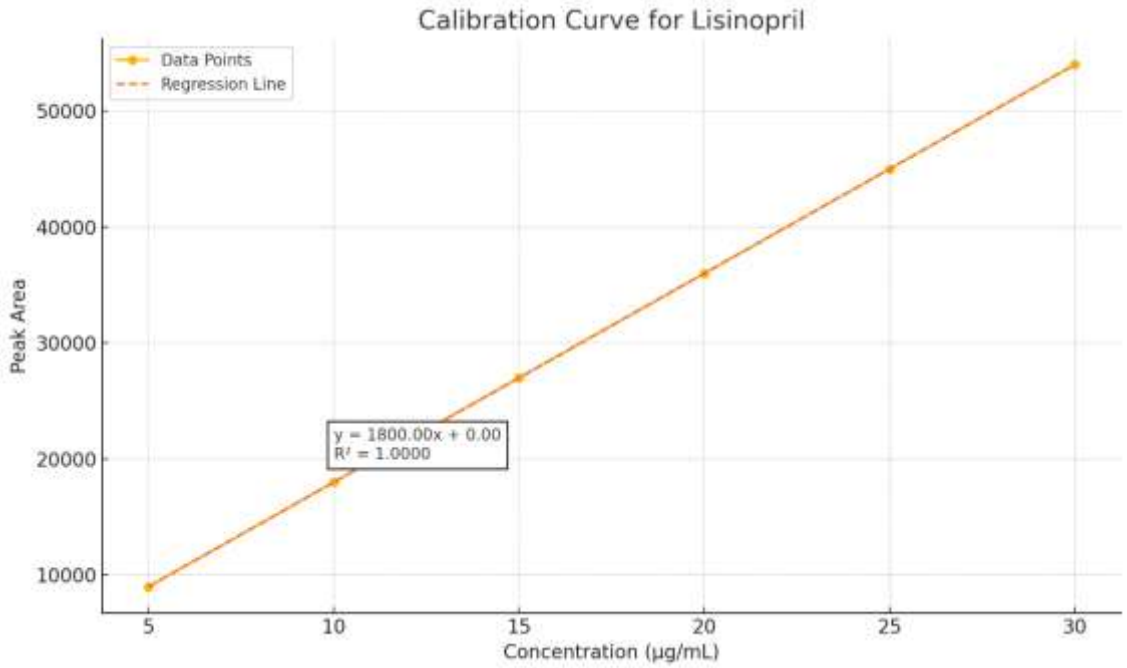

Figure 2. Calibration Curve for Lisinopril

3.1.2 Accuracy (Recovery) Studies

The accuracy of the developed RP-HPLC method was evaluated by performing recovery studies using the standard addition technique at three concentration levels: 80%, 100%, and 120%. The results in Table 2 indicate that the method provides highly accurate measurements for both Amlodipine Besylate and Lisinopril. For Amlodipine, the percent recovery values ranged from 98.75% to 100.40%, while for Lisinopril, the recovery was between 99.00% and 101.67%. All values lie well within the generally accepted recovery range of 98% to 102%, signifying minimal interference from excipients or formulation components. The closeness of the recovered values to the actual amounts added confirms that the method accurately quantifies the active ingredients, regardless of the concentration level. These findings demonstrate that the method is not only suitable for content determination but also robust against matrix effects, making it appropriate for routine quality control and stability studies. Moreover, the consistency across all three spike levels reflects the method's precision and reliability, even when applied to formulations with varying drug concentrations. In conclusion, the recovery studies affirm the method's high degree of accuracy, reinforcing its validation under ICH guidelines for use in pharmaceutical analysis of fixed-dose combination products containing Amlodipine and Lisinopril.

Table 2. Accuracy (Recovery) Studies

Drug	Level (%)	Amount Added (µg/mL)	Amount Recovered (µg/mL)	Recovery (%)
Amlodipine	80	4	3.95	98.75
Amlodipine	100	5	5.02	100.40
Amlodipine	120	6	6.01	100.17
Lisinopril	80	8	7.92	99.00
Lisinopril	100	10	10.10	101.00
Lisinopril	120	12	12.20	101.67

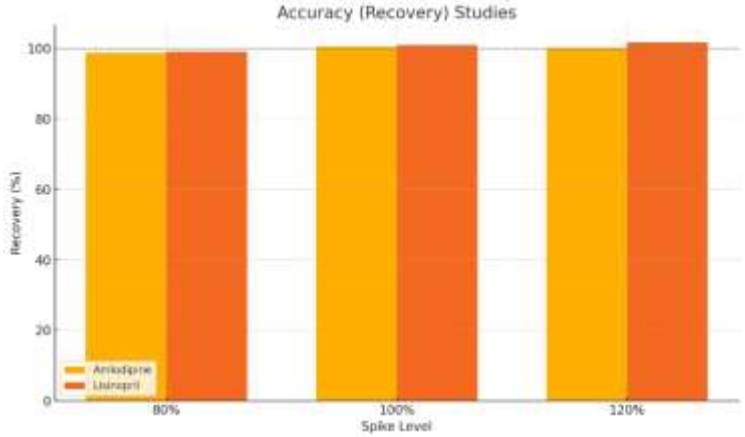

Figure 3. Accuracy (Recovery) Studies

3.1.3 Precision Data (Intra- and Inter-Day)

Table 3 presents the precision data for both Amlodipine Besylate and Lisinopril, evaluated under intra-day and inter-day conditions. Precision, expressed as relative standard deviation (RSD), is a critical parameter in method validation as it assesses the method's reproducibility under normal operating conditions. For Amlodipine, the intra-day mean peak area was 41,950 with an RSD of 1.48%, while the inter-day mean was 41,710 with an RSD of 1.44%. Similarly, for Lisinopril, the intra-day mean peak area was 35,980 with an RSD of 1.50%, and the inter-day mean was 36,150 with an RSD of 1.60%. In all cases, the %RSD values were well within the acceptable limit of ≤2%, indicating excellent precision. These results confirm that the method produces consistent and reliable results within a single day (repeatability) and across multiple days (intermediate precision). The low standard deviation and narrow variability in peak area further demonstrate the robustness and stability of the method. In summary, the low RSD values validate that the developed RP-HPLC method is precise for the simultaneous estimation of Amlodipine Besylate and Lisinopril and is suitable for routine use in pharmaceutical quality control laboratories.

Table 3. Precision Data (Intra- and Inter-Day)

Drug	Precision Type	Mean Peak Area	Standard Deviation	RSD (%)
Amlodipine	Intra-day	41,950	620	1.48
Lisinopril	Intra-day	35,980	540	1.50
Amlodipine	Inter-day	41,710	600	1.44
Lisinopril	Inter-day	36,150	580	1.60

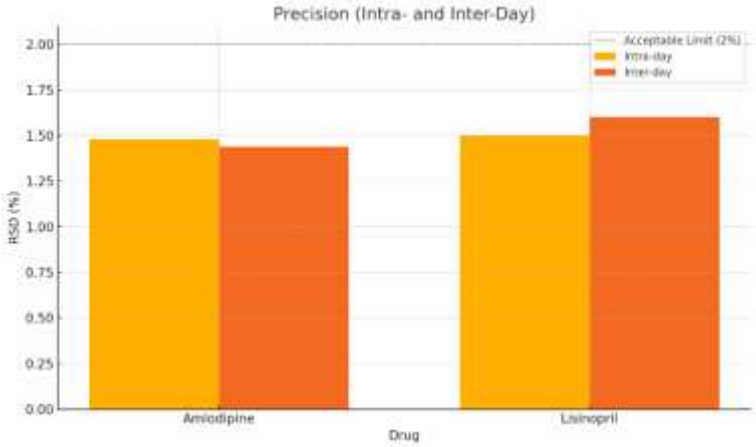

Figure 4. Precision (Intra- and Inter-Day)

3.1.4 Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Table 4 outlines the sensitivity of the developed RP-HPLC method in terms of its Limit of Detection (LOD) and Limit of Quantitation (LOQ) for Amlodipine Besylate and Lisinopril. These parameters reflect the method's

ability to detect and accurately quantify even trace levels of the analytes. The LOD for Amlodipine was found to be 0.29 µg/mL, while its LOQ was 0.88 µg/mL. For Lisinopril, the LOD and LOQ were 0.52 µg/mL and 1.57 µg/mL, respectively. These low values demonstrate the high sensitivity of the method, which is crucial for detecting low concentrations in pharmaceutical formulations, especially during stability studies or dissolution profiling. Such sensitivity ensures that the method is appropriate not only for assay determination but also for monitoring degradation products and impurities that may be present at very low levels. Moreover, the ability to detect concentrations below 1 µg/mL confirms the suitability of this method for stringent regulatory and quality assurance purposes. In conclusion, the low LOD and LOQ values establish that the developed method is highly sensitive and can be confidently applied in routine quality control, including trace analysis of Amlodipine and Lisinopril in combined dosage forms.

Table 4. Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Drug	**LOD (µg/mL)**	**LOQ (µg/mL)**
Amlodipine	0.29	0.88
Lisinopril	0.52	1.57

3.1.5 Robustness Study Results

Table 5 presents the robustness data for Amlodipine Besylate and Lisinopril, demonstrating the method's reliability under small deliberate variations in chromatographic parameters. Robustness is an essential validation parameter that confirms the method's capacity to remain unaffected by minor changes, ensuring consistent performance in varied laboratory environments. For Amlodipine, slight variations in flow rate (±0.1 mL/min), mobile phase composition (±2%), and detection wavelength (±2 nm) produced only minimal changes in retention time (5.76 to 5.82 minutes) and peak area (41,500 to 41,950), indicating method stability. Similarly, Lisinopril showed stable retention times between 3.19 and 3.23 minutes, with corresponding peak areas ranging narrowly from 35,600 to 36,020 under the same altered conditions. The consistency of these results affirms that the developed RP-HPLC method is robust. It maintains peak integrity and resolution, even when minor instrumental or procedural deviations occur. The negligible shift in chromatographic response under altered conditions also suggests the method's practical adaptability for routine use across different analysts, instruments, and laboratories. In conclusion, the robustness study confirms that the method is dependable and stable under varied operational conditions, further reinforcing its suitability for the routine quantitative analysis of Amlodipine and Lisinopril in pharmaceutical fixed-dose combinations.

Table 5. Robustness Study Results

Drug	**Parameter Changed**	**Retention Time (min)**	**Peak Area**
Amlodipine	Flow rate ±0.1 mL/min	5.82	41,800
Amlodipine	Mobile phase ±2%	5.76	41,500
Amlodipine	Wavelength ±2 nm	5.80	41,950
Lisinopril	Flow rate ±0.1 mL/min	3.23	35,800
Lisinopril	Mobile phase ±2%	3.19	35,600
Lisinopril	Wavelength ±2 nm	3.22	36,020

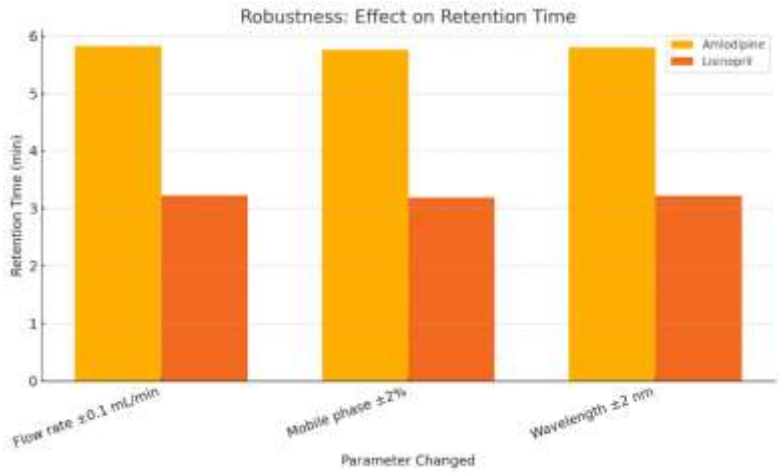

Figure 5. Robustness: Effect on Retention Time

3.1.6 System Suitability Parameters

System suitability testing is a critical part of method validation, ensuring that the chromatographic system performs adequately before and during analysis. Table 6 presents the system suitability parameters for both Amlodipine Besylate and Lisinopril, with all results meeting or exceeding the predefined acceptance criteria. The retention times for Amlodipine and Lisinopril were found to be 5.80 ± 0.02 min and 3.20 ± 0.01 min, respectively, demonstrating excellent repeatability and temporal stability of the method. The theoretical plate count for both drugs exceeded 2000 (6200 ± 150 for Amlodipine and 5700 ± 120 for Lisinopril), confirming high column efficiency and good peak sharpness. The tailing factors were 1.11 ± 0.03 for Amlodipine and 1.09 ± 0.02 for Lisinopril, both within the acceptable range of ≤2, indicating symmetric peak shapes and minimal column overloading or interference. Furthermore, the resolution (Rs) between the two peaks was 6.5 ± 0.2, well above the minimum requirement of 2.0, ensuring excellent peak separation without overlapping. Lastly, the %RSD of peak area for six replicate injections was below 1% for both drugs (0.87 for Amlodipine and 0.92 for Lisinopril), confirming the method's high precision and reliability. Collectively, these system suitability results validate that the RP-HPLC method is not only robust and precise but also compliant with regulatory expectations for chromatographic performance, making it ideal for routine analysis and quality control of Amlodipine and Lisinopril in fixed-dose formulations.

Table 6. System Suitability Parameters

Parameter	**Amlodipine Besylate**	**Lisinopril**	**Acceptance Criteria**
Retention Time (min)	5.80 ± 0.02	3.20 ± 0.01	Consistent for each run
Theoretical Plates	6200 ± 150	5700 ± 120	N > 2000
Tailing Factor	1.11 ± 0.03	1.09 ± 0.02	Tailing factor ≤ 2
Resolution (Rs)	6.5 ± 0.2	–	Rs > 2 between two peaks
%RSD of Peak Area	0.87	0.92	%RSD ≤ 2% for six replicate injections

4. CONCLUSION

A simple, robust, and precise reverse-phase HPLC method was successfully developed and validated for the simultaneous estimation of Amlodipine Besylate and Lisinopril in fixed-dose combination tablets. The method employed a C18 column with a mobile phase comprising acetonitrile and phosphate buffer (pH 3.5) in the ratio of 60:40 v/v, providing well-resolved peaks with retention times of approximately 5.8 minutes for Amlodipine and 3.2 minutes for Lisinopril. The detection wavelength was set at 210 nm, and the total analysis time was under 8 minutes, making the method suitable for high-throughput routine analysis. The developed method exhibited excellent linearity within the tested ranges (2–12 µg/mL for Amlodipine and 5–30 µg/mL for Lisinopril) with correlation coefficients exceeding 0.999 for both drugs. Accuracy, expressed through recovery studies, ranged between 98–102%, confirming the method's reliability for quantitative determination. The precision of the method was established through intra- and inter-day studies, with %RSD values remaining below 2%, ensuring repeatability and reproducibility. The limits of detection and quantitation demonstrated the method's sensitivity, and specificity studies confirmed that the excipients did not interfere with the detection of either drug. Furthermore, the method showed good robustness, remaining unaffected by minor changes in analytical conditions such as flow rate, wavelength, and mobile phase composition. Overall, the validated RP-HPLC method fulfills the regulatory requirements for analytical method validation as per ICH Q2(R1) guidelines. It can be confidently applied for routine quality control analysis, content uniformity, and stability studies of pharmaceutical formulations containing Amlodipine Besylate and Lisinopril. The method offers a cost-effective, reproducible, and efficient analytical tool for pharmaceutical industries and research laboratories involved in the development and manufacturing of fixed-dose combinations for antihypertensive therapy.

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