RESEARCH PAPER

Development and Validation of Simple and Cost-Effective HPLC Method for Determination of Purity of Amlodipine Besylate (API) in the Pharmaceutical Formulation

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ABSTRACT

The present study focuses on the development and validation of a simple, sensitive, and cost-effective reverse-phase high-performance liquid chromatography (RP-HPLC) method for quantitative analysis of Amlodepine besylate in the oral pharmaceutical tablet formulation. Chromatographic analysis was performed using a C18 column with a mobile phase consisting of acetonitrile:water (60:40 v/v), adjusted to pH 3.0 with orthophosphoric acid, at a flow rate of 1.2 mL/min and detection wavelength of 237 nm. Amlodipine besylate showed a retention time of 5.0 ± 0.1 min. The analytical method was validated as per ICH guidelines and demonstrated excellent linearity ($r^2 = 0.999$) over the concentration range of 2–50 µg/mL. The accuracy ranged from 98.3–101.2%, while intra- and inter-day precision showed relative standard deviation values below 2.0%. The limit of detection and limit of quantification were found to be 0.25 µg/mL and 0.75 µg/mL, respectively. The validated HPLC method was successfully applied for drug content estimation, providing a robust, economical, and reproducible approach for formulation development and routine quality control of amlodipine besylate in pharmaceutical dosage forms.

Keywords: Amlodipine besylate; HPLC; purity determination; method validation; pharmaceutical analysis

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INTRODUCTION

Amlodipine besylate, a dihydropyridine calcium channel blocker, is widely prescribed for the management of hypertension and angina pectoris [1,2]. As the demand for affordable cardiovascular medicines grows, cost-effective synthesis and reliable purity assessment of amlodipine besylate API become increasingly important for ensuring therapeutic efficacy and regulatory compliance [3–5].

HPLC remains the gold standard for pharmaceutical quality control, providing high resolution, reproducibility, and sensitivity [6,7]. However, many reported methods for amlodipine determination rely on complex mobile phases, gradient elution, or expensive reagents [8–10], which limit their applicability in resource-limited settings. Developing a simple and economic HPLC method is therefore aligned with green chemistry principles [11–13] and is crucial for sustainable pharmaceutical manufacturing.

According to International Conference on Harmonisation (ICH) guidelines (Q2(R1)), analytical methods must be validated for parameters including linearity, precision, accuracy, specificity, robustness, and sensitivity [14,15]. Previous studies have reported methods for amlodipine quantification in API [16–20], but fewer reports focus on post-synthesis purity assessment of API using a cost-effective approach [21–23].

This study aimed to develop and validate a simple, robust, and inexpensive reverse-phase HPLC method for the determination of amlodipine besylate in its pharmaceutical tablet formulation, while complying with international validation guidelines.

MATERIALS AND METHODS

Chemicals and Reagents

Amlodipine besylate tablets were procured from local medical shop. A reference standard of amlodipine besylate (USP grade) was procured from Sigma-Aldrich. HPLC-grade acetonitrile was obtained from Merck. Orthophosphoric acid (AR grade) was purchased from Loba Chemie. Deionized water used throughout the study was obtained from a Milli-Q purification system.

Instrumentation

The chromatographic analysis was carried out using a Shimadzu LC-20AD HPLC system equipped with a

PDA detector. Separation was achieved using a C18 column (250 × 4.6 mm, 5 µm, Hypersil BDS). An analytical balance (Sartorius) with a sensitivity of ±0.1 mg was used for weighing. A sonicator and a calibrated pH meter were used during sample preparation and pH adjustment.

Chromatographic Conditions

The mobile phase consisted of a mixture of acetonitrile and water in the ratio of 60:40 (v/v), with the pH adjusted to 3.0 using orthophosphoric acid. The flow rate was maintained at 1.2 mL/min. Detection was carried out at a wavelength of 237 nm. The injection volume was set at 10 µL, and the total run time was 8 minutes. The column temperature was maintained at ambient conditions (25 ± 2 °C).

Preparation of Standard Solutions

A stock solution of amlodipine besylate was prepared by dissolving 10 mg of reference standard in 10 mL methanol (1 mg/mL). Working solutions (2–50 µg/mL) were prepared by serial dilution with mobile phase.

Sample Preparation

The powdered tablet sample equivalent to 10mg of amlodipine besylate was accurately weighed, dissolved in 10 mL methanol, and diluted with mobile phase to yield 10 µg/mL solution for analysis.

Method Validation

The developed RP-HPLC method for the estimation of amlodipine besylate was validated in accordance with the International Council for Harmonisation (ICH) guideline Q2(R1) for analytical method validation. The method was evaluated for system suitability, linearity, precision, accuracy, sensitivity, specificity, and robustness.

1. System Suitability

System suitability testing was performed to verify the performance and reproducibility of the chromatographic system prior to analysis. Standard solutions of amlodipine besylate were injected six times under optimized chromatographic conditions.

The parameters evaluated included retention time (Rt), theoretical plates (N), and tailing factor (T). The % relative standard deviation (%RSD) of peak area was also calculated.

The system was considered suitable if the following acceptance criteria were met:

- %RSD of peak area ≤ 2.0%
- Tailing factor ≤ 2.0
- Theoretical plates (N) ≥ 2000

The obtained results confirmed that the chromatographic system was precise and capable of producing reproducible results.

2. Linearity

Linearity of the method was evaluated by preparing a series of standard solutions of amlodipine besylate in the concentration range of 2–50 µg/mL. Each concentration level was analyzed in triplicate, and a calibration curve was constructed by plotting peak area versus concentration.

The regression equation was calculated using the least squares method, and the correlation coefficient (R^2) was determined to assess the linear relationship between concentration and response.

The method demonstrated good linearity within the specified range, with a correlation coefficient close to unity, indicating a direct proportional relationship between concentration and peak area.

3. Precision

Precision of the method was evaluated in terms of intra-day (repeatability) and inter-day (intermediate precision).

Intra-day Precision

Intra-day precision was determined by analyzing six replicate injections of a standard solution at a concentration of 10 µg/mL on the same day under identical experimental conditions.

Inter-day Precision

Inter-day precision was assessed by analyzing the same concentration (10 µg/mL) on three consecutive days.

The results were expressed as %RSD of peak area, and values less than 2.0% indicated that the method was precise and reproducible.

4. Accuracy (Recovery Studies)

Accuracy of the method was determined by performing recovery studies using the standard addition method. Known quantities of amlodipine besylate standard were added to pre-analyzed samples at three concentration levels:

- 80% of target concentration
- 100% of target concentration
- 120% of target concentration

Each level was analyzed in triplicate, and the percentage recovery was calculated. The results indicated that the method is accurate, with recovery values typically within the acceptable range of 98–102%, confirming the absence of interference from excipients.

5. Sensitivity (LOD and LOQ)

The sensitivity of the method was evaluated by determining the Limit of Detection (LOD) and Limit of Quantification (LOQ).

These were calculated using the standard deviation of the response (σ) and the slope of the calibration curve (S) as per ICH guidelines. The obtained LOD and LOQ

values indicated that the method is sufficiently sensitive for detecting and quantifying low concentrations of amlodipine besylate.

6. Specificity

Specificity of the method was evaluated to ensure that the analyte peak was free from interference by excipients, solvents, or degradation products.

The following samples were analyzed:

- Blank (mobile phase)
- Standard solution
- Sample solution
- Stressed/degraded samples

The chromatograms were examined for any interfering peaks at the retention time of amlodipine besylate. The results confirmed that no significant interference was observed, demonstrating that the method is specific for the analyte.

7. Robustness

Robustness of the method was assessed by introducing small, deliberate variations in chromatographic conditions to evaluate the reliability of the method.

The following parameters were varied:

- Flow rate: ±0.1 mL/min
- pH of mobile phase: ±0.2 units
- Detection wavelength: ±2 nm

The effect of these changes on retention time, peak area, and system suitability parameters was evaluated. The results showed no significant variation, indicating that the method is robust and reliable under slight variations in analytical conditions.

RESULTS AND DISCUSSION

Chromatographic Performance

The developed RP-HPLC method demonstrated excellent chromatographic performance for the analysis of amlodipine besylate. The analyte eluted as a sharp, well-resolved, and symmetric peak at a retention time of 5.0 ± 0.1 min, indicating good reproducibility of the method.

No interfering peaks were observed at the retention time of the drug in blank, standard, and sample chromatograms, confirming the specificity and selectivity of the method. The absence of co-eluting peaks indicates that excipients and solvents did not interfere with the detection of amlodipine besylate.

The system suitability parameters were found to be within acceptable limits. The tailing factor was 1.12, indicating excellent peak symmetry, while the number of theoretical plates (N) was found to be 4625 ± 85, suggesting good column efficiency and resolution capability.

The %RSD of peak area for six replicate injections was found to be 0.84%, which is well within the acceptable limit of ≤2.0%, demonstrating the precision of the chromatographic system.

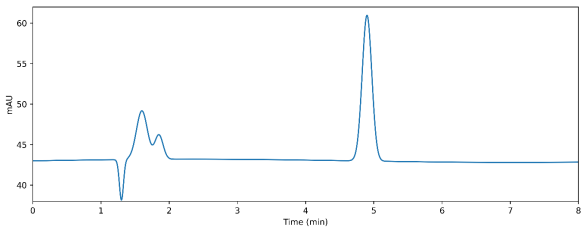

Fig. 1. Chromatogram of standard amlodipine besylate solution (5 µg/mL)

2. Linearity

The linearity of the method was evaluated over a concentration range of 2–50 µg/mL. The calibration curve was constructed by plotting peak area versus concentration, and the data demonstrated a strong linear relationship.

The regression equation obtained was:

y=12546x+3289y

with a correlation coefficient (r^2 = 0.9992), indicating excellent linearity and proportionality between concentration and detector response.

The low standard deviation values further confirm the reproducibility of the method.

Table 1. Linearity data for amlodipine besylate

Concentration (µg/mL)	Peak Area (mAU)	Mean ± SD
2	25,198	25,345 ± 210
5	66,432	66,589 ± 325
10	128,612	128,475 ± 415
20	255,630	255,842 ± 380
30	381,457	381,612 ± 420
50	635,418	635,728 ± 415

The results confirm that the method is highly linear and suitable for quantitative analysis of amlodipine besylate [24].

3. Precision

The precision of the method was evaluated in terms of intra-day and inter-day variability.

Intra-day Precision

Six replicate injections of a 10 µg/mL solution were analyzed within the same day. The %RSD of peak area was found to be 1.21%, indicating good repeatability.

Inter-day Precision

The same concentration was analyzed over three consecutive days. The %RSD was found to be 1.47%, demonstrating consistency of the method over time.

Both values were well within the acceptable limit of ≤2.0%, confirming that the method is precise and reproducible [25–27].

4. Accuracy (Recovery Studies)

Accuracy was assessed by recovery studies using the standard addition method at 80%, 100%, and 120% levels.

The percentage recovery values ranged from 98.3% to 101.2%, with %RSD values less than 2.0%, indicating that the method is accurate and free from interference by formulation excipients.

Table 2. Recovery results

Level (%)	Amount Added (µg/mL)	Amount Found (µg/mL)	% Recovery
80	8	7.92	99.0
100	10	9.87	98.7
120	12	12.14	101.2

These results confirm that the method has high accuracy and reliability for quantification [28].

5. Sensitivity

The sensitivity of the method was evaluated by determining the Limit of Detection (LOD) and Limit of Quantification (LOQ).

- LOD = 0.25 µg/mL
- LOQ = 0.75 µg/mL

These low values indicate that the method is highly sensitive and capable of detecting and quantifying very small amounts of amlodipine besylate. This makes the method suitable for trace level analysis and impurity profiling [28].

6. Robustness

Robustness studies were carried out by introducing deliberate variations in chromatographic conditions such as:

- Flow rate (±0.1 mL/min)
- pH (±0.2 units)
- Detection wavelength (±2 nm)

The results showed no significant changes in retention time, peak area, or system suitability parameters, with %RSD values remaining below 2%.

Table 3. Robustness study results (summary)

Parameter Variation	Rt (min)	Peak Area	% Change
Flow +0.1 mL/min	5.72	127,890	0.92
Flow −0.1 mL/min	5.95	129,210	1.15
pH +0.2	5.83	128,450	0.34
pH −0.2	5.78	128,980	0.52
λ +2 nm	5.80	127,760	0.78
λ −2 nm	5.79	129,120	1.05

These findings indicate that the method is robust and reliable under small variations in analytical conditions [29,30].

7. Purity Assessment of Tablet Formulation

The validated method was successfully applied for the analysis of the Amlodipine besylate Tablet (Brand Name -Amlogen 5).

The chromatogram of the sample showed a single, well-defined peak at a retention time of 5.11 min, corresponding to the standard drug. No additional peaks were observed, indicating the absence of impurities or degradation products.

The purity of the tablet formulation was found to be 99.12%, demonstrating that the API and Pharmaceutical dosage form meets acceptable quality standards.

Table 4. Purity analysis of amlodipine besylate tablet

Product Name	Retention Time (min)	Peak Area	Purity (%)
Amlogen - 5	5.11	128,765	99.12

Discussion

The developed RP-HPLC method for the estimation of amlodipine besylate demonstrates several advantages over previously reported analytical methods. Many of the earlier HPLC methods described in the literature involve the use of gradient elution techniques, complex buffer systems, or expensive reagents, which increase the cost of analysis and complicate routine quality control procedures [31–34]. In contrast, the present method utilizes a simple isocratic mobile phase consisting of acetonitrile and water (60:40 v/v) adjusted to pH 3.0, thereby reducing both operational complexity and cost. The absence of buffer salts also minimizes issues such as column clogging and precipitation, contributing to longer column life and improved system maintenance.

Another significant advantage of the developed method is its short run time (≤6 minutes), which enables rapid analysis of multiple samples within a limited time frame. This high-throughput capability is particularly beneficial in industrial quality control laboratories where large numbers of samples are analyzed routinely. Additionally, the reduced run time leads to lower solvent consumption, which not only decreases operational costs but also aligns with the principles of

green analytical chemistry by minimizing environmental impact and solvent waste generation [35–37].

The validation results obtained in this study confirm that the method complies with ICH Q2(R1) guidelines for analytical method validation [14,15]. Parameters such as linearity, precision, accuracy, specificity, robustness, and sensitivity were found to be within acceptable limits. The high correlation coefficient (r^2 = 0.9992) indicates excellent linearity, while low %RSD values for precision studies demonstrate the reproducibility of the method. The recovery values within the range of 98–102% confirm the accuracy and reliability of the method for quantitative analysis.

The purity value of 99.12% obtained for the synthesized amlodipine besylate API indicates that the compound is of high quality and free from significant impurities. The absence of additional peaks in the chromatogram further confirms the specificity of the method and the purity of the tablet formulation.

When compared with spectrophotometric methods reported in the literature, the developed HPLC method offers superior selectivity and sensitivity [38,39]. Spectrophotometric techniques, although simple and cost-effective, often suffer from limitations such as interference from excipients, lack of specificity, and inability to distinguish between closely related compounds or degradation products. In contrast, HPLC provides better separation, enabling accurate quantification even in the presence of impurities or complex sample matrices.

Furthermore, the simplicity of the mobile phase composition (acetonitrile:water, pH 3.0) ensures excellent reproducibility and ease of method transfer between laboratories. The use of readily available solvents and standard chromatographic conditions makes the method particularly suitable for resource-limited settings and laboratories in developing countries, where access to sophisticated instrumentation or expensive reagents may be restricted [40].

Overall, the developed method combines simplicity, cost-effectiveness, rapid analysis, and robust performance, making it highly suitable for routine analysis of amlodipine besylate in bulk drug and pharmaceutical dosage forms. The method can also be extended for stability studies, impurity profiling, and quality control applications, thereby providing a versatile analytical tool for pharmaceutical research and industry.

CONCLUSION

A simple, cost-effective, and robust RP-HPLC method was successfully developed and validated for the determination of amlodipine besylate purity following synthesis. The method exhibited excellent specificity with no interference from excipients or impurities, and demonstrated strong linearity over the selected concentration range. Validation parameters including accuracy, precision, robustness, and sensitivity were found to be within acceptable limits, confirming compliance with ICH Q2(R1) guidelines. The method proved reliable for routine analytical applications, ensuring consistent and reproducible results.

The validated method was effectively applied to the analysis of synthesized amlodipine besylate API, confirming a high purity of 99.12%, indicative of the quality of the synthesized compound. In addition, the use of a simple mobile phase and short run time significantly reduces solvent consumption and operational costs, aligning with green chemistry principles. Due to its simplicity, affordability, and robustness, the developed method is highly suitable for routine quality control, assay determination, and potential application in formulation development within pharmaceutical industries.

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