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Original Article

QbD Assisted Method Development and Validation of Stability Indicating UV Spectrophotometric Method for Azelnidipine

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ABSTRACT

Background: Azelnidipine, calcium channel blocker used in hypertension management, requires reliable analytical method to ensure its stability, quality, and regulatory compliance. Stability-indicating methods are essential for detecting and quantifying degradation products under stress conditions. Traditional spectrophotometric methods, however, often lack systematic approaches, robustness, and sensitivity. Integrating Quality by Design (QbD) principles into method development provides a structured approach to address these challenges, enhancing method performance and regulatory compliance. **Materials and Methods:** The UV spectrophotometric technique was developed using a 50:50 diluent ratio, and the λ_{max} was found to be 258 nm. In the proposed study, QbD-assisted approach was employed to develop and validate stability-indicating spectrophotometric method for Azelnidipine. Risk assessment tools were used to identify Critical Quality Attributes (CQAs) and Critical Method Parameters (CMPs) that influence method performance. A Design of Experiments (DoE) approach was applied to systematically optimize method parameters. Stress degradation studies were conducted under acidic, basic, oxidative, thermal, and photolytic conditions, following International Council for Harmonization (ICH) guidelines. The method was validated for specificity, linearity, accuracy, precision, robustness, ruggedness, and solution stability. **Results:** The developed method demonstrated high accuracy, precision, and sensitivity in quantifying Azelnidipine and its degradation products. Stress testing showed significant degradation under acidic and oxidative conditions, with minimal degradation under basic conditions. The drug remained stable under thermal and photolytic stress. Validation results confirmed the method compliance with ICH Q2(R2), with a linear response observed in the 2-10 µg/mL concentration range and an excellent R^2 value of 0.9998 at 258 nm. Additionally, the area under the curve was measured at wavelength range of 242-276 nm. The QbD approach ensured a robust and reproducible method, minimizing variability and enhancing reliability for routine quality control and stability analysis. **Conclusion:** The QbD-assisted spectrophotometric method developed in this study provides cost-effective, robust, systematic, and regulatory-compliant solution for the stability analysis of Azelnidipine. The proposed study exemplifies how QbD approach can enhance analytical method development, ensuring greater reliability, improved pharmaceutical quality assurance, and compliance with modern regulatory standards.

Keywords: Azelnidipine, UV Spectrophotometry, Quality by Design, Forced Degradation, Central Composite Design.

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INTRODUCTION

Hypertension remains one of the most significant global health challenges, contributing to increased morbidity and mortality due to its association with cardiovascular diseases, stroke, and renal dysfunction.[1,2] Effective management of hypertension relies on pharmacological interventions, with calcium channel blockers being a widely prescribed class of drugs.[3,4] Among them, Azelnidipine has emerged as a preferred choice due to its unique pharmacokinetic profile, offering sustained blood pressure control, improved vascular protection, and fewer side effects

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compared to other antihypertensive agents.[5,6] As Azelnidipine continues to gain importance in clinical practice, ensuring its stability, safety, and efficacy throughout its shelf life becomes a critical aspect of pharmaceutical development. Its IUPAC name is 3-O-(1-benzhydrylazetidin-3-yl) 5-O-propan-2-yl 2-amino-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate, (Figure 1).[7,8] Stability studies form an essential component of drug development, focusing on the impact of environmental factors such as temperature, humidity, and light on drug physical and chemical properties.[9] Stability-indicating analytical methods are indispensable for identifying and quantifying the Active Pharmaceutical Ingredient (API) as well as its degradation products under stressed conditions. These methods ensure that the drug remains within acceptable quality parameters as outlined by regulatory guidelines.[10,11]

Spectrophotometric techniques are among the most commonly employed methods for drug analysis due to their simplicity, cost-effectiveness, and accessibility.[12] These methods are particularly suitable for routine quality control in pharmaceutical manufacturing. However, traditional spectrophotometric approaches often lack the sensitivity and robustness required to address the complexities of stability studies, particularly when degradation products are formed. One of the key challenges in conventional analytical method development is the reliance on trial-and-error approaches, which are often empirical and fail to ensure consistent performance. Such methods frequently result in suboptimal robustness, limited reproducibility, and potential non-compliance with regulatory standards.

To address these limitations, the pharmaceutical industry has increasingly adopted Quality by Design (QbD) principles in method development. QbD is a systematic approach that integrates predefined objectives, product and process understanding, and control based on sound science and quality risk management to ensure built-in quality and meet the inherent demands of regulatory agencies.[13,14] The implementation of QbD in spectrophotometric method development is gaining recognition as promising approach to improve analytical efficiency, shorten development timelines, and reduce costs by incorporating quality principles from the initial stages of the process.[15,16] The integration of Design of Experiments (DoE) within QbD enables the creation of robust design space, ensuring reliable and optimal performance.[17,18]

Despite the widespread application of QbD principles in chromatographic methods, their adoption in spectrophotometric analysis remains relatively limited and less developed. A review of the literature indicates that the majority of analytical methods reported for Azelnidipine are based on HPLC, primarily in combination drug formulations.[19-31] However, these methods often involve longer run times, higher solvent consumption, and greater operational costs. The proposed UV spectrophotometric method developed using QbD approach offers simpler, cost-effective, and eco-friendly alternative. By systematically analyzing the impact of parameters such as scanning speed and diluent composition, the method minimizes unnecessary experimental trials, thereby saving both time and resources. This makes it particularly suitable for routine quality control applications. The approach uses risk assessment tools and Central Composite Design (CCD) to identify and optimize critical method variables, enhancing method robustness and reproducibility within a defined design space.[32,33] Unlike conventional single-wavelength UV methods, this study applies an Area Under the Curve (AUC) analysis over a selected wavelength range to improve sensitivity and specificity in detecting degradation products.

Stress degradation studies were performed according to ICH Q2(R2) under acidic, basic, oxidative, thermal, and photolytic conditions to evaluate the method stability-indicating capability comprehensively. While this research focuses on the pure drug substance, future work will extend the method validation to formulated products to evaluate excipient interference and formulation-specific challenges.

By integrating QbD principles into UV spectrophotometric method development, this study not only overcomes the limitations of traditional UV methods but also offers a feasible alternative to chromatographic methods for early-stage analysis and routine quality control, particularly in settings with limited access to advanced instrumentation. The outcomes of this research aim to provide a validated, regulatory-compliant analytical tool that advances pharmaceutical quality control of Azelnidipine and sets a precedent for similar applications across other drug substances.

MATERIALS AND METHODS

The standard Azelnidipine drug was kindly provided as a gift sample by Precise Biopharma Pvt. Ltd., India. All reagents used in the study were of analytical grade, ensuring the accuracy of the experimental procedures. Methanol (HPLC grade) and Milli-Q water were employed as solvents. The Azusa-8 mg tablet was procured from a local market in Belgaum, India, for the analysis.

Instrumentation

The analysis was performed using a Shimadzu UV-1800 spectrophotometer (version 2.62), outfitted with 1 cm matched quartz cells. All chemicals and reagents used in the study were of analytical grade.

Selection of Solvents

Different analytical solvents were systematically tested through trial-and-error experiments to identify the most appropriate solvent for the study. Solvents including water, methanol, and DMSO were evaluated. Based on the drug profile and empirical findings, methanol was selected as the optimal solvent. In accordance with the principles of the Design of Experiments

(DOE), 50:50 methanol-water mixture was adopted as the diluent for the preparation of the working stock solution.

Preparation of standard stock solution

A standard stock solution of Azelnidipine (1000 µg/mL) was precisely prepared by dissolving 10 mg of Azelnidipine in 10 mL of methanol. From this concentrated solution, 1 mL was transferred to 10 mL volumetric flask and further diluted to 100 µg/mL with methanol. Subsequently, 1 mL of the 100 µg/mL solution was diluted to 10 µg/mL using a 50:50 methanol-water mixture to facilitate the initial UV spectral analysis.

Determination of wavelength of maximum absorption

A standard working solution of Azelnidipine (10 µg/mL) was analyzed by UV spectrophotometry over the wavelength range of 200-400 nm to determine the optimal analytical wavelength. Azelnidipine exhibited maximum absorbance (λ_{max}) at 258 nm, which was initially selected as the analytical wavelength for subsequent analyses.

However, the absorbance was integrated over the 242-276 nm range using the Area Under the Curve (AUC) technique. This approach was employed to enhance method sensitivity and robustness by capturing a broader spectral profile, thereby minimizing the impact of minor spectral shifts or fluctuations caused by degradation products. The selected wavelength range was chosen based on optimization of the signal-to-noise ratio and demonstrated consistent spectral stability across multiple replicates, ensuring reliable quantification in both stressed and non-stressed samples.

DoE based Method Development and optimization

The adoption of an AQbD paradigm for systematic and science driven method development

The AQbD framework to Carefully analyze cause-and-effect interaction and enable the development of robust risk management strategies. A cause-and-effect showing the relation between CMVs and CAAs. Furthermore, the solvent for UV spectrophotometric analysis of Azelnidipine was selected to be methanol, and subsequent validation was performed by optimizing the methanol-to-water ratio, as methanol is less commonly utilized, thereby enhancing the cost-efficiency of the method. Then spectra were taken by scanning 10 µg/mL standard solution in the chosen solvent from 200 to 400 nm using UV spectrophotometer. Variables influencing the method are represented in the Ishikawa diagram, and resulting data were systematically analyzed to assess optimization and ensure reproducibility.

Creation Analytical target profile

Thorough literature review and analyte profiling helped define the method goal, which was aimed at the development of robust, cost-efficient analytical strategy for the precise quantification of Azelnidipine. The UV spectrophotometric method was chosen because it is simple, fast, and cost-effective compared to more complex methods. To align with the ATP, the absorbance of Azelnidipine was selected as the CAA.[34,35]

Optimization of the Method with Central Composite Design

CCD served as a statistical approach to identify the optimal method parameters and assess the robustness of the analytical procedure.[36,37] Nine experiments were systematically conducted, incorporating three center points, in alignment with the face-centered CCD methodology.[38] This approach aimed to optimize Critical Method Variables (CMVs), such as scanning speed (A) and the proportion of diluent (B), as driven by comprehensive risk assessment. According to the CCD framework, the response variable for the study was the absorbance of the standard Azelnidipine solution (10 µg/mL) measured at its maximum absorbance wavelength of 258 nm. Although Azelnidipine exhibited maximum absorbance at 258 nm, absorbance at this wavelength was selected as the primary response variable for CCD optimization. The Area Under the

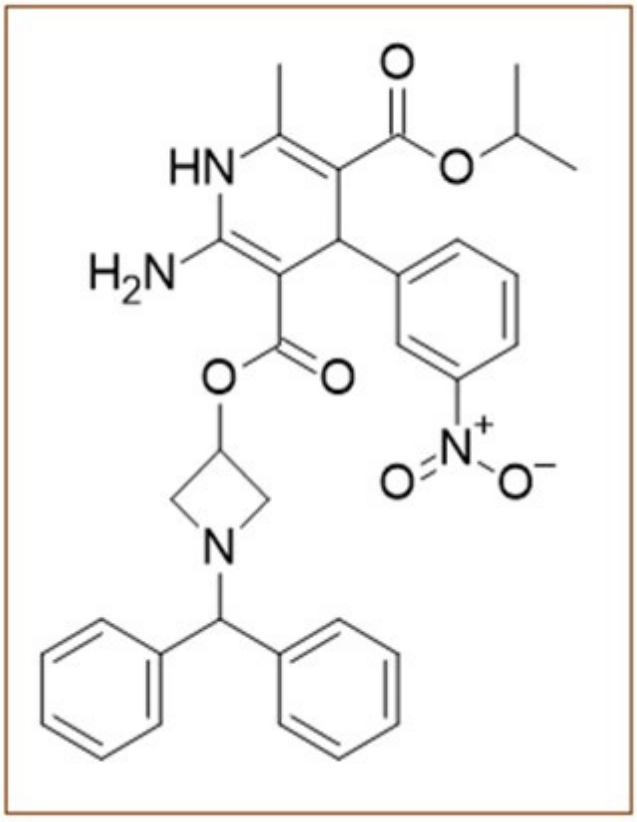


Figure 1: Chemical structure of Azelnidipine.

Curve (AUC) from 242-276 nm was additionally assessed during method validation to improve robustness against spectral fluctuations caused by stress degradation. While AUC was not used as the CCD response, it served as supporting metric to confirm method sensitivity and stability across stressed and non-stressed conditions.

The observed data were rigorously analyzed and modeled using the Design Expert software version 13.Polynomial equations were derived for the significant model terms (p-values ≤ 0.05), as determined through ANOVA.[39] Model relevance was assessed using lack-of-fit tests and correlation coefficient analysis, while contour and response surface plots were employed to thoroughly explore the relationship between the CMVs and Critical Analytical Attributes (CAA).[40] Method optimization was carefully conducted using both advanced numerical and graphical approaches via DoE.

Method Validation

In the validation method, the important parameters of an analytical method must be in line with its expected performance features to meet certain standards such as ATP.[41,42] Moreover, International Council for Harmonisation has set inclusive guidelines to ensure that these methods meet global performance standards. Consequently, for this analytical method, it is crucial to validate the method in accordance with ICH Q2(R2), with focus on the carefully defined key parameters, to effectively apply the AQbD approach. Hence, by utilizing the chosen critical parameters, the developed method was subsequently validated in compliance with ICH Q2(R2).[43,44] The assessed aspects included specificity, selectivity, system suitability, linearity, precision, accuracy, Limit of Detection (LOD), Limit of Quantification (LOQ), robustness, ruggedness, solution stability, forced degradation studies, and the assay of the marketed formulation.[45-47]

Specificity and Selectivity

When the solvent was run alone, its spectrum showed no interference with the absorbance of the analytes.Azelnidipine exhibited maximum absorbance at 258 nm. The UV spectrum of the analytes is shown in (Figure 2A).

The method was found to be selective for Azelnidipine when analyzed using a marketed formulation, with no interference observed from excipients.

Linearity

Prepare concentration range from 2 to 10 µg/mL by diluting the 100 µg/mL standard solution of Azelnidipine. UV absorbance was recorded at 258 nm for each concentration. Furthermore, the AUC method was utilized, considering wavelength range of 242-276 nm, which corresponds to the absorption spectrum. Calibration curves were then generated to define the correlation between concentration and absorbance.

LOD & LOQ

The LOD refers to the smallest concentration of the drug that can be accurately detected, though not necessarily quantified, whereas the LOQ is the minimum concentration that can be reliably quantified with acceptable precision and accuracy.

System Suitability

System suitability for UV spectrophotometry, as per ICH Q2(R2) guidelines, ensures the reliability and precision of the analytical system by assessing parameters such as absorbance consistency,

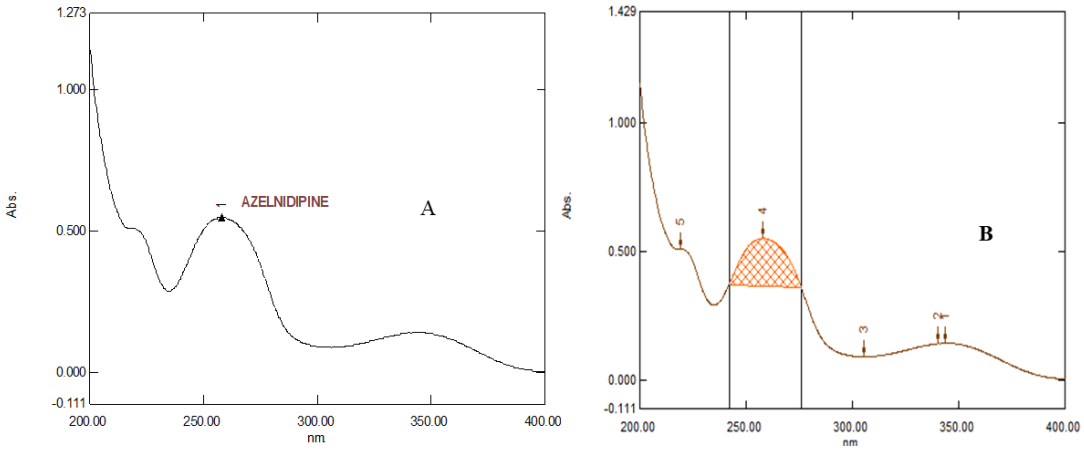


Figure 2: (A) UV absorption spectrum of Azelnidipine using the zero-order method, (B) The area under curve method.

Table 1: **Experimental Design Matrix Demonstrating the Spectrophotometric Range Investigated and the Corresponding Response.**

Run. No	Factor A %Diluents(A)	Factor B Scanning speed(B)	Absorbance
1	40	-1	0.525
2	50	0	0.545
3	60	0	0.586
4	50	-1	0.543
5	50	1	0.546
6	60	1	0.584
7	40	0	0.523
8	60	-1	0.587
9	40	1	0.521
Coded level -1(Low) 0 (Nominal) 1 (High)	Percentage of Diluents(A) Low 40% Medium 50% High 60%	Scanning speed(B) Slow Medium Fast	

Table 2: **Interday Precision.**

Intraday precision (*n*=6)			
Concentration (µg/mL)	Azelnidipine (258 nm)		
	Morning	Morning	Morning
	Absorbance	Absorbance	Absorbance
2	0.119	0.126	0.152
2	0.12	0.124	0.153
2	0.12	0.124	0.152
2	0.119	0.121	0.148
2	0.12	0.123	0.148
2	0.121	0.124	0.152
%RSD	0.628	1.320	1.477
6	0.334	0.339	0.369
6	0.337	0.342	0.369
6	0.337	0.341	0.37
6	0.332	0.34	0.368
6	0.332	0.341	0.367
6	0.33	0.341	0.367
%RSD	0.861	0.303	0.328
10	0.552	0.558	0.584
10	0.553	0.554	0.584
10	0.552	0.558	0.584
10	0.551	0.557	0.583
10	0.551	0.557	0.583
10	0.552	0.558	0.583
%RSD	0.136	0.278	0.0856

Table 3: Interday Precision.

Interday precision (*n*=6)			
Concentration (µg/mL)	Azelnidipine (258 nm)		
	Morning	Morning	Morning
	Absorbance	Absorbance	Absorbance
2	0.119	0.145	0.147
2	0.12	0.143	0.147
2	0.12	0.145	0.147
2	0.119	0.145	0.148
2	0.12	0.142	0.147
2	0.121	0.142	0.147
%RSD	0.628	1.047	0.277
6	0.334	0.352	0.356
6	0.337	0.352	0.356
6	0.337	0.352	0.357
6	0.332	0.351	0.357
6	0.332	0.352	0.357
6	0.33	0.352	0.357
%RSD	0.861	0.116	0.144
10	0.552	0.563	0.565
10	0.553	0.563	0.566
10	0.552	0.564	0.565
10	0.551	0.563	0.565
10	0.551	0.563	0.566
10	0.552	0.564	0.566
%RSD	0.136	0.0916	0.0968

wavelength accuracy, and %RSD. This is evaluated by analyzing six replicates of 6 µg/mL solution and calculating the %RSD of absorbance to validate the method performance.

Precision

In accordance with ICH Q2(R2), the precision of an analytical procedure involves to the degree of reproducibility in results obtained from multiple measurements of an identical, homogeneous sample. Both intra-day and inter-day precision are systematically assessed to evaluate the method reliability and consistency over time. To evaluate intra-day precision, six replicates of 2, 6, and 10 µg/mL concentrations was measured on single day, for inter-day precision, six replicates of the same concentrations was assessed across three sequential days by uv spectroscopy. Then % RSD was subsequently determined.

Accuracy

Accuracy is determined by comparing the observed concentration of sample to its known or expected value. proposed study, accuracy was evaluated through recovery experiments at three levels, 80%,100% and 120% of the standard solution. The sample were spiked accordingly and analyzed using UV spectrophotometer. The percentage recovery was calculated from six trials analyses.

Robustness

Robustness was evaluated by varying the wavelength. Six replicates of Azelnidipine were prepared at concentrations of 2, 6, and 10 µg/mL, then the absorbance will be recorded at wavelength (256, 258 and 260 nm), accurately calculated the percentage Relative Standard Deviation (%RSD).

Ruggedness

The method of ruggedness was assessed by introducing two variations: (A) changing the analyst, and (B) changing the instrument. Three sets of solutions, containing concentrations of 2, 6, and 10 µg/mL of Azelnidipine, were prepared. The absorbance of each solution was measured by different analysts. Additionally, for variation (B), change in the instrument was made, using the Shimadzu UV-1900 UV-Spectrophotometer to measure this variation. Furthermore, each concentration for both variation levels of Azelnidipine was analyzed in six replicates, and the (%RSD) was then calculated

Solution Stability

The stability of the solvent and standard stock solutions was evaluated by preparing fresh stock solutions. These newly prepared solutions were then used to prepare dilutions at concentrations of 2, 6, and 10 µg/mL for analyte. The absorbance of these newly prepared dilutions was compared with that of the stock solutions prepared earlier. To assess any potential variations, the percentage Relative Standard Deviation (%RSD) was calculated.

Forced Degradation Study

The FDA and ICH Q2(R2) mandate the collection of stability testing data to evaluate the quality of drug substances. Forced degradation studies are designed to generate degradation products, which are critical for developing stability-indicating methods for the analysis of pharmaceutical samples.[48] These studies involve various stress conditions, including acidic, basic, oxidative, thermal, and photolytic degradation, providing valuable insights into the degradation mechanisms and by products of active pharmaceutical ingredients.[49] In this study, degradation was assessed by measuring the absorbance of Azelnidipine before and after stress exposure, as well as by evaluating any potential interference with the analyte peak, in accordance with ICH Q2(R2). Such data is essential for understanding the stability profile and ensuring the integrity of the drug throughout its shelf life. In deviation from ICH Q1B guidelines, the photolytic exposure was restricted to 2 hr using available conventional light sources, owing to limited instrument accessibility.

Condition of Forced Degradation

Acid Degradation

In 10 mL volumetric flask, 1 mL of 0.1N HCl was added to 1 mL of the second stock solution of Azelnidipine (100 µg/mL). Then solution was diluted to the mark with working diluent. After that mixture was heated at 70°C for 2 hr, then allowed to cool. After cooling, it was analyzed using spectrophotometry.

Base Degradation

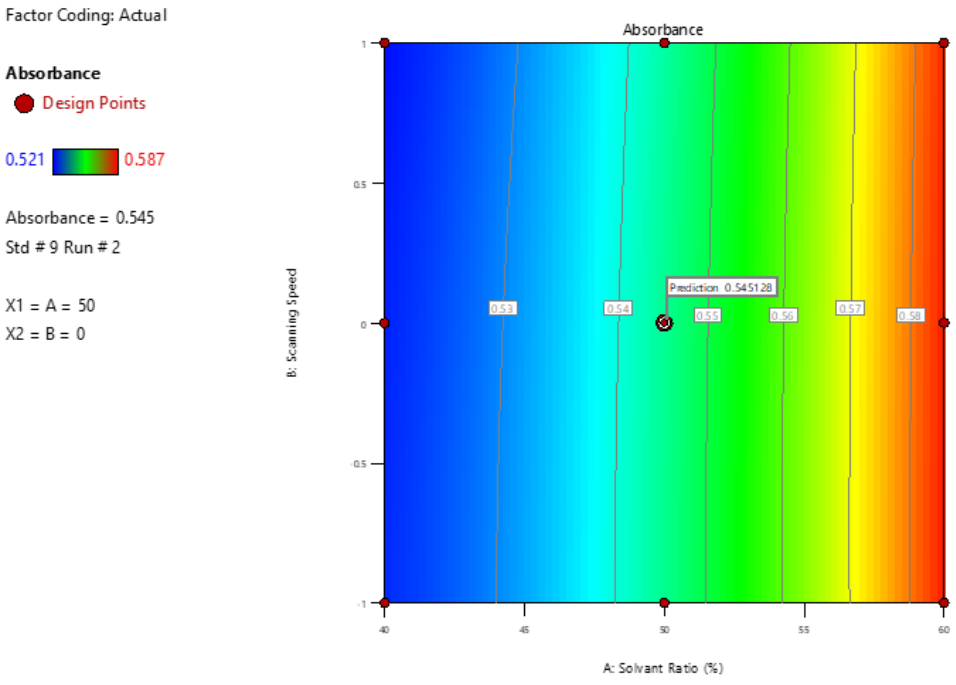
In 10 mL volumetric flask, 1 mL of the second stock solution of Azelnidipine (100 µg/mL) was combined with 1 mL of 0.1N NaOH. The solution was then subsequently diluted to the calibration mark with an appropriate diluent. The mixture was then subjected to heating at 70C for a duration of 2 hr, followed by cooling. Once the solution had cooled, it was analyzed using UV spectroscopy.

Oxidative Degradation

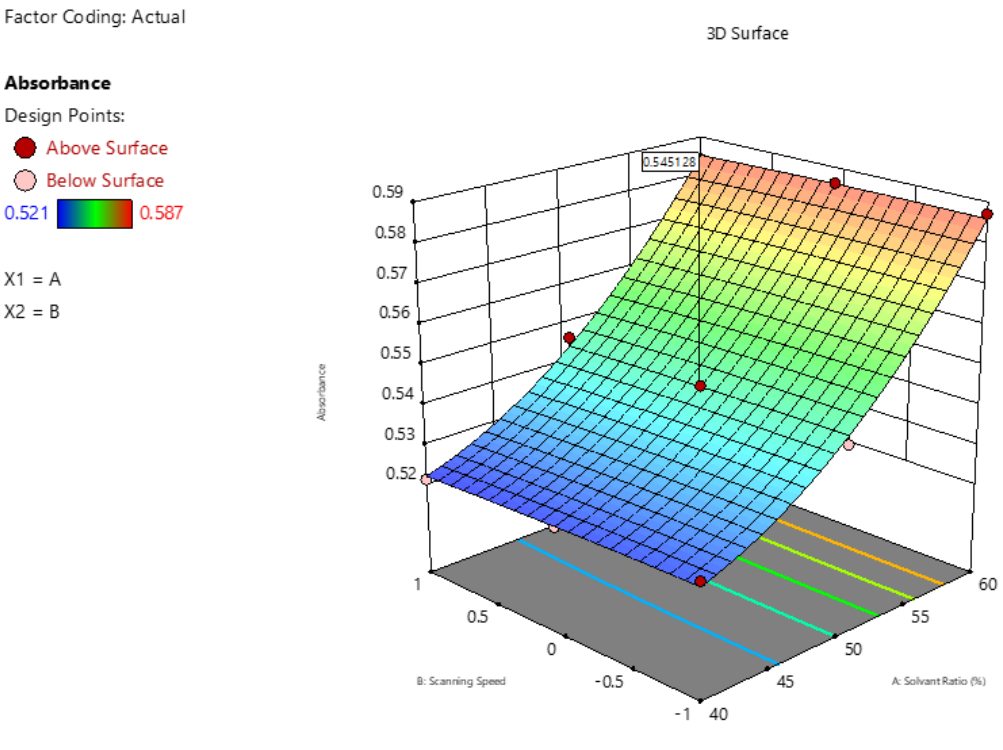
1 mL of the second stock solution of Azelnidipine (100 µg/mL) was mixed with 1 mL of 3% hydrogen peroxide (H_2O_2) in 10 mL volumetric flask. The resulting mixture was subjected to heating at 70°C for duration of 2 hr, followed by dilution to the desired volume with the appropriate diluent. Once the solution had cooled, it was analyzed using spectrophotometric methods to assess its characteristics.

Thermolytic Degradation

1 mL of the 100 µg/mL Azelnidipine solution was carefully pipetted into 10 mL volumetric flask, then diluted with the



(A)



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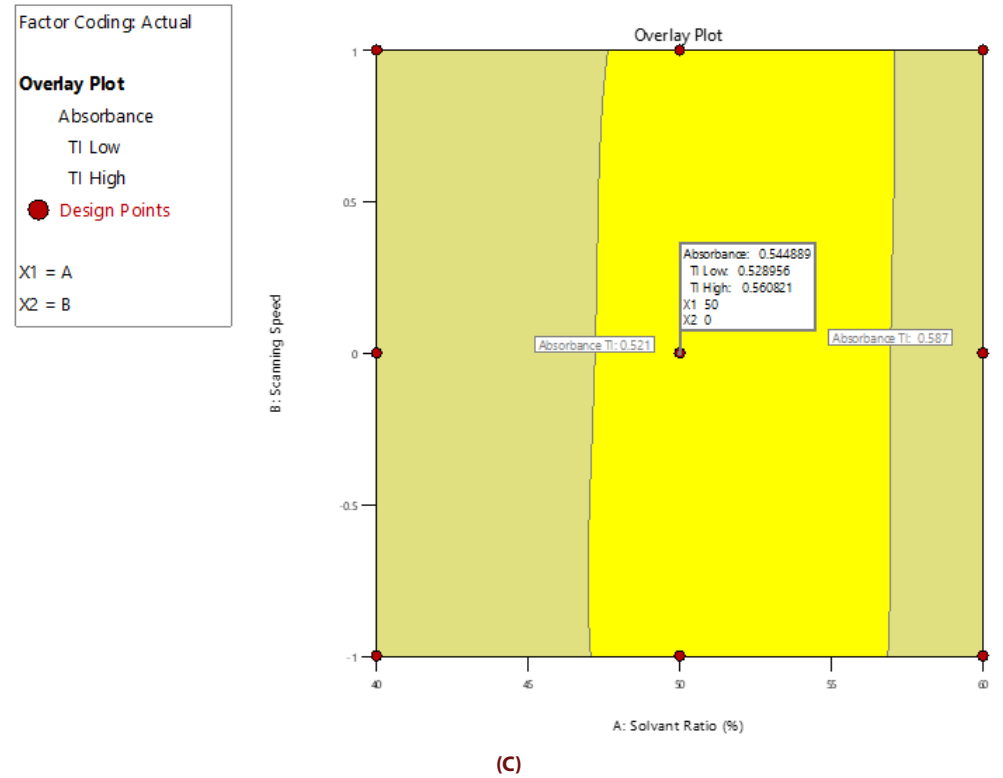


(C)

Figure 3: (A) Counter plot showing the correlation between the selected variables i.e., percentage of Diluents and scanning speed and response. (B) 3D plot of response of Azelnidipine. (C) Overlay plot showing the design space from experimental area.

designated solvent. The mixture was subjected to heating at 70°C for period of 2 hr. After cooling, the solution was adjusted to the final volume and subsequently analyzed using spectrophotometric techniques to evaluate its properties.

Photolytic Degradation

1 mL of the 100 µg/mL Azelnidipine stock solution was aliquoted into 10 mL volumetric flask, followed by dilution with working diluent. The solution was then subjected to direct sunlight exposure for duration of 2 hr. Afterward, the sample underwent analysis via spectrophotometric methods to assess its characteristics.

Estimation in Marketed formulation

Twenty Azelnidipine tablets (8 mg each) were weighed, powdered, and placed in 50 mL volumetric flask. Then, 30 mL of methanol was added, and the mixture was shaken and sonicated for 5 min. The solution was then diluted to the 50 mL mark, resulting in a concentration of 80 µg/mL of Azelnidipine.

Working Sample Preparation

For the assay, pipette 1 mL from the 80 µg/mL solution and transfer it into 10 mL volumetric flask. Then, add the working diluent to bring the volume up to the desired final level. The solution yielding final Azelnidipine concentration of 8 µg/mL, was subsequently subjected to UV spectral analysis for further evaluation.

RESULTS

The proposed study, developed through UV spectrophotometric method. Where the drug Azelnidipine was found to be soluble in methanol with reference to the BCS class II. The percentage of diluent and scanning speed were considered independent variables, while absorbance was the dependent variables. The optimal values for the percentage of diluent ratio and scanning speed were determined to be 50:50 methanol-water and nominal scan speed, respectively, with the assistance of (DoE) software. Azelnidipine solution showed peak at 258 nm represented in (Figure 2B), which was selected as the detection wavelength for the study. Additionally, the Area Under the Curve (AUC) was measured in the range of 242-276 nm.

Method Development and optimization

A QbD approach was utilized, identifying two key factors based on CMA and CPP. The effect of CMVs on CAA was evaluated utilizing (CCD). A total of nine randomized experiments were performed on a UV spectrophotometer, incorporating at least three center points to enhance the robustness of the design and ensure the reliability and impartiality of the results. The responses derived from each experiment, along with the corresponding spectrophotometric range investigated, are presented in Table 1.

Where, A represents the percentage of diluent ratio, B represents the scanning speed.

The response surface analysis was performed using 3D response surface plot and 2D contour plot to elucidate the interaction effects and optimize the analytical parameters. In the 2D contour plot (Figure 3A), absorbance exhibited direct proportionality to both the percentage of diluent and the scanning speed, highlighting their significant role in the analytical response. It was observed that the absorbance at 258 nm exhibited meaningful increase when both, the ratio of diluent in percentage and scanning speed was increased, indicating strong correlation between these variables and the analytical response as shown in (Figure 3B). The design space displayed in (Figure 3C), for the optimized conditions suggested employing mid-level values for both factors to facilitate the progression of the study, thereby ensuring balanced and controlled experimental approach.

Method Validation

Linearity

Azelnidipine displayed linear absorbance within the 2 to 10 µg/mL concentration range, with regression coefficients of 0.9998 for respectively. The linearity data is provided in Table 4, and the standard overlay spectra calibration curves and for drug are shown in Figures 4A and 4B. Additionally, the AUC for the linearity range of 2 to 10 µg/mL.The graphs are represented in Figure 5A, and the linearity data is provided in Table 4.

LOD and LOQ

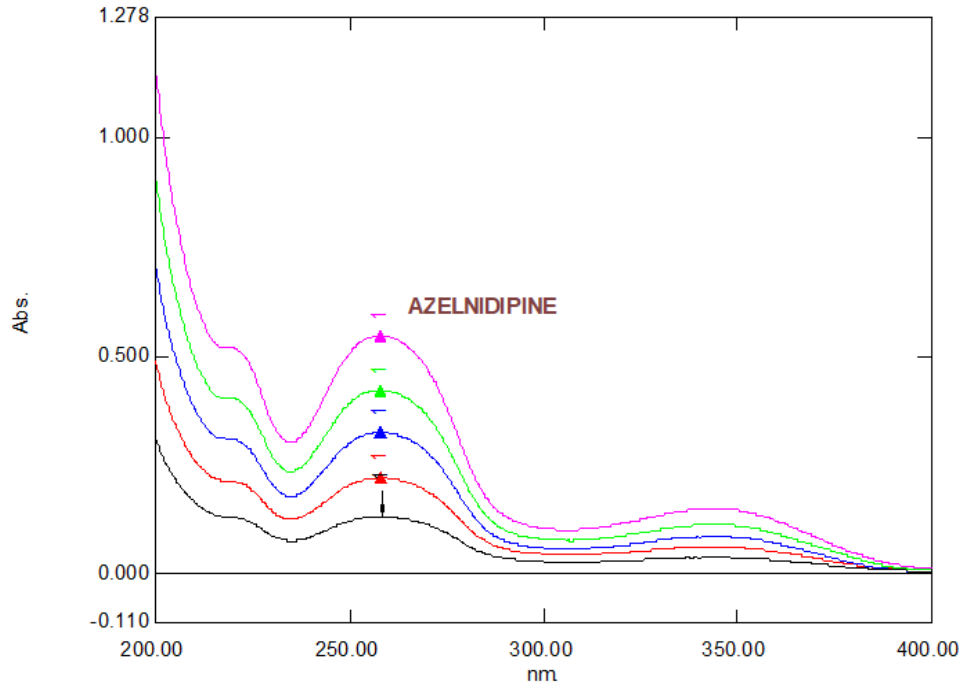
The LOD and LOQ were determined based on the concentrations used in the linearity assessment, specifically 2 to 10 µg/mL. The absorbance responses for these concentrations were recorded, and the standard deviation of the intercept and slope of the calibration curve was used for LOD and LOQ calculations, adhering to the formulas prescribed by ICH Q2(R2). The resulting LOD (0.187 µg/mL) and LOQ (0.567 µg/mL), as well as the AUC-based LOD (0.179 µg/mL) and LOQ (0.544 µg/mL), comply with the acceptance criteria defined by ICH, confirming the sensitivity and reliability of the method.

Precision

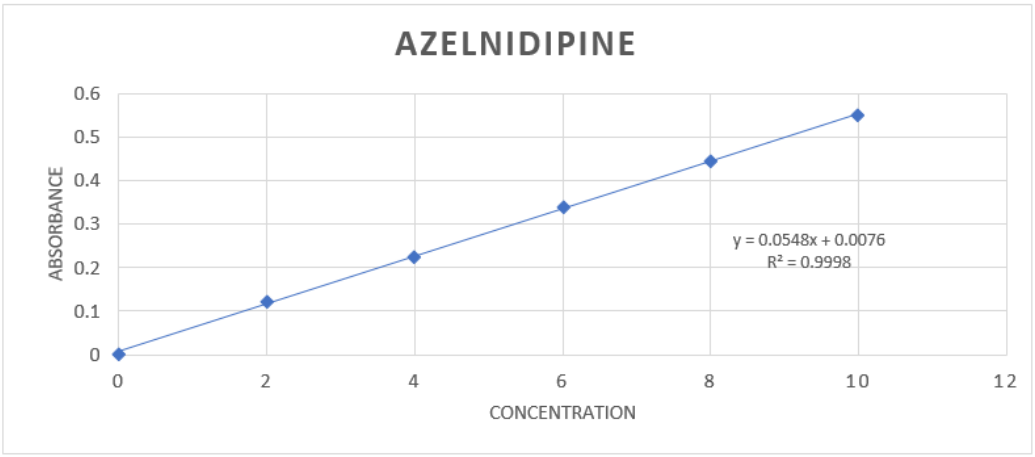
According to ICH Q2(R2), the precision studies for both intraday and interday assessments demonstrated that the percentage RSD was less than 2%, as presented in Tables 2 and 3.

System Suitability

System suitability was assessed by measuring the absorbance of six replicate standard solutions of Azelnidipine at a concentration of 2, 6, 10 µg/mL. As per system suitability criteria for UV spectrophotometry, the (%RSD) of absorbance values should not exceed 2.0%. The observed %RSD was within the acceptable limit,



(A)



(B)

Figure 4: (A) Overlay spectra of Azelnidipine for linearity, (B) Calibration curve plot of Azelnidipine at 258 nm.

as reported in Table 4, indicating consistent instrument response and confirming the suitability of the method for routine analysis.

Accuracy

The proposed study demonstrates a recovery range of 99.87% to 100.15%, it obeys with ICH Q2(R2), with the results presented in Table 4.

Robustness

This study demonstrates that variations in wavelength were evaluated by measuring absorbance at different wavelengths. A percentage RSD of less than 2% confirms the robustness of the method. The results are presented in Table 4.

Ruggedness

Ruggedness was evaluated by employing different analysts and varying the instruments used. The results, showing a percentage RSD of less than 2%, confirm the method reliability. The data are presented in Table 4.

Solution Stability

The solvent and standard stock solution demonstrated stability for up to 10 days, with the %RSD for Azelnidipine between the 10-day-old and freshly prepared stock solutions remaining below 2%. The solution stability data are detailed in Table 4.

Table 4: Summary of Validation parameter.

Sl. No.	Parameter	Concentration *n=6*	Result
1.	Linearity (r^2)	2-10 (µg/mL)	0.9998
2.	AUC Linearity (r^2)	2-10 (µg/mL)	0.9998
3.	System sutability	2,6,10 (µg/mL)	% rsd ≤2%
		Normal range	
3.	LOD	≥2 times base line	0.18 & 0.17-AUC
4.	LOQ		0.56 & 0.54-AUC
5.	Accuracy	98-102%	100.04%
6.	Robustness	% rsd ≤2%	0.642%
7.	Ruggedness	% rsd ≤2%	1.709%
8.	Solution Stability	2,6,10 (µg/mL)	% rsd ≤2%
9.	Assay	% Purity	101.25%

Table 5: Azelnidipine degradation under different conditions of exposure.

Percentage of Degradation	
Parameters	**% Degradation**
30% H_2O_2	10.90%
1N HCl	18%
0.1N NaOH	6.90%
70C THERMOLYTIC	5%
PHOTOLYTIC	2.9

Forced Degradation Study

Degradation studies was performed by subjecting the product to various physical stress conditions. The forced degradation results are shown in Table 5, The findings demonstrated that the degradation of Azelnidipine remained within pharmaceutically acceptable limits. Acid degradation (18%): Azelnidipine degraded the most under acidic conditions, with 18% of the drug breaking down. This suggests that the drug is somewhat sensitive to acidic environments. Base degradation (6.90%): Under basic (alkaline) conditions, only 6.9% of the drug degraded. This is a lower degradation rate compared to acid, indicating that Azelnidipine is more stable in basic conditions. Oxidative degradation (10.90%): Under oxidative stress used reagent 3% H_2O_2 the drug degraded by 10.9%. This shows moderate sensitivity to oxidation. Thermal degradation (5.90%): When exposed to heat, 5.9% of the drug degraded, indicating that it is relatively stable under thermal conditions Photolytic degradation (2.90%): Exposure to light led to a 2.9% degradation, suggesting that Azelnidipine is quite stable when exposed to light. The degradation percentages were quantitatively determined by comparing the absorbance values of stressed samples with those of the unstressed control sample. No spectral interference from degradation products was observed, ensuring the specificity and reliability of the analytical method (Figure 5B).

Estimation of Azelnidipine in marketed formulation

The method effectively evaluated Azelnidipine, with clear spectrophotometric separation and no excipient interference Table 4, confirms compliance with the 98% to 102% acceptance criteria.

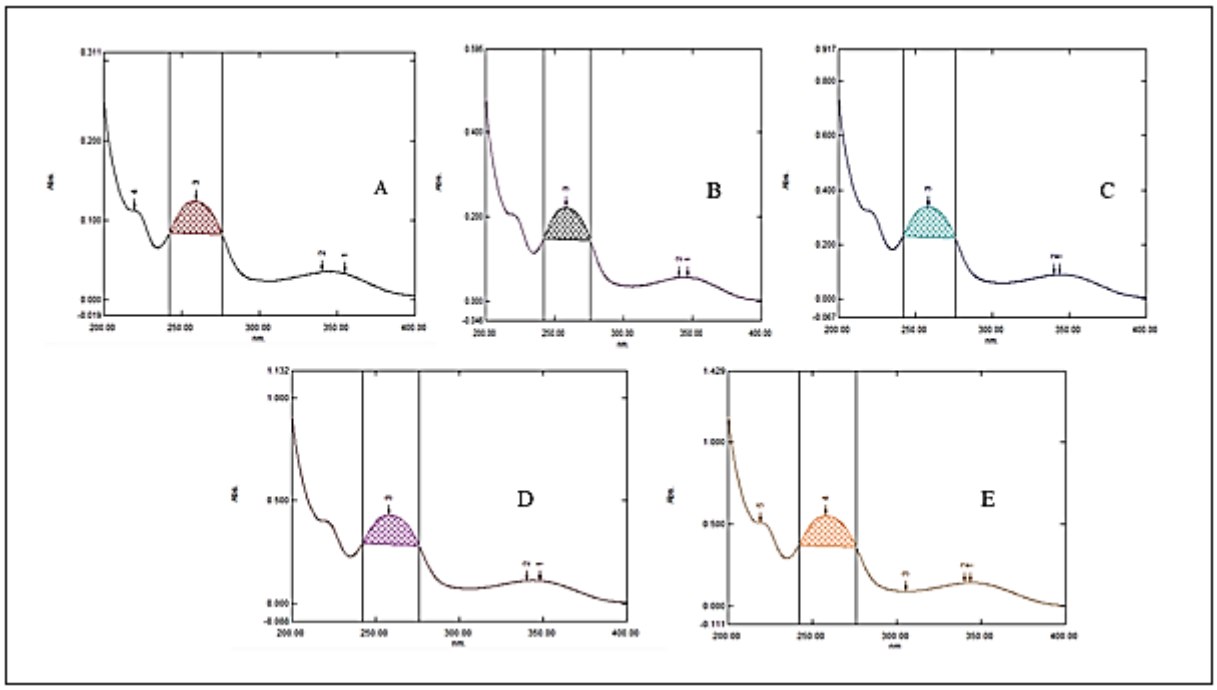
DISCUSSION

The results of the proposed study demonstrate that the UV spectrophotometric method developed for Azelnidipine, optimized using Analytical Quality by Design (AQbD) principles, is robust,accurate, precise, and linear within the tested concentration range. Method robustness was confirmed through Design of Experiments® (DoE), showing minimal variation under deliberately altered conditions, indicating the method suitability for stable and reproducible analytical applications.

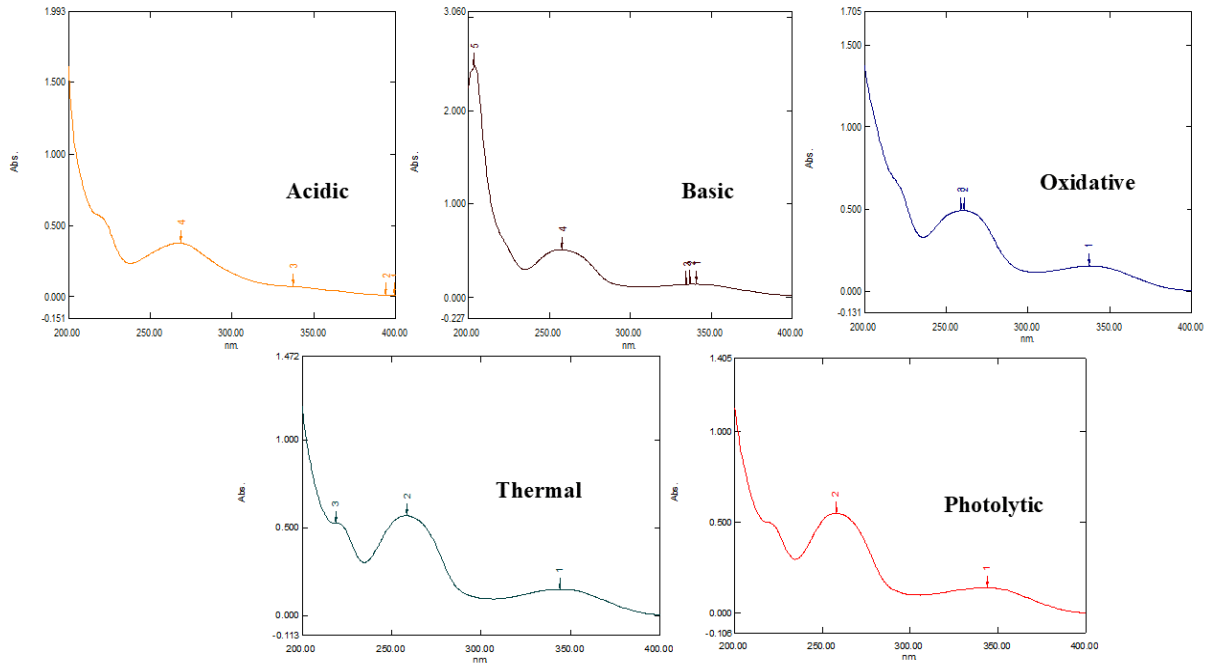
Limitations and Scope

We acknowledge that method validation using marketed formulations was not performed in this study, although it is mandatory according to ICH Q2(R2) guidelines. However, the assay parameter (% purity) of the marketed formulation was evaluated as an initial step.We recognize this limitation and recommend that future studies include comprehensive matrix-based validation using marketed products to confirm selectivity and recovery under real-world conditions. Additionally, while forced degradation studies followed ICH-recommended stress conditions for acid, base, oxidation, and thermal degradation, and the photolytic degradation exposure was limited to 2 hr due to practical constraints. We acknowledge this as a methodological limitation and suggest that future studies include extended photolytic degradation using standardized light sources, as outlined in ICH Q1B guidelines.

Although the degradation results under acidic and oxidative conditions are consistent with prior reports on dihydropyridine analogs, the novelty of this study lies in the application this newly



(A)



(B)

Figure 5: (A) Spectra representing the Area Under the Curve (AUC) for Azelnidipine in the linearity range of 2-10 µg/mL: (A) 2 µg/mL, (B) 4 µg/mL, (C) 6 µg/mL, (D) 8 µg/mL, and (E) 10 µg/mL. (B) Forced Degradation Study of Azelnidipine.

developed method using less costly solvents like Methanol:Water (50:50% v/v) as compared to exsiting methods. Unlike conventional method-based approaches, this method offers more eco-friendly,resourse efficient, and simplified analytical alternative, making it highly suitable for routine stability testing.

CONCLUSION

In conclusion, QbD-assisted UV spectrophotometric method was successfully developed and validated for Azelnidipine, demonstrating robustness, precision, and cost-effectiveness. Compared to conventional methods, this UV approach utilized significantly less solvent, aided by optimization of the diluent ratio based on QbD principles, thereby reducing solvent consumption

and overall analytical costs.The application of Quality by Design (QbD) principles enabled the systematic identification and control of critical method parameters, ensuring method reliability and reproducibility. Validation confirmed the accordance with ICH Q2(R2) guidelines, while forced degradation studies demonstrated the method stability-indicating capability under acid, base, oxidative, thermal, and photolytic stress.

While the current validation was limited to the pure drug, the absence of excipient interaction studies is acknowledged as limitation. In accordance with ICH Q2(R2) guidelines, matrix-based validation using marketed formulations is recommended to assess method selectivity and robustness in the presence of common tablet excipients, ensuring more comprehensive evaluation of method performance.

The method simplicity, low environmental impact, and scalability make it a valuable tool for routine quality control of Azelnidipine. Furthermore, the QbD foundation of this method is expected to support regulatory compliance and may facilitate broader applications, such as combination products, real-time stability testing, and possible integration with chromatographic techniques for improved impurity profiling.

This study contributes meaningfully to the advancement of analytical methodologies in pharmaceutical sciences, reinforcing the role of QbD in developing reliable, regulatory-aligned, and future-ready solutions for drug analysis.

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ABBREVIATIONS

UV: Ultra Violet Spectrophotometry; **LOD:** Limit of Detection; **LOD:** Limit of Quantification; **QbD:** Quality by Design; **CCD:** Central Composite Design; **CMV:** Critical Method Variable; **CMP:** Critical Method Parameter; **CQA:** Critical Quality Attributes; **ATP:** Analytical Target Profile; **CAA:** Critical Attributes Assessment; **DOE:** Design of Expert: **RSD:** Relative Standard.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICAL APPROVAL

This research project does not need ethics approval since it does not involve human participants, and therefore, consent from participants is not required.

SUMMARY

A robust, QbD-assisted UV spectrophotometric method was successfully developed and validated for the stability analysis of Azelnidipine. Addressing a critical gap in current literature, this study applies Quality by Design (QbD) principles to enhance method reliability, control critical method parameters, and ensure analytical performance. The method utilized a methanol:water solvent system, with λ_{max} determined at 258 nm, and incorporated Area Under the Curve (AUC) analysis from 242-276 nm for improved quantitation. Validation was performed as per ICH Q2(R2) guidelines, confirming linearity, specificity,precision, accuracy, robustness,ruggdness, and solution stability for the bulk drug. While current validation focused on the pure form, the importance of excipient interference is acknowledged, and future studies encouraged commercial formulations to ensure broader applicability. Forced degradation studies under acidic, basic, oxidative, thermal, and photolytic conditions confirmed the method stability-indicating capability. Given its simplicity, low cost, and reliability, this method is suitable for routine quality control of Azelnidipine and may be extended to other antihypertensive agents. This work demonstrates the potential of QbD-based UV methods in advancing analytical quality and regulatory compliance in pharmaceutical development.

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