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Optimization and Validation of An Eco-friendly Stability Indicating UPLC Analytical Method for Amlodipine Besylate Determination

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ABSTRACT

Amlodipine besylate (AB) is commonly used to manage high blood pressure and cardiovascular diseases. Several HPLC assays were developed to analyze AB alone or in combination with other drugs, with a lack of eco-friendly properties. Therefore, this work aims to develop and optimize an efficient and eco-friendly Ultra Performance Liquid Chromatography (UPLC) analytical method for the precise determination of AB. A 32 full factorial experimental design was utilized to analyze the impact of Methanol percentage (X1; 60%, 65%, and 70%) in the mobile phase and column temperature (X_2; 15, 20, and 25°C) on retention time (Y_1), peak area (Y_2), and resolution (Y_3). The optimized method (60% methanol and 25°C column temperature) accurately determined AB peaks at 5.37±0.21 min, with excellent separation and resolution of 2.17±0.07. Moreover, the calculated HPLC-EAT (Environmental Assessment Tool) value was 2.91, which is considered a low value on a scale of 1-10. The developed analytical assay for AB was simple, sensitive, linear (R_2=0.994), sustainable, and suitable for routine analysis of pure drug form and pharmaceutical formulations with RSD% for accuracy and precision less than 2%. Economically, the developed AB assay could be eco-friendly based on flow rate, running time, and the HPLC-EAT value.

Keywords: Amlodipine besylate, Optimization, Validation, UPLC, Quality control, Stability.

INTRODUCTION

Analytical methods are in demand not only during the development of pharmaceutical products but also for post-marketing assessments and patient drug analysis to optimize dosage regimens. Therefore, developing a rapid, robust, simple, sensitive, and precise analysis will save cost and time. This can be done by implementing an analytical quality-by-design (AQbD) approach. As a tool of an AQbD, the Design of Experiment (DoE) plays a crucial role in designing methods for assessing primary factors and their interactions. Several factors can manipulate the results of chromatography

analysis. The mobile phase composition and column temperature could be chromatographic set points during the screening and optimization stage[2-4].

Most guidelines recommend the use of calcium channel blockers alone or associated with other agents to treat hypertension. Amlodipine besylate (AB) is a dihydropyridine compound (Fig. 1) that inhibits the influx of calcium into cardiac muscle[5-9]. It is a white, crystalline compound designed as capsules or tablets for oral administration in varying dosage strengths of 2.5 mg, 5 mg, and 10 mg[10].

Several chromatographic methods have been published for analyzing AB alone or simultaneously with other drugs. Zarghi et al. focused on amlodipine using a high-performance liquid chromatographic (HPLC) system with a UV detector set at 239 nm using a mobile phase consisting of sodium dihydrogen phosphate buffer (0.01 M) and acetonitrile (63:37, v/v) at pH 3.5[11]. In another study, Vojta and other researchers analyzed AB and atorvastatin using a liquid chromatographic system with a mobile phase composed of acetonitrile[12]. Several other studies have been conducted to analyze AB in fixed-dose combinations containing AM, valsartan, and Hydrochlorothiazide[4,13-15]. Different methods have been developed to analyze AB and enalapril in pharmaceutical dosage forms[16-18]. However, as the green analytical chemistry approach recommended, most of these works used acetonitrile in the mobile phase, which should be used in reduced amounts.

Moreover, during the stability study, the developed method should be able to separate the drug from the degradation product successfully. In the literature, several studies developed an analytical method that can specify the AB from its degradation product[19-27]. The stability-indicating method can be referred to as any validated analytical method that can detect minor changes in the drug substance or pharmaceutical product quantitatively without interference[28].

Therefore, this work aims to analyze AB using a green analytical method, in addition to implementing DoE, to study the impact of a multiparameter approach rather than a one-factor-at-a-time design. The study focuses on developing and refining an environmentally friendly analytical technique for detecting AB in its pure form and in commercially available products. The study utilized a 3^2 full factorial experimental design to explore the effects of methanol concentration (A) and column temperature (B) on critical parameters of AB, including retention time (Y_1), peak area (Y_2), and resolution (Y_3).

MATERIALS AND METHODS

Materials

SPIMACO Pharmaceutical company (Qassim, KSA) supplied the raw material for amlodipine besylate (AB). Formic acid ≥ 98% SIGMA- ALDRICH (Steinheim, Germany). Methanol (Riedelde Haën Laboratory Chemicals, Selzer, Germany). Milli-Q purification system to obtain deionized water (Millipore, USA).

Experimental Design

To assess the effects of Methanol percentage in the mobile phase and column temperature on the attributes of AB, a 32 full factorial experimental design was implemented. These attributes were considered responses, including retention time, peak area, and resolution. Design Expert® Software was employed for this study. For each factor, three levels were defined accordingly, as presented in Table 1. A series of nine experimental runs were conducted, as depicted in the design matrix shown in Table 1.

Table 1: Experimental design and run conditions for the analytical procedure of amlodipine besylate using UPLC.

Independent factors	Level			Dependent Parameters					
	-1	0	+1						
X_1 : Methanol (%)	60	65	70	Retention time (min)					
				Peak area (mAU/min)					
X_2 : Column Temperature (°C)	15	20	25	Resolution from mobile phase Peak					
Condition/Run Number	1	2	3	4	5	6	7	8	9
Methanol (%)	60	65	70	60	65	70	60	65	70
Column Temperature (°C)	15	15	15	20	20	20	25	25	25

Preparation of Standard Stock Solution, Calibration and Quality control samples

A concentration of 15 ppm was prepared by diluting 1 mL (150 ppm) with methanol ten times to prepare the working stock solution. Subsequently, a serial dilution was prepared using the previous stock solution in methanol, ranging from 0.3 to 15 ppm. Triplicate injections were implemented for each concentration. The instrument peak area response was plotted versus the analyte concentration to determine the linearity.

UPLC System and Conditions

The investigation utilized a highly sensitive UHPLC system (Ultimate 3000® binary solvent manager) from Thermo Scientific (Bedford, MA, USA) equipped with an automatic sampler and an ultraviolet detector. Separation was accomplished via reverse-phase Isocratic elution, employing a mobile phase composed of % Water containing 0.1% formic acid mixed with methanol at different percentages, as shown in Table 4, delivered at a flow rate of 0.05 mL/min through an Acquity UHPLC column (HSS C18, 2.1 x 50 mm, 1.7 µm). The total run time was 8.0 minutes; detection was performed at 238 nm. Different temperature levels were implemented throughout the process according to the design. A working solution of 15 µg/mL of AB was utilized for the analytical assay.

HPLC- Environmental Assessment Tool (EAT)

HPLC-EAT is a summation of the safety, health, and environmental scores using EHS sheets developed by Koller and coworkers. It can be calculated as per the following equation;

$$\text{HPLC-EAT Score}=m_1S+m_1H+m_1E+m_2S+m_2H+m_2E+\ldots+m_nS+m_nH+m_nE$$

Where S refers to safety, H to health, and E to environmental factors for n solvents, respectively, and m is the mass of the solvents. The m (solvent's mass) calculation depends on the retention time and flow rate.

ULC Analytical Validation

The validation of the analytical method followed the guidelines outlined by the USFDA for Bioanalytical methods[19,20].

Linearity and Range

Optimal volumes of the AB stock solution (15 µg/mL) were employed to create six standard concentrations, spanning the calibration range from 0.3 to 15 ppm. For validation purposes, each standard solution (0.3, 0.6, 1.5, 3, 6, 15 ppm) was injected three times. In each run, the calibration solutions were analysed in an ascending manner. Statistical linearity assessment was conducted using linear regression equations and the R-squared (R^2) correlation coefficient.

Accuracy and Precision

Accuracy and Intra-day precision assessments were conducted by performing triplicate determinations of four AB standards within a single day. Inter-day precision was evaluated through triplicate measurements conducted over three consecutive days. The relative standard deviation (%RSD) indicated the method's overall precision, while accuracy was interpreted as the percentage of drug recovered.

Robustness

Robustness refers to the ability of an analytical method to maintain its integrity despite minor variations in method and environmental parameters. Herein, the effect of varying three process parameters of the developed method flow rate, wavelength, and temperature was explored. Specifically, three slightly different flow rates (0.048, 0.05, and 0.052 mL/min), three wavelengths (236, 238, and 240 nm), and three temperatures (23, 25, and 27°C) were employed for this purpose.

Limit of Detection (LOD) and Lower Limit of Quantitation (LLOQ)

The Limit of Detection (LOD) and Lower Limit of Quantitation (LLOQ) were established through serial dilutions of AB stock solutions, aiming to achieve a signal-to-noise (S/N) ratio of approximately 3:1 for LOD and approximately 10:1 for LLOQ.

Application

Quality control tests for capsules

Content uniformity

Uniformity of dosage units of the marketed

product (Amlor capsules) containing 5 mg AB was assessed according to the USP30-NF25 harmonized monograph[21]. Briefly, the content of each capsule was emptied into a 100-mL volumetric flask. The powder was dispersed in methanol (30 mL) and sonicated for 10 minutes. The volume was then adjusted to 100 mL with 0.1 N HCl. The dispersion was filtered and analyzed at 238 nm using the optimized UPLC analytical procedures. The experiment was conducted for ten capsules separately by individual assay, and the acceptance value (AV) was computed according to the equation:

$$AV = |\bar{x} - M| + K.S,$$

Where, the mean value of the drug content is (x), S is the standard deviation, M is dependent on x, and K is a constant value of 2.4 for ten dosage units and 2 for 30 dosage units.

The content uniformity is acceptable if the AV is not more than 15% of the first 10 tested capsules (Stage I). In case of AV higher than 15, an additional 20 capsules will be used (stage II), and in such case, AV should not exceed 25 for the 30 dosage units.

***In vitro* dissolution**

In vitro AB dissolution from Amlor® capsules (5 mg) was conducted for six capsules according to the USP 30-NF[22]. The dissolution procedures were monitored using an apparatus II (paddle method) dissolution tester (Electrolab, ED-21, Mumbai, India. Dissolution was carried out in 500 mL 0.01 N hydrochloric acid. The paddle was adjusted to spin 75 rpm at 37±0.5°C for 30 minute. Dissolution samples were taken at specified time intervals (5, 15, and 30 min) and analyzed at 238 nm using the optimized UPLC analytical procedures.

Forced degradation studies

To study the validity of the developed method in AB stability studies under various harsh conditions, a drug concentration of 15 ppm was subjected to stress conditions, including acid/base hydrolysis and oxidative stress, as well as the effect of elevated temperature. One mL of AB solution in methanol (15 ppm) was added to 4 mL of 0.1 M HCl, 0.1 M NaOH, and 30% hydrogen peroxide (H_2O_2) separately, mixed, and then incubated in a hot air oven (50°C) for two days. In the study of the effect of thermal stress, 5 mL of 15 ppm solution was incubated directly in a hot air oven for two days at 50°C23.

RESULTS AND DISCUSSION

Effect of independent parameters on retention time (RT)

The standardized Pareto chart (Fig. 1(a)) shows that methanol exhibited an agonistic effect on retention time. Still, this effect was insignificant due to the antagonistic mean effect of column temperature on the attributes, with P-values of 0.1999 and 0.6162, respectively. Also, the methanol quadratic effect and the quadratic effect of column temperature showed an agonistic but insignificant impact on the response. The three-dimensional response surface plot depicted in Fig. 1(b) illustrates the effects of temperature and methanol concentration on the retention time of the AB peak. The data revealed that rising column temperature was accompanied by a decrease in the retention time of the drug peak, while increasing methanol concentration increased the retention time. Therefore, these two opposite actions of column temperature and methanol concentration in the mobile phase are responsible for the overall insignificant effects on the response. As shown in Table 2, the shortest retention time detected for AB peak (3.94 min) was observed using high column temperature and a high mobile phase methanol level, in comparison, the longest retention time of the drug (5.88 min) resulted from applying the lowest column temperature with the medium level of methanol. The results showed that the impact of column temperature on the elution time of the UPLC chromatogram's peak was deemed insignificant. Still, numerous studies have demonstrated that increasing the column temperature has a significant effect on the UPLC chromatogram. This effect reduces the mobile phase solvent's viscosity, lowering the backpressure[24-29]. Moreover, Joseph *et al.,*[27] showed that as the percentage of methanol increased, the retention times of benzoic acid and phenol decreased at all tested pH values.

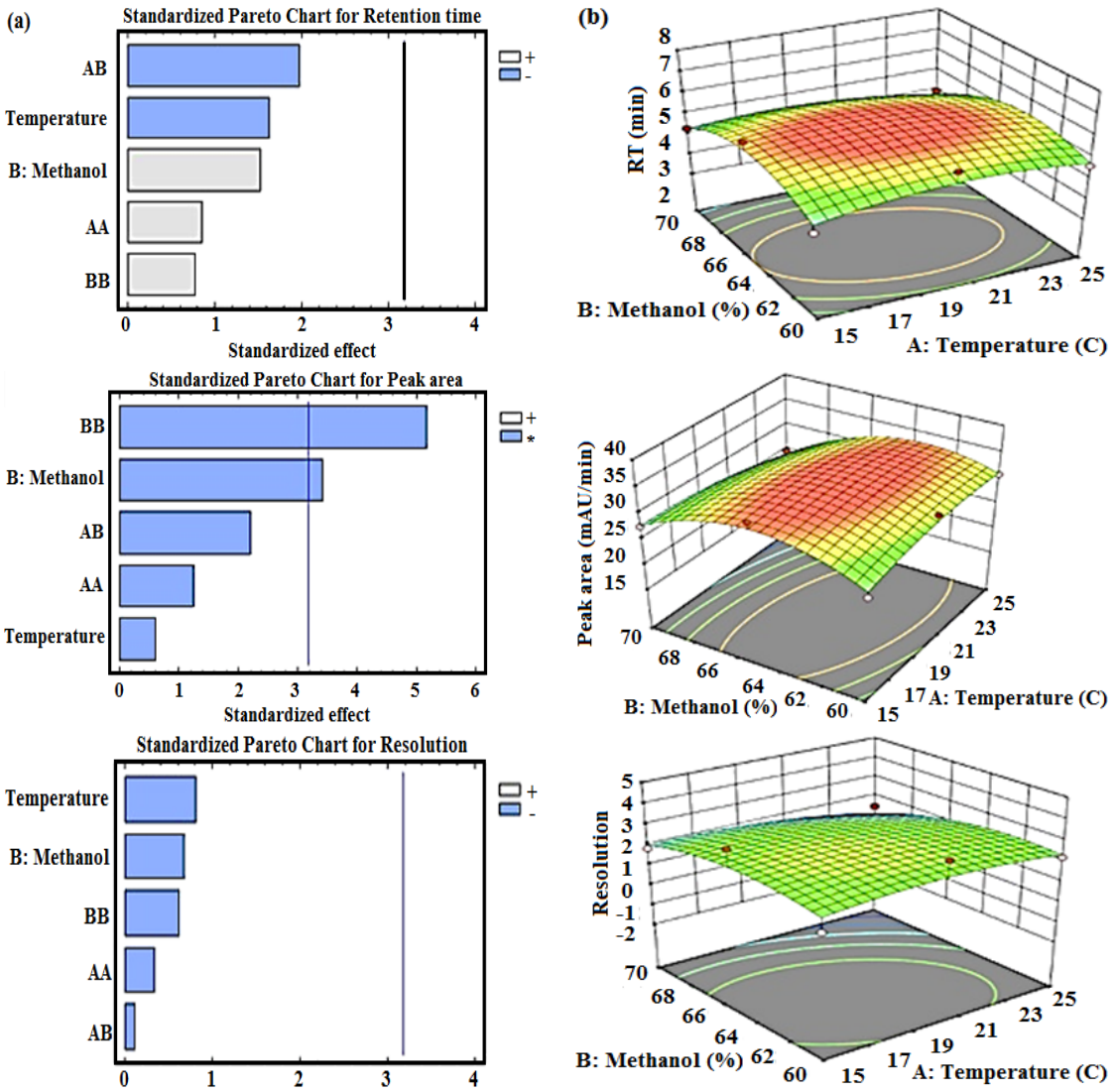

Fig. 1. Analysis of independent factors on Amlodipine besylate's retention time, peak area, and resolution, (a) Pareto chart, (b) 3D Response surface plot

Table 2: The Observed chromatogram attributes for analytical procedure of Amlodipine Besylate using UPLC

Run	A Methanol %	B Column Temperature °C	Retention Time (min)	Peak Area (mAu/min)	Resolution	HPLC-EAT	Peak Symmetry
1	60	15	4.20±0.02	27.46±1.23	1.71±0.007	2.27±0.07	1.12±0.009
2	65	15	5.88±0.03	34.54±0.81	3.55±0.007	3.37±0.03	0.94±0.006
3	70	15	4.73±0.02	27.57±1.07	1.83±0.003	2.60±0.03	1.07±0.007
4	60	20	5.62±0.06	32.31±0.97	3.44±0.005	3.04±0.01	0.94±0.006
5	65	20	5.66±0.01	33.56±1.35	1.68±0.004	3.24±0.07	0.93±0.007
6	70	20	4.14±0.03	26.40±1.54	1.68±0.003	2.50±0.04	1.08±0.006
7	60	25	4.45±0.04	30.76±0.87	1.56±0.007	2.40±0.06	1.04±0.002
8	65	25	5.14±0.05	32.08±0.64	1.98±0.004	2.94±0.01	0.97±0.003
9	70	25	3.94±0.02	24.60±1.08	1.44±0.006	2.38±0.03	0.96±0.007

Effect on Peak Area

The Pareto chart in Fig. 1(a) investigates the impact of methanol and column temperature on the AB peak area. The Table indicates that methanol exhibited a significant antagonistic effect on the peak, along with an insignificant antagonistic impact

of column temperature on the response. Additionally, the temperature quadratic term and the interaction between methanol and temperature (X_1X_2) have an insignificant antagonistic effect on the peak area of AB, with p-values of 0.584 and 0.115, respectively. In contrast, the methanol level in the mobile phase and its quadratic mode exhibited a significant antagonistic effect on the peak area, with P-values of 0.042 and 0.014, respectively.

The three-dimensional surface plots depicted in Fig. 1(b) indicate that an increase in methanol concentration in the mobile phase results in a reduction in the drug peak area on the UPLC chromatogram, particularly at low and medium concentrations. In addition, the highest levels of drug peak area were noticed at the medium levels of the two tested independent factors. This finding is also assured by Table 2, which indicates that the lowest area for the AB peak (24.6 mAU/min) was detected when using the highest methanol levels with the highest column temperature. The highest peak area value (32.31 mAU/min) was observed for the analytical run that applied medium levels of methanol and column temperature. Other analytical investigations show that the retention time and area of the peak for the analyte are significantly affected by the percentage of organic solvent in the eluent. Dural *et al.,* showed that the choice of the mobile phase is considered the cornerstone of the analytical separations based on chromatographic procedures[30]. They also indicated that increasing the concentration and the content of the mobile phase affected the peak area of clozapine. In addition, Ibrahim *et al.,* showed that increasing column temperature could increase the chromatogram's peak area[24].

Effect on Peak Resolution

The effect of the two independent factors (column temperature, A, and methanol, B) on the resolution of the AB peak from the adjacent mobile phase peak (appeared around 3 min) was studied, and the data are in the Pareto chart in Fig. 1(a). The results indicated that temperature and methanol, their quadratic, and their interactive effects have an insignificant antagonistic effect on resolution, with p-values higher than 0.05. The 3D response surface plot in Fig. 1(b) illustrates the impact of temperature and methanol concentration on resolution at a given time. It shows that variations in temperature or methanol concentration have an insignificant effect on the resolution of AB peak from mobile phase one. The results of peak resolution are depicted in Table 2, showing that the lowest peak separation value (1.44) was observed in run # 9 based on using the highest methanol level along with medium column temperature, while the highest value of peak separation (3.55) was observed in case of the analytical run based on using 65% methanol with the 15°C column temperature. The results showed that the peak separation range was from 1.44 to 3.55. These values of resolutions between AB and mobile phase peaks could be considered as reasonable values to prevent peak overlap[31,32].

This agrees with the basic knowledge that increasing the temperature leads to faster separation, albeit with reduced efficiency or resolution[33]. Still, the significance could vary based on the eluted compound. In contrast, Prabaningdyah *et al.,*[34] found that the column temperature has a positive effect on resolution. It is imperative to remember that the effect here is not due to a single parameter but an interactive one.

Peak symmetry

In Table 2, the peak symmetry factors derived from the instrument for all nine analytical runs were in the range of 0.97-1.12, which is within acceptable range[32].

Environmental assessment (HPLC-EAT)

By applying the HPLC Environmental Assessment Tool (HPLC-EAT), the resulting score below 5 denotes the environmental friendliness or greenness nature of the evaluated UPLC method. In the current study, the HPLC-EAT for all tested analytical runs ranged from 2.27 to 3.38. These results indicate that the method has a minimal environmental impact across various factors, including solvent utilization, analytical time, waste production, and energy consumption, as evaluated by the HPLC-EAT. Additionally, these lower EAT scores (<5) indicate an enhanced focus on sustainability and environmental consciousness within HPLC operations, reflecting adherence to green chemistry principles and sustainable practices in analytical methodologies[35].

Optimization of UPLC conditions for Amlodipine besylate analysis

The responses obtained for critical quality attributes (CQAs; including retention time, peak area, and Resolution) after evaluating the effects of independent factors are recorded in Table 3. Use one-way analysis of variance (ANOVA) to study statistical differences in means, assuming significance when the p-value is less than 0.05. Optimization was performed based on specific desired properties, including minimum retention time, maximum peak area, and maximum Resolution (Table 3). Accordingly, the optimized values suggested by the statistical software program were a column temperature of 25°C and a mixture of 60% methanol and 40% water containing 0.1% formic acid. The observed data for the analytical responses were almost identical to the observed values. According to the optimized analytical procedures, the drug peak was detected at 5.37±0.21 min, which is close to the expected value of 4.72 minute. In contrast, its area was 30.97±1.98 mAU/min, which was highly correlated with the predicted value of 29.53 mAU/min. Additionally, the resolution between the AB and mobile phase peaks was 2.17±0.07, which is slightly lower than the predicted resolution of 2.31. Moreover, the AB peak derived from the optimized analytical procedure exhibited an acceptable symmetry factor (0.95±0.01) and an HPLC-EAT value of 2.91.

Table 3: The predicted and investigated values of the optimized UPLC condition for the Amlodipine besylate analysis

Optimized independent parameters	Response Type	Desirability	Predicted	Investigated
A. Temperature = 25	Y_1: Retention time (min)	Minimum	4.72	5.37±0.21
B. Methanol = 60%	Y_2: Peak area (mAU/min)	Maximum	29.53	30.97±1.98
	Y_3: Resolution	Maximum	2.31	2.17±0.07
Other analytical parameters	Peak symmetry: 0.95±0.01	HPLC-EAT: 2.91		

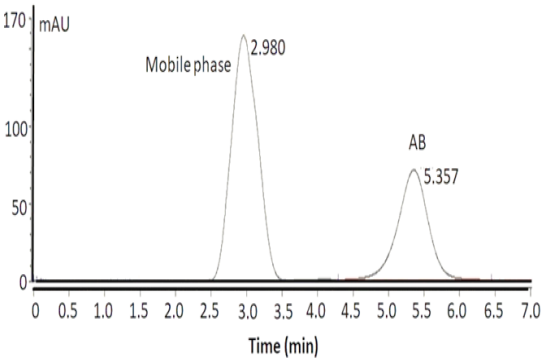

Fig. 2. UPLC chromatogram of optimized conditions for Amlodipine besylate analysis using UPLC

Figure 2 shows the UPLC chromatogram according to the optimized analytical method. The drug was extracted at a retention time of 5.37 min with a peak area of 30.97 mAU/min and resolution of 2.17 from the mobile phase peak. In addition, the drug peak showed acceptable symmetry with a value of 1.01, which is considered in the acceptable range (0.8-1.2)[19].

Validation procedure

Calibration of the optimized UPLC green analytical procedure for Amlodipine besylate. A linear calibration curve was generated using the developed UPLC green analytical method, incorporating various concentrations of AB ranging from 0.3 to 15 ppm. Fig. 3 showcases the linear regression data obtained and calculated.

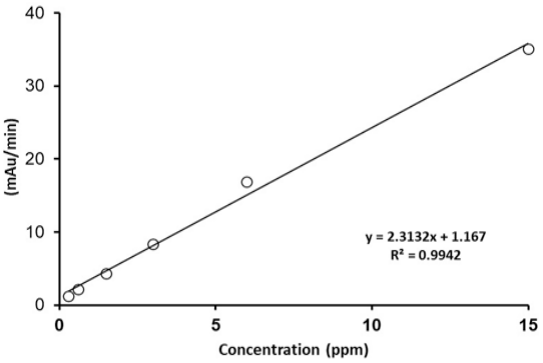

Fig. 3. The calibration curve of Amlodipine besylate obtained from the optimized analytical procedures

Linearity

The linearity of the detector's response to AB was assessed by plotting the peak area of the pure drug against its concentration. The calibration curve exhibited linearity within the 0.3-15 ppm range, demonstrating a strong r^2 value of 0.9942. The regression line equation was y = 2.3132x+1.167 (Figure 3).

Another study separated AB from the

pharmaceutical dosage form, and the method was linear over the range of 10-100 µg/mL. This could indicate the sensitivity of the presently developed process, which can detect the drug at 0.3 ppm concentration.[36]

Limit of Detection and Limit Quantitation

From the constructed calibration curve and the linear regression equation, the LOD and LOQ were calculated. The calculated data indicated that the smallest analyte concentration detectable was 0.117 ppm, and the lowest concentration that could be precisely detected was 0.39 ppm.

Compared to the Chinese Pharmacopeia 2015, the present developed UPLC analytical method was found to be more responsive, with a limit of quantification (LOQ) of 0.117 ppm, compared to 18 ppm for the Chinese Pharmacopeia assay37. Compared to other studies, the results also showed sensitivity in detecting small amounts of the drug, which is consistent with other studies that detect the drug with a limit of quantification (LOQ) of 2.6 ppm and a limit of detection (LOD) of 0.8 ppm[32].

Accuracy

The accuracy verification was assessed by analyzing three samples of AB reference drug (0.6, 6, and 15 µg/mL), prepared according to the methodology described earlier. The %recovery range from 94.38-116.06%, which indicates a high level of accuracy. Detailed analytical data are offered in Table 4.

Table 4: Percentage Recovery and Inter- and Intra-Day Precision of the Optimized UPLC Green Analytical Assay for Amlodipine Besylate

Nominal concentration (ppm)		0.6	6	15
		(PeaK Area (mAu))/(RSD%)		
Intra-day	Day-1	2.13	16.12	35.12
		1.65	2.34	1.79
	% Recovery	94.38	116.06	98.87
Inter-Day	Day-1	2.13	16.12	35.12
		1.65	2.34	1.79
	Day-2	2.4	17.38	33.51
		2.01	2.13	2.46
	Day-3	2.33	17.51	34.15
		2.21	1.88	1.78

Precision

To verify the precision of the proposed UPLC method, it was evaluated by analyzing the selected standard solutions of AB at the same concentration levels as those used for the accuracy assessment. Precision was determined by conducting the assay three times within the same day and on three separate days. In both cases, low relative standard deviation (RSD) %values were obtained, indicating a high level of method precision. Detailed analytical data are presented in Table 4.

Robustness

The robustness study aimed to demonstrate that the method remained unaffected by minor intentional changes in the analytical conditions. Triplicate samples were analyzed for each parameter, including flow rate (ml/min), detection wavelength (nm), and temperature (°C). The low %relative standard deviation (RSD) values, as shown in Table 5, indicate the method's durability.

Table 5: Robustness of the Optimized UPLC Condition for Amlodipine Besylate Analysis

Parameters	Responses			
Flow rate (mL/min)	Retention time (min)	RSD%	Peak Area (mAU)	RSD%
0.048	6.44	0.89	37.99	0.52
0.05	5.43	0.58	35.12	1.79
0.052	5.97	0.07	36.52	0.87
Wavelength (nm)				
236	5.34	0.45	34.24	2.40
238	5.34	0.45	34.40	1.79
240	5.34	0.45	33.84	0.99
Temperature °C				
23	6.35	0.45	35.4	1.55
25	5.43	0.58	35.12	1.79
27	6.01	0.39	37.34	2.24

Quality control tests for capsules

Content uniformity

The content uniformity analysis for AB in Amlor® with a dosage of 5 mg was conducted following the guidelines outlined in the USP Pharmacopeia. The findings revealed that Amlor® (Fig. 4(b)) has an Acceptance Value (AV) of 10.12. Consequently, the calculated acceptance values for Amlor® were below 15, indicating compliance with the specified requirement.

***In vitro* dissolution**

The dissolution data for AB from the branded product (Amlor®) with a 5 mg dosage is presented in Fig. 4(a). The drug showed a dissolution rate of 80.23±2.65% after 30 min, meeting the requirements specified in its monograph. At least 75% of the claimed amount of AB is dissolved within 30 minutes.

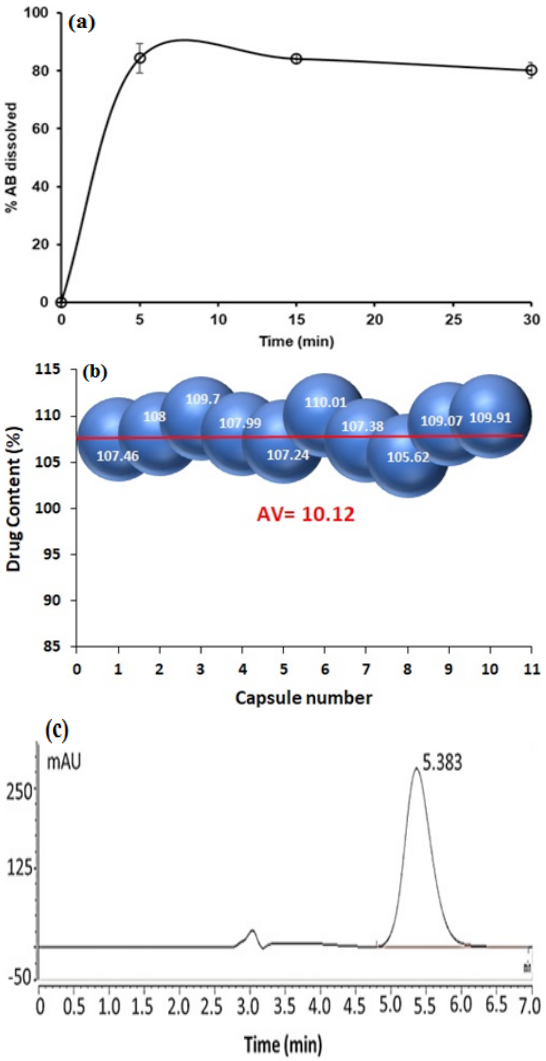

Fig. 4. Analysis of Amlodipine besylate from marketed product (Amlor®); (a) Dissolution profile, (b) content uniformity for 10 capsules, and C) UPLC chromatogram

Stability studies

Stability studies for AB solutions under various harsh conditions (0.1 N HCl, 0.1 N NaOH, 30% H_2O_2, and at a high temperature of 50°C) were conducted, and the results are presented in Fig. 5. The obtained data indicated that the thermal stress condition was tolerated by AB (% of remaining AB was 101.2±3.5), and no degradation was noticed on the chromatogram. However, the drug was completely degraded under alkaline stress conditions, and no traces of the intact AB could be detected in the UPLC chromatogram. Also, the drug exhibited extensive degradation in the acidic stress condition, where only 42.07±0.19% of the initial drug remained. The peak of the degraded drug was detected at a retention time of 3.74 min, adjacent to the mobile phase peak. Furthermore, the drug was subjected to extensive degradation in H_2O_2 solution, where only 16.5±0.05 of the initial AB concentration remained unchanged after the incubation period. Additionally, H_2O_2 exhibited a considerable peak that interfered with the mobile phase peak at a retention time of 3.4 minutes. These results agree with the finding of Jain *et al.,* that AB is extensively degraded in oxidative conditions (75%) and highly stable in thermal conditions[38]. In contrast, Mhaske *et al.,*[39] and Runja *et al.,*[40] found that AB was highly stable in thermal and oxidative conditions, with only 11% and 25% of the drug degraded, respectively. For acid/base degradation, Shah *et al.,* found that AB is highly degraded in acidic conditions and stable in alkaline degradation conditions[41]. This finding contrasts with our results, which indicate AB's instability under alkaline degradation conditions.

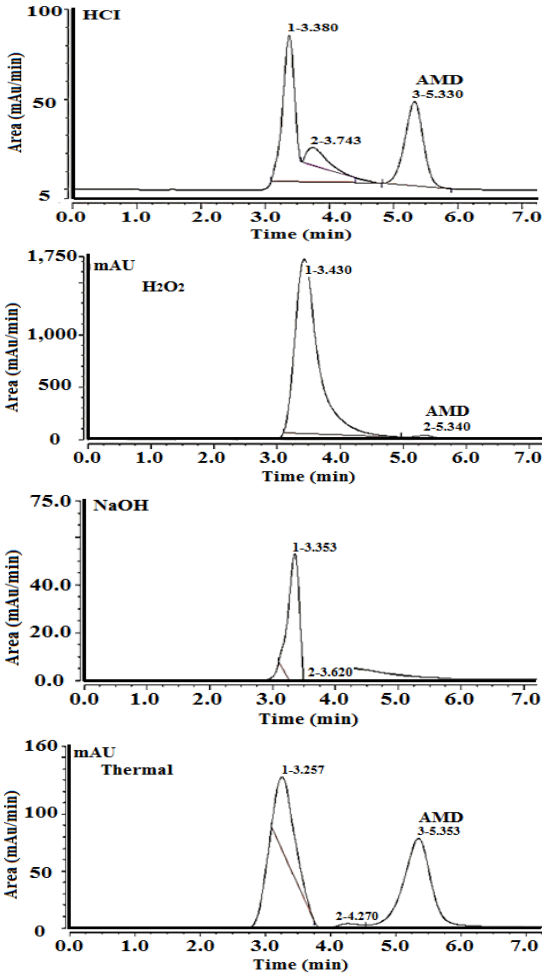

Fig. 5. UPLC chromatogram for the Effects of forced degradation conditions; 0.1 N HCl, 0.01 N NaOH, 30% H_2O_2, and thermal stress at 50°C on AB stability

CONCLUSION

The developed UPLC analytical method was valid and considered simple, accurate, sensitive, rapid, robust, precise, selective, and environmentally friendly due to several factors, such as using less hazardous substances, short run time, and rapid flow rate. Utilizing the Quality by Design (QbD) approach, the optimized values recommended by statistical software closely match the observed values. This method effectively quantifies AB in its pure form and marketed products. Therefore, it can be implemented for routine analysis of Amlodipine besylate in pure and pharmaceutical formulations as an eco-friendly alternative to more hazardous substances commonly used in pharmaceutical analysis. The optimized analytical method was suitable for application in analyzing the routine quality control samples for the assessment of commercial AB product. In addition, this method was found applicable for stability studies on the pure AB. However, future work should be proposed in order to analyze AB in complex formulations and combination active ingredients dosage, in addition to stability in the dosage forms.

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Conflicts of Interest

The authors declare no conflict of interest.

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