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Sustainable QbD-Driven RP-HPLC Method for the Simultaneous Estimation of Azelnidipine and Metoprolol Succinate with Green and Red Analytical Assessments

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Abstract: A sustainable, stability-indicating Reverse Phase High Performance Liquid Chromatography method was developed and validated for the simultaneous estimation of Azelnidipine and Metoprolol Succinate using an Analytical Quality by Design approach. Method optimization was performed using Central Composite Design, focusing on critical method parameters including flow rate, mobile phase composition, and detection wavelength. The optimized method employed a C18 column with a mobile phase of acetonitrile: water (70:30, v/v) under gradient elution and detection at 230 nm. The method showed excellent linearity, accuracy, precision, and robustness across a defined concentration range and was confirmed to be stability-indicating through forced degradation studies. To assess the environmental and safety performance of the method, two modern evaluation tools were applied: Analytical Green Star Area and Rapid Assessment of Performance Indicators. The Analytical Green Star Area tool confirmed strong adherence to the 12 Principles of Green Analytical Chemistry, while the Rapid Assessment of Performance Indicators score reflected high analytical reliability and occupational safety in line with Red Analytical Chemistry principles. The integration of Quality by Design with green and red assessments ensures both regulatory compliance and sustainable laboratory practice. This validated method offers a practical and comprehensive solution for the routine quality control of Azelnidipine (AZL) and Metoprolol Succinate (MET) in pharmaceutical formulations.

Keywords: Azelnidipine, Metoprolol succinate, RP-HPLC, Analytical Quality Design, Stability-indicating method, Red Analytical Chemistry and Green Analytical Chemistry

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Introduction

The highest risk factor for cardiovascular disease (CVD) and mortality is hypertension; treatment with this condition can significantly reduce the higher risk associated with blood pressure elevation. Antihypertensive drugs reduce blood pressure and associated harm to target organs (Kain *et al.*, 2010). The long-acting dihydropyridine-based calcium antagonist Azelnidipine (AZL) was just approved and is used to treat myocardial infarction-related cardiac remodeling and ischemic heart disease; however, its impact on hyperglycemia-induced cardiac damage has not been investigated (Fig. 1) (Morris *et al.*, 2024).A common β-adrenergic antagonist, metoprolol is essential to cardiovascular pharmacology. Heart failure, arrhythmias, angina pectoris, and hypertension are the main conditions for which metoprolol is prescribed. The drug works by specifically blocking β-1 adrenergic receptors, which lowers blood pressure, heart rate, and cardiac contractility (Fig. 1) (Darji and Dedania, 2023).

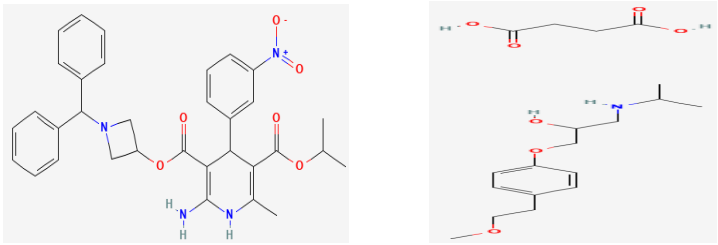
In contrast, Red Analytical Chemistry is a relatively recent concept that emphasizes the safety, health, and well-being of analysts and laboratory personnel. While GAC addresses ecological sustainability, Red Analytical Chemistry highlights human-centric concerns, focusing on reducing occupational exposure to toxic reagents, improving laboratory ergonomics, and ensuring safer analytical practices (Miladinović, 2024; Nowak *et al.*,2025; Mansourv *et al.*, 2025). It underscores the ethical responsibility of chemists to design not only environmentally friendly but also safe and risk-conscious analytical procedures. Together, GAC and Red Analytical Chemistry offer a comprehensive framework for developing modern analytical methods that are both eco-efficient and human-safe. Their integration is particularly vital in pharmaceutical analysis, where large volumes of solvents and reagents are routinely used. GAC's main objective is to reduce or eliminate the use of dangerous chemicals in analytical procedures in order to improve health and the environment without compromising method performance (Galuszka *et al.*, 2013; Koel and Kaljurand, 2021).

The most popular analytical method in pharmaceutical quality control (QC) for characterizing active pharmaceutical ingredients (APIs) and their contaminants in biological fluids and pharmaceutical formulations is high performance liquid chromatography (HPLC). These methods are ideal for regular analysis, allowing for the accurate determination of multiple components in pharmaceutical formulations (Ravi Sankar *et al.*, 2019; Dhull *et al.*, 2025).

To improve accuracy and robustness, the International Council for Harmonization (ICH) places a strong emphasis on Analytical Quality by Design (AQbD) in method development. The concepts of AQbD aid in the development of trustworthy techniques, allowing for ongoing improvement and lowering the need for revalidation. In contrast to conventional trial-and-error techniques, AQbD combines quality to reduce failures and results that are not up to par and guarantees resilience early in the development process (Agrawal and Kotadita, 2024; Mevada *et al.*, 2024a, b, 2025a, b).

A detailed literature survey revealed that numbers of method have been reported in literature for the individual analysis of Azelnidipine (Agrawal and Nizami, 2021; Darji and Dedania, 2023; Author and Mane, 2024; Mevada *et al.*, 2024a;) and Metoprolol succinate (Pekamwar *et al.*, 2014; Zhang and Liu, 2016; Shaikh *et al.*, 2017; Bawane *et al.*, 2018; Marie *et al.*, 2023; Krittanawong *et al.*, 2024) by various analytical methods. However, no Stability indicating RP-HPLC method has been reported with Green and Redassessment for simultaneous estimation of AZL and MET utilizing the Analytical Quality by Design approach.

The objective of this study is to develop reliable and accurate analytical techniques for figuring out how much AZL and MET are present



(a) (b)

Fig. 1: Structure of (a) Azelnidipine and (b) Metoprolol.

in mixes made in a lab. These methods offer an economical and effective choice for simultaneous analysis, making a substantial contribution to pharmaceutical research, upholding quality control, and streamlining accurate dosage calculation. We used AGSA and RAPI tools to do a green and red profile assessment in order to investigate the environmental impact of the recently developed Stability indicating RP-HPLC method utilizing the Analytical Quality by Design approach. Taking into account variables including solvent usage, chemical compounds, energy consumption, and waste formation, this evaluation confirmed the methods' environmental friendliness.

Materials and Methods

Reagents and chemicals

Reference standard of AZL and MET were procured from Zydus Life Science, Ahmedabad, India. Acetonitrile and Methanol (HPLC grade) was used as solvents in this method. All the glass wares were calibrated before using.

Instrumentation

Chromatographic analysis was carried out on a prominence HPLC Method: LC-2010AHT series binary pump systems, Auto sampler injection, temperature controller (column oven) system controller and a UV detector (LC-2010). CLASS-VP (version 2.42) software was used to acquire and process the data.

Sample and standard preparation

Standard solution of AZL (8 µg/ml) and MET (25 µg/ml) were prepared separately by dissolving 8 mg of AZL and 25 mg of MET in 10 ml of mobile phase, respectively. The standard solution was subsequently diluted to prepare different concentrations 4 – 20 µg/ml and 12.5-62.5 µg/ml of AZL and MET, respectively.

Selection of elution mode

Reverse phase chromatography is recommended for ionic and moderate to polar compounds. It is not only easy to use and convenient, but it also performs better in terms of efficiency, stability, and reproducibility. The C18 column was chosen because it is less polar than the C4 and C8 columns, allowing polar compounds to be eluted more quickly than non-polar ones. As a foundation for method development, a 250 × 4.6 mm column of 5 µm particle packing was chosen. Because of its resilience with regard to extended column stability and ease of application, gradient mode was selected. For the majority of separations, this arrangement offers a wide range of potential plate values.

Preparation of mobile phase

The mobile phase consisted of mixture of Acetonitrile:Water (70: 30% v/v) the mode for gradient. The mobile phase was filtered through a 0.22 µm nylon membrane filter and degassed prior to use.

Chromatographic separation

Standard and sample solutions were injected in column using ultrafast autosampler. The chromatogram was run for appropriate time

duration with degassed mobile Acetonitrile: Water (70: 30% v/v) using UV detector (LC-2010) at wavelength 230 nm. The chromatogram was stopped after separation was achieved completely.

Method development using AQbD framework

Establishing Analytical Target Profiles and Critical Quality Attributes: In the context of AQbD, the term "Analytical Target Profile" (ATP) refers to a thorough explanation of the intended performance characteristics and requirements of an analytical technique. It serves as a road map for the methodical advancement and improvement of analytical techniques, guaranteeing that they satisfy established quality standards and legal obligations. By carefully selecting elements like Critical Quality Attributes (CQAs), which have a direct impact on the analytical procedure's quality and safety, the ATP serves as a first step in implementing a QbD-oriented approach. The ATP element targets and their rationale are shown in Table 2. The retention duration, tailing factor, theoretical plates, and resolution are the CQA components that correspond to ATPs and are in charge of effective HPLC analysis.

Risk assessment studies: The primary risk factors influencing the advancement of analytical techniques are to be identified and addressed by this study. To determine how different situations would affect the approach's performance, a thorough risk analysis was conducted. To identify possible hazards, an analysis was done to look at the relationship between ATP and Critical Method Parameters (CMP).

Factor screening study: To find CMPs that had a major influence on the CAAs, screening study was carried out using a Central Composite design (CCD) with Design-Expert® Software version 13. Three center points and two levels—represented by the symbols +1 and -1 for high and low levels, respectively—were used to alter all dependent variables. In order to screen 4 CMPs, the CCD recommended fifteen experiments: flow rate (0.8–1.2 min), acetonitrile volume (65–75), injection volume (15–25 µl), and detection wavelength (229–232 nm). Retention duration, peak area, number of theoretical plates, tailing factor, and resolution were among the factors whose effects on CAAs were examined.

Optimization study: Critical parameters influencing the HPLC method's critical quality attributes (CAAs) were determined by the screening research and risk assessment. Central Composite Design (CCD) was then used to do an optimization analysis. The critical material parameters (CMPs) for the CAAs, which comprised retention duration, number of theoretical plates, and resolution, were determined to be the Flow Rate (ml/min) (X1), Mobile Phase (Volume of Acetonitrile (70 ml)) (X2), and Detection wavelength (nm) (X3). A design matrix with 17 trial runs with chosen CAAs ranging at three levelshigh (+1), midrange (0), and low (-1) was suggested by Design-Expert® version 13.

Force Degradation

In order to demonstrate the stability of both the standard and sample solutions during analysis, both solutions were analyzed over a period of 24 h at room temperature. The results indicated that for both the solutions, the retention time and peak area of AZL and MET did not show much % difference. There was no significant degradation within the indicated period. Hence, it was concluded that both the solutions were stable for 24 h at room temperature.

Method validation

System suitability studies: The system suitability was evaluated by five replicate analyses of AZL and MET mixture at concentration of 16 µg/ml of AZL and 50 µg/ml of MET. The column efficiency, resolution, and peak asymmetry were calculated for the standard solutions.

Linearity: Linearity of the proposed method was assessed by scanning concentrations at five equidistance levels 6 - 20µg/ml and 12.5-62.5µg/ml for HPLC method for Azelnidipine and Metoprolol succinate, respectively. A graph of Concentration vs. Peak Area and Concentration vs. Absorbance was plotted for HPLC method,

respectively and regression equation was obtained.

Precision

Repeatability: The Repeatability precision was performed by evaluating lowest concentration for six replicates; 16 µg/ml and 50 µg/ml for HPLC for Azelnidipine and Metoprolol succinate respectively.

Intraday precision: The Intraday precision was performed by evaluating three concentration levels on same day for three replicates; 4, 12 and 20 µg/ ml and 12.5, 37.5 and 62.5 µg/ml for HPLC method for Azelnidipine and Metoprolol succinate, respectively.

Interday precision: The Interday precision was performed by considering three concentration levels (same as intraday precision) on three different days for three replicates. All solutions were prepared form different stocks prepared on different days. The standard deviation and% RSD were calculated.

Recovery Studies: It was determined by calculating the recovery of AZL and MET by standard addition method. Accuracy was performed by % recovery study at 50%, 100% and 150% by spiking the API to the placebo.

Limit of quantification (*LOQ*) *and Limit of detection* (*LOD*): As per ICH guideline, limit of detection and quantitation of the developed method were calculated from the standard deviation of the response (σ) and slope of the calibration curve (S) of drug using the formula; Limit of detection=3.3* σ/S and Limit of quantitation=10* σ/S.

Robustness: Robustness of the method was determined by subjecting the method to slight change in the method condition individually. For HPLC method, robustness parameters were Pump flow rate (1 ml/min ± 0.2 ml/min) and Mobile Phase Composition. The % RSD was calculated for all the parameters.

Analysis of marketed formulation: Take HPMC (4 mg), MCC (190 mg), Magnesium stearate (4 mg), Talc (2 mg). Role of HPLC-Film forming agent, MCC- Directly compressible material, MS, Gliding agent, Talk, Lubricating agent, AZL (80 mg), and MET (250 mg) was taken into the volumetric flask (100 ml) and volume of the flask was made to 100 ml with methyl alcohol to give stock solution containing 800 µg/ml of AZL, and 250 µg/ml of MET. Withdraw 100 µl from above filtrate in 100 ml volumetric flask; make up the volume with mobile phase, which contain AZL+MET = 8+25 µg/ml.

Results and Discussion

Selection of wavelength

The 230 nm isobestic wavelength of AZL and MET was selected as the detection wavelength for HPLC

Mobile phase selection

Asymmetric peaks and a delayed retention time of MET were the results of using multiple mobile phases with varying ratios of different solvents and pH. The ideal polarity for appropriate migration, separation, and resolution of AZL and MET peaks was supplied by the 70:30 v/v acetonitrile: water mixture. The eluted peaks were clear, distinct, and tailing-free under these circumstances.

Utilization of CCD method for Optimization of RP-HPLC method

Retention duration and resolution were the analytical target profiles chosen for HPLC condition optimization. To further optimize several factors inside the design space, the Central Composite Design was employed. The quadratic model for main and interaction effects was chosen in order to analyze the data. Three primary points and twenty experimental designs were implemented. 20 optimized experimental runs, the mobile phase [volume of acetonitrile (70 ml)], the detection wavelength (nm), and the flow fate (ml/min) are among the variables. Table 1 provides a summary of the replies that were obtained. The model was validated using the provided ANOVA. The significance level was less than 0.05. The corrected R2 was found to have a high coefficient of variation (≥10%). This

Table 1: Central Composite rotatable design arrangement and responses

	Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3
Run	A:Flow Rate	B:Mobile Phase (Volume of Acetonitrile (70 ml))	C:Detection wavelength	Rt of AZE	Rt of MET	Resolution AZE & MET
	min/ml	ml	Nm	Min	Min	
1	1.2	75	232	5.993	3.862	6.79
2	1.2	75	228	5.836	4.256	6.38
3	1	70	233.364	5.582	3.315	5.25
4	1	70	230	5.884	3.218	6.78
5	1.2	65	228	6.002	3.589	6.36
6	1	61.591	230	5.586	3.836	7.59
7	0.8	65	232	4.982	3.998	6.79
8	1	70	230	5.589	3.325	6.71
9	1	70	230	5.398	3.359	6.98
10	0.8	75	232	5.962	3.986	6.61
11	1.2	65	232	5.981	3.058	6.93
12	1	70	230	5.584	3.319	6.73
13	1.33636	70	230	5.984	3.325	6.58
14	0.8	65	228	5.298	3.698	6.28
15	0.663641	70	230	5.289	3.389	6.48
16	1	70	230	5.514	3.198	7.14
17	1	78.409	230	5.589	4.296	6.98
18	1	70	226.636	5.189	3.398	5.98
19	0.8	75	228	5.369	3.456	6.45
20	1	70	230	5.525	3.302	6.75

Table 2: Summary of results of regression analysis for models and responses

	Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	%CV	P-value	Precision (Adequate)
Y1	Quadratic	0.1835	0.8044	0.6284	-0.0692	3.27	0.0132	7.8262
Y2		0.0873	0.9693	0.9417	0.8183	3.48	0.0001	22.2434
Y3		0.2974	0.7925	0.6058	-0.3608	4.49	0.0170	8.8649

demonstrates a strong correlation between the models and the experimental data that was acquired. The collected experimental data fits the equations with the components and factors listed in Table 2 as indicated by the R2 values adjusted with limits of R≥ 0.70, which are within acceptable bounds.

Each variable's 3D response surface plots illustrate how the CMPs affect CAAs. Peak area is significantly influenced by critical parameters like flow fate (ml/min), mobile phase (volume of acetonitrile 70 ml), and detection wavelength

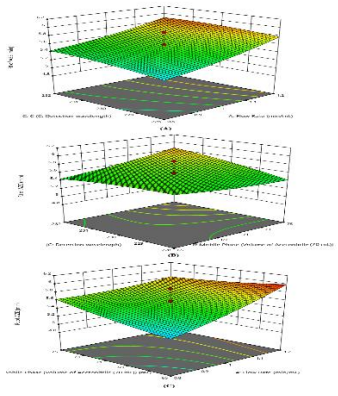


Fig. 2: 3D "plots of Rt of AZE against C and B (A)", 3D "plots of Rt of AZE against A and C (B)" and Contour "plots of Rt of AZE against A and B (C)".

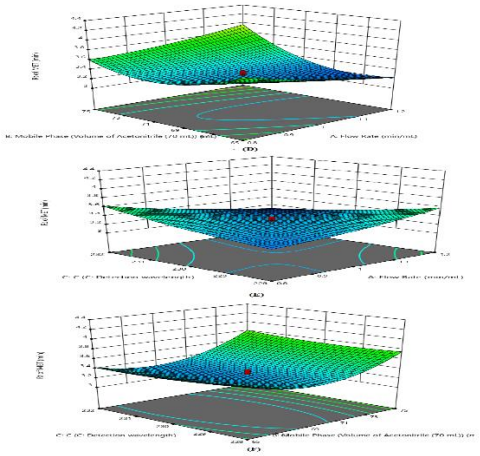


Fig. 3: 3D "plots of Rt of MET against C and B (D)", 3D "plots of Rt of MET against A and C (E)" and 3D "plots of Rt of MET against A and B (F)".

(nm). The curve graphs show how each of the three parameters significantly affects resolution and retention time.

Equation for Retention time of AZL (Y1): +5.58+0.2468 A+ 0.0661 B+0.0786 C – 0.1506 AB – 0.0176 AC + 0.1359 BC + 0.0496 A^2+ 0.0322 B^2 – 0.0392 C^2. The retention period of AZL appears to be directly related to the flow rate (A) and detection wavelength (C), as indicated by the positive coefficients of factors A, B, and C. B has a less significant effect when the Mobile Phase [volume of Acetonitrile (70 ml)] (B) factor is lower. Therefore, as the flow rate (A) and detection wavelength (C) increase, the AZL retention duration also increases (Fig. 2).

Equation for Retention time of MET (Y2): +3.28 – 0.0352 A +0.1458 B – 0.0172 C + 0.2156 AB – 0.2194 AC + 0.0459 BC + 0.0514 A^2 + 0.3021B^2 + 0.0512 C^2. The retention period of MET appears to be directly proportional to the mobile phase (volume of acetonitrile 70 ml), as indicated by the positive coefficients of factors A, B, and C (B). The flow rate (A) and detection wavelength (C) variables' negative or lower values suggest that A and C have little to no effect. Therefore, as the volume of acetonitrile (70 ml) in the mobile phase increases, so does the retention period of MET (B) (Fig. 3).

Equation for Resolution between AZL and MET (Y3): +6.84 + 0.0365 A + 0.0846 B + 0.0309 C –

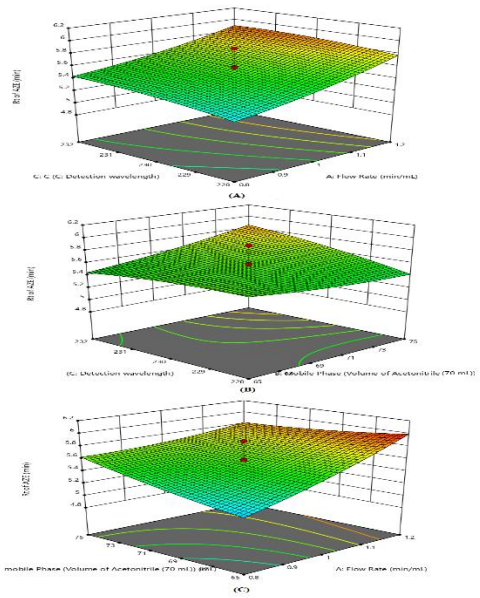


Fig. 4: 3D "plots of Resolution AZE & MET against C and B (G)", 3D "plots of Resolution AZE & MET against A and B (H)" and 3D "plots of Resolution AZE & MET against C and A (I)".

Table 3: Comparison of experimental and predictive value of different experimental runs under optimum conditions

Optimum Condition	Response	Responses (predicted)	Responses (observed)	Predicted error %
	Retention time of AZL	5.587	5.584	-0.053
	Retention time of MET	3.312	3.318	-0.181
	esolution between AZL and MET	6.77	6.7	1.033

0.0317 AB + 0.0388 AC – 0.0637 BC – 0.0873 A^2 + 0.1796 B^2 – 0.4108 C^2. Resolution between AZL and MET is directly proportional to Flow Rate (A) and Detection wavelength (C), according to the positive coefficient of factors A, B, and C. Thus, Resolution between AZL and MET increases as Flow Rate (A) and Detection wavelength (C) increase. A negative or less value of the Mobile Phase [volume of Acetonitrile (70 ml)] (B) factor indicates less or no significant effect of B (Fig. 4).

Under ideal circumstances, the percentage anticipated error displayed a desirability value (D = 1), which provided a set of coordinates provided in Table 3.

The mobile phase composition of the optimized solution, which had a 70:30% v/v mixture of acetonitrile and water, at a detection wavelength of 230 nm and a flow rate of 1 ml/min, produced a desirability that was nearly 1.0 and all of the CAAs were within the intended range (Table 4). Standard AZL and MET have demonstrated distinct peaks and satisfactory separation under the chromatographic conditions (Fig. 5).

Force Degradation study

The capacity of the optimized approach to separate all degradation products in the presence of the active ingredient was determined to be a stability indicator (Fig. 6-9). In stressed samples,

Table 4: Optimized Chromatographic condition for the estimation of AZL and MET Succinate by HPLC

Drug	Retention time (min)	Tailing Factor	Theoretical Plates	Resolution
AZL	3.318 min	1.03	19821.00	6.78
MET	5.584 min	1.13	13751.67	6.78

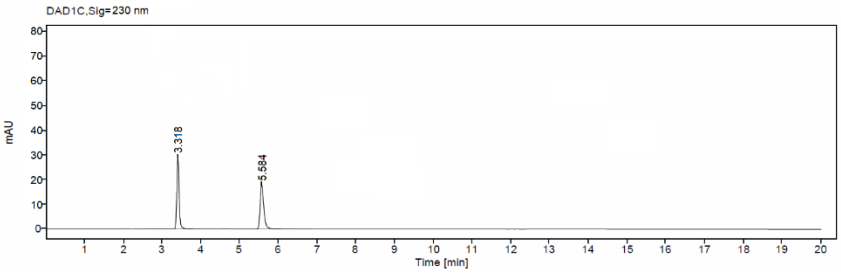


Fig. 5: High performance liquid chromatography of standard AZE + MET (16+50 µg/ml).

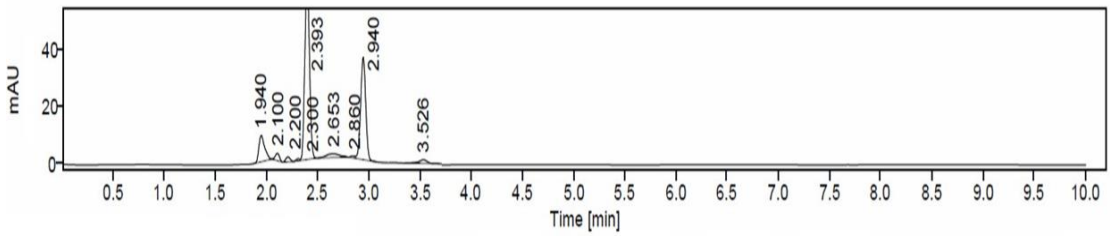


Fig. 6: Acid Degradation.

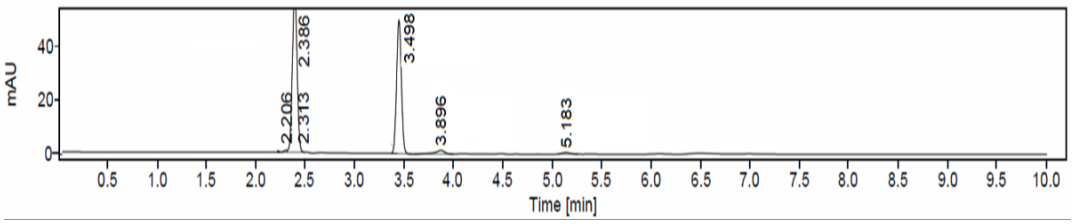


Fig. 7: Base degradation.

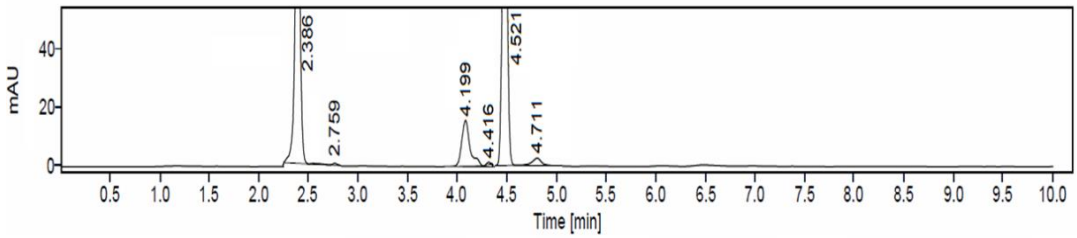


Fig. 8: Oxidation degradation.

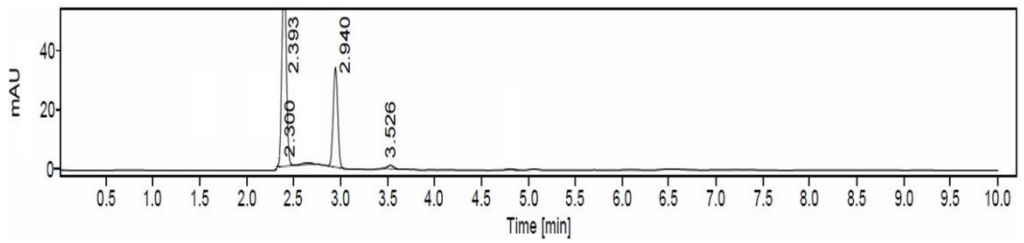


Fig. 9: Thermal degradation.

Table 5: Evaluation Table of Forced Degradation Studies

Stress Condition	Area	AZL	MET	% Degradation (AZL)	% Degradation (MET)
Acid Hydrolysis	Standard Area	34587	72856	15.79 %	14.23 %
	Observed Area	29124	62485		
Base Hydrolysis	Standard Area	34587	72856	13.99 %	13.19 %
	Observed Area	29745	63241		
Oxidative Stress	Standard Area	34587	72856	12.58 %	11.02 %
	Observed Area	30234	64827		
Thermal Degradation	Standard Area	34587	72856	10.66 %	10.13 %
	Observed Area	30897	65471		

no degradation product was discovered to obstruct the estimate of MET and AZE. imposed that the percentage of degradation seen was predictive in nature (below 15%), even the stress imposed was determined to be optimal (Table 5).

Method validation

System suitability data: The standard solutions' column efficiency, resolution, and peak asymmetry were computed and compiled in Table 4.

Linearity: The HPLC overlain chromatogram for AZL succinate and MET succinate is displayed in Table 6 and ranged from 4 to 20 µg/ml and 12.5 to 62.5 µg/ml, respectively, with retention times of 5.58 and 3.32 min.

Precision: Table 6 summarized the results of the HPLC and Absorbance Correction UV method's repeatability, intraday precision, and interday precision.

Accuracy: According to the results described in Table 6, the recovery percentage, which was determined to be between 98% and 102%, validates the correctness of the devised HPLC and Absorbance Correction UV method.

Limit of quantification (LOQ) and Limit of detection (LOD)

AZL's LOD and LOQ were determined to be 0.24 µg/ml and 0.73 µg/ml, while MET's were 0.44 µg/ml and 1.35 µg/ml, respectively (Table 6).

Robustness: The devised HPLC technique was found to be robust when the percentage RSD value for all robustness parameters was less than 2%. Table 7 illustrates a summary of the findings.

Table 6: Validation parameter for RP-HPLC method

S. No.	Parameter	AZL	MET
1	Linearity Range	4-20 µg/ml	12.5-62.5 µg/ml
2	Co- relation Coefficient	0.998	0.998
3	Precision 1. Repeatability (n=5) 2. Intra-day precision (n=3) 3. Inter-day precision (n=3)	 1.04 0.47-1.02 0.63-1.16	 0.85 0.59-0.82 0.62-1.03
4	Accuracy (% Recovery)	98.75 – 99.31	98.60-99.56
5	Limit of detection (LOD) (µg/ml)	0.24	0.44
6	Limit of quantification (LOQ) (µg/ml)	0.73	1.35

Table 7: Robustness data for AZL and MET

Parameter	Level of Change	Effect on assay volume			
		AZL		MET	
		Assay ± SD	RSD	Assay ± SD	RSD
Flow rate (± 0.1)	0.9 ml/min	98.52 ± 0.24	0.25	99.47 ± 0.32	0.32
	1.1 ml/min	98.41 ± 0.42	0.42	99.49 ± 0.26	0.26
Mobile Phase Composition (70:30)	72:28	98.35 ± 0.20	0.20	99.52 ± 0.26	0.26
	68:32	98.77 ± 0.53	0.53	98.94 ± 0.97	0.98

Table 8: Assay of Marketed Formulation

Drug	Amount taken (µg/ml)	Amount found (µg/ml)	% Assay
AZL	8	7.95 ± 0.10	99.42 ± 1.26
MET	25	24.74 ± 0.11	98.96 ± 0.43

Assay: By adding standard excipients to a synthetic mixture, the assay was estimated and examined. Table 8 presents the assay results, which fell between 98% and 102% of the acceptance criteria.

Green Assessment profile: Analytical Green Star Area (*AGSA*)

The Analytical Green Star Area (AGSA) is a novel tool developed to assess the environmental impact of analytical chemistry methods in a structured, visual, and objective way. Unlike existing metrics such as the Analytical Eco-Scale (AES) and GAPI, AGSA integrates both a total scoring system and a visual star-shaped diagram to evaluate how well a method aligns with the 12 Principles of Green Analytical Chemistry (GAC). Each principle is assessed through specific questions with standardized scoring from 1 to 3, allowing for consistent and reproducible evaluations. AGSA

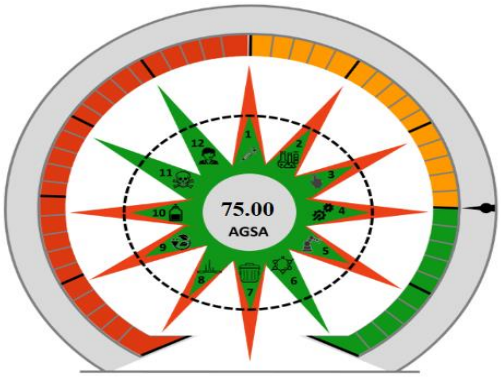


Fig. 10: Result of Analytical Green Star Area (AGSA).

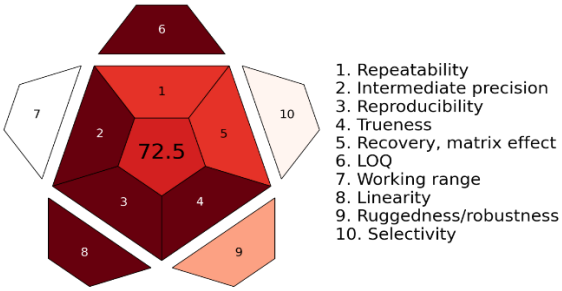


Fig. 11: Result of Red Analytical Procedure Index (RAPI) matrix.

reduces user bias by providing clear guidelines and combines both numeric results and visual outputs to make comparison between methods easier and more intuitive. The final greenness score is out of 36 points, representing 100% alignment with green practices. A larger star area on the AGSA diagram indicates a greener method. Additionally, AGSA builds upon the Green Star Area concept used in green chemistry, making it suitable for cross-disciplinary evaluations. AGSA represents a practical, effective, and user-friendly solution for promoting greener, safer, and more sustainable analytical procedures. The output score of the suggested method was 75 according to the AGSA tool. Fig. 10 depicts explicit reference to it. The chemical community will pay more attention to trust and accept the AGSA tool.

Red Assessment profile: Rapid Assessment of Performance Indicators (*RAPI*)

RAPI (Rapid Assessment of Performance Indicators) is a Python-based, open-source tool designed to assess the quantitative performance of analytical methods quickly and objectively. It follows the structure of BAGI and operates through a simple interface where users select values from drop-down menus. RAPI focuses on ten key criteria based on ICH validation guidelines and general good laboratory practices: repeatability, intermediate precision, reproducibility, trueness, recovery and matrix effects, LOQ, working range, linearity (R^2), ruggedness, and selectivity. Each parameter is scored on a five-level scale from 0 to 10, with 0 also assigned when a parameter has not been tested. The overall method score, ranging from 0–100, is shown at the center of a star-shaped pictogram, where the color intensity visually reflects the score for each criterion. RAPI ensures fairness by giving equal weight to all criteria and adjusts expectations based on analyte concentration using the Horwitz

model. It encourages comprehensive validation by penalizing missing data, promoting better laboratory practices. While it provides valuable comparative insight, RAPI should not be the only factor in determining method suitability. It is most useful when comparing methods applied to the same analyte and matrix, ensuring context-appropriate decisions in method development and selection. The tool thus supports laboratories in reducing their environmental footprint while maintaining the high standards of accuracy and reliability required in analytical chemistry (Fig. 11).

Conclusion

A robust, eco-friendly, and cost-effective RP-HPLC method was successfully developed and validated for the simultaneous estimation of Azelnidipine and Metoprolol Succinate using a Quality by Design (QbD) approach. The method demonstrated excellent precision, accuracy, linearity, robustness, and stability, making it suitable for routine pharmaceutical quality control. Notably, the greenness and safety of the developed method were comprehensively evaluated using Analytical Green Star Area (AGSA) and Rapid Assessment of Performance Indicators (RAPI), confirming its strong environmental and occupational health profile. This integrated green–red assessment underscores the method's alignment with modern analytical goals, combining sustainability, performance, and analyst safety. The proposed method not only supports regulatory compliance and reliability but also contributes to sustainable laboratory practices, offering a valuable tool for the pharmaceutical industry.

Ethical Statement

No animal or microbes have been used or sacrificed for this study, hence Ethical Approval not required.

Author Contributions

Shukla Saurabh: Conceptualization, methodology, software, validation, investigation, writing original draft preparation editing; *Patel Ravikumar*: Supervision and visualization. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest

The authors declare no conflicts of interest.

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