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Research Article

# Stability-Indicating and RP-HPLC Method for Analyzing Amlodipine Besylate in Commercially Available Drug Products in Kurdistan, Iraq

**Faroq O. Qasim[1], Nidhal M. Sher Mohammed[2], Nzar I. Yousi[3], Muhammed H. Fattah[1]**

*[1]Department of Horticulture, Akre Technical College, Akre University for Applied Sciences, Kurdistan Region, Iraq, [2]Department of Medicinal Chemistry, College of Pharmacy, University of Duhok, Duhok City, Kurdistan Region, Iraq, [3]Department of Medical Laboratory Analysis, Cihan University - Erbil, Erbil, Kurdistan Region, Iraq*

## ABSTRACT

This study aims to develop and validate a stability-indicating HPLC method for the simultaneous determination of Amlodipine (Amlo) in bulk and tablet dosage forms. The method's goal is to evaluate the stability and degradation of Amlo under various stress conditions. The method was optimized using high-performance liquid chromatography (HPLC) with a UV detector. The mobile phase consisted of water and methanol in a 50:50 (v/v) ratio. An Eclipse XDB-C18 column (4.6 mm × 250 mm, 5 µm) was used for separation. The analysis was carried out at room temperature (25°C) with a 10 µL injection volume, a flow rate of 1.2 mL/min, and detection at 360 nm. Method validation was conducted in terms of linearity, precision, accuracy, limit of detection (LOD), and limit of quantification (LOQ). The stability of Amlodipine in tablet forms, under various stress conditions (acidic, alkaline, oxidative, thermal, and photolytic), was also evaluated. The finding showed excellent linearity ($R^2$ = 0.994) in the concentration range of 5–25 µg/mL. Precision was demonstrated by a relative standard deviation (RSD) of less than 0.8%. The LOD and LOQ were found to be 0.65 µg/mL and 2.16 µg/mL, respectively. Accuracy was assessed at three different levels, yielding recoveries above 98%. The method successfully detected the degradation products of Amlo under the applied stress conditions. The developed and validated HPLC method is simple, precise, and accurate for the simultaneous determination of Amlodipine in bulk and tablet forms. It is reliable for evaluating the stability and degradation of Amlo under different stress conditions, making it suitable for routine quality control and stability testing of Amlo-based pharmaceutical products.

**Keywords:** Quality control, reversed-phase high-performance liquid chromatography, development and validation, amlodipine besylate, stability-indicating

## INTRODUCTION

Blood pressure refers to the measurement of the pressure exerted by the heart as it circulates blood throughout the body. When the heart pumps blood, it creates two forms of pressure: Systolic pressure (measured at around 140 mmHg) when the heart contracts, and diastolic pressure (measured at around 90 mmHg) when the heart is in a resting phase between beats. When the range is over 140/90 the blood pressure is considered to be higher. The pressure is often written as "140/90" and measured by (mmHg). As getting older the risk of developing high blood pressure increases.[1]

Amlodipine besylate (amlo) is an antihypertensive calcium channel blocker medication, The chemical structure depicted in Figure 1 is that of 3-ethyl (3RS)-2(2-aminoethoxy)-4-(2-chlorophenyl)-methyl-1-hydro-pyredine-3-di-carboxylate benzene-sulfonate.[2,3] This drug, part of the dihydropyridine class, is a calcium channel blocker that stimulates the arterial wall muscular relaxation and lowers blood pressure.[3] Many methods have been developed and validated for the determination of amlo drug, alone or with other medications, including high-performance liquid chromatography (HPLC)[4-8] spectrophotometry,[9-12] thin-layer chromatography,[13,14] and kinetic.[15-17]

The stability indicating research investigates the degradation of medical drug products under specific circumstances, such as photo light, acid, alkaline, oxidative, and thermal.[18] It is crucial for demonstrating the specificity of techniques that indicate

**Corresponding Author:**
Faroq O. Qasim, Department of Horticulture, Akre Technical College, Akre University for Applied Sciences, Kurdistan Region, Iraq.
E-mail: faroq.omer@auas.edu.krd

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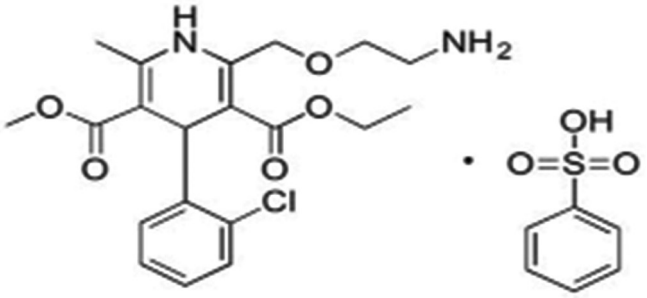

**Figure 1:** Chemical structure of amlodipine besylate

stability, providing insight into drug substance breakdown pathways and products, and explaining their structure.[19,20] The current research aims to develop and evaluate a novel analytical technique for routine analysis of quality control using HPLC, and a forced degradation investigation of the drug amlodipine besylate in the tablet form in pharmacy.

## MATERIALS AND METHODS

### Chemicals

The pharmaceutical active component of amlodipine besylate with a purity of 99% was obtained from Awamedica Company in Kurdistan, Iraq. Commercial drugs including awalodipine, amloneer, amlo-denk, amlodipine-accord, amipine bioactive, almacor antibiotic, amaday ajanta, and lowvasc-Hikma, which are randomly purchased from local pharmacies, are made in Kurdistan-Iraq (Erbil and Sulaymaniyah), Germany, America, British, India, and Jordan, respectively. Grade reagents namely sodium hydroxide (NaOH) and hydrochloric acid (HCl) were purchased from Scharlau (Spain), whereas hydrogen peroxide ($H_2O_2$) was purchased from Roth (Germany). Solvents, acetonitrile, methanol, and water HPLC grade, were purchased from Merck (Germany).

### Preparing Stock and Working Solutions

To prepare a stock standard solution, precisely 100 mg of pure drug was weighed and dissolved in methanol as solvent. The result was then transferred to a volumetric flask of 100 mL, resulting in a concentration of 1000 μg/mL. Subsequently, the entire volume was supplemented with solvent till reaching the desired level. The stock standard solution was maintained in a refrigerator to make various concentrations of working solutions.

### Chromatographic Conditions

The Nanodrop spectrophotometer, developed by Thermo Fisher Scientific, has revolutionized ultraviolet (UV)-visible spectroscopy by enabling absorbance measurements from very small sample volumes without cuvettes. The Nanodrop quickly gained popularity in labs for its ease of use, accuracy, and minimal sample requirements, becoming essential in fields such as molecular biology and analytical chemistry.[21] A Nanodrop spectrophotometer was used to measure the wavelength ($\lambda_{max}$) of amlo in the range of 200–700 nm. For this particular situation, it is advisable to use a variety of solvents including methanol, water, acetonitrile, ethanol, and acetone.

The system used for analysis is an Agilent high-performance liquid chromatography (HPLC) instrument supplied with a detector for UV radiation. The chromatographic column used is an Eclipse C18 column. The system also includes an autosampler and a binary pump. The specific column used is XDB-C18 with a diameter of 4.6 mm and length of 250 mm, with a specific size of 5 μm. A mobile phase consisting of a 50:50 v/v combination of water with methanol was prepared. 10 min were spent sonicating the mixture and then passed through a 0.45-μm. A 1.2 mL/min flow rate adjustment was made. At a wavelength of 360 nm, the medication showed remarkable absorption. Consequently, this wavelength was selected for further investigation, and the study was conducted at 25°C, corresponding to room temperature.

### Method Validation

The HPLC technique for the simultaneous determination of amlo has been evaluated by the International Council for Harmonization (ICH) criteria. The validation characteristics that must be considered include robustness, limit of detection (LOD), limit of quantification (LOQ), specificity, linearity, accuracy, and precision.

### Forced Degradation Study

An HPLC system was used to conduct a forced degradation analysis of amlo in the pharmaceutical formulation, subjecting it to various conditions such as acidity, alkaline, oxidation, thermal, and exposure to different types of light (sunlight, UV, and darkness).

*Preparing the stock solution of the formulation*

Accurate weighing was performed on 10 tablets (1.0 g) of commercial forms, where every single tablet contained 10 mg of pure drug. The tablets were then powdered, and an equivalent of 100 mg of amlo powder was placed in a conical flask of 100 mL. The mixture underwent a 10-min sonication after the addition of 25 mL of solvent. The resultant mixture was passed through 0.2 μm of Whitman filter paper and transferred into a volumetric flask of 100 mL. The methanol as solvent was added to the flask until it reached the mark, producing a stock solution that included 1000 μg/mL of amlo for each of the eight medication formulations. We used the stock solution of this formulation to create working solutions at various concentrations.

*Preparing the blank solution*

To prepare the blank solution, 25 mL of each degradation reagent (0.1 and 0.5 N of HCl, 0.1 and 0.5 N of NaOH, and 5% and 20% of $H_2O_2$), were added into the volumetric flask of 50 mL. Methanol was added to fill the flask, and the solution was heated at 80°C. 4 mL of an aliquot was obtained from this solution at various time intervals in separate 25 mL volumetric flasks and neutralized with an appropriate reagent. Finally, the volumetric flask was filled to the mark with a methanol.

*Acid and alkaline conditions*

Research on the forced degradation of pharmacological compounds by exposure to acid and base was carried out. 25 mL of the stock solution (100 μg/mL) of drugs was transferred to a volumetric flask of 100 mL, along with 25 mL

of 0.1 N and 0.5 N of HCl and NaOH, respectively. Initially, 4 mL aliquot of this solution was collected and maintained at room temperature for the duration of 4 h. Subsequently, another 4 mL aliquot of the same solution was heated at 80°C for the same duration of 4 h. The solvent was added to complete volume after neutralizing with a reagent in a volumetric flask of 25 mL, to obtain a 10 µg/mL concentration of amlo product.

*Oxidation condition*

Follow the same process as described in section 2.5.3. However, $H_2O_2$ was used instead of HCl and NaOH.

*Photo degradation*

25 mL of a working solution of formulations (1000 µg/mL) was added to volumetric flasks of 250 mL to make three solutions with 100 µg/mL of amlo of the two products. These solutions of the two products were left under the sun, dark, and UV light. After that, 5 mL of the exposed solutions were transferred into a volumetric flask of 50 mL. The final volume was made up of solvent to make a solution with 10 µg/mL of the products.

*Thermal degradation*

A precise amount of 1.0 g (10 tablets) of each product was taken, crushed up, and placed in an oven at 80°C for 10 h. 5 mg of these two products were dissolved in a conical flask with a solvent and sonicating for 10 min. Then, put into a volumetric flask 25 mL, and the final volume was made with solvent to make a solution with 10 µg/mL of amlo.

## RESULT AND DISCUSSION

### Optimization of Chromatographic Conditions

Figure 2 illustrates the highest absorbance and the $\lambda_{max}$ of amlo at 360 nm. Therefore, this specific wavelength was employed for all measurements throughout the study.

The objective of applying HPLC analysis is to provide a precise method for quantifying amlodipine besylate and its degradation products in pharmaceutical formulations. Different ratios of mobile phase were tested to select the best one. A ratio of 50:50 water and methanol was selected as shown in [Figure 3], and suitable chromatographic conditions are shown in Table 1. Several mobile phases were assessed to develop a precise and reliable RP-HPLC method for the simultaneous estimation of Amlo in local pharmaceutical formulations of awalodipine and amloneer drugs.

The impact of different solvents, including water, acetonitrile, and methanol, was examined to determine their influence on the solubility of pure amlo. A quantity of 20 mL of each solvent was utilized to assess the solubility of the pure drug. It was noticed that the pure medication exhibited greater solubility in a mixture of methanol and water compared to other mixtures.

### Method Validation

Method validation is a systematic process of gathering and analyzing data to confirm the reliability and applicability of the analytical method. The proposed method was validated by the requirements established by the ICH to assess its performance in terms of linearity, precision, specificity accuracy, LOD, LOQ, and robustness.

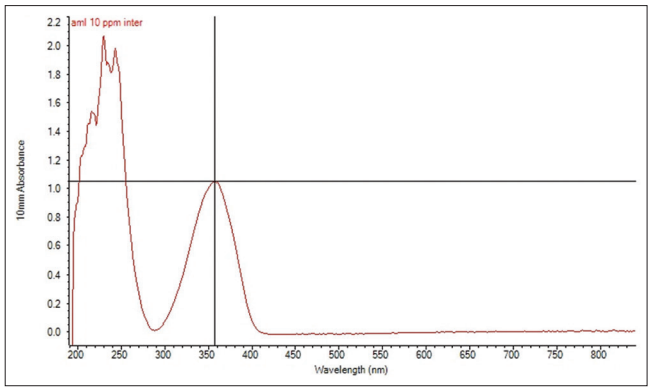


**Figure 2:** Ultraviolet-visible spectrum of amlodipine besylate

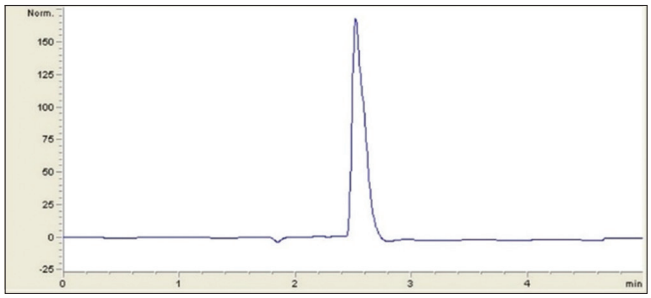


**Figure 3:** Chromatogram of amlodipine besylate

**Table 1:** Optimized chromatographic conditions

| Parameter | Condition |
|---|---|
| Column | Eclipse C18-column (XDB-C18, 4.6 mm 250 mm, 5 µm) |
| Flow rate | 1.2 mL/min |
| Injection volume | 10 µL |
| Wavelength | 360 nm |
| Column temp. | 25°C |
| Pump flow | Binary |
| Run time | 5 min |
| Mobile phase | 50:50 water: methanol |

*Linearity*

The linearity method was established by analyzing five distinct concentrations, ranging from 5 to 25 µg/mL as shown in Figure 4. It was discovered that the amlo regression formulas were y = 42.183x + 15.009, with a correlation of determination ($R^2$) of 0.994.

*Precision*

The precision study was assessed by administering five injections of amlo at amounts of 5, 10, and 15 µg/mL, each at three different levels. According to the standard criteria

set by the ICH, Table 2 demonstrates good precision, with the RSD values being below 0.4% indicating that the method was stable at the three levels of precision.

*Accuracy*

The standard addition method was applied for accuracy assessment at the three levels 50%, 100%, and 150%. The method involved adding known concentrations of standard drug solutions to a fixed concentration of the pre-analyzed solution. Solutions were prepared in triplicate at each level and measured. Table 3 shows that the values of recovery studies were in the acceptance levels of 99.1–99.59, with a low % RSD indicating that the method is accurate.

*LOD and LOQ*

LOD and LOQ were determined by calculating the signal-to-noise ratio, with a threshold of 3:1 for LOD and 10:1 for LOQ. The minimum LOD threshold for amlo was 0.65 µg/mL, and the minimum LOQ was 2.16 µg/mL, as shown in Table 4.

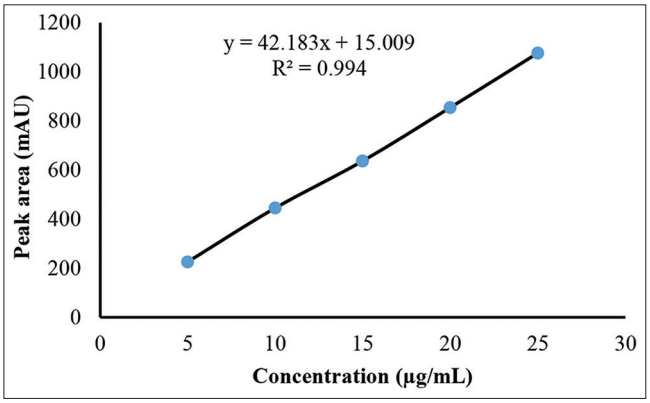


**Figure 4:** Calibration curve of amlodipine besylate

*Specificity*

In this study, we introduced starch and lactose into the standard solution of amlo. Subsequently, we determined the recovery percentage for three repeated experiments, which yielded a value exceeding 99.00% with a RSD of 0.32. The results show that the presence of common excipients in the formulation did not affect the method's accuracy and reliability.

*Robustness*

To evaluate the robustness of the method, small changes in the chromatographic conditions were performed. The flow rate was changed by ±0.2 mL/min, the wavelength was changed by ±0.3 nm, and the temperature of the column oven was changed by ±5°C. As shown in Table 5, the method was unaffected by simple changes and has sufficient robustness according to the standard criteria of ICH.

The proposed method has been validated showing linear, precise, accurate, specified, and robust, with good LOD and LOQ values. This method satisfied with standard requirements of the ICH recommendations, making it suitable for use in amlodipine analysis in quality control laboratories and stability indicators.

## Forced Degradation Study

The experiment is designed to apply the forced degradation method to pharmaceutical drugs in Kurdistan, Iraq, to determine whether the active ingredient will be decomposed when exposed to specific experimental circumstances, involving acid, alkaline, oxidation, thermal, and light. This method has been used to measure the stability of amlodipine in two local commercial products, awalodipine and amloneer, respectively.

*Acid degradation*

In an acidic condition, the two products were exposed to 0.1 N and 0.5 N of HCl at ambient temperature for up to 4 h at 80°C.

**Table 2:** Evaluation of precision study

| No. of Injection | Intra-day (µg/mL) | | | Inter-day (µg/mL) | | | Repeatability (µg/mL) | | |
|---|---|---|---|---|---|---|---|---|---|
| | 5 | 10 | 15 | 5 | 10 | 15 | 5 | 10 | 15 |
| 1 | 207.67 | 416.45 | 626.49 | 208.4 | 417.7 | 627.9 | 212.89 | 420.13 | 632.44 |
| 2 | 208.12 | 418.34 | 626.24 | 208.3 | 418.9 | 627.35 | 211.3 | 416.75 | 631.35 |
| 3 | 207.92 | 417.76 | 625.09 | 209 | 417.1 | 628.9 | 211.76 | 424.64 | 628.9 |
| 4 | 207.74 | 420.74 | 622.81 | 207.84 | 418.4 | 628.99 | 209.91 | 422.4 | 631.98 |
| 5 | 209.11 | 418.11 | 624.56 | 208 | 417.9 | 627.67 | 210.46 | 423.9 | 629.39 |
| Mean (%) | 208.11 | 418.28 | 625.04 | 208.31 | 418.00 | 628.16 | 211.264 | 421.56 | 630.81 |
| Standard deviation | 0.58 | 1.56 | 1.48 | 0.452 | 0.69 | 0.74 | 1.16 | 3.2 | 1.58 |
| % Relative standard deviation | 0.28 | 0.37 | 0.24 | 0.21 | 0.16 | 0.12 | 0.55 | 0.76 | 0.25 |

**Table 3:** Evaluation of accuracy

| Level of recovery (%) | Conc. given (µg/mL) | Recovery | Relative standard deviation % |
|---|---|---|---|
| 50 | 5 | 99.10 | 0.28 |
| 100 | 10 | 99.59 | 0.37 |
| 150 | 15 | 99.21 | 0.24 |

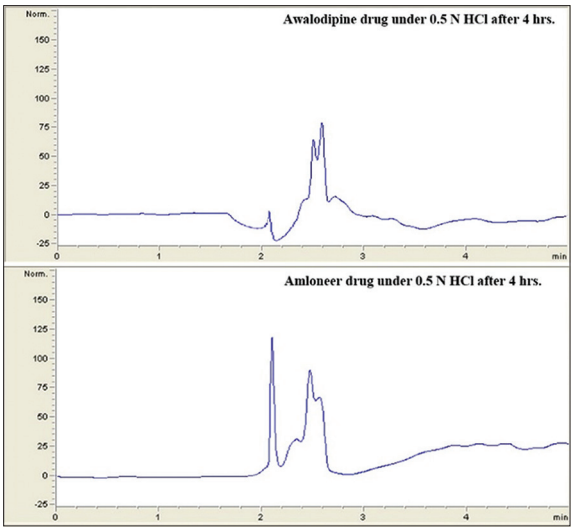


**Figure 5:** High-performance liquid chromatography chromatograms of acid degradation of awalodipine and amloneer

**Table 4:** LOD and LOQ values

| Parameters | Value |
|---|---|
| LOD PA | 42.36 |
| LOQ PA | 106.19 |
| LOD | 0.65 µg/mL |
| LOQ | 2.16 µg/mL |

LOD: Limit of detection, LOQ: Limit of quantification

Table 6 shows the degradation of amlo in both products at room temperature before heating. Degradation was increased rapidly at 80°C than at room temperature for both products when the incubation period was extended up to 4 h, as shown in Figure 5. There was a correlation between the amount of deterioration and the time when medications were exposed to acidic and heat conditions.

*Alkaline degradation*

In a basic condition, the two drugs were exposed to 0.1 N and 0.5 N of NaOH at ambient temperature for up to 4 h at 80°C. Table 7 shows the degradation of amlo in both products at room temperature. Degradation was increased rapidly at 80°C than at room temperature for both products when the incubation period was extended up to 4 h, as shown in Figure 6. The results also showed that under basic and heated conditions, the amlo in the amloneer product degraded less slowly than the awalodipine product.

*Oxidation degradation*

According to the results of this study, the two products show the highest degradation under oxidative conditions, with 5% and 20% of $H_2O_2$ as shown in Figure 7. At the ambient temperature, amlo in both drugs began to degrade. The degradation percentage increased up to 4 h after heating at 80 °C, as shown in Table 8.

*Photolytic degradation*

A stress degradation study has been conducted to study the effects of sunlight, UV light, and darkness on amlodipine

**Table 5:** Changes in flow rate, wavelength, and temperature

| Flow rate (mL/min) | % Assay | Wavelength (nm) | %Assay | Column oven temp (°C) | % Assay |
|---|---|---|---|---|---|
| 1.0 | 99.1 | 363 | 99.8 | 20 | 99.9 |
| 1.2 | 100.1 | 360 | 99.95 | Ambient (25) | 100.01 |
| 1.4 | 98.3 | 357 | 99.4 | 35 | 99.95 |
| Average | 99.83 | Average | 99.7 | Average | 99.95 |
| RSD% | 0.30 | RSD% | 0.28 | RSD% | 0.05 |

RSD: Relative standard deviation

**Table 6:** Impact of acid hydrolysis on amlodipine in two products

| Sample | Conditions of Exposure | Given Conc. (µg/mL) | Founded Conc. (µg/mL) | Percentage of degradation% | Observation |
|---|---|---|---|---|---|
| Awalodipine | 0.1N | | | | |
| | RT | 10 | 9.77 | 2.29 | No change |
| | 4h 80°C | 10 | 7.10 | 29.04 | Degrade |
| | 0.5N | | | | |
| | RT | 10 | 8.18 | 18.22 | Degrade |
| | 4h 80°C | 10 | 5.01 | 49.88 | Degrade |
| Amloneer | 0.1N | | | | |
| | RT | 10 | 9.06 | 9.38 | Degrade |
| | 4h 80°C | 10 | 7.54 | 24.64 | Degrade |
| | 0.5N | | | | |
| | RT | 10 | 8.08 | 19.16 | Degrade |
| | 4h 80°C | 10 | 6.31 | 36.95 | Degrade |

**Table 7:** Impact of alkaline hydrolysis on amlodipine in two products

| Sample | Conditions Exposure | Given Conc. (µg/mL) | Founded Conc. (µg/mL) | Percentage of Degradation% | Observation |
|---|---|---|---|---|---|
| Awalodipine | 0.1N | | | | |
| | RT | 10 | 9.24 | 7.62 | Degrade |
| | 4h 80°C | 10 | 7.20 | 28.04 | Degrade |
| | 0.5N | | | | |
| | RT | 10 | 7.54 | 24.64 | Degrade |
| | 4h 80°C | 10 | 3.89 | 61.08 | Degrade |
| Amloneer | 0.1N | | | | |
| | RT | 10 | 9.68 | 3.23 | No change |
| | 4h 80°C | 10 | 7.89 | 21.15 | Degrade |
| | 0.5N | | | | |
| | RT | 10 | 8.23 | 17.73 | Degrade |
| | 4h 80°C | 10 | 6.16 | 38.36 | Degrade |

**Table 8:** Oxidative effect on amlodipine in two products

| Sample | Conditions Exposure | Given Conc. (µg/mL) | Founded Conc. (µg/mL) | Percentage of Degradation% | Observation |
|---|---|---|---|---|---|
| Awalodipine | 5% | | | | |
| | RT | 10 | 8.29 | 17.14 | Degrade |
| | 4h 80°C | 10 | 3.93 | 60.72 | Degrade |
| | 20% | | | | |
| | RT | 10 | 7.00 | 29.98 | Degrade |
| | 4h 80°C | 10 | 2.42 | 75.79 | Degrade |
| Amloneer | 5% | | | | |
| | RT | 10 | 7.96 | 20.37 | Degrade |
| | 4h 80°C | 10 | 5.88 | 41.22 | Degrade |
| | 20% | | | | |
| | RT | 10 | 7.10 | 28.97 | Degrade |
| | 4h 80°C | 10 | 4.94 | 50.63 | Degrade |

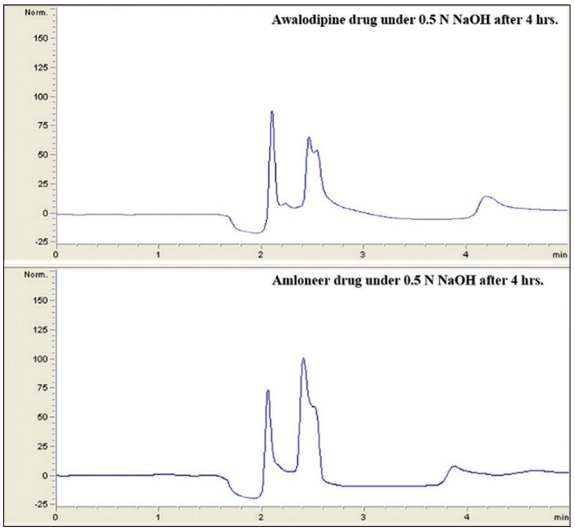


**Figure 6:** High-performance liquid chromatography chromatograms of alkaline degradation of awalodipine and amloneer

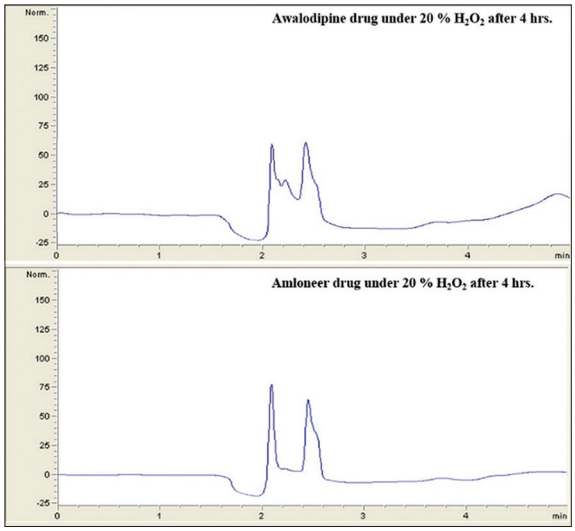


**Figure 7:** High-performance liquid chromatography chromatograms of oxidative degradation awalodipine and amloneer

**Table 9:** Photolytic effect on amlodipine in two products

| Sample | Conditions Exposure | Given Conc. (µg/mL) | Found Conc. (µg/mL) | Percentage of Degradation% | Observation |
|---|---|---|---|---|---|
| Awalodipine | UV | | | | |
| | 1 day | 10 | 9.19 | 8.10 | Degrade |
| | 5 days | 10 | 9.2 | 8.2 | Degrade |
| | SUN | | | | |
| | 1 day | 10 | 8.79 | 12.08 | Degrade |
| | 5 days | 10 | 8.51 | 14.89 | Degrade |
| | DARK | | | | |
| | 1 day | 10 | 9.59 | 4.08 | No change |
| | 5 days | 10 | 9.52 | 4.84 | No change |
| Amloneer | UV | | | | |
| | 1 day | 10 | 9.42 | 5.79 | No change |
| | 5 days | 10 | 9.18 | 8.16 | Degrade |
| | SUN | | | | |
| | 1 day | 10 | 9.65 | 3.48 | No change |
| | 5 days | 10 | 8.72 | 12.83 | Degrade |
| | DARK | | | | |
| | 1 day | 10 | 9.84 | 1.56 | No change |
| | 5 days | 10 | 9.60 | 3.98 | No change |

**Table 10:** Application of amlo in tablets dosage form

| Tablets | Added Conc. µg/mL | Founded Conc. µg/mL | Recovery% | Relative standard deviation % |
|---|---|---|---|---|
| Pure drug | 10 | 9.99 | 99.9 | 0.063 |
| Awalodipine | 10 | 9.91 | 99.1 | 0.072 |
| Amloneer | 10 | 9.83 | 98.3 | 0.063 |
| Amlo-denk | 10 | 10.1 | 100.1 | 0.050 |
| Amlodipine-accord | 10 | 9.91 | 99.1 | 0.063 |
| Amipine-bioactive | 10 | 9.93 | 99.3 | 0.033 |
| Almacor-antibiotic | 10 | 9.94 | 99.4 | 0.144 |
| Amaday ajanta | 10 | 9.89 | 98.9 | 0.138 |
| Lowvasc-Hikma | 10 | 9.99 | 99.94 | 0.044 |

products (awalodipine and amloneer). The exposure period was from 1 to 5 days. Two products showed slight degradation in UV and sunlight, with <15% degradation over 5 days. The overall results are shown in Table 9.

*Thermal degradation*

The two products of awalodipine and amloneer were exposed to 80°C for 48 h in an oven to study the thermal degradation. The percent of degradation was found to be <5% for both drugs. According to the ICH recommendation, these studies showed that amlo remains stable in both products under heating conditions.

## Application to Analysis of Commercial Samples

To verify the efficacy of the proposed method, amlo in many commercial formulations was assessed. The determination findings are presented in Table 10, indicating a strong agreement between the results obtained using the offered method and the labeled claim. However, In terms of comparison, drug products (awalodipine and amloneer) manufactured in Kurdistan/Iraq can compete with other companies with their high quality.

## CONCLUSION

An RP-HPLC method was developed to determine amlodipine besylate in pure form and commercial products. Its rapidity, linearity, precision, accuracy, specificity, robustness, and cost-effectiveness make it suitable for routine analysis and stability testing. A forced degradation study on awalodipine and amloneer revealed that time is the main factor influencing degradation. The outcomes of the present investigation demonstrated the quality of the pharmaceutical specimens

and the method's implementations for quality control analysis. The method can be employed for drug analysis during stability studies, improving quality control.

## ACKNOWLEDGMENTS

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