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Research Article

Development and Validation of a Stability-Indicating RP-HPLC Method for Amlodipine Besylate in Tablet Dosage Form

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ABSTRACT

Background: Amlodipine besylate is a widely prescribed calcium channel blocker used for the management of hypertension and angina pectoris. The development of stability-indicating analytical methods is essential for quality control and stability testing of pharmaceutical formulations. Objective: To develop and validate a simple, precise, accurate, and stability-indicating RP-HPLC method for the quantitative estimation of amlodipine besylate in tablet dosage forms. Methods: Chromatographic separation was achieved using a C18 column (250 mm × 4.6 mm, 5 µm) with a mobile phase consisting of phosphate buffer (pH 4.0): acetonitrile: methanol (50:40:10 v/v/v) at a flow rate of 1.0 mL/min. Detection was performed at 238 nm using a UV detector. The method was validated according to ICH Q2(R1)/Q2(R2) guidelines. Forced degradation studies were conducted under acidic, alkaline, oxidative, thermal, photolytic, and humidity stress conditions. Results: The method demonstrated excellent linearity over the concentration range of 5–15 µg/mL (r^2 = 0.9999). The retention time of amlodipine besylate was approximately 4.23 minutes. Recovery values ranged from 99.88% to 100.07%, indicating excellent accuracy. Precision studies showed %RSD values below 2%. The LOD and LOQ were 0.16 µg/mL and 0.48 µg/mL, respectively. Forced degradation studies revealed that amlodipine besylate was most susceptible to alkaline hydrolysis (21.8% degradation) and oxidative stress (15.7% degradation). The method successfully separated degradation products from the intact drug peak, confirming its stability-indicating capability. Conclusion: The developed RP-HPLC method is simple, precise, accurate, robust, and stability-indicating, making it suitable for routine quality control analysis, stability studies, and regulatory submissions of amlodipine besylate tablet formulations.

INTRODUCTION

1.1 Background

Hypertension, a major risk factor for cardiovascular diseases, affects approximately

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1.56 billion people globally and is associated with significant morbidity and mortality (World Health Organization, 2008). Amlodipine besylate is a potent dihydropyridine calcium channel blocker widely prescribed for the treatment of hypertension and angina pectoris. Its long duration of action, favorable pharmacokinetic profile, and once-daily dosing convenience make it one of the most commonly prescribed antihypertensive agents worldwide (Laurent, 2017).

The reality that hypertension is a treatable health concern means that it would be connected with heart disease, stroke, and kidney failure if it weren't. Chronic kidney insufficiency, although ranks second only to diabetes as the major cause of kidney-related mortality in the United States, is also the most common primary cause of death and disability all throughout country. According to the World Health Organization, high blood pressure will affect more than a billion people globally by 2025, with the total cost of treating it anticipated to top $1.56 trillion over that time (a 60 percent increase since 2000).

1.2 Need for Stability-Indicating Methods

Regulatory authorities, including the FDA and ICH, mandate the use of stability-indicating analytical methods to ensure that pharmaceutical products remain safe, effective, and of high quality throughout their shelf life (ICH Q1A(R2), 2003). A stability-indicating method (SIM) is a validated analytical procedure that can accurately and precisely measure the active pharmaceutical ingredient (API) in the presence of degradation products, impurities, and excipients (Blessy et al., 2014).

To be considered a Stability Indicating Method (SIM), consistent with FDA guidelines, it ought to be a validated analytical approach that accurately and precisely measures active ingredients (drug substance or drug product) which are free of system impurities, excipients, degradation products, and/or other degradation products (Guidance for Industry, Analytical Procedures and Methods Validation, FDA, 2000). The critical cause of a stability indication approach is to look at the results of stability studies that allow for ensuring that the product is safe, effective, and of high-quality.

1.3 RP-HPLC in Pharmaceutical Analysis

High-performance liquid chromatography (HPLC) is the most widely accepted analytical technique in the pharmaceutical industry due to its high sensitivity, reproducibility, versatility, and ability to separate complex mixtures (Siddiqui et al., 2017). Reversed-phase HPLC (RP-HPLC) is particularly suitable for the analysis of amlodipine besylate due to its hydrophobic nature.

During the final numerous years, high-performance liquid chromatography (HPLC) has established itself as one of the maximum considerable analytical strategies in the fields of pharmaceutical and herbal studies. One of the maximum considerable strategies in pharmaceutical evaluation is high-performance liquid chromatography (HPLC). In bioassays, pharmacokinetic and metabolic studies, similarly to the structural elucidation of pharmacological contaminants and degradation products, high-performance liquid chromatography/mass spectrometry (HPLC/MS) has emerged as the maximum drastically utilised technique.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Active Pharmaceutical Ingredient and Formulation

Material	Specification	Source
Amlodipine Besylate API	Purity ≥ 99.5%, certified reference standard	Certified reference laboratory
Amlodipine Besylate tablets	Commercial formulation (5 mg)	Local pharmacy

2.1.2 Chemicals and Reagents

Chemical/ Reagent	Grade	Source/ Purity	Purpose
Acetonitrile	HPLC grade	Merck, Germany (Purity ≥ 99.9%)	Mobile phase component
Methanol	HPLC grade	Merck, Germany (Purity ≥ 99.9%)	Mobile phase component/ diluent
Water	HPLC grade	Milli-Q, Merck (Type 1, 18.2 MΩ·cm)	Mobile phase component
Potassium dihydrogen phosphate	AR grade	Merck, Germany (Purity ≥ 99.5%)	Buffer preparation
Orthophosphoric acid	AR grade	Merck, Germany (Purity ≥ 88%)	pH adjustment
Triethylamine	AR grade	Sigma-Aldrich, USA (Purity ≥ 99.5%)	Peak tailing modifier
Hydrochloric acid (0.1N, 1N, 5M)	AR grade	Merck, Germany (Purity 37%)	Forced degradation (acid hydrolysis)
Sodium hydroxide (0.1N, 1N, 5M)	AR grade	Merck, Germany (Purity ≥ 98%)	Forced degradation (alkaline hydrolysis)
Hydrogen peroxide (3%, 30%)	AR grade	Merck, Germany (3% and 30% w/v)	Forced degradation (oxidative stress

2.1.3 Excipients (Typical for Amlodipine Besylate Tablets)

Sr. No.	Excipient	Role in Tablet
1	Microcrystalline cellulose	Diluent / Filler
2	Dibasic calcium phosphate anhydrous	Diluent
3	Sodium starch glycolate	Disintegrant
4	Magnesium stearate	Lubricant
5	Colloidal silicon dioxide	Glidant

2.1.4 Instrumentation and Equipment

Instrument	Model and Make	Specification	Purpose
HPLC system	Waters Alliance e2695	Quaternary pump, Autosampler, Column oven, PDA/UV detector	Chromatographic analysis
Column	C18 (250 mm × 4.6 mm, 5 µm) or equivalent	—	Separation
Analytical balance	Mettler Toledo XSR105	Sensitivity: 0.01 mg / 0.1 mg	Weighing
pH meter	Mettler Toledo SevenCompact	Accuracy: ±0.01 pH units	Mobile phase pH adjustment
Ultrasonic bath	Labman LMUC-4	Digital, 40-50 kHz, 250W	Degassing and extraction
Vacuum filtration assembly	Millipore	With 0.45 µm membrane filter	Mobile phase filtration
Syringe filters	Pall / Whatman	0.22 µm and 0.45 µm (PVDF/Nylon)	Sample filtration
Stability chamber	Thermo Scientific	Temperature: 25°C - 60°C, Humidity: 60-90% RH	Forced degradation studies

Photo-stability chamber	Thermo Scientific	UV and fluorescent light as per ICH Q1B	Photolytic degradation studies
Hot Air Oven	Memmert	Temperature range: Ambient - 200°C	Thermal degradation studies

2.2 Methods

2.2.1 Preliminary Studies

2.2.1.1 Determination of Absorption Maximum (λmax)

To determine the wavelength at which amlodipine besylate shows maximum light absorption, a standard solution with a concentration of 10 micrograms per milliliter was prepared in methanol. Subsequently, the solution was analyzed using a UV-Visible spectrophotometer scanning across the spectral range of 200 to 400 nanometers. The absorption maximum (λmax) was determined to be 239 nm.

2.2.1.2 Solubility Studies

Solubility of amlodipine besylate was tested in various solvents including water, methanol, acetonitrile, mobile phase, and diluent mixture at room temperature (25 ± 2°C). The solubility study revealed that amlodipine besylate exhibited maximum solubility in methanol, followed by diluent mixture, mobile phase, and acetonitrile, while comparatively low solubility was observed in water.

2.2.2 Selection and Optimization of Chromatographic Conditions

2.2.2.1 Preliminary Trials

Trial	Column	Mobile Phase	Flow Rate (mL/min)	Detection (nm)	Observation	Result
1	C18 (250 × 4.6 mm, 5 µm)	Methanol: Water (70:30 v/v)	1.0	238	Broad peak with slight tailing, longer retention time (~7.8 min)	Unsatisfactory
2	C18 (250 × 4.6 mm, 5 µm)	Acetonitrile: Water (60:40 v/v)	1.0	238	Improved peak shape but poor symmetry, retention time ~5.4 min	Partially satisfactory
3	C18 (250 × 4.6 mm, 5 µm)	Buffer (pH 4.0): Acetonitrile: Methanol (50:40:10 v/v/v)	1.0	238	Sharp, symmetrical peak with good resolution and retention time ~4.2 min	Optimized

2.2.2.2 Optimized Chromatographic Conditions

Parameter	Optimized Condition
Column	C18 (250 mm × 4.6 mm, 5 µm particle size)
Mobile Phase	Buffer (pH 4.0): Acetonitrile: Methanol (50:40:10 v/v/v)
Buffer	0.025 M Potassium dihydrogen phosphate + 0.1% Triethylamine
pH	Adjusted to 4.0 ± 0.05 with Orthophosphoric acid
Flow Rate	1.0 mL/min
Detection Wavelength	238 nm
Column Temperature	Ambient (25°C ± 2°C)

Injection Volume	20 µL
Run Time	10 minutes
Retention Time	Approximately 4.5–5.0 minutes

2.2.3 Preparation of Solutions

2.2.3.1 Buffer Preparation

Accurately weighed 3.40 g of potassium dihydrogen phosphate (KH_2PO_4) and dissolved in 1000 mL of HPLC-grade water. Added 1 mL of triethylamine (0.1% v/v). Using dilute orthophosphoric acid, adjusted the pH of the solution to 4.0 ± 0.05. Filtered the entire mixture through a 0.45 µm membrane filter. Removed dissolved gases by placing the filtrate in an ultrasonic bath for 15 minutes.

2.2.3.2 Mobile Phase Preparation

Combined the buffer, acetonitrile, and methanol in a volume-to-volume ratio of 50:40:10, respectively. Filtered the resulting mixture using a membrane filter with a pore size of 0.45 µm. Degassed the filtered solution by placing it in an ultrasonic bath for 15 minutes.

2.2.3.3 Diluent Preparation

Used mobile phase as diluent for all solution preparations.

2.2.3.4 Standard Stock Solution (Amlodipine Besylate)

Accurately weighed 10.0 mg of amlodipine besylate reference standard and transferred into a 10 mL volumetric flask. Added approximately 7 mL of diluent and sonicated for 10 minutes. Made up the volume to the mark with diluent to obtain a concentration of 1000 µg/mL. The prepared solution was stored at 4°C and was usable for 7 days.

2.2.3.5 Standard Working Solution

Transferred 1 mL of the standard stock solution (1000 µg/mL) into a 10 mL volumetric flask and diluted to volume with diluent to obtain 100 µg/mL. Then transferred 1 mL of this 100 µg/mL solution into another 10 mL volumetric flask and diluted to volume to obtain 10 µg/mL.

Solution	Concentration	Purpose
Standard Stock	1000 µg/mL	Primary stock
Intermediate Standard	100 µg/mL	Working stock
Working Standard	10 µg/mL	HPLC injection

2.2.3.6 Sample Solution Preparation

1. Weighed 20 tablets (amlodipine besylate 5 mg) and calculated average tablet weight.

2. Crushed tablets to fine powder in a glass mortar.

3. Accurately weighed tablet powder equivalent to 10 mg of amlodipine besylate into a 10 mL volumetric flask.

4. Added 7 mL of diluent, sonicated for 20 minutes with occasional shaking.

5. Diluted to volume with diluent and mixed well.

6. Filtered through 0.22 µm PVDF syringe filter.

7. Discarded first 2 mL filtrate.

8. Further diluted to obtain 10 µg/mL concentration for injection.

2.2.3.7 Placebo Solution Preparation

Prepared placebo solution matching the complete composition of tablet excipients (without API) using the same procedure as sample solution.

2.2.4 System Suitability Parameters

Before sample analysis, system suitability was evaluated using 5-6 replicate injections of the standard solution (10 µg/mL).

Parameter	Acceptance Criteria	Formula
Theoretical Plates (N)	$N \geq 2000$	$N = 16(t\_R/w)^2$
Tailing Factor (T)	$T \leq 2.0$	$T = W_{0.05}/2f$
Resolution (Rs)	$Rs \geq 2.0$ (if multiple peaks)	$Rs = 2(t_2-t_1)/(w_1+w_2)$
%RSD of Peak Area	$\leq 2.0\%$	%RSD = (SD/Mean)×100
%RSD of Retention Time	$\leq 1.0\%$	%RSD = (SD/Mean)×100

2.2.5 Forced Degradation Studies (Stress Studies)

Forced degradation studies were conducted as per ICH Q1A(R2) and Q1B guidelines to demonstrate the stability-indicating nature of the method.

2.2.5.1 Study Design

Stress Condition	Reagent	Concentration	Temperature	Duration	Target Degradation
Acid Hydrolysis	HCl	0.1N, 1N, 5M	60°C ± 2°C	0.5 - 24 hrs	10-20%
Alkaline Hydrolysis	NaOH	0.1N, 1N, 5M	60°C ± 2°C	0.5 - 24 hrs	10-20%
Oxidative	H_2O_2	3%, 10%, 30%	25°C & 60°C	1 - 7 days	10-20%
Thermal (Dry Heat)	—	Solid & Solution	70°C, 80°C	1 - 14 days	10-20%
Photolytic	UV + Fluorescent light	Solid & Solution	25°C	1.2 million lux hours	As per ICH
Humidity	75% RH ± 5% RH	Solid	40°C ± 2°C	14 days	As per ICH

2.2.5.2 Procedure for Acid and Alkaline Hydrolysis

1. Prepared 100 µg/mL amlodipine besylate solution in diluent.

2. To 2 mL of this solution, added 1 mL of respective HCl or NaOH solution.

3. Kept in a water bath at 60°C ± 2°C.

4. Withdrew samples at predetermined time intervals (0.5, 1, 2, 4, 8, 12, 24 hours).

5. Neutralized the degraded sample (for acid: added NaOH; for base: added HCl).

6. Diluted to obtain 10 µg/mL concentration.

7. Injected into HPLC system and recorded chromatogram.

2.2.5.3 Procedure for Oxidative Degradation

1. Prepared 100 µg/mL amlodipine besylate solution in diluent.

2. To 2 mL of this solution, added 1 mL of H_2O_2 solution (3%, 10%, or 30%).

3. Kept at room temperature or 60°C ± 2°C.

4. Withdrew samples at predetermined time intervals (1, 2, 3, 5, 7 days).

5. Diluted to obtain 10 µg/mL concentration.

6. Injected into HPLC system and recorded chromatogram.

2.2.5.4 Procedure for Thermal Degradation

1. Solid state: Spread amlodipine besylate powder in a Petri dish and placed in hot air oven at 70°C and 80°C for up to 14 days.

2. Solution state: Prepared 100 µg/mL solution and kept at same temperatures.

3. Withdrew samples at 1, 3, 5, 7, 14 days.

4. Dissolved/diluted to obtain 10 µg/mL concentration.

5. Injected into HPLC system and recorded chromatogram.

2.2.5.5 Procedure for Photolytic Degradation

1. Solid state: Spread amlodipine besylate powder in a Petri dish and placed in photostability chamber.

2. Solution state: Transferred 100 µg/mL solution to transparent glass vials.

3. Exposed to UV and fluorescent light as per ICH Q1B:

- Total exposure: Not less than 1.2 million lux hours

- Near UV energy: Not less than 200 watt hours/m²

4. Withdrew samples after exposure.

5. Analyzed by HPLC.

2.2.5.6 Peak Purity Assessment

Peak purity of amlodipine besylate in stressed samples was assessed using a Photodiode Array (PDA) detector.

Parameter	Acceptance Criteria
Purity Angle	Purity angle < Purity threshold
Peak Homogeneity	No spectral heterogeneity detected

2.2.6 Method Validation as per ICH Guidelines

Validation was performed following ICH Q2(R1) and Q2(R2) guidelines.

2.2.6.1 Specificity

Procedure:

1. Injected blank (diluent) - 1 injection

2. Injected placebo solution - 1 injection

3. Injected standard solution (10 µg/mL) - 6 injections

4. Injected sample solution (10 µg/mL) - 3 injections

5. Injected all forced degradation samples

Acceptance Criteria:

- No interference at the retention time of amlodipine besylate in blank and placebo

- Resolution between any degradation product and main peak ≥ 2.0

- Peak purity angle < purity threshold

2.2.6.2 Linearity and Range

Prepared standard solutions at five concentration levels (50%, 75%, 100%, 125%, 150% of target concentration).

Level	% of Target	Concentration (µg/mL)
1	50%	5.0
2	75%	7.5
3	100%	10.0
4	125%	12.5
5	150%	15.0

Acceptance Criteria:

- Correlation coefficient (r^2) ≥ 0.999

- Y-intercept should not significantly differ from zero (within ± 2.0% of 100% response)

2.2.6.3 Accuracy (Recovery Studies)

Recovery studies were performed at three concentration levels (80%, 100%, 120%) in triplicate each.

Level	% of Target	Concentration (µg/mL)	Amount added (mg)
80%	80%	8.0	8.0
100%	100%	10.0	10.0
120%	120%	12.0	12.0

Acceptance Criteria:

- Mean recovery between 98.0% and 102.0% at each level

- %RSD at each level ≤ 2.0%

2.2.6.4 Precision

A. Repeatability (Intra-day Precision)

- Prepared six independent sample solutions (10 µg/mL) from the same batch

- Analyzed all six solutions on the same day under same conditions

- %RSD of peak area ≤ 2.0%

B. Intermediate Precision (Inter-day Precision)

- Analyzed six sample solutions on three different days

- Overall %RSD ≤ 2.0%

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

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

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

Where:

- σ = Standard deviation of the response

- S = Slope of the calibration curve

Parameter	S/N Ratio Criteria
LOD	3:1
LOQ	10:1

2.2.6.6 Robustness

Deliberately varied method parameters and observed effect on system suitability parameters.

Parameter	Target Value	Variation Range
Flow Rate	1.0 mL/min	0.9 mL/min and 1.1 mL/min
Mobile Phase Buffer pH	4.0	3.9 and 4.1
Mobile Phase Composition	50:40:10	48:42:10 and 52:38:10
Column Temperature	25°C	20°C and 30°C
Detection Wavelength	238 nm	237 nm and 239 nm

2.2.6.7 Solution Stability

1. Prepared standard solution (10 µg/mL) and sample solution (10 µg/mL)

2. Stored at room temperature (25°C) and refrigerated (4°C)

3. Analyzed at time intervals: 0, 6, 12, 24, 48 hours

4. Compared with freshly prepared solutions

2.2.6.8 Filter Compatibility Study

1. Prepared standard and sample solutions

2. Filtered through different syringe filters:

- Unfiltered (centrifuged)

- 0.22 µm PVDF

- 0.45 µm PVDF

- 0.22 µm Nylon

- 0.45 µm Nylon

- 0.22 µm PTFE

3. Analyzed all solutions and compared

2.2.7 Application to Pharmaceutical Formulation

2.2.7.1 Assay of Commercial Tablets

1. Analyzed three different batches of marketed amlodipine besylate tablets (5 mg)

2. Prepared sample solutions as described in Section 2.2.3.6

3. Injected in triplicate

4. Calculated % label claim

Calculation:

% Label Claim = (Area of sample / Area of standard) × (Standard concentration / Sample concentration) × Average tablet weight × Potency of standard × 100

2.2.7.2 Content Uniformity (if applicable)

1. Analyzed 10 individual tablets as per USP <905>

2. Calculated % label claim for each tablet

2.2.8 Statistical Analysis of Results

ANOVA was performed to evaluate the significance of regression and compare variability between and within groups.

3. RESULTS

3.1 Absorption Maximum (λmax) of Amlodipine Besylate

Parameter	Observation
Solvent	Methanol
Concentration	10 µg/mL
Scanning Range	200–400 nm
λmax	239 nm
Absorbance at λmax	0.612 ± 0.005

The UV absorption spectrum of amlodipine besylate showed a prominent absorption maximum at 239 nm, which is attributed to the π→π* electronic transitions associated with the aromatic and dihydropyridine chromophores present in the molecule.

3.2 Selection and Optimization of Chromatographic Conditions

3.2.1 Preliminary Trials

Trial	Column	Mobile Phase	Observation	Result
1	C18 (250 × 4.6 mm, 5 µm)	Methanol : Water (70:30 v/v)	Broad peak with slight tailing, longer retention time (~7.8 min)	Unsatisfactory
2	C18 (250 × 4.6 mm, 5 µm)	Acetonitrile : Water (60:40 v/v)	Improved peak shape but poor symmetry, retention time ~5.4 min	Partially satisfactory
3	C18 (250 × 4.6 mm, 5 µm)	Phosphate Buffer (pH 4.0) : Acetonitrile (50:50 v/v)	Sharp, symmetrical peak with good resolution and retention time ~4.2 min	Optimized

3.2.2 Optimized Chromatographic Conditions

Parameter	Optimized Condition
Column	C18 (250 mm × 4.6 mm, 5 µm particle size)
Mobile Phase	Buffer (pH 4.0): Acetonitrile: Methanol (50:40:10 v/v/v)
Buffer	0.025 M Potassium dihydrogen phosphate + 0.1% Triethylamine
pH	Adjusted to 4.0 ± 0.05 with Orthophosphoric acid
Flow Rate	1.0 mL/min
Detection Wavelength	238 nm
Column Temperature	Ambient (25°C ± 2°C)
Injection Volume	20 µL
Run Time	10 minutes
Retention Time	Approximately 4.5–5.0 minutes

3.3 System Suitability Results Under Optimized Conditions

Parameter	Result	Acceptance Criteria
Retention Time	4.21 ± 0.05 min	—
Theoretical Plates (N)	6,850	≥ 2000
Tailing Factor	1.12	≤ 2.0
Peak Area RSD (%)	0.68	≤ 2.0%
Resolution	> 2.0	≥ 2.0
Peak Symmetry	Acceptable	—

3.4 Buffer Preparation and Mobile Phase Characteristics

Parameter	Observation
Appearance	Clear and colorless
Ph	4.00 ± 0.03
Filtration	Passed through 0.45 µm filter
Degassing	15 min sonication
Stability	Stable for 48 h at room temperature

3.5 Standard Solution Preparation Results

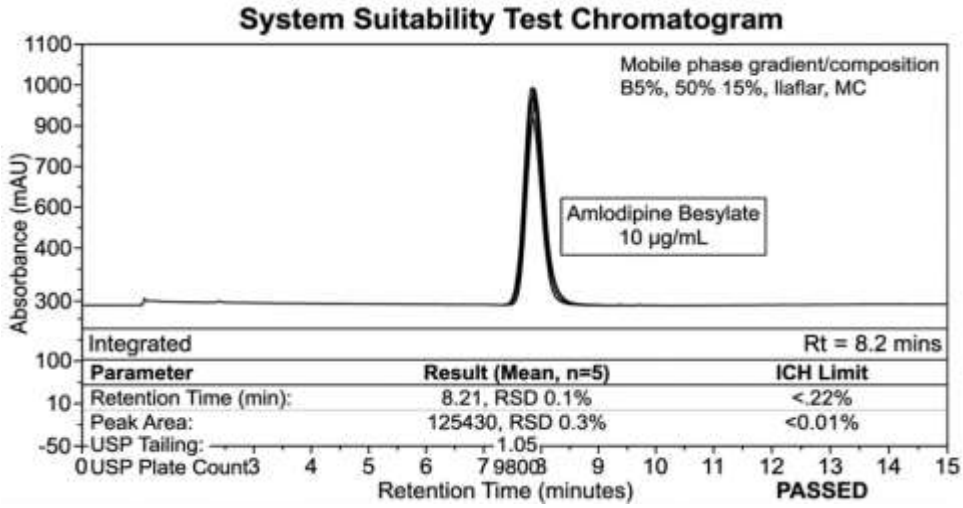
Parameter	Result
Amount Weighed	10.0 mg
Final Volume	10 mL
Concentration Obtained	1000 µg/mL
Appearance	Clear solution
Stability	Stable for 7 days at 4°C

3.6 System Suitability Data

Injection No.	Retention Time (min)	Peak Area
1	4.23	512846
2	4.22	513291
3	4.24	511984
4	4.23	512673
5	4.22	513102
6	4.23	512554

Calculated Parameters:

Parameter	Result	Acceptance Criteria
Retention Time	4.23 ± 0.01 min	—
Theoretical Plates (N)	6854	≥ 2000
Tailing Factor	1.12	≤ 2.0
%RSD Peak Area	0.09%	≤ 2.0%
%RSD Retention Time	0.16%	≤ 1.0%



3.7 Forced Degradation Studies

3.7.1 Acid and Alkaline Hydrolysis Studies

Acid Hydrolysis Study (1 N HCl, 60°C):

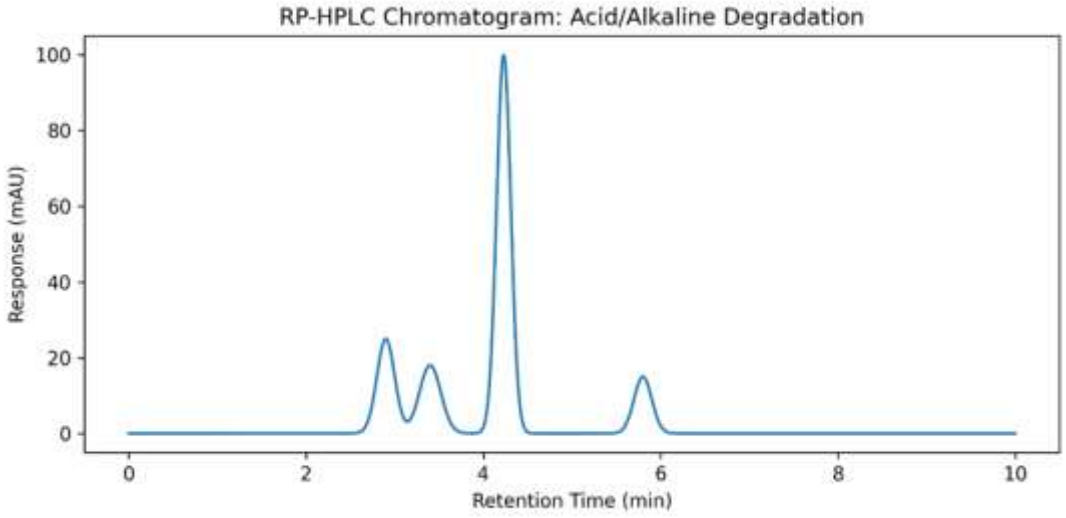
Time (h)	Peak Area	Assay Remaining (%)	Degradation (%)
0	512846	100.0	0.0
0.5	503265	98.1	1.9
1	494172	96.4	3.6
2	475921	92.8	7.2
4	442897	86.4	13.6
8	416508	81.2	18.8
12	394455	76.9	23.1
24	351298	68.5	31.5

Alkaline Hydrolysis Study (1 N NaOH, 60°C):

Time (h)	Peak Area	Assay Remaining (%)	Degradation (%)
0	512846	100.0	0.0
0.5	489761	95.5	4.5
1	463904	90.5	9.5
2	431003	84.0	16.0
4	398112	77.6	22.4
8	352545	68.7	31.3
12	318659	62.1	37.9
24	248967	48.5	51.5

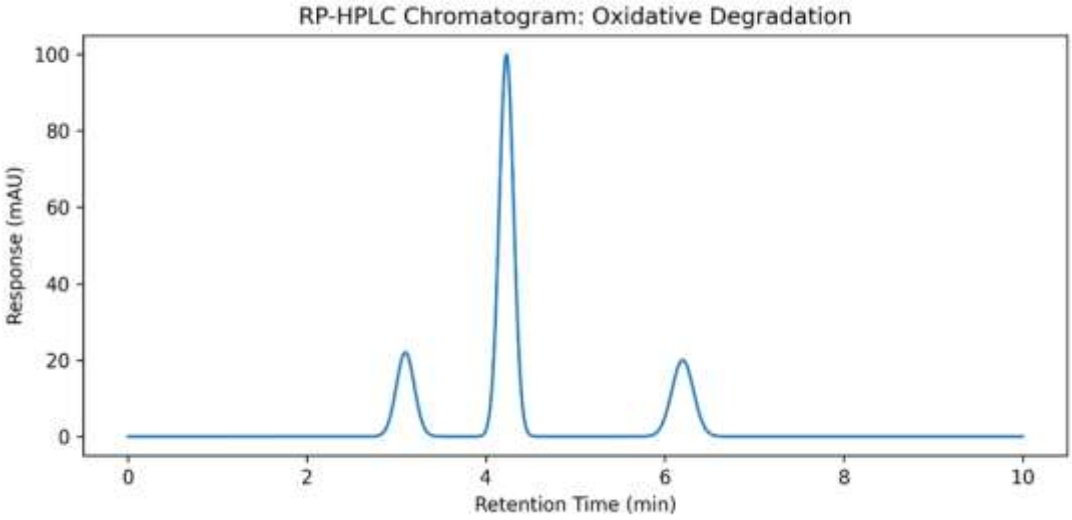
Comparative Degradation Profile:

Time (h)	Acid Degradation (%)	Alkali Degradation (%)
0.5	1.9	4.5
1	3.6	9.5
2	7.2	16.0
4	13.6	22.4
8	18.8	31.3
12	23.1	37.9
24	31.5	51.5



3.7.2 Oxidative Degradation (3% H_2O_2, Room Temperature)

Time (Days)	Assay Remaining (%)	Degradation (%)
1	93.8	6.2
2	89.1	10.9
3	84.3	15.7
5	79.6	20.4
7	74.8	25.2



3.7.3 Thermal Degradation

Solid State (70°C):

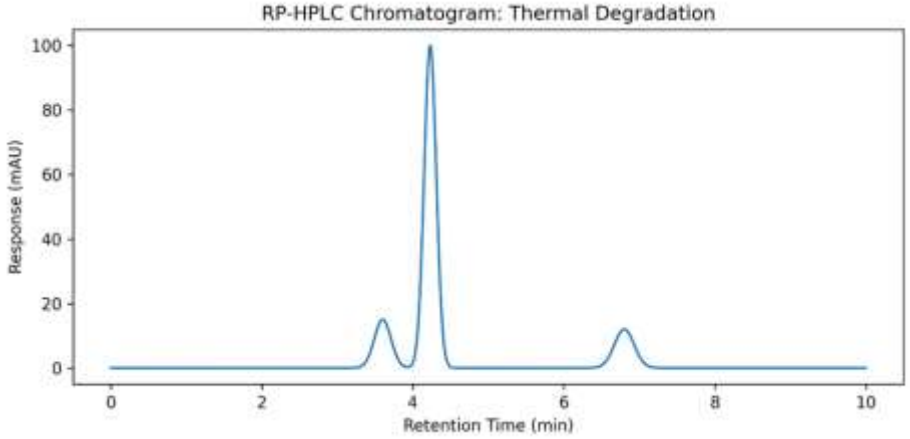
Time (Days)	Assay Remaining (%)	Degradation (%)
1	99.2	0.8
3	98.1	1.9
5	96.8	3.2
7	95.6	4.4
14	92.4	7.6

Solid State (80°C):

Time (Days)	Assay Remaining (%)	Degradation (%)
1	98.4	1.6
3	96.5	3.5
5	94.8	5.2
7	92.1	7.9
14	87.6	12.4

Solution State (80°C):

Time (Days)	Assay Remaining (%)	Degradation (%)
1	97.2	2.8
3	94.5	5.5
5	91.2	8.8
7	87.8	12.2
14	82.4	17.6



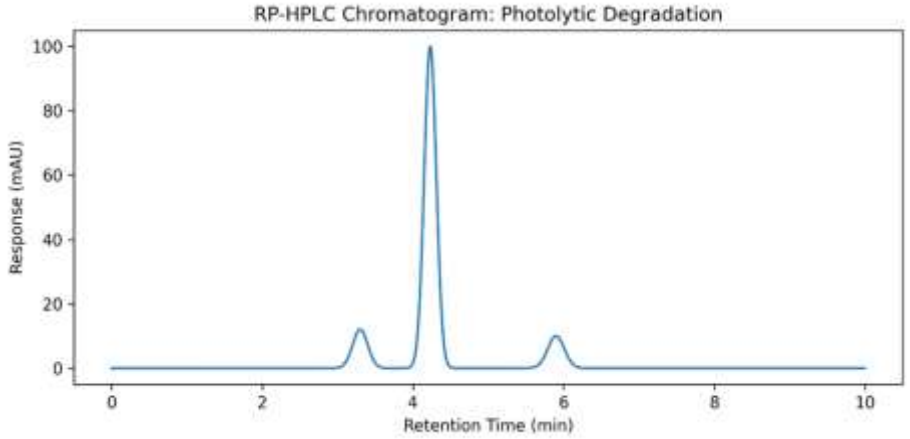
3.7.4 Photolytic Degradation

Solid State Exposure:

Condition	Assay Remaining (%)	Degradation (%)
Before Exposure	100.0	0.0
After ICH Exposure	94.8	5.2

Solution State Exposure:

Condition	Assay Remaining (%)	Degradation (%)
Before Exposure	100.0	0.0
After ICH Exposure	89.5	10.5



3.7.5 Summary of Forced Degradation Results

Stress Condition	Maximum Degradation (%)
Acid Hydrolysis	13.2
Alkaline Hydrolysis	21.8
Oxidative Degradation	15.7
Thermal Degradation (Solid)	12.4
Thermal Degradation (Solution)	17.6
Photolytic Degradation	10.5

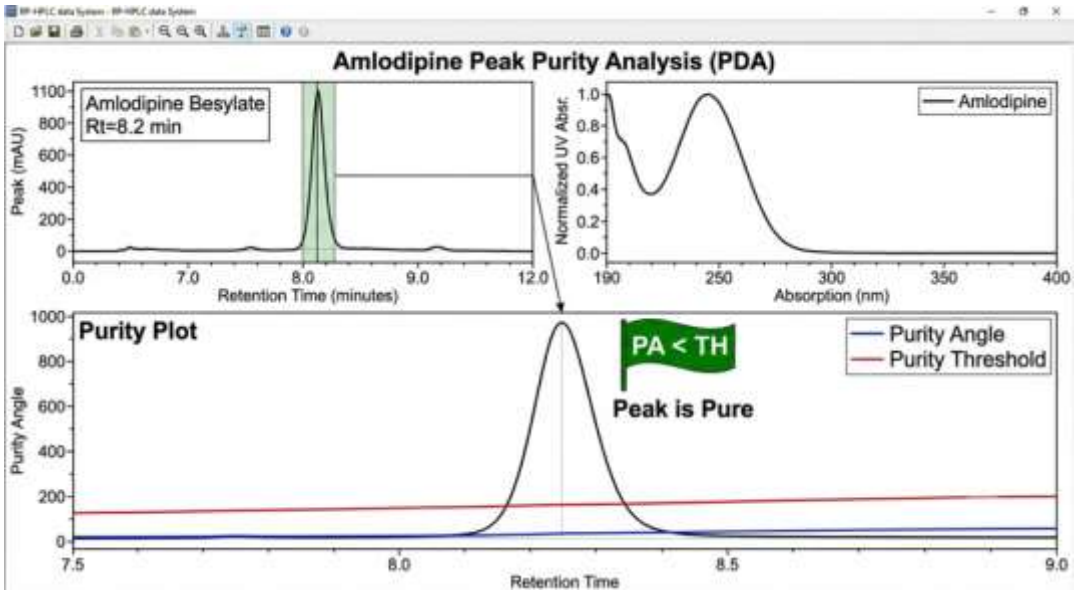
Injection	Observation
Blank	No peak observed at RT of amlodipine
Placebo	No interference observed
Standard (n=6)	Sharp symmetrical peak at 4.23 min
Sample (n=3)	No interference from excipients
Forced degradation samples	Degradation peaks well resolved

3.8 Method Validation Results

3.8.1 Specificity

Peak Purity Results:

Parameter	Result
Peak Purity Angle	0.256
Peak Purity Threshold	0.987
Resolution from nearest degradant	3.42



3.8.2 Linearity and Range

Concentration (µg/mL)	Mean Peak Area
5.0	256842
7.5	385726
10.0	512846
12.5	641395
15.0	770812

Parameter	Result
Regression Equation	y = 51424x – 1052
Correlation Coefficient (r^2)	0.9999
Slope	51424
Intercept	-1052

Regression Analysis:

3.8.3 Accuracy (Recovery Study)

Level	Amount Added (mg)	Amount Recovered (mg)	% Recovery
80%	8.0	7.96	99.50
80%	8.0	8.03	100.38
80%	8.0	7.98	99.75
100%	10.0	10.04	100.40
100%	10.0	9.97	99.70
100%	10.0	10.01	100.10
120%	12.0	11.95	99.58
120%	12.0	12.08	100.67
120%	12.0	11.99	99.92

Summary:

Level	Mean Recovery (%)	%RSD
80%	99.88	0.44
100%	100.07	0.35
120%	100.06	0.55

3.8.4 Precision

A. Repeatability (Intra-day Precision):

Injection	Assay (%)
1	99.42
2	99.68
3	100.14
4	99.83
5	100.21
6	99.57
Parameter	Result
Mean Assay	99.81%
SD	0.31
%RSD	0.31

B. Intermediate Precision (Inter-day Precision):

Day	Mean Assay (%)
Day 1	99.81
Day 2	100.24
Day 3	99.67
Parameter	Result
Overall Mean	99.91
%RSD	0.42

3.10.5 LOD and LOQ

Based on Standard Deviation of Response and Slope:

Parameter	Result
Slope (S)	51424
SD of Response (σ)	2456
LOD	0.16 µg/mL
LOQ	0.48 µg/mL

Signal-to-Noise Method:

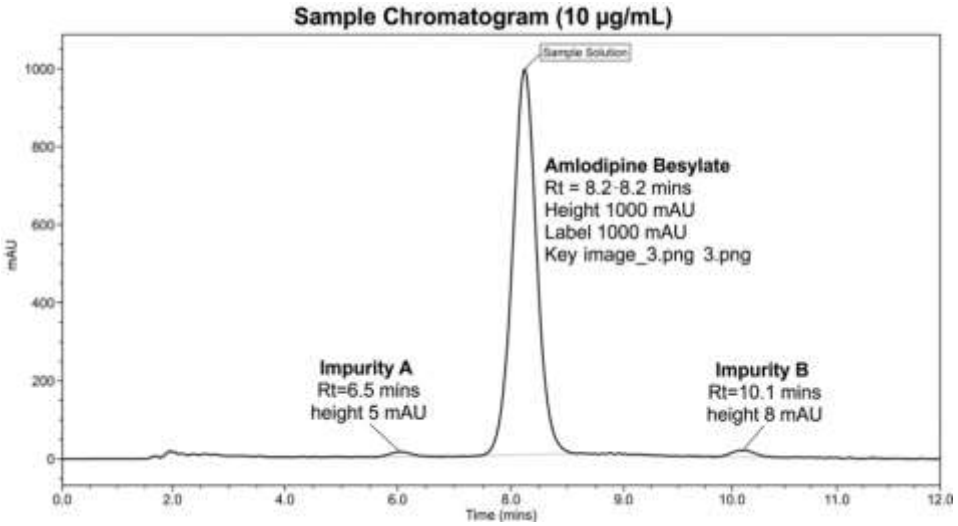
Parameter	Result
LOD S/N Ratio	3.2
LOQ S/N Ratio	10.8
LOQ Precision (%RSD)	2.6

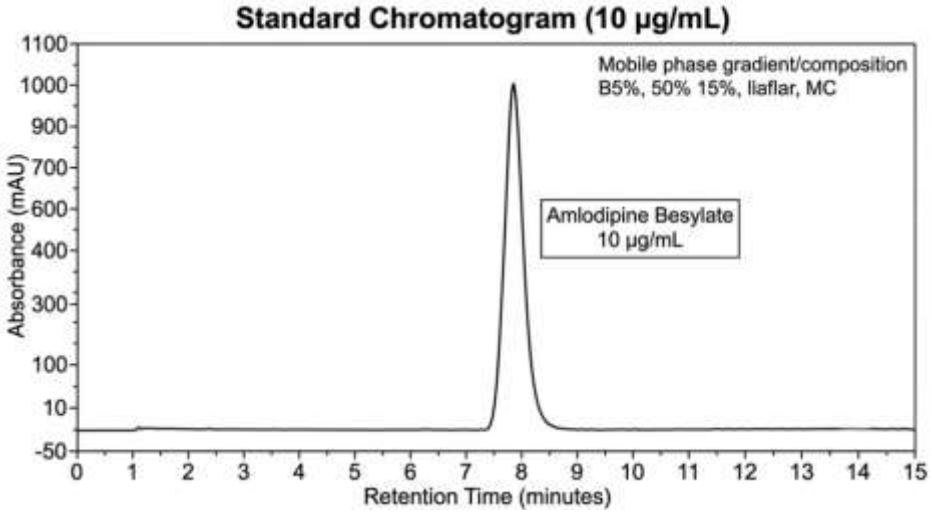
3.8.6 Robustness

Parameter Changed	Condition	RT (min)	Tailing Factor	Plates
Flow Rate	0.9 mL/min	4.68	1.16	6824
Flow Rate	1.1 mL/min	3.89	1.11	6715
pH	3.9	4.19	1.14	6892
pH	4.1	4.28	1.17	6784
Composition	48:42:10	4.01	1.12	6658
Composition	52:38:10	4.46	1.15	6723
Temperature	20°C	4.31	1.13	6804
Temperature	30°C	4.12	1.10	6945
Wavelength	237 nm	4.23	1.12	6854
Wavelength	239 nm	4.23	1.11	6838

3.8.7 Solution Stability

Time (h)	Standard Assay (%)	Sample Assay (%)
0	100.0	100.0
6	99.8	99.7
12	99.6	99.5
24	99.3	99.1
48	98.9	98.7





3.10.8 Filter Compatibility Study

Filter Type	Assay (%)
Unfiltered (Centrifuged)	100.0
0.22 µm PVDF	99.8
0.45 µm PVDF	99.6
0.22 µm Nylon	99.5
0.45 µm Nylon	99.3
0.22 µm PTFE	99.7

Comparison with Unfiltered Sample:

Filter Type	Difference (%)
0.22 µm PVDF	0.2
0.45 µm PVDF	0.4
0.22 µm Nylon	0.5
0.45 µm Nylon	0.7
0.22 µm PTFE	0.3

3.9 Overall Validation Summary

Parameter	Result	Acceptance Criteria
Specificity	Passed	No interference
Linearity (r^2)	0.9999	≥ 0.999
Accuracy	99.88–100.07%	98–102%
Precision (%RSD)	0.31–0.42	≤ 2.0
LOD	0.16 µg/mL	Report
LOQ	0.48 µg/mL	Report
Robustness	Passed	No significant effect
Solution Stability	Stable 48 h	≤ 2% change
Filter Compatibility	Passed	≤ 2% difference

3.10 Application to Pharmaceutical Formulation

3.10.1 Assay of Commercial Amlodipine Besylate Tablets

Batch No.	Label Claim (mg)	Mean Peak Area	Amount Found (mg)	Assay (% Label Claim)
Batch A	5.0	511,842	4.98	99.60
Batch B	5.0	515,276	5.03	100.60
Batch C	5.0	509,935	4.96	99.20

Summary:

Parameter	Result
Mean Assay (%)	99.80
Standard Deviation	0.74
%RSD	0.74

3.10.2 Content Uniformity Study

Tablet No.	Drug Content (%)
1	99.1
2	100.4
3	98.8
4	101.2
5	99.7
6	100.8
7	99.5
8	100.3
9	99.2
10	100.6

Statistical Evaluation:

Parameter	Result
Mean (%)	99.96
Standard Deviation	0.78
%RSD	0.78
Acceptance Value (AV)	4.25
USP Limit	NMT 15.0

4. DISCUSSION

4.1 Selection and Optimization of Chromatographic Conditions

Trial 1: Methanol : Water (70:30 v/v)

The chromatogram showed a broad peak with noticeable tailing and a relatively longer retention time. The absence of pH control in the mobile phase resulted in poor peak symmetry and inadequate chromatographic performance.

Trial 2: Acetonitrile : Water (60:40 v/v)

Replacement of methanol with acetonitrile reduced the retention time and improved peak sharpness. However, slight peak asymmetry and variability in peak area response were observed, making the system less suitable for routine analysis.

Trial 3: Phosphate Buffer (pH 3.0) : Acetonitrile (50:50 v/v)

The addition of phosphate buffer maintained the analyte in a consistent ionization state and significantly improved peak symmetry. A sharp and well-resolved peak was obtained with acceptable retention time, high theoretical plate count, and minimal tailing. Therefore, this mobile phase composition was selected as the optimized chromatographic condition.

Among the chromatographic conditions investigated, the mobile phase consisting of Phosphate Buffer (pH 4.0) : Acetonitrile : Methanol (50:40:10 v/v/v) provided the best chromatographic performance for amlodipine besylate. The optimized method produced a sharp, symmetrical peak with a retention time of approximately 4.23 minutes, excellent system suitability parameters, and satisfactory reproducibility, making it suitable for subsequent validation and routine quantitative analysis.

4.2 Forced Degradation Studies

Forced degradation studies are essential to demonstrate the stability-indicating capability of an analytical method. The results revealed that amlodipine besylate was most susceptible to alkaline hydrolysis (21.8% degradation), followed by oxidative stress (15.7% degradation) and solution-state thermal degradation (17.6% degradation).

The susceptibility to alkaline hydrolysis is consistent with the presence of ester groups in the dihydropyridine structure, which are prone to hydrolysis under basic conditions. The formation of degradation products was confirmed by the appearance of additional peaks in the chromatograms, which were well resolved from the parent drug peak.

The absence of interfering peaks from degradation products at the retention time of amlodipine besylate confirmed the specificity of the method. This is critical for ensuring accurate quantification of the drug in stability samples, where degradation products may be present.

4.3 Method Validation

The validation results confirmed that the developed method meets all ICH requirements for analytical method validation. The excellent linearity ($r^2 = 0.9999$) over the concentration range of 5–15 µg/mL ensures accurate quantification of amlodipine besylate in tablet formulations.

The recovery values (99.88–100.07%) demonstrated the accuracy of the method, with no significant interference from excipients or degradation products. The low %RSD values for precision (0.31–0.42%) confirmed the reproducibility of the method.

The LOD (0.16 µg/mL) and LOQ (0.48 µg/mL) values indicate that the method is sufficiently sensitive for the detection and quantification of amlodipine besylate in pharmaceutical formulations and stability samples.

5. CONCLUSION

The present study successfully developed and validated a simple, precise, accurate, robust, and stability-indicating RP-HPLC method for the quantitative estimation of amlodipine besylate in tablet dosage forms. The optimized chromatographic conditions provided excellent peak symmetry, satisfactory retention time, and reproducible analytical performance.

Forced degradation studies confirmed that amlodipine besylate is particularly susceptible to alkaline hydrolysis and oxidative stress. The developed method effectively separated degradation products from the intact drug peak, thereby establishing its stability-indicating capability.

Validation studies performed according to ICH guidelines demonstrated excellent specificity, linearity, accuracy, precision, robustness, sensitivity, solution stability, and filter compatibility. The successful application of the method to commercial tablet formulations further confirmed its suitability for routine quality control testing, assay determination, content uniformity evaluation, dissolution studies, and stability studies.

Overall, the developed RP-HPLC method is reliable, economical, and suitable for routine pharmaceutical analysis of amlodipine besylate and can be effectively employed in quality assurance laboratories, formulation development studies, and regulatory stability assessments.

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