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Stability-Oriented RP-HPLC Method Development for Simultaneous Quantification of Azelnidipine and Telmisartan in Pharmaceutical Dosage Forms

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ABSTRACT

A reversed-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for simultaneous quantification of Azelnidipine (AZN) and Telmisartan (TMN) in combined tablet dosage forms. Chromatographic separation was achieved using a C_{18} column (100 × 4.6 mm, 2.5 µm particle size) with a mobile phase consisting of Acetonitrile: pH 3 phosphate buffer (75:25, v/v) at a flow rate of 0.9 mL/min, with detection occurring at 237 nm.

The method was validated according to International Conference on Harmonisation (ICH) guidelines, demonstrating linearity over the concentration ranges of 6.4-32 µg/mL for AZN and 16-80 µg/mL for TMN. The limits of detection (LOD) and quantitation (LOQ) were determined to be 0.4399 and 1.3332 for AZN, and 0.882 and 2.6729 for TMN, respectively. The method also exhibited precision, repeatability, and accuracy, with relative standard deviations (RSD) ≤ 2% and accuracy ranging from 99-101%.

To assess the method's stability-indicating capability, forced degradation studies were conducted under four stress conditions: acidic hydrolysis, alkaline hydrolysis, oxidative degradation, and thermal degradation. The results confirmed the method's ability to accurately quantify AZN and TMN in the presence of degradation products.

Keywords: Azelnidipine, Telmisartan, Development, Forced Degradation, Reverse Phase High performance liquid chromatography (RP-HPLC), Validation.

INTRODUCTION

Hypertension, or high blood pressure, is a pervasive health concern globally, particularly affecting individuals over middle age. Characterized by elevated blood vessel pressure, hypertension itself isn't a disease, but it significantly increases cardiovascular mortality and morbidity risk. Effective management is crucial due to its widespread prevalence and associated health risks.

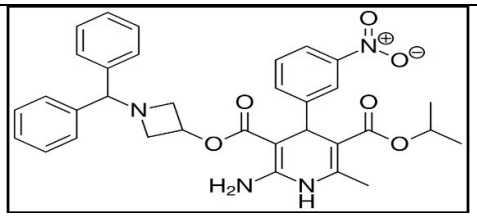
The pharmaceutical industry has introduced combination therapies to enhance treatment efficacy, especially for Stage II Hypertension. One prominent combination is Azelnidipine and Telmisartan. This combination targets multiple blood pressure regulation pathways, providing improved hypertension management.

Azelnidipine, a dihydropyridine calcium channel blocker, inhibits calcium influx through voltage-dependent calcium channels in vascular smooth muscle cells, causing vasodilation and blood pressure reduction. Telmisartan, an angiotensin II receptor blocker (ARB), selectively antagonizes the angiotensin II type 1 receptor, promoting vasodilation and decreasing systemic vascular resistance.

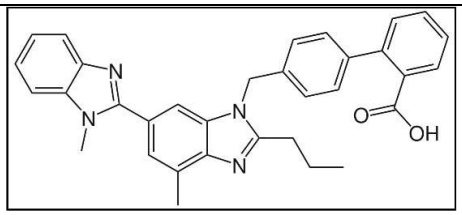
The Azelnidipine-Telmisartan combination leverages complementary mechanisms, offering a synergistic

approach to hypertension management. By collectively affecting vascular tone and blood pressure regulation, this combination is a promising therapeutic strategy for Stage II Hypertension.

Azelnidipine (3-[1-(Benzyldrylazetidin-3-yl] 5-isopropyl- 2-amino-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate) is a dihydropyridine derivative, and its molecular structure is shown in Figure 1. Telmisartan (2-{4-[[4-methyl-6-(1-methylbenzimidazol-2yl)-2-propylbenzimidazol-1yl]methyl]biphenyl)-benzoic acid) is a benzimidazole derivative, with its molecular structure represented in Figure 1.



(a) Azelnidipine



(b) Telmisartan

Figure 1: Structure of AZN and TMN

MATERIALS AND METHODS:

Reagents and Chemicals:

Azelnidipine (AZN) and Telmisartan (TMN) reference standards were procured from Reliable Lab, Jalgaon. The pharmaceutical dosage form, Telma AZ tablet, containing AZN 16 mg and TMN 40 mg, was obtained commercially. Acetonitrile and o-phosphoric acid, both of HPLC grade, were used as solvents.

Standard Stock Solution of AZN and TMN (Mixed):

A standard stock solution, designated as Stock Solution-I, was prepared by accurately weighing 16 mg of Azelnidipine (AZN) and 40 mg of Telmisartan (TMN) and dissolving them in a suitable diluent to a final volume of 10 mL. This resulted in a solution with concentrations of 1600 µg/mL of AZN and 4000 µg/mL of TMN. This stock solution served as the primary source for further dilutions and analysis, providing a consistent and precise starting point for the quantification of AZN and TMN.

Chromatographic Equipment:

The chromatographic analysis was conducted utilizing an Agilent gradient system, comprising a UV detector, a reverse-phase C_{18} column (4.6 mm × 100 mm; 2.5 µm particle size), a SP930d pump, and a 20 µL injection loop. The system was controlled and data processed using Chemstation software. To ensure accuracy and precision, Class 'A' borosilicate glassware was employed for all volumetric measurements and general laboratory purposes throughout the study.

Preparation of Mobile Phase:

The mobile phase was prepared by mixing acetonitrile with pH 3 phosphate buffer, containing o-phosphoric acid, in a ratio of 75:25 (v/v). To ensure optimal chromatographic performance, the mobile phase was filtered through a 0.45 µm membrane filter and degassed by sonication prior to use, removing any dissolved gases and particulate matter that could potentially interfere with the analysis.

Preparation of Standard Solution:

A standard stock solution of Azelnidipine (AZN) and Telmisartan (TMN) was prepared by dissolving 16 mg of AZN and 40 mg of TMN in a 10 mL volumetric flask, and then diluting to volume with a suitable solvent. This resulted in a standard stock solution with concentrations of 1600 µg/mL AZN and 4000 µg/mL TMN, serving as the primary source for subsequent dilutions and analytical experiments.

Optimized Chromatographic Conditions:

The HPLC analysis was performed using the following conditions:

- Column: Reverse-phase C_{18} (100 mm × 4.6 mm ID, 2.5 µm particle size)
- Mobile Phase: Acetonitrile: pH 3 phosphate buffer (containing o-phosphoric acid) (75:25, v/v)
- Flow Rate: 0.9 mL/min
- Detection Wavelength: 237 nm
- Column Temperature: Ambient (room temperature)
- Run Time: 11.36 minutes

- Injection Volume: 20 µL (using a 20 µL injection loop)

These optimized chromatographic conditions enabled efficient separation and accurate quantification of Azelnidipine (AZN) and Telmisartan (TMN).

Calibration of Standards:

Calibration curves for Azelnidipine (AZN) and Telmisartan (TMN) were constructed by preparing a series of standard solutions across concentration ranges of 6.4 to 32 µg/mL and 16 to 80 µg/mL, respectively. Aliquots from the standard stock solutions were accurately pipetted and diluted to volume with the mobile phase to prepare solutions of varying concentrations. Each calibration level was prepared in triplicate. The resulting calibration curves were plotted as peak area versus concentration, and the linearity of the response was evaluated using regression analysis. This process enabled the establishment of a reliable and accurate quantitative method for AZN and TMN, facilitating precise determination of their concentrations in pharmaceutical formulations.

METHOD VALIDATION:

The developed HPLC method for simultaneous quantification of Azelnidipine (AZN) and Telmisartan (TMN) was rigorously validated through evaluation of multiple parameters, encompassing linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), and robustness. This comprehensive validation ensured the reliability, specificity, and sensitivity of the method, confirming its suitability for accurate and precise determination of AZN and TMN concentrations in pharmaceutical formulations.

Linearity: The linearity of the HPLC method was assessed through least squares linear regression analysis of the calibration curves for Azelnidipine (AZN) and Telmisartan (TMN) across their respective concentration ranges. The calibration standards were chromatographed, and the resulting peak areas were plotted against concentrations to construct the linearity plots, which are illustrated in Figures 2 and 3. The linearity evaluation results, including correlation coefficients (r2), slopes, intercepts, and residual plots, are summarized in Table 1.

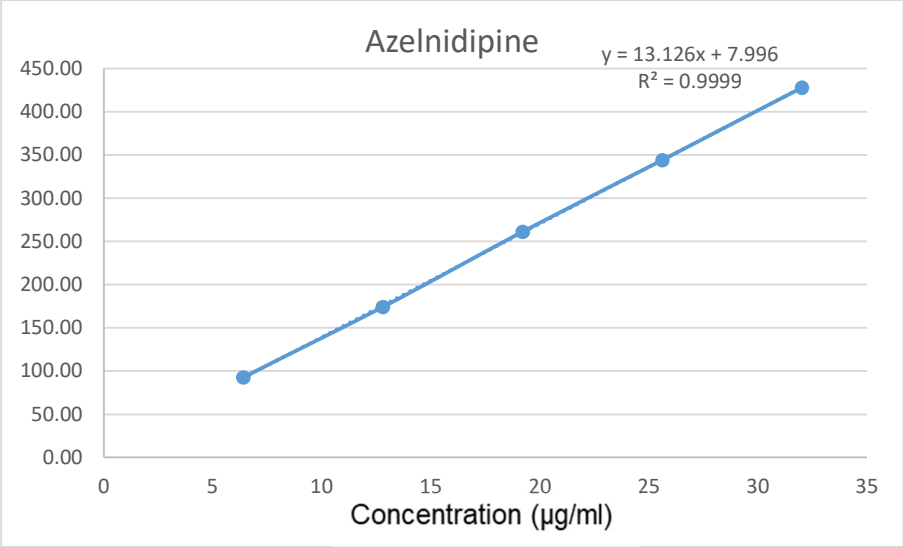


Fig 2: Calibration curve of AZN

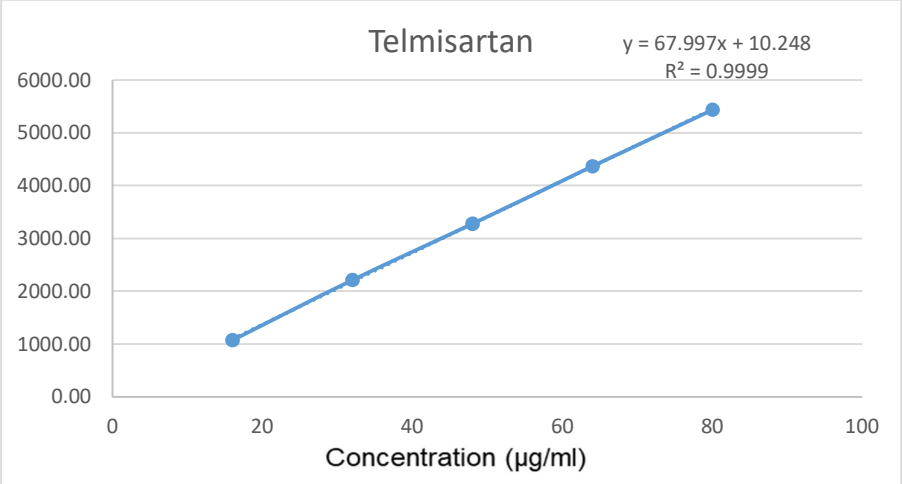


Fig 3: Calibration curve of TMN

Table 1: Linearity data for AZN and TMN

Sr. No.	AZN (µg/ml)	Peak Area± S.D.(n=5)	TMN (µg/ml)	Peak Area± S.D.(n=5)
1	6.4	92.80±0.92	16	1073.57±1.72
2	12.8	175.22±1.67	32	2213.76±30.62
3	19.2	261.03±3.14	48	3278.98±47.35
4	25.6	344.12±0.75	64	4367.96±0.98
5	32	427.87±2.25	80	5436.22±10.19

Table 2: Regression analysis data for AZN and TMN

Regression Analysis	AZN	TMN
Regression equation	y = 13.126x + 7.996	y = 67.997x + 10.248
Correlation co-efficient (R^2)	0.9997	0.9999
Slope(S)	13.126	67.997
Intercept (б)	7.996	10.248

Accuracy:

To assess the accuracy of the developed HPLC method, recovery experiments were conducted using the standard addition technique. Known quantities of pure Azelnidipine (AZN) and Telmisartan (TMN) standards were spiked into pre-analyzed sample solutions at three concentration levels: 80%, 100%, and 120% of the target concentration. The recovery of AZN and TMN was calculated for each level, and the results are presented in Table 3.

Table 3. Accuracy Data for AZN and TMN

Drug	% Level	Amt. of sample (µg/ml)	Amt. of drug added (µg/ml)	Amt. Recovered Mean ± S.D (µg/ml)	%Recovery± S.D.	% RSD
AZN	80	2	1.6	1.62±0.014	101.64±0.92	0.92
	100	2	2	2.01±0.016	100.67±0.83	0.83
	120	2	2.4	2.42±0.011	100.88±0.47	0.47

Drug	% Level	Amt. of sample (µg/ml)	Amt. of drug added (µg/ml)	Amt. Recovered Mean ± S.D (µg/ml)	%Recovery± S.D.	% RSD
TMN	80	16	12.8	12.83±0.20	100.27±1.62	1.62
	100	16	16	15.96±0.17	99.73±1.10	1.10
	120	16	19.2	19.51±0.38	101.61±1.95	1.95

Precision:

The precision of the developed HPLC method was comprehensively evaluated through intra-day (repeatability) and inter-day variation studies. Intra-day precision was assessed by analyzing drug solutions on the same day, whereas inter-day precision was evaluated by analyzing samples over three consecutive days.

For both studies, six replicates of samples at 100% concentration (12.8, 19.2, 25.6 µg/mL of AZN and 32, 48, 64 µg/mL of TMN) were injected. The percentage relative standard deviation (% RSD) values of the peak areas are presented in Table 4.

Table 4: Intraday Precision for AZN and TMN

Conc. (µg/ml)		Mean Peak Area*± S.D.		Mean %Assay* ± S.D.		%RSD	
AZN	TMN	AZN	TMN	AZN	TMN	AZN	TMN
12.8	32	176.52±2.73	2245.14±2.34	99.57±1.24	101.39±0.46	1.55	0.10
19.2	48	262.67±2.09	3299.53±3.18	100.74±0.84	100.25±0.78	0.80	0.10
25.6	64	350.31±0.33	4394.96±2.26	101.77±0.69	99.83±1.04	0.09	0.05

Interday Precision for AZN and TMN

Conc. (µg/ml)		Mean Peak Area*± S.D.		Mean %Assay* ± S.D.		%RSD	
AZN	TMN	AZN	TMN	AZN	TMN	AZN	TMN
12.8	32	176.95±2.14	2244.31±3.89	99.83±1.03	101.47±1.64	1.21	0.17
19.2	48	264.81±4.94	3278.46±2.82	101.59±1.78	101.35±1.79	1.87	0.48
25.6	64	347.76±5.15	4453.76±3.42	101.00±1.24	101.29±1.28	1.48	1.03

Repeatability data for estimation of AZN and TMN

Sr. No.	Drug	Conc. (µg/ml)	Mean Peak Area ± S.D.	Mean % Assay ± S.D.	%RSD
1	Azelnidipine	25.6	348.31±4.24	101.33±0.54	1.22
2	Telmisartan	64	4413.25±2.83	101.11±0.14	0.06

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

The LOD and LOQ values were determined from the calibration curves. The standard deviation (SD) represents the variability of the response of the minimum detectable drug, and the slope of the calibration curve indicates the sensitivity of the method. The formulas used for calculating LOD and LOQ are as follows:

LOD = 3.3 × avg SD / slope

LOQ = 10 × avg SD / slope

Table 5: Limit of Detection and Limit of Quantitation of AZN and TMN

Sr No.	Drug	LOD	LOQ
1	Azelnidipine	0.4399	1.3332
2	Telmisartan	0.882	2.6729

Robustness:

The robustness of the developed HPLC method was assessed by systematically introducing minor variations in three critical method parameters:

1. Flow rate (±0.1 mL/min)
2. Organic phase ratio (±5%)

3. Detection wavelength (±1 nm)

This evaluation employed solutions containing 1600 µg/mL Azelnidipine (AZN) and 4000 µg/mL Telmisartan (TMN). The effects of these deliberate variations on the method's performance were investigated.

The results of the robustness study, presented in Table 6, demonstrate the method's reliability and insensitivity to minor fluctuations in these parameters.

Table 6: Robustness study for AZN and TMN

Condition	Variation	AZN			TMN		
		Area Mean	SD	% RSD	Area Mean	SD	% RSD
Change in Flow Rate(±0.1ml/min)	1	279.71	1.87	0.67	3004.80	10.53	0.35
	0.8	235.68	1.69	0.72	3367.33	13.86	0.41
Change in Mobile Phase (±5)	80:20	279.71	1.48	0.54	3429.83	2.26	0.07
	70:30	266.4	1.42	0.53	3420.9	2.12	0.06
Change in Wavelength(±1nm)	238	255.16	0.30	0.12	3125.28	1.41	0.05
	236	265.9	0.64	0.24	3508.2	6.94	0.20

System Suitability:

System suitability tests were conducted to evaluate the performance of the developed HPLC method in generating accurate and precise results. Following method development and validation, these tests assessed critical chromatographic parameters, including plate number (N), resolution (R), tailing factor, capacity factor, height equivalent to a theoretical plate (HETP), and peak symmetry for Azelnidipine (AZN) and Telmisartan (TMN). The results, presented in Table 7, confirm that the method meets the required standards, ensuring reliable and consistent analysis of AZN and TMN in pharmaceutical formulations.

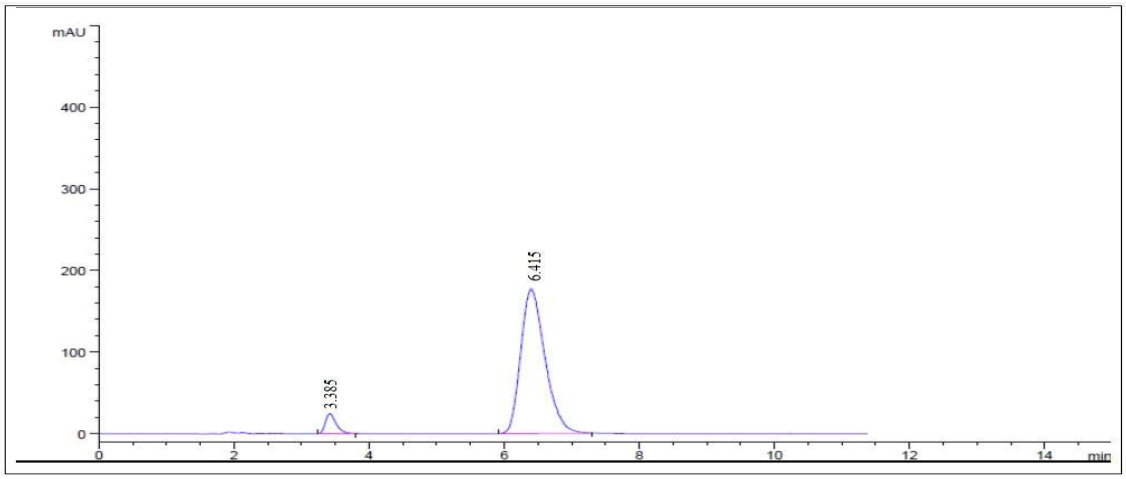


Fig. 4: Optimized mobile phase trial for AZN and TMN

Table 7. System Suitability for AZN and TMN

Drug	Resolution	Retention Time	Area (mAU x min) (USP)	Asymmetry (USP)	Theoretical Plate
Azelnidipine	0.00	3.385	255.87	0.93	4247
Telmisartan	6.325	6.415	3576.35	0.76	5768

Specificity:

The specificity of the developed HPLC method was evaluated by spiking commonly used tablet excipients into pre-weighed quantities of Azelnidipine (AZN) and Telmisartan (TMN), followed by measurement of peak areas and calculation of drug quantities. This study aimed to investigate potential interferences from excipients on the chromatographic analysis of AZN and TMN.

Assay of Marketed Formulation:

The assay results for the marketed formulation of Azelnidipine (AZN) and Telmisartan (TMN) tablets, determined

by comparing peak areas of test samples to standard solutions using the developed HPLC method, revealed that the content of AZN and TMN was 100.51% and 99.74%, respectively, of the labeled claim. These findings indicate that the marketed formulation meets the acceptable limits for pharmaceutical products, confirming its quality and potency, as both values fall within the 90-110% acceptance criteria. The results demonstrate the accuracy and reliability of the developed HPLC method for quantifying AZN and TMN in pharmaceutical formulations.

Table 8: Analysis of market formulation

Tablet	Label Claim (in mg/ml)	Conc.	Amount of Recovered ± S.D.	% Assay ± S.D.	%RSD
Telma AZ	Azelnidipine 16 mg	6 ppm	6.03±0.16	100.51±0.27	0.27
	Telmisartan 40 mg	48 ppm	47.87±0.30	99.74±0.64	0.64

Forced Degradation Sample Preparation

Forced degradation studies were conducted to evaluate the stability-indicating properties of the developed method for separating Azelnidipine (AZN) and Telmisartan (TMN). The studies aimed to identify potential degradation products and assess the method's robustness under various environmental conditions.

Stress conditions employed:

1. Acidic degradation (0.1N HCl)
2. Basic degradation (0.1N NaOH)
3. Oxidative degradation (3% H_2O_2)
4. Photolytic degradation (sunlight exposure)
5. Thermal degradation (80°C)

The degraded samples were analyzed using the developed method, monitoring chromatographic peaks corresponding to AZN and TMN. Additional peaks were investigated for potential degradation products.

This comprehensive assessment ensures the reliability and stability of the analytical method for quantifying AZN and TMN in pharmaceutical formulations under various stress conditions, confirming its suitability for routine analysis and quality control.

Acid Degradation

An acidic degradation study was conducted by transferring 1 mL of standard stock solution containing Azelnidipine and Telmisartan to a volumetric flask, followed by the addition of 1 mL of 0.1 M Hydrochloric Acid (HCl) and dilution to the mark with solvent (mobile phase). The solution was then allowed to stand for 24 hours, after which it was filtered using a syringe filter, and a chromatogram was recorded. This study aimed to evaluate the stability and degradation pattern of Azelnidipine and Telmisartan under acidic conditions (0.1 M HCl, 24 hours, ambient temperature), ensuring the developed HPLC method's ability to accurately quantify the drugs in the presence of potential degradants.

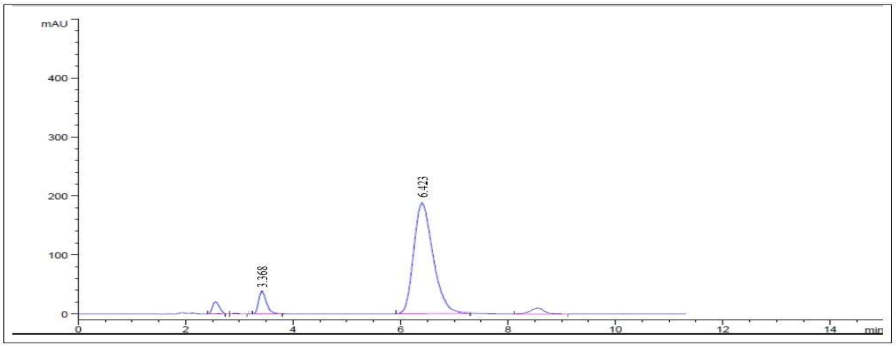


Fig.5: Chromatogram of degradation study in Acid Degradation sample

Basic Degradation

A basic degradation study was conducted by transferring 1 mL of stock solution-I containing Azelnidipine (AZN) and Telmisartan (TMN) to a 10 mL volumetric flask, followed by the addition of 5 mL of 0.1 N sodium hydroxide

(NaOH). The mixture was allowed to stand for 5 hours, then neutralized with 2 mL of 0.1 N hydrochloric acid (HCl) to halt degradation, and diluted to the mark with mobile phase. After neutralization, the chromatogram of the resulting solution was recorded to analyze changes or degradation products that occurred under basic conditions, assessing the stability of AZN and TMN in the presence of 0.1 N NaOH for 5 hours.

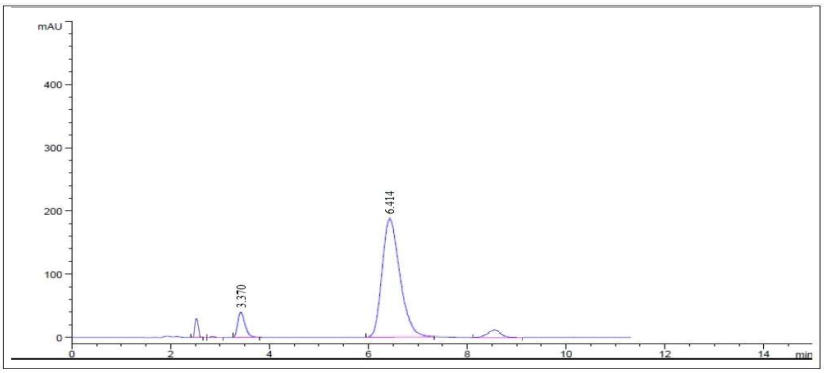


Fig. 6: Chromatogram of degradation study in Base Degradation sample

Oxidative Degradation

An oxidative degradation study was conducted by transferring 1 mL of stock solution-I containing Azelnidipine (AZN) and Telmisartan (TMN) to a 10 mL volumetric flask, followed by the addition of 5 mL of 3% hydrogen peroxide (H_2O_2). The volume was then made up to the mark with mobile phase. The solution was allowed to stand for 4 hours, after which the chromatogram of the resulting solution was recorded to analyze changes or degradation products that occurred under oxidative conditions, evaluating the stability of AZN and TMN when exposed to 3% H_2O_2 for 4 hours.

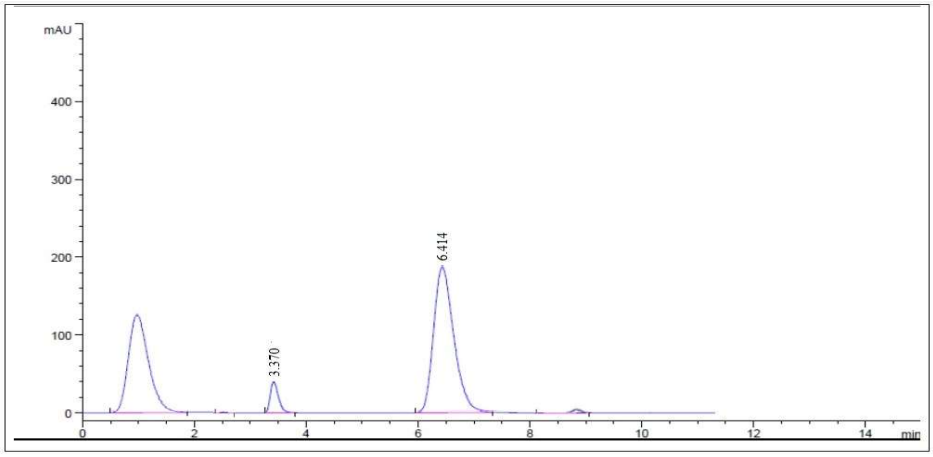


Fig. 7: Chromatogram of stability for AZN and TMN in Oxidation Degradation sample

Photolytic Degradation

A photolytic degradation study was conducted by transferring 1 mL of stock solution-I containing Azelnidipine (AZN) and Telmisartan (TMN) to a 10 mL volumetric flask. The solution was then exposed to sunlight for 12 hours. Post-exposure, the solution was adjusted with diluent to achieve concentrations of 160 µg/mL (AZN) and 400 µg/mL (TMN) for analysis. The adjusted solution was then analyzed to detect changes or degradation products resulting from photolytic degradation, assessing the stability of AZN and TMN when exposed to sunlight for 12 hours.

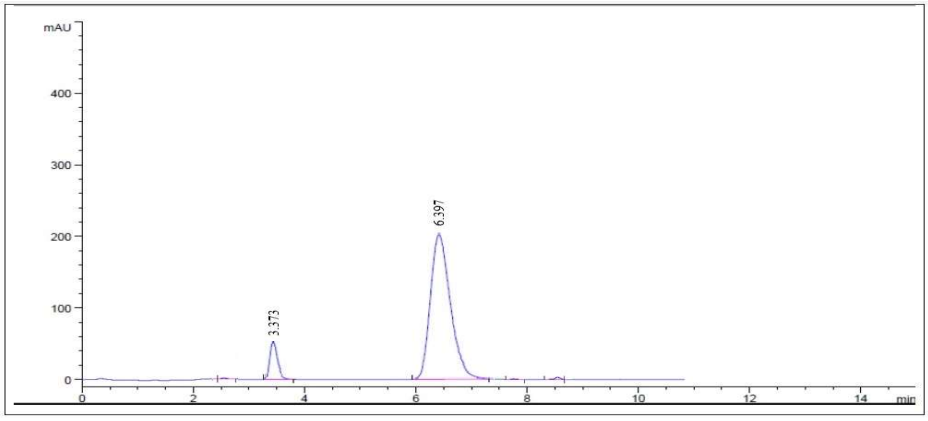


Fig. 8: Chromatogram of stability for AZN and TMN Photo Degradation sample

Thermal Degradation

A thermal degradation study was conducted by transferring 1 mL of stock solution-I containing Azelnidipine (AZN) and Telmisartan (TMN) to a 10 mL volumetric flask, adding 5 mL of water, and diluting to the mark with mobile phase. The solution was then heated at 80°C for 4 hours, followed by chromatographic analysis to evaluate changes or degradation products resulting from thermal degradation, assessing the stability of AZN and TMN when exposed to elevated temperature (80°C) for 4 hours.

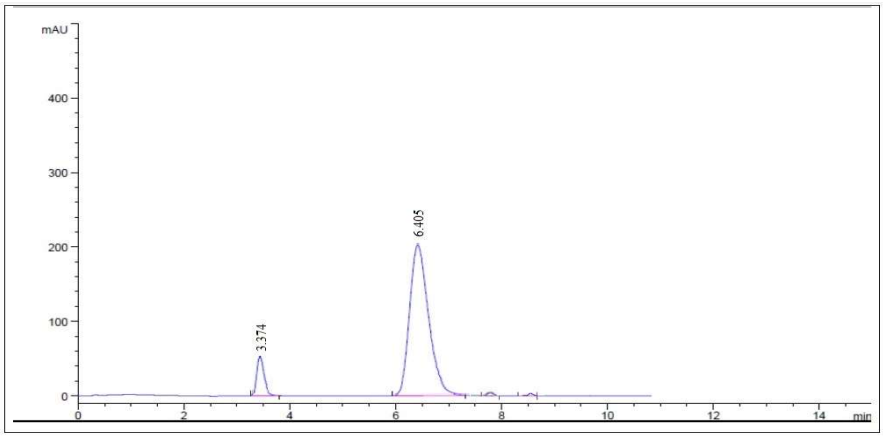


Fig. 9: Chromatogram of stability for AZN and TMN Thermal Degradation sample

Method Validation for Azelnidipine (AZN) and Telmisartan (TMN)

A method validation was conducted for the assay and stability studies of Azelnidipine (AZN) and Telmisartan (TMN), adhering to the International Council for Harmonisation (ICH) guidelines outlined in the Q2 (R1) document from 2005. This validation process comprehensively evaluated the method's precision, linearity, accuracy, robustness, limit of detection (LOD), and limit of quantitation (LOQ) in analytical solutions, ensuring compliance with established international standards for analytical method validation [ICH, 2005].

The validation parameters were assessed as follows:

1. **Precision:** The method's precision was evaluated through both intra-day and inter-day variations, ensuring consistency in the measurement of AZN and TMN concentrations.
2. **Linearity:** The linearity of the method was determined by analyzing calibration curves constructed over specified concentration ranges for AZN and TMN.
3. **Accuracy:** Accuracy was assessed by recovery experiments, where known amounts of AZN and TMN were added to pre-analyzed samples to evaluate the method's ability to accurately quantify the drugs.
4. **Robustness:** Robustness was evaluated by introducing small variations in critical method parameters such as flow rate, mobile phase composition, and detection wavelength to assess the method's reliability under different conditions.

5. **Limit of Detection (LOD) and Limit of Quantitation (LOQ):** LOD and LOQ were determined to establish the method's sensitivity by calculating the lowest concentration of AZN and TMN that could be reliably detected and quantified.

This comprehensive validation process ensures that the developed method is reliable, accurate, and suitable for the intended analytical purposes, in accordance with international guidelines set forth by the ICH.

RESULTS AND DISCUSSION

A reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the simultaneous determination of Azelnidipine (AZN) and Telmisartan (TMN) in pharmaceutical formulations. The RP-HPLC method was chosen for its simplicity and suitability, considering sample nature, molecular weight, and solubility. Optimal separation was achieved using Acetonitrile: pH Buffer pH 3 (OPA) in a 75:25 %v/v ratio. The method's sensitivity was established through Limit of Detection (LOD) and Limit of Quantitation (LOQ) calculations, yielding values of 0.4399 and 1.3332 for AZN, and 0.882 and 2.6729 for TMN, respectively.

Forced degradation studies were conducted on AZN and TMN standard samples under various stress conditions, including acid, base, oxidation, photolytic, and thermal degradation. The results showed degradation percentages within acceptable criteria, ranging from 4.98% to 18.97% for AZN and from 4.01% to 11.16% for TMN, demonstrating the method's stability-indicating properties. Overall, the developed RP-HPLC method proved suitable for the accurate determination and stability assessment of AZN and TMN in pharmaceutical formulations, underscoring its potential for routine analysis and quality control applications due to its robust performance in separating AZN, TMN and degradation products under various conditions.

Table 9: Stability data

Sr. No.	Degradation	AZN		TMN	
		Degraded upto %	Actual % Degradation	Degraded upto %	Actual % Degradation
1	Acid Degradation	81.02	18.97	94.05	5.95
2	Base Degradation	84.55	16.56	88.83	11.16
3	H_2O_2 Degradation	94.93	05.07	95.98	4.01
4	Photolytic Degradation	91.11	08.89	95.52	4.47
5	Thermal Degradation	95.01	04.98	95.07	4.93

Table 10: Summary of Development and validation of RP-HPLC method for AZN and TMN in pharmaceutical dosage form

Sr. No.	Parameters	AZN	TMN
1	Linearity Range	6.4-32 µg/mL	16-80 µg/mL
2	Co-relation coefficient	0.9999	0.9999
3	Precision 1. Repeatability (n=6) 2. Intra-day precision (n=6) 3. Inter-day precision (n=6)	 1.22% 0.09-1.55 1.21-1.48	 0.06% 0.05-0.10 0.17-1.03
4	Accuracy (% Recovery)	100.67-101.64%	99.73-101.61%
5	Limit of Detection (LOD)	0.4399	0.882
6	Limit of Quantification (LOQ)	1.3332	2.6729
7	% Assay	100.51	99.74

CONCLUSION

In conclusion, this study successfully developed and validated a stability-indicating RP-HPLC method for the simultaneous determination and stability assessment of Azelnidipine (AZN) and Telmisartan (TMN) in pharmaceutical formulations. The method exhibited desirable sensitivity, with low Limit of Detection (LOD) and Limit of Quantitation (LOQ) values of 0.4399 µg/mL and 1.3332 µg/mL for AZN, and 0.882 µg/mL and 2.6729 µg/mL for TMN, indicating its capability to detect and quantify lower concentrations accurately. Forced degradation studies demonstrated the method's robustness, as observed degradation percentages remained within acceptable criteria under various stress conditions, including acid, base, oxidation, photolytic, and thermal

degradation. The selected mobile phase and chromatographic conditions proved effective in separating AZN and TMN, highlighting the method's suitability for routine analysis and quality control applications. Overall, the developed RP-HPLC method offers a reliable and efficient means for pharmaceutical analysts to assess the stability and quality of AZN and TMN formulations, ensuring drug safety and efficacy, and ultimately benefiting patient health and well-being.

Conflicts of interest

The authors hereby declare that they do not have any conflicts of interest.

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