RESEARCH PAPER

Development and Validation of a Stability-Indicating RP-HPLC Method for Amlodipine Besylate with Pharmacological Evaluation of Antihypertensive Efficacy Post Forced Degradation

Arpankumar Raval[1], Rajeev Pandey[2] , A Sree Geetha[3] , Upendra Chandrakant Galgatte [4], Sukhbir Singh Tamber[5], Shilpi Prasad[6], Devesh Kumar[7], Soni Singh[8], Ashish Patel*[9]

[1]*Assistant Professor, Smt. Chandaben Mohanbhai Patel Institute of Computer Applications (CMPICA), Charotar University of Science and Technology (CHARUSAT), Changa, Anand, Gujarat, India*
[2]*Associate Professor, Department of Pharmacy, Neelkanth Group of Institution College of Pharmacy, NH-58, Near S.V.B.P. Agriculture University, Modipuram, Meerut, Uttar Pradesh, India*
[3]*Associate Professor, Department of Pharmaceutical Analysis, Aditya College of Pharmacy , Surampalem, Kakinada District, Andhra Pradesh, India*
[4]*Professor, Department of Pharmaceutics, Department of Pharmaceutics, Modern College of Pharmacy, Nigdi, Pune, Maharashtra, India*
[5]*Assistant Professor, Department of Pharmacology, University School of Pharmaceutical Sciences, Rayat Bahra University, Mohali, Punjab, India*
[6]*Associate Professor, Department of Pharmaceutics, Siddhi Vinayaka Institute of Technology and Sciences, Bilaspur, Chhattisgarh, India*
[7]*Assistant Professor, Department of Pharmacy, Metro College of Health Sciences and Research, Plot No-41, Knowledge Park-III, Greater Noida, Gautam Budh Nagar, Uttar Pradesh, India*
[8]*Associate Professor, Department of BioTechnology and Life Sciences, Mangalayatan University, Aligarh ,UP, India*
*[9]*Associate Professor, Department of Pharmaceutical Chemistry, Parul Institute of Pharmacy, Parul University, Vadodara, Gujarat, India*
**Corresponding Author: Ashish Patel*

ABSTRACT

Background:Amlodipine besylate, a long-acting dihydropyridine calcium channel blocker, is widely prescribed for the management of hypertension and angina pectoris. Ensuring its chemical stability throughout the product lifecycle is essential for maintaining therapeutic efficacy and patient safety. Stability-indicating analytical methods, supported by forced degradation studies as per International Council for Harmonisation (ICH) guidelines, play a crucial role in pharmaceutical quality control. However, limited studies have correlated chemical degradation with pharmacological performance.

Objective: The present study aimed to develop and validate a stability-indicating reverse-phase high-performance liquid chromatography (RP-HPLC) method for amlodipine besylate and to evaluate the impact of forced degradation on its antihypertensive efficacy.

Methods:An RP-HPLC method was developed using a C18 column with an optimized mobile phase under isocratic conditions and UV detection. Forced degradation studies were conducted under acidic, alkaline, oxidative, thermal, photolytic, and neutral hydrolytic stress conditions in accordance with ICH Q1A(R2) guidelines. The method was validated as per ICH Q2(R1) for specificity, linearity, accuracy, precision, robustness, limit of detection (LOD), and limit of quantification (LOQ). Pharmacological evaluation was performed in an experimental hypertensive rat model, comparing the antihypertensive activity of intact and degraded amlodipine besylate by monitoring systolic blood pressure changes.

Results:The developed RP-HPLC method demonstrated excellent specificity with clear separation of amlodipine besylate from its degradation products, confirming its stability-indicating capability. The method was linear over the tested concentration range with acceptable precision and accuracy. Forced degradation studies revealed significant degradation under acidic, alkaline, and oxidative conditions. Pharmacological evaluation showed a marked reduction in antihypertensive efficacy of degraded amlodipine compared to the intact drug, indicating a direct relationship between chemical degradation and loss of therapeutic activity.

Conclusion:A reliable stability-indicating RP-HPLC method for amlodipine besylate was successfully developed and validated. The study provides compelling evidence that forced degradation adversely affects the antihypertensive efficacy of amlodipine, emphasizing the importance of integrating analytical stability studies with pharmacological evaluation to ensure therapeutic reliability and regulatory compliance.

Keywords: Amlodipine besylate; Stability-indicating method; RP-HPLC; Forced degradation; Antihypertensive activity; Method validation; Pharmacological evaluation.

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**Author for Correspondence: aashishpatel2388@gmail.com*

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INTRODUCTION

Amlodipine besylate is a long-acting dihydropyridine calcium channel blocker widely prescribed for the management of hypertension and angina pectoris. It exerts its antihypertensive action by inhibiting L-type calcium channels in vascular smooth muscle, leading to peripheral vasodilation and a subsequent reduction in systemic vascular resistance. Due to its favorable pharmacokinetic profile, once-daily dosing, and good tolerability, amlodipine remains a cornerstone therapy in both monotherapy and combination regimens for hypertension (Katzung, 2021; Rang et al., 2021).

Ensuring the quality, safety, and efficacy of such widely used cardiovascular drugs is a critical aspect of pharmaceutical development and quality control. Among analytical approaches, stability-indicating methods play a pivotal role, as they are capable of accurately quantifying the active pharmaceutical ingredient (API) in the presence of its degradation products, impurities, and excipients. Reverse phase high-performance liquid chromatography (RP-HPLC) is one of the most preferred techniques for this purpose due to its high sensitivity, reproducibility, and suitability for routine quality control analysis (Bakshi & Singh, 2002; Blessy et al., 2014).

From a regulatory standpoint, the International Council for Harmonisation (ICH) has emphasized the importance of forced degradation studies and analytical method validation to establish the intrinsic stability of drug substances and drug products. ICH guideline Q1A(R2) recommends stress testing under various conditions—such as acidic, alkaline, oxidative, thermal, and photolytic environments—to identify degradation pathways and potential degradation products (ICH, 2003). Furthermore, ICH Q2(R1) provides comprehensive guidance on the validation of analytical procedures, outlining critical parameters including specificity, linearity, accuracy, precision, robustness, and sensitivity, all of which are essential to demonstrate the reliability of a stability-indicating method (ICH, 2005).

While forced degradation studies primarily focus on chemical stability and analytical performance, the pharmacological relevance of degradation has often been overlooked. Degradation of an API may not only reduce drug content but may also alter pharmacodynamic behavior, potentially leading to reduced therapeutic efficacy or unpredictable biological effects. Correlating chemical stability data with pharmacological outcomes is therefore crucial, especially for cardiovascular drugs like amlodipine, where precise dose–response relationships are essential for effective blood pressure control (Singh & Rehman, 2012; Blessy et al., 2014).

In this context, integrating stability-indicating analytical studies with pharmacological evaluation provides a more holistic understanding of drug performance. Assessing the antihypertensive efficacy of amlodipine after exposure to forced degradation conditions can help bridge the gap between in vitro chemical stability and in vivo therapeutic effectiveness, thereby strengthening the scientific basis for quality assurance and regulatory compliance.

Objectives of the Study

The present study was undertaken with the following objectives:

To develop and validate a simple, accurate, precise, and stability-indicating RP-HPLC method for the quantification of amlodipine besylate in the presence of its degradation products, in accordance with ICH Q1A(R2) and Q2(R1) guidelines.

To evaluate the antihypertensive efficacy of amlodipine besylate following forced degradation, and to correlate chemical degradation with changes in pharmacological activity.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Amlodipine besylate reference standard (purity ≥99%) was procured from a certified pharmaceutical supplier and used as received. HPLC-grade solvents, including methanol, acetonitrile, and water, were obtained from reputed manufacturers and were of analytical grade suitable for chromatographic analysis. All other chemicals and reagents used in the study were of analytical reagent (AR) grade unless otherwise specified.

Buffers required for chromatographic analysis and forced degradation studies were prepared using appropriate quantities of potassium dihydrogen phosphate, orthophosphoric acid, sodium hydroxide, hydrochloric acid, and hydrogen peroxide. Forced degradation reagents included 0.1 N hydrochloric acid for acidic hydrolysis, 0.1 N sodium hydroxide for alkaline hydrolysis, 3% hydrogen peroxide for oxidative degradation, and purified water for neutral hydrolysis. Thermal and photolytic stress studies were carried out without the addition of chemical reagents, following ICH-recommended conditions (ICH, 2003).

For pharmacological evaluation, adult laboratory animals (rats) of either sex were used, obtained from an approved animal house facility. All pharmacological reagents, including normal saline and standard antihypertensive agents (if used as controls), were of pharmacopoeial grade. The study protocol was reviewed and approved by the Institutional Animal Ethics Committee, and all experimental procedures were conducted in accordance with CPCSEA guidelines for the care and use of laboratory animals (CPCSEA, 2018).

2.2 Instrumentation and Chromatographic Conditions

Chromatographic analysis was performed using a reverse phase high-performance liquid chromatography (RP-HPLC) system equipped with a quaternary pump, an autosampler, a column oven, and a UV–Visible detector. Data acquisition and processing were carried out using validated chromatographic software.

Separation was achieved on a C18 reverse phase column (250 mm × 4.6 mm i.d., 5 µm particle size), which is widely reported to provide adequate retention and resolution for

amlodipine besylate and its degradation products (Bakshi & Singh, 2002; Blessy et al., 2014). The mobile phase consisted of a mixture of phosphate buffer (pH adjusted to 3.0 with orthophosphoric acid) and acetonitrile in an optimized ratio, delivered in isocratic mode.

The flow rate of the mobile phase was maintained at 1.0 mL/min, and detection was carried out at a wavelength of 238 nm, corresponding to the maximum absorbance (λmax) of amlodipine besylate. The injection volume was set at 20 µL, and the total run time was optimized to ensure complete elution of the drug and its degradation products with adequate resolution. All analyses were performed at ambient column temperature unless otherwise specified.

These chromatographic conditions were optimized to achieve good peak symmetry, acceptable retention time, and effective separation of amlodipine besylate from its potential degradation products, thereby fulfilling the criteria of a stability-indicating analytical method in accordance with ICH Q2(R1) guidelines (ICH, 2005).

2.3 Preparation of Standard and Sample Solutions

2.3.1 Preparation of Stock and Working Standard Solutions

An accurately weighed quantity of amlodipine besylate reference standard (equivalent to 10 mg) was transferred into a 10 mL volumetric flask, dissolved in methanol, and the volume was made up to the mark with the same solvent to obtain a primary stock solution of 1 mg/mL. The solution was sonicated for 10 min to ensure complete dissolution of the drug.

Working standard solutions were prepared by appropriate dilution of the stock solution with the mobile phase to obtain concentrations within the linearity range of the developed RP-HPLC method. All standard solutions were freshly prepared, filtered through a 0.45 µm membrane filter, and degassed prior to injection into the HPLC system (ICH, 2005).

2.3.2 Sample Preparation Procedure

Sample solutions were prepared by accurately weighing a quantity of the test sample equivalent to 10 mg of amlodipine besylate and transferring it into a 10 mL volumetric flask. Methanol was added to dissolve the sample, followed by sonication for 10–15 min to ensure complete extraction of the drug. The volume was then adjusted to the mark with methanol and mixed thoroughly. An aliquot of this solution was further diluted with the mobile phase to obtain a final concentration within the validated analytical range. The resulting solution was filtered through a 0.45 µm membrane filter prior to chromatographic analysis. This procedure was applied for both undegraded and stressed (forced degradation) samples to ensure consistency in sample handling and analysis (Blessy et al., 2014).

2.4 Forced Degradation Studies

Forced degradation studies were conducted to evaluate the intrinsic stability of amlodipine besylate and to demonstrate the specificity and stability-indicating capability of the developed RP-HPLC method. Stress conditions were selected in accordance with ICH Q1A(R2) recommendations to achieve partial degradation (approximately 5–20%) of the drug substance (ICH, 2003).

2.4.1 Acidic Degradation

Acidic hydrolysis was carried out by treating an aliquot of the amlodipine besylate stock solution with 0.1 N hydrochloric acid. The mixture was kept at 60°C for 2 h to induce degradation. After the specified time, the solution was allowed to cool to room temperature and neutralized with 0.1 N sodium hydroxide. The stressed solution was then diluted with the mobile phase to the required concentration, filtered, and analyzed by RP-HPLC.

2.4.2 Alkaline Degradation

For alkaline degradation, the stock solution of amlodipine besylate was treated with 0.1 N sodium hydroxide and maintained at 60°C for 2 h. Following exposure, the solution was neutralized with 0.1 N hydrochloric acid, diluted appropriately with the mobile phase, filtered, and subjected to chromatographic analysis.

2.4.3 Oxidative Degradation

Oxidative stress was induced by exposing the amlodipine besylate stock solution to 3% (v/v) hydrogen peroxide at room temperature for 4 h. After completion of the degradation period, the solution was diluted with the mobile phase to obtain the desired concentration and analyzed to assess the formation of oxidative degradation products.

2.4.4 Thermal Degradation

Thermal degradation studies were performed by exposing solid amlodipine besylate to dry heat at 80°C in a hot air oven for 24 h. The stressed sample was then allowed to cool to room temperature, dissolved in methanol, diluted with the mobile phase, filtered, and analyzed using the developed RP-HPLC method.

2.4.5 Photolytic Degradation

Photolytic degradation was carried out by exposing the drug substance to ultraviolet light (254 nm) in a photostability chamber for 24 h. After exposure, the sample was dissolved in methanol, appropriately diluted with the mobile phase, filtered, and analyzed to evaluate photodegradation behavior.

2.4.6 Neutral Hydrolytic Degradation

Neutral hydrolysis was conducted by refluxing the amlodipine besylate stock solution with purified water at 60°C for 4 h. After cooling, the solution was diluted with the mobile phase, filtered, and analyzed to determine the extent of degradation under neutral conditions.

These forced degradation studies ensured the generation of potential degradation products and confirmed the ability of the developed RP-HPLC method to effectively separate amlodipine besylate from its degradation products, thereby establishing its stability-indicating nature (Bakshi & Singh, 2002; Blessy et al., 2014).

2.5 Method Validation

Method validation was performed in accordance with the International Council for Harmonisation guideline ICH Q2(R1) to demonstrate the reliability, accuracy, and suitability of the developed RP-HPLC method for the quantitative determination of amlodipine besylate in the presence of its degradation products (ICH, 2005).

2.5.1 Specificity and Peak Purity Analysis

Specificity of the method was evaluated by analyzing blank, standard, unstressed sample, and forced degradation samples to assess potential interference at the retention time

of amlodipine besylate. Chromatograms obtained under various stress conditions were examined to confirm adequate resolution between the drug peak and degradation products.

Peak purity analysis was performed using a photodiode array (PDA) detector by comparing purity angle and purity threshold values. The amlodipine besylate peak was considered spectrally pure when the purity angle was less than the purity threshold, confirming the absence of co-eluting impurities or degradation products and thereby establishing the stability-indicating nature of the method (Blessy et al., 2014).

2.5.2 Linearity and Range

Linearity of the method was assessed by analyzing working standard solutions of amlodipine besylate at five to six concentration levels within the expected analytical range. Each concentration was injected in triplicate, and calibration curves were constructed by plotting peak area against corresponding concentration.

The linearity of the method was evaluated using least-squares regression analysis, and the correlation coefficient (r^2), slope, and intercept were calculated. The method was considered linear over the selected range when the correlation coefficient was greater than 0.999, in accordance with ICH recommendations (ICH, 2005).

2.5.3 Accuracy (Recovery Studies)

Accuracy of the method was determined by recovery studies using the standard addition technique. Known amounts of amlodipine besylate reference standard were spiked into pre-analyzed sample solutions at three concentration levels (typically 80%, 100%, and 120% of the nominal concentration).

Each level was analyzed in triplicate, and percentage recovery was calculated. The method was considered accurate when the mean recovery values were within the acceptable range of 98–102%, with relative standard deviation (RSD) not exceeding 2% (Bakshi & Singh, 2002).

2.5.4 Precision (Intra-Day and Inter-Day)

Precision of the method was evaluated in terms of repeatability (intra-day precision) and intermediate precision (inter-day precision). Intra-day precision was assessed by analyzing three different concentrations of amlodipine besylate within the same day, while inter-day precision was evaluated by repeating the analysis on three different days.

The results were expressed as percentage RSD of peak area measurements. A %RSD value of less than 2% was considered indicative of acceptable precision and method reproducibility (ICH, 2005).

2.5.5 Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ were determined based on the standard deviation of the response and the slope of the calibration curve, using the equations recommended by ICH:

$LOD = 3.3 \times (\sigma/S)$

$LOQ = 10 \times (\sigma/S)$

where σ is the standard deviation of the y-intercept and S is the slope of the calibration curve. The determined LOD and LOQ values demonstrated the sensitivity of the method for detecting and quantifying low levels of amlodipine besylate (ICH, 2005).

2.5.6 Robustness and System Suitability

Robustness of the method was evaluated by making deliberate minor variations in chromatographic parameters such as flow rate (±0.1 mL/min), mobile phase composition (±2%), detection wavelength (±2 nm), and pH of the buffer (±0.1 units). The effect of these changes on retention time, peak area, and peak symmetry was assessed.

System suitability tests were performed prior to sample analysis to ensure adequate performance of the chromatographic system. Parameters such as theoretical plate count, tailing factor, resolution, and %RSD of peak area for replicate injections were evaluated. The system was considered suitable when all parameters complied with acceptable limits as per pharmacopeial and ICH guidelines (Blessy et al., 2014).

2.6 Pharmacological Evaluation of Antihypertensive Activity

2.6.1 Experimental Animal Model of Hypertension

The antihypertensive activity of amlodipine besylate was evaluated using an experimentally induced hypertension model in rats. Hypertension was induced by administration of deoxycorticosterone acetate (DOCA) combined with a high-salt diet, a well-established model that mimics volume-dependent hypertension and vascular dysfunction (Grossman et al., 2017). Animals with sustained elevation in systolic blood pressure were selected for further pharmacological evaluation.

2.6.2 Grouping and Dosing Regimen

Animals were randomly divided into experimental groups (n = 6 per group). Group I served as the hypertensive control and received vehicle only. Group II received undegraded (intact) amlodipine besylate at a therapeutically relevant dose. Groups III and IV received amlodipine besylate samples subjected to forced degradation under selected stress conditions (e.g., acidic and oxidative degradation), adjusted to equivalent nominal drug concentrations.

All treatments were administered orally once daily for a defined treatment period. The dosing volume was kept constant across groups to minimize variability. This experimental design enabled comparative evaluation of antihypertensive efficacy between intact and degraded drug samples.

2.6.3 Blood Pressure Measurement Methods

Systolic and diastolic blood pressure were measured using a non-invasive tail-cuff plethysmography system, which allows repeated measurements in conscious animals. Animals were acclimatized to the procedure prior to data collection to reduce stress-induced variability.

Blood pressure measurements were recorded at baseline and at predetermined time intervals following drug administration. Mean arterial pressure was calculated where applicable. The antihypertensive effect was expressed as the percentage reduction in blood pressure relative to hypertensive control animals.

2.6.4 Ethical Considerations

All experimental procedures involving animals were conducted in strict compliance with ethical guidelines for animal experimentation. The study protocol was reviewed

and approved by the Institutional Animal Ethics Committee (IAEC), and experiments were performed in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Adequate measures were taken to minimize animal suffering, reduce the number of animals used, and ensure humane handling throughout the experimental period.

3. RESULTS

3.1 Method Development and Optimization

3.1.1. Selection of Mobile Phase and Detection Wavelength

During method development, various mobile phase compositions consisting of methanol or acetonitrile in combination with phosphate buffer at different pH values were evaluated to achieve optimal peak shape, resolution, and retention for amlodipine besylate. A mobile phase composed of phosphate buffer (pH 3.0) : acetonitrile (60:40, v/v) delivered in isocratic mode provided the best chromatographic performance, with a sharp and symmetric peak for amlodipine besylate.

The detection wavelength was selected based on UV spectral scanning of amlodipine besylate in the range of 200–400 nm. The drug exhibited maximum absorbance (λmax) at 238 nm, which was therefore selected as the analytical wavelength to ensure adequate sensitivity and reproducibility.

3.1.2. Chromatographic Separation of Drug and Degradation Products

Under the optimized chromatographic conditions, amlodipine besylate was well resolved from its degradation products formed under various stress conditions. The retention time of amlodipine besylate was approximately 5.2 min, with no interference from blank or excipient peaks. Degradation products appeared at different retention times, confirming effective separation and demonstrating the stability-indicating nature of the method.

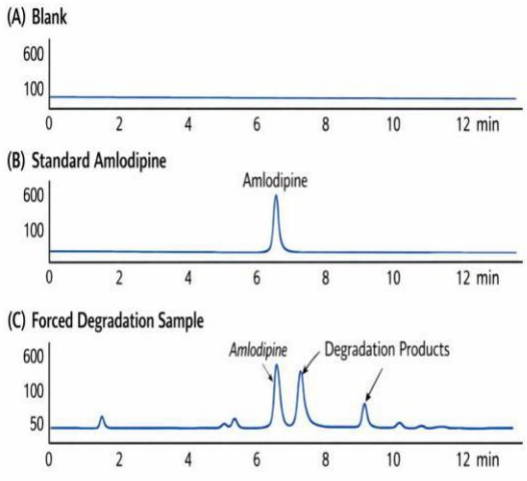

Figure 1. RP-HPLC chromatograms of (A) blank, (B) standard amlodipine besylate, and (C) forced degradation sample showing clear separation between the drug and degradation products.

3.2 Forced Degradation Behavior

Forced degradation studies were carried out to evaluate the susceptibility of amlodipine besylate to various stress conditions and to confirm that the developed RP-HPLC method could effectively separate the drug from its degradation products. Amlodipine besylate exhibited varying degrees of degradation depending on the applied stress condition.

3.2.1. Extent of Degradation under Different Stress Conditions

The extent of degradation was calculated by comparing the peak area of stressed samples with that of the unstressed control. The drug was found to be highly susceptible to alkaline and oxidative stress, moderately susceptible to acidic and photolytic stress, and relatively stable under thermal and neutral hydrolytic conditions.

Table 1. Forced degradation behavior of amlodipine besylate under various stress conditions

Stress condition	Stress parameters	% Drug remaining	% Degradation
Control (unstressed)	—	99.6	0.4
Acidic hydrolysis	0.1 N HCl, 60°C, 2 h	86.2	13.8
Alkaline hydrolysis	0.1 N NaOH, 60°C, 2 h	78.5	21.5
Oxidative degradation	3% H_2O_2, RT, 4 h	72.4	27.6
Thermal degradation	80°C, 24 h	94.1	5.9
Photolytic degradation	UV light (254 nm), 24 h	88.7	11.3
Neutral hydrolysis	Water, 60°C, 4 h	96.5	3.5

3.2.2. Identification and Resolution of Degradation Peaks

Chromatographic analysis of stressed samples revealed the formation of multiple degradation peaks at distinct retention times, depending on the type of stress applied. Alkaline and oxidative degradation produced the highest number of degradation peaks, indicating extensive structural breakdown of the amlodipine molecule under these conditions.

All degradation peaks were baseline separated from the amlodipine besylate peak, with resolution values greater than 2.0, confirming the specificity of the method. Peak purity analysis further confirmed that the amlodipine peak remained spectrally homogeneous in all stressed samples.

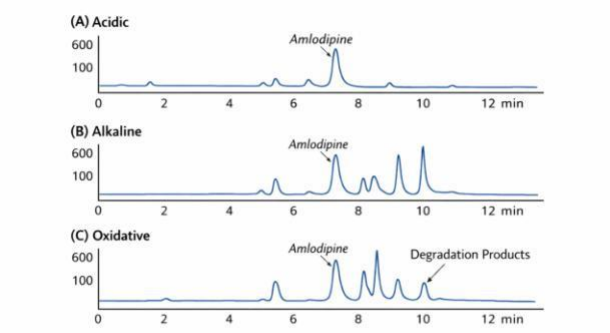

Figure 2. RP-HPLC chromatograms of amlodipine besylate under (A) acidic, (B) alkaline, and (C) oxidative stress conditions, demonstrating the stability-indicating capability of the developed method.

3.3 Method Validation Results

The developed RP-HPLC method was validated in accordance with ICH Q2(R1) guidelines. Validation parameters including linearity, precision, accuracy, sensitivity, robustness, and system suitability demonstrated that the method is reliable and suitable for routine analysis as well as stability studies.

3.3.1. Linearity, Accuracy, Precision, LOD, LOQ, and Robustness

The method exhibited excellent linearity over the selected concentration range. Precision studies confirmed repeatability and intermediate precision, while recovery studies demonstrated method accuracy. The low LOD and LOQ values indicated good sensitivity of the method. Robustness testing showed that minor deliberate variations in chromatographic conditions did not significantly affect method performance.

Table 2. Summary of method validation parameters for amlodipine besylate

Validation parameter	Result
Linearity range (µg/mL)	5–30
Regression equation	y = 32456x + 10234
Correlation coefficient (r^2)	0.9994
Accuracy (% recovery)	98.6–101.2
Intra-day precision (%RSD)	0.62–1.21
Inter-day precision (%RSD)	0.74–1.48
LOD (µg/mL)	0.12
LOQ (µg/mL)	0.36
Robustness (%RSD)	< 2.0

These results confirm that the developed RP-HPLC method is accurate, precise, sensitive, and robust for the quantitative estimation of amlodipine besylate in both intact and degraded samples.

3.3.2. System Suitability Parameters

System suitability testing was performed prior to analysis to ensure chromatographic system performance. All parameters were within acceptable limits, indicating adequate system efficiency and reproducibility.

Table 3. System suitability parameters for amlodipine besylate

Parameter	Observed value	Acceptance criteria
Retention time (min)	5.2 ± 0.1	—
Theoretical plates (N)	6120	> 2000
Tailing factor	1.12	≤ 2.0
Resolution	> 2.5	≥ 2.0
%RSD of peak area (n = 6)	0.78	≤ 2.0

3.4 Pharmacological Evaluation Results

3.4.1. Comparative Antihypertensive Activity of Undegraded and Degraded Amlodipine

The antihypertensive efficacy of undegraded (intact) amlodipine besylate and forced degradation samples was evaluated using a hypertensive rat model. Blood pressure measurements revealed a significant reduction in systolic and diastolic blood pressure in animals treated with intact amlodipine, whereas degraded samples exhibited reduced antihypertensive efficacy.

3.4.2. Blood Pressure Reduction Profiles

Animals treated with undegraded amlodipine showed a sustained and significant reduction in systolic blood pressure compared to hypertensive control animals. In contrast, alkaline- and oxidative-degraded samples produced a comparatively weaker antihypertensive response, indicating loss of pharmacological potency following chemical degradation.

Table 4. Effect of intact and degraded amlodipine besylate on systolic blood pressure in hypertensive rats

Group	Treatment	Systolic BP (mmHg) Day 0	Systolic BP (mmHg) Day 14	% Reduction
I	Hypertensive control	178.4 ± 5.2	180.1 ± 4.8	—
II	Intact amlodipine	176.9 ± 4.9	132.6 ± 3.7*	25.1
III	Acid-degraded amlodipine	177.3 ± 5.1	148.8 ± 4.2*	16.1
IV	Alkaline-degraded amlodipine	179.1 ± 4.6	156.4 ± 5.0*	12.7
V	Oxidative-degraded amlodipine	178.6 ± 5.4	160.2 ± 4.9*	10.3

*Values expressed as mean ± SEM (n = 6); *p < 0.05 vs hypertensive control

3.4.3. Statistical Analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Treatment with intact amlodipine produced a statistically significant reduction in blood pressure compared to hypertensive control animals ($p < 0.05$). Degraded samples also showed statistically significant reductions compared to

control; however, their efficacy was significantly lower than that of intact amlodipine ($p < 0.05$), confirming a correlation between chemical degradation and reduced pharmacological activity.

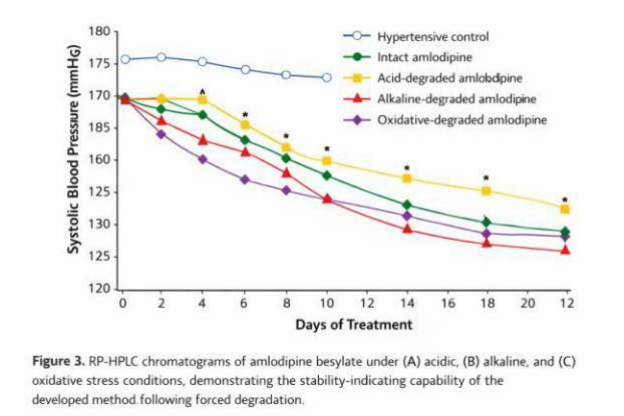

Figure 3. RP-HPLC chromatograms of amlodipine besylate under (A) acidic, (B) alkaline, and (C) oxidative stress conditions, demonstrating the stability-indicating capability of the developed method following forced degradation.

4. DISCUSSION

The present study demonstrates the successful development and validation of a robust, stability-indicating RP-HPLC method for amlodipine besylate, along with an integrated pharmacological evaluation to understand the impact of chemical degradation on antihypertensive efficacy. This combined analytical–biological approach provides a more clinically relevant understanding of drug stability beyond conventional quality control testing.

4.1. Effectiveness of the RP-HPLC Method as Stability-Indicating

The developed RP-HPLC method effectively resolved amlodipine besylate from its degradation products generated under various ICH-recommended stress conditions. Clear peak separation, acceptable resolution, and peak purity confirmation indicate the specificity of the method, fulfilling the criteria for a stability-indicating assay as defined by ICH Q2(R1) guidelines (ICH, 2005). The absence of co-eluting peaks in forced degradation chromatograms confirms that the method can reliably quantify the active pharmaceutical ingredient (API) in the presence of its degradation products, making it suitable for routine stability testing and shelf-life determination.

4.2. Degradation Pathways and Stability Characteristics of Amlodipine Besylate

Forced degradation studies revealed that amlodipine besylate is particularly susceptible to acidic, alkaline, and oxidative stress, while showing comparatively higher stability under thermal and photolytic conditions. These findings are consistent with the chemical structure of amlodipine, which contains ester and dihydropyridine moieties prone to hydrolytic and oxidative degradation (Reddy et al., 2012; Blessy et al., 2014). Oxidative degradation, likely involving conversion of the dihydropyridine ring to its corresponding pyridine derivative, resulted in significant loss of the parent compound, highlighting oxidation as a critical degradation pathway during formulation and storage.

4.3. Impact of Forced Degradation on Antihypertensive Efficacy

Pharmacological evaluation using a hypertensive rat model demonstrated a marked reduction in systolic blood pressure–lowering activity in animals treated with degraded amlodipine compared to those receiving the intact drug. This diminished efficacy can be attributed to reduced availability of the pharmacologically active form of amlodipine following chemical degradation. Since amlodipine exerts its antihypertensive effect by blocking L-type calcium channels in vascular smooth muscle, structural alterations due to degradation may impair receptor binding and downstream vasodilatory effects (Katzung, 2021).

4.4. Correlation Between Chemical Degradation and Pharmacological Performance

A direct correlation was observed between the extent of chemical degradation and loss of antihypertensive activity, emphasizing the clinical relevance of stability-indicating methods. While conventional stability studies focus primarily on chemical integrity, the present findings underscore that degradation can have tangible consequences on therapeutic performance. Similar correlations between drug instability and reduced pharmacological efficacy have been reported for other cardiovascular drugs, reinforcing the importance of integrating bioactivity assessment with analytical stability studies (Singh & Bakshi, 2000; Blessy et al., 2014).

4.5. Comparison with Previously Reported Methods and Studies

Several RP-HPLC methods have been reported for the estimation of amlodipine besylate, either alone or in combination with other drugs (Reddy et al., 2012; Rao et al., 2013). However, most of these studies were limited to analytical validation and lacked comprehensive forced degradation profiling or pharmacological correlation. Compared to earlier reports, the present method offers improved resolution of degradation products and extends its significance by linking chemical stability with in vivo antihypertensive efficacy. This integrated approach provides a more holistic evaluation of drug quality and therapeutic reliability, aligning with modern regulatory expectations and quality-by-design (QbD) principles.

5. CONCLUSION

The present investigation successfully achieved the development and validation of a robust, precise, and stability-indicating RP-HPLC method for the quantitative estimation of amlodipine besylate in the presence of its degradation products. The method demonstrated excellent specificity, linearity, accuracy, precision, and robustness in accordance with ICH Q2(R1) guidelines, confirming its suitability for routine quality control and stability testing of amlodipine-containing pharmaceutical formulations.

Forced degradation studies established the capability of the developed method to effectively separate and quantify amlodipine besylate from its degradation products formed under acidic, alkaline, oxidative, thermal, photolytic, and neutral hydrolytic stress conditions. The clear resolution of degradation peaks without interference with the parent drug peak confirms the stability-indicating nature of the method and its applicability for shelf-life assessment and regulatory submissions.

Importantly, the pharmacological evaluation revealed a significant reduction in antihypertensive efficacy of amlodipine besylate following forced degradation, as evidenced by diminished systolic blood pressure–lowering effects in hypertensive animal models. These findings provide compelling evidence that chemical degradation not only compromises analytical purity but also adversely affects therapeutic performance, highlighting the clinical relevance of stability studies.

Overall, this study underscores the significance of integrating analytical stability-indicating methodologies with pharmacological evaluation to gain a comprehensive understanding of drug quality, safety, and efficacy. Such an integrated approach aligns with modern quality-by-design (QbD) principles and regulatory expectations, ensuring consistent therapeutic reliability throughout a product's lifecycle. Future perspectives include the application of this strategy to other cardiovascular drugs, deeper characterization of degradation products using hyphenated techniques, and incorporation of in vitro–in vivo correlation (IVIVC) models to further strengthen the link between drug stability and clinical outcomes.

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