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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF AZELNIDIPINE AND METOPROLOL SUCCINATE IN TABLET DOSAGE FORM

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ABSTRACT

The present research describes the development and validation of a novel, stability-indicating RP-HPLC method for the simultaneous estimation of Azelnidipine and Metoprolol Succinate in a combined tablet dosage form. Given the DCGI approval of this synergistic combination in January 2024 and the lack of documented stability-indicating protocols, this study provides a necessary analytical solution. Chromatographic separation was performed on a Waters 2695 Alliance HPLC system using a C8 column (250×4.6 mm, 5 µm) with an optimized mobile phase consisting of Phosphate buffer (pH adjusted to 3.25 with OPA) and Acetonitrile in a 50:50 (v/v) ratio. The method was rigorously validated according to ICH guidelines and subjected to forced degradation studies under acidic, alkaline, thermal, and photolytic stress conditions. The results demonstrated effective resolution of the active pharmaceutical ingredients from their degradation products, confirming the method's specificity. This precise, accurate, and robust analytical tool is highly suitable for routine quality control and stability monitoring, ensuring the efficacy and safety of the formulation in the pharmaceutical industry.

KEYWORDS: Azelnidipine, Metoprolol Succinate, RP-HPLC, Stability-indicating, Method Validation, Simultaneous Estimation.

INTRODUCTION

The therapeutic alliance of Azelnidipine and Metoprolol Succinate is highly effective in treating high blood pressure and various heart-related ailments. Although these drugs are often prescribed together, there is a notable absence of a validated analytical procedure capable of measuring both components alongside their degradation impurities. Consequently, the current investigation aims to develop a robust RP-HPLC method that meets ICH criteria for stability-indicating assays, ensuring reliable quality control of this combination.[1,2]

Azelnidipine: Azelnidipine is a modern calcium channel blocker characterized by its prolonged duration of action and a gradual onset of effect. It primarily functions by inhibiting L-type calcium channels, which results in significant peripheral vasodilation and is widely utilized for the therapeutic management of persistent hypertension.[3]

Metoprolol Succinate: Metoprolol Succinate serves as a selective ß$_1$ - adrenergic receptor antagonist, frequently prescribed to regulate heart rate and reduce blood pressure. By specifically targeting the cardiac receptors, it provides cardio-protective benefits, making it a cornerstone treatment for various cardiovascular disorders, including angina pectoris and heart failure.[4]

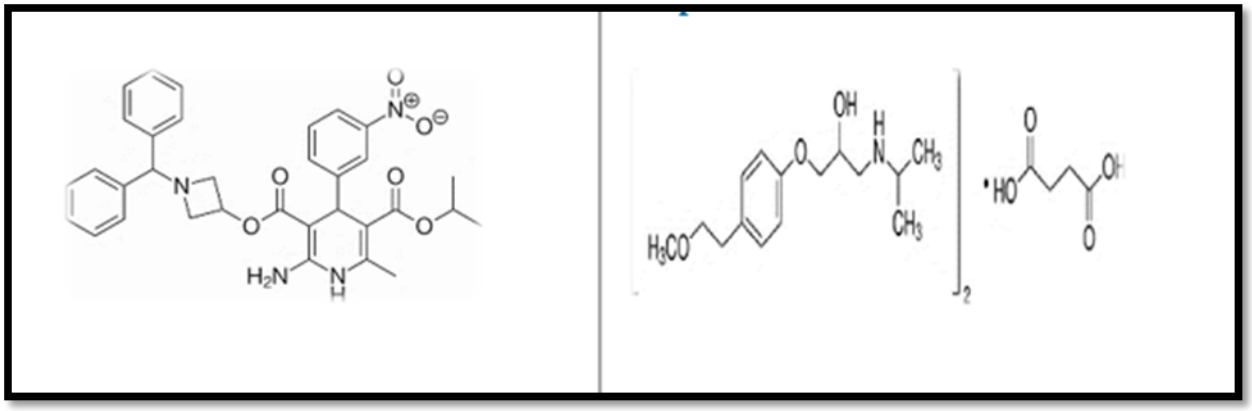

Fig. 1: Structure of Azelnidipine and Metoprolol Succinate.

A systematic survey of the existing scientific literature reveals that various analytical methodologies have been documented for the estimation of Azelnidipine and Metoprolol Succinate. These include diverse RP-HPLC techniquesm,[5-8] spectrophotometric

approaches,[9,10] and advanced HPTLC[11,12] and UPLC[13] studies, focusing on these molecules either as standalone entities or in combination with other active ingredients in bulk and varied dosage forms.

Furthermore, a review of official compendia indicates that while individual monographs may exist in certain pharmacopoeias,[14,15] there is a lack of an official multicomponent method for their simultaneous estimation. While some stability-indicating studies have been reported, there remains a significant research gap regarding a unified, validated, and stability-indicating RP-HPLC method specifically optimized for their concurrent analysis in Tablet Dosage Form. Therefore, the present investigation was initiated to bridge this gap by developing a sensitive and robust stability-indicating RP-HPLC strategy for the dual analysis of Azelnidipine and Metoprolol Succinate, ensuring high accuracy for routine industrial quality control and stability monitoring.

MATERIALS AND METHODS

Chemicals and Reagents

The reference standards of Azelnidipine and Metoprolol Succinate (Uniaz Beta) were graciously provided as gift samples by Torrent Pharmaceuticals Ltd., Gujarat. High-purity Methanol, Acetonitrile, and Milli-Q water (HPLC grade) were utilized for mobile phase preparation and as diluents. Analytical reagent (AR) grade Dipotassium hydrogen phosphate and Orthophosphoric acid were used for buffer formulation and pH adjustment. All chemicals and solvents were procured from Rankem and Merck Life Science Pvt. Ltd. to ensure the highest purity for chromatographic analysis.

Preparation of standard Stock solution

Azelnidipine (160 µg/mL): 16 mg of Azelnidipine was dissolved in 100 mL of diluent using sonication. 10 mL of this solution was further diluted to 100 mL with diluent to obtain the final concentration.

Metoprolol Succinate (500 µg/mL): 50 mg of Metoprolol Succinate was dissolved in 100 mL of diluent with sonication. Further, 10 mL of this stock was diluted to 100 mL to achieve the final concentration.

Preparation of Working Standard (Binary Mixture)

Accurately weighed 16 mg of **Azelnidipine** and 50 mg of **Metoprolol Succinate** were transferred into a 100 mL volumetric flask, dissolved in 70 mL of diluent using sonication, and diluted to volume. From this stock, 10 mL was further diluted to 100 mL with diluent to prepare the final working standard solution of binary mixture (**16 µg/mL AZL** and **50 µg/mL METO).**

Preparation of mobile phase

Mobile Phase: A mixture of the prepared Phosphate Buffer and Acetonitrile was prepared in a ratio of 50:50 % v/v. The mixture was sonicated for 10 minutes and filtered through a 0.45 µm membrane filter.

Forced Degradation Study

1) Acid Degradation

Tablet powder equivalent to two units was treated with 20 mL diluent and 5 mL 0.1N HCl in a 100 mL flask. The mixture was sonicated for 10 min, heated at 60°C for 30 min, cooled, and neutralized with 5 mL 0.1N NaOH. After making up the volume and filtering through a 0.45 µm nylon filter, 10 mL of the filtrate was diluted to 100 mL with diluent.

2) Base Degradation

Tablet powder equivalent to two units was stressed with 5 mL 0.1N NaOH and 20 mL diluent, then sonicated (10 min) and heated at 60°C (30 min). Following cooling and neutralization with 5 mL 0.1N HCl, the solution was diluted to 100 mL and filtered (0.45 µm). A 10 mL aliquot of this filtrate was further diluted to 100 mL with diluent for the final preparation.

3) Oxidation Degradation

Tablet powder (2 units eq.) was treated with 20 mL diluent and 10 mL 3% H_2O_2 in a 100 mL flask, followed by 10 min sonication. The mixture was kept at room temperature for 30 min, then diluted to volume with diluent and filtered through a 0.45 µm nylon filter. A 10 mL aliquot of the filtrate was further diluted to 100 mL with diluent for final analysis.

4) Photolytic Degradation

Tablet powder (2 units eq.) was exposed to UV light for 24 hours, then dissolved in 70 mL diluent using 15 minutes of sonication. After adjusting the volume to 100 mL and filtering

through a 0.45 µm nylon membrane, a 10 mL aliquot was diluted to 100 mL. The resulting solution was then analyzed to evaluate the light stability of the formulation.

5) Thermal Degradation

Tablet powder equivalent to two units was exposed to dry heat at 100°C for 1 hour, then cooled and dispersed in 70 mL diluent via 15-minute sonication. The mixture was diluted to 100 mL, filtered using a 0.45 µm nylon membrane, and a 10 mL aliquot was further diluted to 100 mL. This prepared solution was then utilized for stability-indicating analysis.

METHOD VALIDATION

1) Linearity and Range

The linearity for Metoprolol Succinate and Azelnidipine was evaluated using combined standards ranging from 25–75 µg/mL and 8–24 µg/mL, respectively. The calibration curves yielded excellent correlation coefficients of 0.9999 and 0.9998, confirming a highly proportional response across the tested range.

2) Precision

The method's precision was confirmed via repeatability, intraday, and interday studies. The %RSD for repeatability was found to be 0.34% for Metoprolol and 0.33% for Azelnidipine. All intermediate precision results remained well below the 2.0% acceptance limit, demonstrating that the proposed method is highly consistent and rugged for simultaneous estimation.

3) Limit of Detection and Limit of Quantitation

The sensitivity of the method was determined by calculating the LOD and LOQ based on the standard deviation of the response (σ) and the slope (s) of the calibration curve. The LOD values for Metoprolol and Azelnidipine were found to be 1.13 µg/mL and 0.45 µg/mL, while the LOQ values were 3.42µ g/mL and 1.36µg/mL, respectively. These low values signify the high sensitivity of the developed RP-HPLC method.

4) Accuracy

The accuracy of the method was validated through recovery studies at 50%, 100%, and 150% levels using the standard addition method. Mean recovery values were found to be 99.1%–99.9% for Metoprolol and 100.0%–100.4% for Azelnidipine. These results, with %RSD

values well within the acceptable limits, confirm the method's reliability and the absence of interference from formulation excipients.

5) Robustness

The robustness of the developed method was evaluated by introducing deliberate small variations in critical chromatographic parameters, including flow rate (±0.15mL/min), mobile phase composition (±2\%), temperature (± 5°C), and pH (± 0.2). Under these varied conditions, the %RSD for peak area, tailing factor, and theoretical plates remained within acceptable limits for both Metoprolol and Azelnidipine. These results demonstrate that the analytical performance of the method is stable and unaffected by minor procedural changes, ensuring its reliability.

6) Solution Stability

The stability of Metoprolol and Azelnidipine in both standard and sample solutions was investigated at room temperature over a 24-hour period. The results indicated that the solutions remained stable, with the percentage difference in peak areas being significantly below th**e 2.0%** acceptance limit at all-time intervals (0, 5, 8, 16, and 24 hours). This confirms that the prepared solutions are suitable for analysis within a 24-hour window without any significant degradation.

7) Filter Compatibility

Filter compatibility was assessed using 0.45 µ PVDF and 0.45 µ Nylon filters. The percentage difference in peak areas compared to centrifuged samples was less than 2.0% for both drugs. These results confirm that neither filter causes significant drug adsorption, making them suitable for sample preparation.

RESULT AND DISCUSSION

Optimized Chromatographic Conditions

The RP-HPLC analysis was performed using a Waters 2695 Alliance HPLC system with Empower software and a UV detector. Separation was achieved on a C8 column (250 × 4.6 mm, 5 µm) maintained at 55°C. The optimized mobile phase consisted of Phosphate Buffer (pH 3.25) and Acetonitrile (50:50 v/v), delivered at a flow rate of 1.5 mL/min. A detection wavelength of 230 nm and an injection volume of 5 µl were employed, resulting in a total run time of 10.0 minutes. The method was validated as per ICH Q2 (R1) guidelines, demonstrating excellent linearity, precision (%RSD < 2.0%), and accuracy (recovery 99.1%–

100.4%), confirming its reliability for the simultaneous estimation of Metoprolol and Azelnidipine.

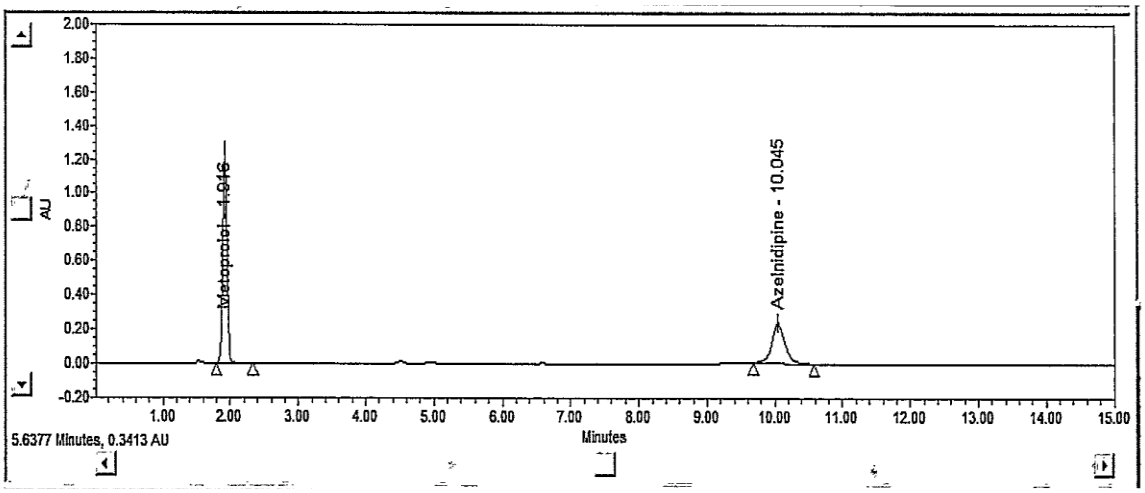

Fig. 2 Optimized Chromatogram of Azelnidipine & Metoprolol Succinate mobile phase: phosphate buffer pH 3.25 (K_2HPO_4): acetonitrile 50:50.

Forced Degradation Study

1) Acid Degradation

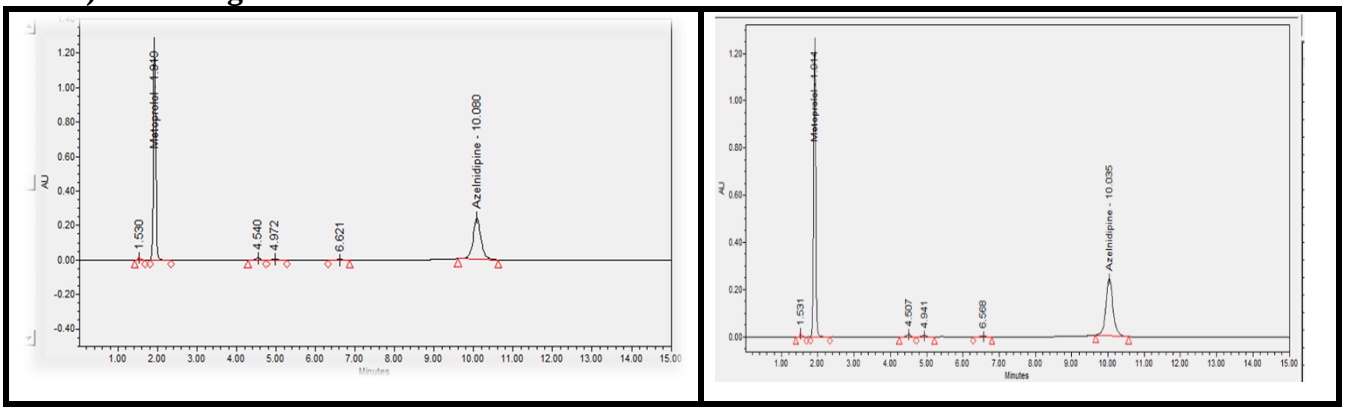

Fig. 3 Chromatogram of Acid Degradation Standard and Sample.

2) Base Degradation

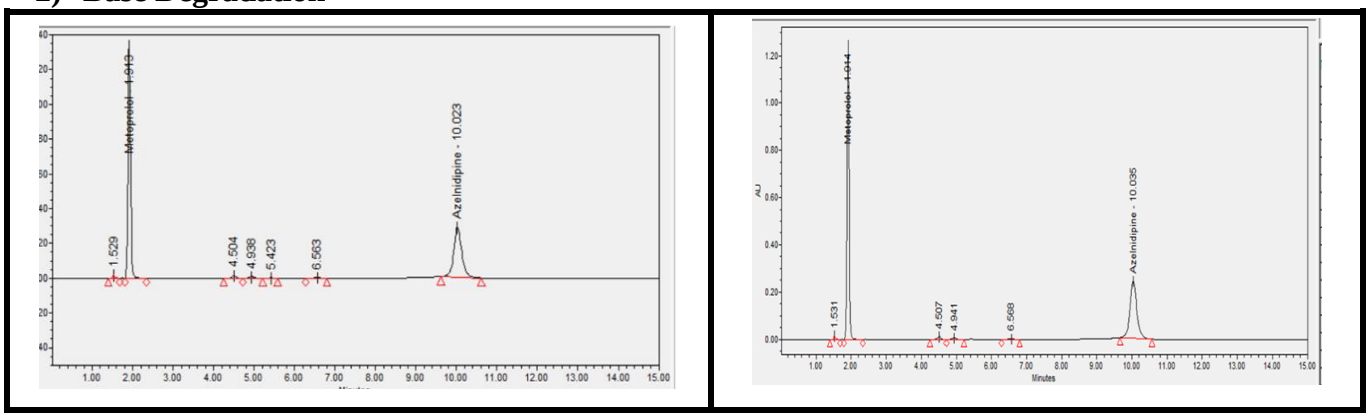

Fig. 4: Chromatogram of Base Degradation Standard and Sample.

3) Oxidation Degradation

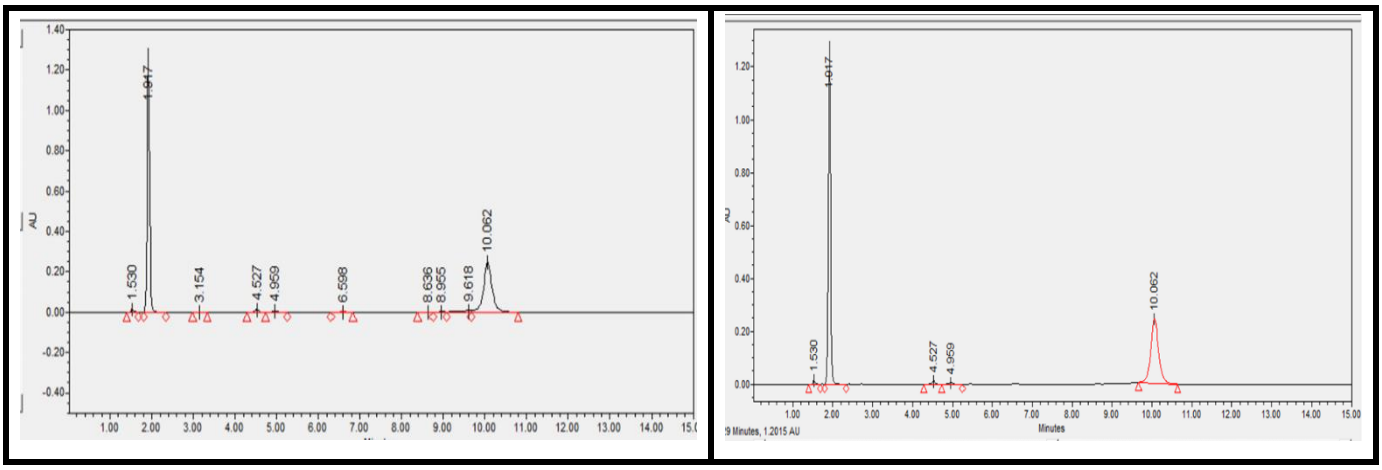

Fig. 5: Chromatogram of Oxidative Degradation Standard and Sample.

4) Photolytic Degradation

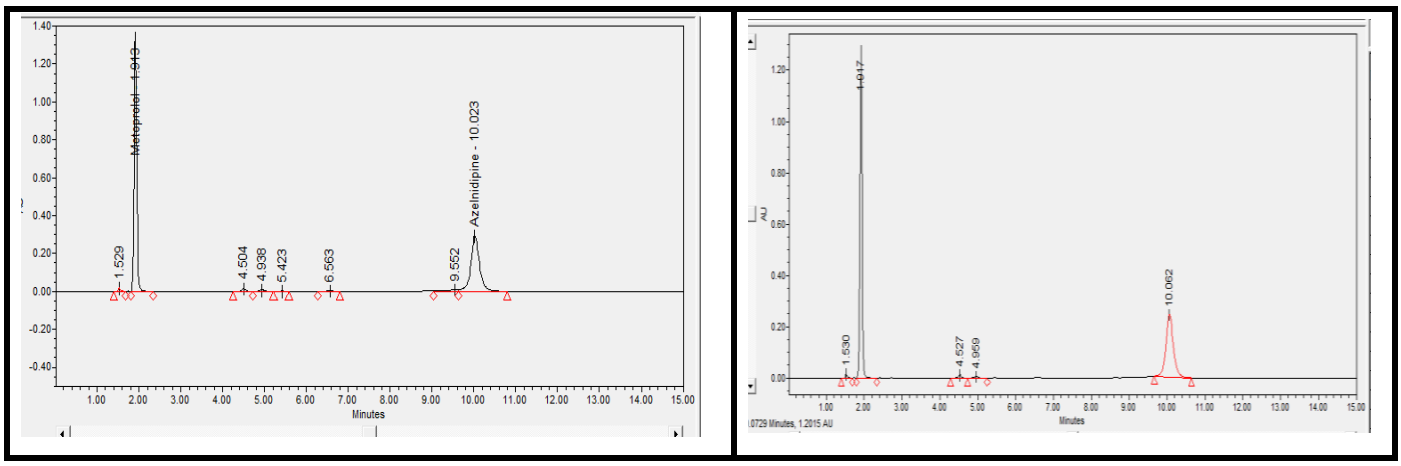

Fig. 6: Chromatogram of Photolytic Degradation Standard and Sample.

5) Thermal Degradation

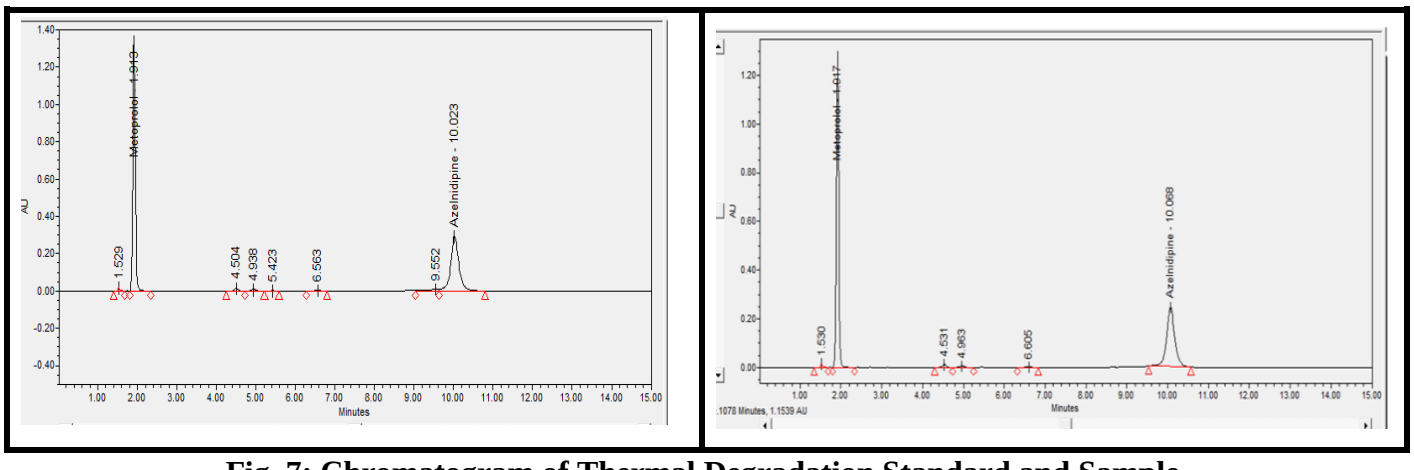

Fig. 7: Chromatogram of Thermal Degradation Standard and Sample.

Table 1: Forced degradation summary metoprolol succinate and Azelnidipine.

% Degradation of Metoprolol Succinate				
Condition	**Sample**		**Standard**	
	Area	**% Degradation**	**Area**	**% Degradation**
Acid	4838270	6.2	4774796	7.4
Base	4916379	4.7	4913875	4.7
Oxidation	5001970	3.0	5013875	2.8
Thermal	5147082	0.18	5152037	0.09
Photo	5097819	1.14	5101690	1.06
% Degradation of Azelnidipine				
Condition	**Sample**		**Standard**	
	Area	**% Degradation**	**Area**	**% Degradation**
Acid	3113510	5.8	3228822	2.3
Base	3240924	1.9	3237529	2.0
Oxidation	3241958	1.9	3230731	2.2
Thermal	3280612	0.7	3298036	0.2
Photo	3290685	0.4	3292205	0.4

Validation of RP-HPLC method

Linearity

Precisely weighed amounts of Metoprolol (50 mg) and Azelnidipine (16 mg) were transferred into a 100 mL volumetric flask. The solids were dissolved in 70 mL of diluent using sonication, and the final volume was adjusted to the mark. The resulting solution was thoroughly mixed before use.

Table 2: Linearity Data for Metoprolol Succinate and Azelnidipine.

Linearity			
Concentration of Metoprolol (µg/mL)	**Area of Metoprolol**	**Concentration of Azelnidipine (µg/mL)**	**Area of Azelnidipine**
25	2602799	8	1666215
40	4151288	12.8	2622329
50	5155404	16	3281324
60	6138549	19.2	3970102
75	7682011	24	4947379

	Metoprolol	**Azelnidipine**
R2 Value	0.9999	0.9998
Slope of curve	101277.87	205833.69
Y Intercept	82117	4130.8

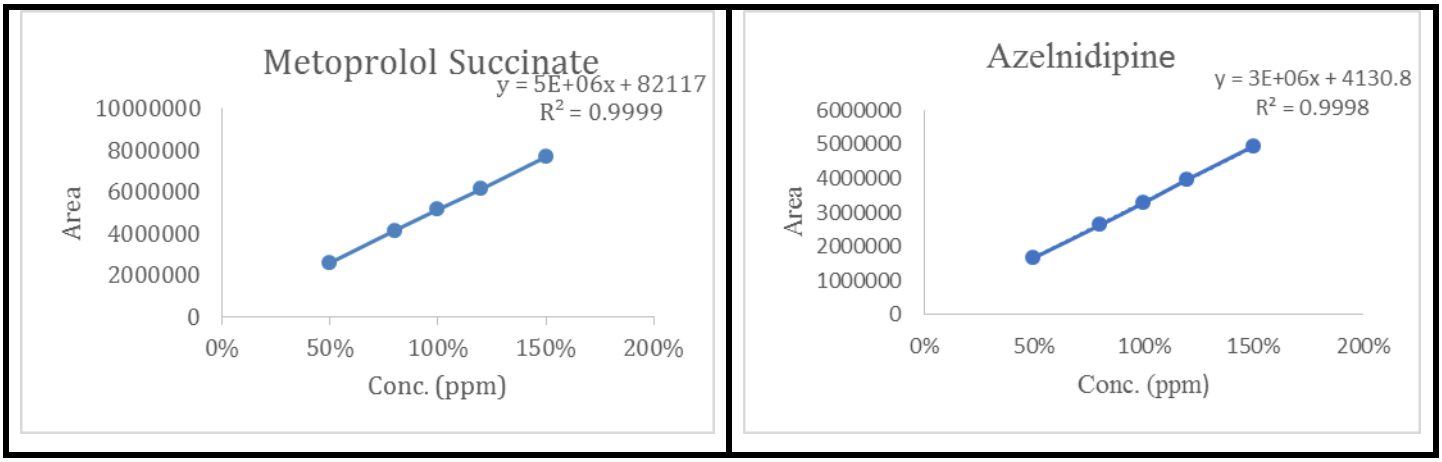

Fig. 8: Calibration Curve of Metoprolol Succinate and Azelnidipine.

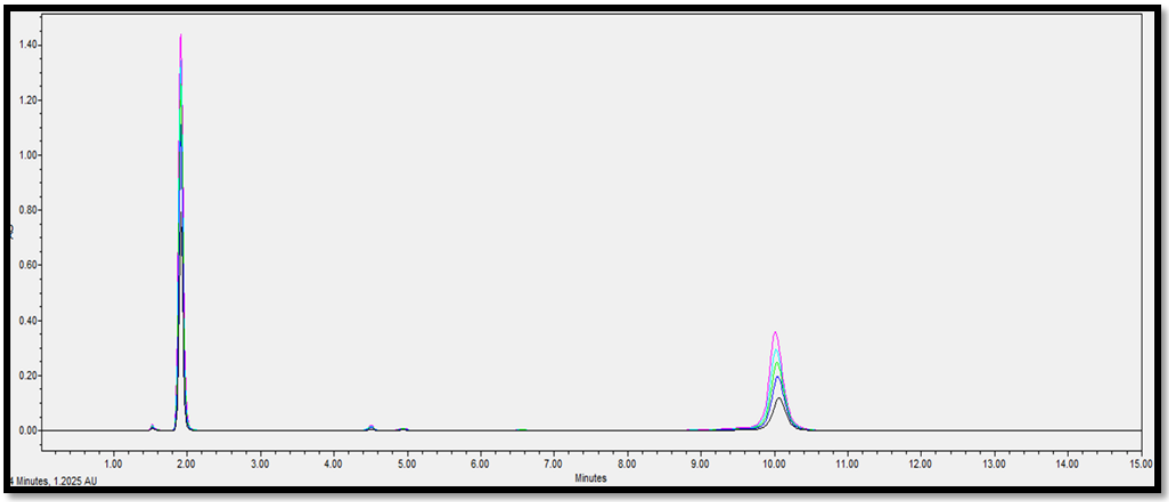

Fig. 9: Overlaid linearity chromatogram of Metoprolol Succinate and Azelnidipine Precision.

An amount of powder equivalent to two capsules was accurately weighed and transferred into a 100 mL volumetric flask. After adding 70 mL of diluent, the mixture was sonicated for 15 minutes to ensure complete drug extraction. The solution was cooled to room temperature, diluted to volume, and filtered through a 0.45 µm nylon filter. A 10 mL aliquot of the filtrate was further diluted to 100 mL with diluent and homogenized. This procedure was repeated to prepare six replicates (n=6).

Table 3: Repeatability data Metoprolol Succinate and Azelnidipine.

Method Precision (Repeatability)		
Sr No.	**Metoprolol**	**Azelnidipine**
Set-01	99.5	99.8
Set-02	99.4	99.4
Set-03	99.8	99.6

Set-04	99.9	99.9
Set-05	100.1	100.1
Set-06	99.2	100.3
Avg	**99.7**	**99.9**
SD	**0.34**	**0.33**
RSD	**0.34**	**0.33**
Intermediate Precision (Reproducibility)		
Sr No.	**% Assay (Metoprolol)**	**% Assay (Azelnidipine)**
Set-01	98.9	100.1
Set-02	99.7	98.9
Set-03	98.8	99.7
Set-04	100.4	99.9
Set-05	99.8	99.6
Set-06	99.1	99.7
Avg	**99.5**	**99.7**
SD	**0.62**	**0.46**
RSD	**0.63**	**0.46**

Table 4: Intraday and interday Precision data of Metoprolol Succinate and Azelnidipine.

Intraday Precision Metoprolol (MPL)			**Intraday Precision Azelnidipine (AZL)**		
Conc. (µg/mL)	**Area Mean ± SD (n=3)**	**% RSD**	**Conc.(µg/mL)**	**Area Mean ± SD (n=3)**	**%RSD**
50	915840 ± 3205.44	0.35	16	435620 ± 1481.10	0.34
75	1369200 ± 4518.36	0.33	24	658900 ± 2108.48	0.32
100	1829500 ± 5671.45	0.31	32	881200 ± 2643.60	0.30
Inter Day Precision Metoprolol (MPL)			**Inter Day Precision Azelnidipine (AZL)**		
Conc. (µg/mL)	**Area Mean ± SD (n=3)**	**% RSD**	**Conc. (µg/mL)**	**Area Mean ± SD (n=3)**	**% SD**
50	912450± 5748.43	0.63	16	432150 ± 1987.89	0.46
75	1365800 ± 8331.38	0.61	24	654820 ± 2881.20	0.44
100	1824100 ± 10762.1	0.59	32	875410 ± 3676.72	0.42

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

The sensitivity of the method was evaluated by determining the Limit of Detection (LOD) and Limit of Quantitation (LOQ) using the calibration curve's slope and intercept. For Metoprolol, the LOD and LOQ were found to be 1.13 µg/mL and 3.42 µg/mL, respectively. For Azelnidipine, the values were determined as 0.45 µg/mL for LOD and 1.36 µg/mL for LOQ. These low values confirm the high sensitivity of the developed method.

Accuracy

The Accuracy of the test method was demonstrated by %recovery across its range by making different concentrations at 50%, 100%, and 150% levels using the standard addition method.

Table 5: Accuracy data of Metoprolol Succinate and Azelnidipine.

Metoprolol Succinate				
Level	**Amount Added (mg)**	**Amount recovered (mg)**	**% Recovery**	**% Mean ± % RSD**
50%	25.3	25.1	99.2	99.1 ± 0.23
	25.4	25.2	99.2	
	25.2	24.9	98.8	
100%	50.1	49.8	99.4	99.3 ± 0.4
	50.6	50.4	99.6	
	50.5	49.9	99.8	
150%	75.0	74.7	99.6	99.9 ± 0.6
	75.6	75.2	99.5	
	75.5	75.9	100.5	
Azelnidipine				
50%	8.1	8.4	101.2	100.4 ± 1.4
	8.3	8.4	98.8	
	8.3	8.6	101.2	
100%	16.1	16.0	99.4	100.0 ± 1.6
	16.4	16.2	98.8	
	16.2	16.5	101.9	
150%	24.5	24.3	99.2	100.1 ± 0.85
	24.0	24.1	100.4	
	24.1	24.3	100.8	

Robustness

Table 6: Robustness data of Metoprolol Succinate and Azelnidipine.

Robustness data for Metoprolol Succinate								
Drug	**Area at Temp. (-5°C)**	**Area at Temp. (+5°C)**	**Area at Flow rate (-0.15 mL/mi)**	**Area at Flow rate (+0.15 mL/mi)**	**Area at Mobile Phase (-2%)**	**Area at Mobile Phase (+2%)**	**Area at pH (-0.2)**	**Area at pH (+0.2)**
	513248	512708	514631	510642	514102	514516	515000	513964
METO	513180	512640	514580	510590	514050	514450	514950	513910
	513306	512756	514692	510694	514164	514572	515050	514028
Mean	5132458	5127018	5146371	5106432	5141072	5145106	5150040	5139654
%RSD	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01

Robustness data for Azelnidipine								
Drug	**Area at Temp. (-5°C)**	**Area at Temp. (+5°C)**	**Area at Flow rate (-0.15 mL/min)**	**Area at Flow rate (+0.15 mL/min)**	**Area at Mobile Phase (-2%)**	**Area at Mobile Phase (+2%)**	**Area at pH (-0.2)**	**Area at pH (+0.2)**
	3244132	3310628	3284321	3344751	3340003	3418507	3350006	3327996
AZEL	3243500	3310000	3283800	3344200	3339500	3418000	3349500	3327400
	3244764	3311256	3284842	3345302	3340506	3419014	3350512	3328592
Mean	3244132	3310628	3284321	3344751	3340003	3418507	3350006	3327996
%RSD	0.02	0.02	0.02	0.02	0.01	0.01	0.01	0.02

Solution Stability

Table 7: Solution stability data of Metoprolol Succinate and Azelnidipine.

Standard				
Time (Hr.)	**Area of Metoprolol**	**% Difference**	**Area of Azelnidipine**	**% Difference**
0	5147819	NA	3319919	NA
5	5145475	0.05	3310825	0.27
8	5140116	0.15	3309994	0.30
16	5139207	0.17	3308538	0.34
24	5137431	0.20	3307652	0.37
Sample				
Time (Hr.)	**Area of Metoprolol**	**% Difference**	**Area of Azelnidipine**	**% Difference**
0	5156809	NA	3327232	NA
5	5149386	0.14	3299278	0.84
8	5142531	0.28	3296315	0.93
16	5139664	0.33	3289879	1.12
24	5138509	0.35	3287673	1.19

Filter Compatibility

Table 8: Filter compatibility data of Metoprolol Succinate and Azelnidipine.

0.45 µ PVDF				
mL Discarded	**Area of Metoprolol**	**% Difference**	**Area of Azelnidipine**	**% Difference**
Centrifuge	5198265	NA	3352684	NA
5	5157279	0.8	3331780	0.6
0.45 µ Nylon				
mL Discarded	**Area of Metoprolol**	**% Difference**	**Area of Azelnidipine**	**% Difference**
Centrifuge	5198265	NA	3352684	NA
5	5171471	0.5	3342148	0.3

Assay of Marketed Formulation

The applicability of the proposed RP-HPLC method was verified by performing an assay of the marketed formulation containing Azelnidipine and Metoprolol Succinate. The results, expressed as % Assay, indicate that the method is accurate and reliable for the routine analysis of these drugs in their combined dosage form. The findings are summarized in the table below.

Table 9: Assay of marketed formulation.

Sr No	**Formulation (Tablet)**	**Label Claim (mg)**	**Sample Peak Area**	**% Assay**	**Mean % Assay**
1	Metoprolol Succinate	50 mg	1542800	99.6	99.57%
2	Metoprolol Succinate		1542800	99.5	
3	Metoprolol Succinate		1541500	99.6	

1	Azelnidipine	16 mg	865400	99.8	99.93%
2	Azelnidipine		868200	100.1	
3	Azelnidipine		866500	99.9	

The validation data demonstrates that the proposed analytical procedure is highly specific, accurate, and precise. Its simplicity, robustness, and speed ensure efficient results. Additionally, favorable findings from solution stability and filter compatibility studies confirm the method's reliability for the routine analysis of tablet dosage forms.

CONCLUSION

The developed RP-HPLC approach is accurate, novel, and highly sensitive, displaying excellent linearity and precision for the simultaneous determination of AZL and METO. The method proved to be robust and reliable for analyzing both the API and its tablet dosage form. Furthermore, forced degradation studies established the method's stability-indicating capability, as it effectively separated the parent drugs from their various degradation products. Due to its simplicity and efficiency, this analytical procedure is highly recommended for routine quality control and stability-indicating analyses in the pharmaceutical industry.

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