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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF AZELNIDIPINE AND TELMISARTAN IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Aim: To develop and validate a simple, rapid and accurate RP-HPLC method for the simultaneous estimation of Azelnidipine and Telmisartan in pharmaceutical dosage forms. **Methodology:** Separation was achieved on Phenomenex Luna C18 column (250 mm × 4.6 mm, 5 µm) using an isocratic mobile phase of Methanol: Phosphate Buffer (37:63 % v/v, pH 4.2) at 1.0 mL/min, detected at 260 nm via PDA. Acetonitrile was entirely replaced by methanol, reducing cost and toxicity versus ACN-based methods (60–80% v/v). Detection at 260 nm was selected over 215–242 nm used in prior methods for superior signal-to-noise ratio. pH 4.2 suppresses ionization of Telmisartan carboxylate group (pKa ≈4.1), ensuring sharp peak symmetry. The method was validated as per ICH Q2(R1) guidelines. **Results:** AZL AND TEL are eluted at 2.13 and 3.69 min respectively. Linearity was found at concentration ranges of 20-60µg/ml for AZL and 10-30µg/ml for TEL. The recoveries obtained were 98–102% for AZL, and 99.62 – 99.88% for TEL. **Conclusion:** The method was validated according to ICH guidelines for accuracy, precision, linearity, specificity, limit of detection, limit of quantification and robustness. This fully validated, eco-friendly, isocratic RP-HPLC method with superior resolution and no complex sample pre-treatment is superior alternative for routine quality control of the AZL-TEL.

KEYWORDS: Azelnidipine, Telmisartan, RP-HPLC, Pharmaceutical Dosage Form.

1. INTRODUCTION

Telmisartan (TEL) is an angiotensin II receptor antagonist (AT1) used in the treatment of high blood pressure. It is a selective antagonist for angiotensin II binding at AT1 subtype receptors. Inhibition of AT1 receptors induces vasodilation and inhibits the angiotensin II mediated aldosterone production, which in turn leads to decrease in sodium and water excretion and also increases potassium excretion and thereby causes a reduction in blood pressure.[1-4] TEL chemically is2-(4-{[4-methyl-6-(1-methyl-1H-1,3-benzodiazol-2-propyl-1H-1,3benzodiazol-1-yl] methyl}phenyl)benzoicacid (Figure-1). It is a white, slightly yellowish solid with a molecular formula $C_{33}H_{30}N_4O_2$. The molecular weight was found to be 514.6 g/mol. It is insoluble in water, sparingly soluble in strong acid and soluble in strong base. It is generally administered through oral route.

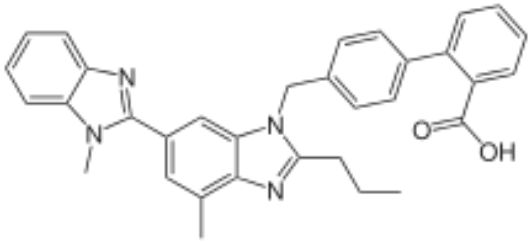

Figure-1: Structure of Telmisartan.

Azelnidipine[5-8] (AZL) chemically (±)-3-(1-diphenylmethylazetidin3-yl) 5-isopropy12-amino-1, 4-dihydro-6-methyl-4-(3-nitrophenyl) 3,5-pyridinedicarboxylate (Figure-2). It is a dihydropyridine (DHP) type of calcium channel blocker (CCB) used for the management of high blood pressure. The presence of asymmetric carbon at the 4-position of the 1,4 dihydropyridine ring enable the Azelnidipine exists in two enatiomeric form. Azelnidipine acts at voltage dependent channels of smooth muscle in vascular walls by inhibiting the influx Ca^{+2} and results in lower peripheral vascular resistance and arterial pressure. It is used in the management of essential hypertension and angina pectoris. The molecular formula was $C_{33}H_{34}N_4O_6$ with molecular weight 582.646g/mol. It is slightly soluble in methanol, freely soluble in acetone, soluble in ethyl acetate, sparingly soluble in water.

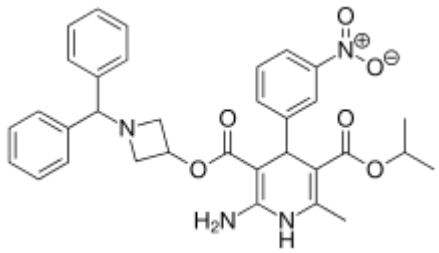

Figure-2: Structure of Azilnidipine.

Recently CDSCO approved the fixed dose combination of Telmisartan and azelnidipine for the treatment of hypertension. As this combination is available in the market and for qualitative analysis the analytical methods is a need. Literature survey reveals there is no analytical method reported yet for the simultaneous estimation of telmisartan and azelnidipine in combined dosage form.

Literature survey revealed that a number of methods like RP-HPLC.[9-21] Stability indicating[22-24] that have been reported for estimation of Telmisartan and Azelnidipine individually or in combination with other drugs. A thorough comparison of the present method with previously reported RP-HPLC methods for simultaneous estimation of Telmisartan and Azelnidipine reveals several advantages. The following summarises the most significant published methods and highlights the improvements offered by the current approach.

Agrawal and Nizami et.al (2021) reported an RP-HPLC method using predominantly acetonitrile (80% v/v), making it considerably more expensive and toxic than the present formulation. Additionally, the use of triethylamine as a buffer modifier has been associated with column contamination and irreproducible peak shapes over prolonged use. The retention times reported (6.0 min for AZL, 3.8 min for TEL) are also longer compared to the present method.

Ahmad et.al (2022) proposed a method using acetonitrile:phosphate buffer (70:30) at pH 4.6 on an Intersil C18 column, detected at 255 nm. The high acetonitrile proportion increases solvent consumption and operating costs.

2. MATERIALS AND METHODS

2.1. Instruments used

The HPLC study was carried out on Waters Alliance 2695 with a Photo diode array detector (PDA) 996 model. Phenomenex Luna (250 mm × 4.6 mm × 5µm particle size) was used at temperature of 35ºC. Other equipments are Sonicator (Labman), Analytical balance (Sartorius), pH meter (Lab India) was used.

2.2. Chemicals used

The working standard of Telmisartan and Azelnidipine pure drugs ((API)was provided as a gift sample from Sura Labs and combination of Azelnidipine and Telmisartan tablets (Glenmark), Methanol (MeOH) Acetonitrile (ACN), Potassium dihydrogen phosphate and milli-Q water (HPLC grade) were used for the preparation of mobile phase.

3. METHODOLOGY

3.1. Preparations of Solutions

3.1.1. Preparation of Standard Stock Solution

Accurately weighed 10mg of Telmisartan and Azelnidipine was transferred to 10mL volumetric flasks, 7ml of diluent (Methanol) was added and sonicated for 10 minutes. Flasks were made up with diluents and labelled as Standard stock solution of Telmisartan and Azelnidipine). 0.2mL of Telmisartan and 0.4ml of Azelnidipine from each Standard stock solution was pipetted out and taken into a 10mL volumetric flask and made up with diluent.

3.1.2. Preparation of 0.001N Potassium dihydrogen Phosphate (KH2PO4) buffer (pH-4.2)

Accurately weighed 6.8063gms of Potassium dihydrogen Ortho phosphate in a 1000mL of Volumetric flask add about 900mL of milli-Q water and adjust the p^H 4.2 with dilute orthophosphoric acid. Filter and sonicate the solution by vacuum filtration and ultrasonication.

3.1.3. Preparation of Mobile Phase

Accurately measured 370 ml of Methanol and 630 ml of Phosphate buffer with the p^H of 4.2 in the ratio of 37:63 v/v were mixed and degassed in a digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter.

3.2. Determination of Detection Wavelength

Between 200 and 400 nm, the standard solution was scanned. The wavelength of maximum absorption for drug was determined to be 260 nm.

4. METHOD DEVELOPMENT

4.1. Optimization of Mobile Phase

A variety of solvents with different compositions were screened to find out the ideal mobile phase given in Table 1.

Table 1: list of mobile phase compositions screened.

Sr. no.	Mobile phase	Ratio(V/V)	Remark
1	Methanol: Water	50: 50	peak was not eluted
2	Methanol: Acetonitrile	60: 40	excess noise was observed
3	ACN: Water	70: 30	less USP plate count was observed
4	Methanol: Phosphate Buffer (pH-4.2)	7:63	eak was symmetric

4.2. Chromatographic Conditions

Phenomenex Luna C18 column (250mm × 4.6 mm having 5µm particle size equilibrated with a mobile phase consisting of mixture of Methanol: Phosphate Buffer in the ratio of 37:63% v/v (pH-4.2) was found to be an ideal column. The flow rate was kept at 1mL/min, and column was set at 35ºC. Eluents were supervised using a PDA detector at 260nm.

In summary, the present method offers advantages: (a) use of methanol as a cost-effective, less toxic organic modifier; (b) isocratic elution mode for operational simplicity; (c) detection at 260 nm for better specificity and reduced background interference; (d) excellent chromatographic performance with resolution Rs = 9.8, USP plate counts exceeding 5600 and 8600 for AZL and TEL respectively, and tailing factors within acceptable limits; (e) concentration ranges and dilution factors matched to the approved FDC dose ratio; (f) full ICH Q2(R1) validation including accuracy, precision, linearity, specificity, LOD, LOQ, and robustness; and (g) suitability for direct application to commercially available pharmaceutical tablets without complex sample pre-treatment. These attributes collectively justify the development and reporting of the present method as a practically superior alternative to existing approaches for routine quality control of the Telmisartan-Azelnidipine fixed-dose combination.

5. METHOD VALIDATION

To confirm that the developed method is suitable for routine quality control of AZL and TEL pharmaceutical formulations it was validated according to ICH guidelines for accuracy, precision, linearity, specificity, sensitivity, limit of detection, limit of quantification and robustness.

5.1 system suitability

System suitability study was performed prior to method validation. The standard solution was injected five times and recorded the chromatograms for all five injections in HPLC and %RSD was calculated. %RSD of five different sample solutions should not more than 2.

5.2. Assay of drug

5.2.1. Preparation of Sample Stock Solution

Average weight of one tablet is taken and crushed in a mortar by using pestle and 10mg equivalent weight of Azelnidipine and Telmisartan samples was transferred into a 10 mL clean dry volumetric flask and 7mL of diluent was added and sonicated and the volume was made up with diluent and filtered by HPLC filters Further pipette out 0.4ml of Azelnidipine and 0.2ml of Telmisartan from the above stock solution into 10ml volumetric flask and dilute upto mark with diluent.

Procedure

Inject the three replicate injections of standard and sample solutions and calculate the %assay using the formula given below.

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

= 99.89%

5.2. Specificity

To demonstrate the method's precision, the following three replicate solutions will be prepared and injected as Blank (diluent), standard solution, sample solution and the chromatograms was recorded.

5.3. Linearity

The linearity of the method was studied over five different concentrations of Telmisartan and Azelnidipine in the range of 20-60ppm and 10- 30ppm. The calibration curve was constructed by plotting peak area on y axis versus concentration on x axis. The regression line equation and correlation coefficient values were determined.

5.4. Precision

The Precision is reported in terms of Relative Standard deviation (RSD). There are two levels of precision repeatability and intermediate precision which are calculated.

5.4.1. Repeatability

The standard solution was injected for five times and chromatograms was recorded under same operating conditions. The %RSD for the area of five replicate injections was calculated.

5.4.2. Intermediate Precision

The standard solution was injected for Six times and measured the area for all Six injections in HPLC. This was performed on different days under the same operating conditions. Chromatograms was recorded and %RSD was calculated.

5.5. Accuracy

Accuracy of the method was confirmed by a recovery study from marketed formulation at three replicate injections of individual concentrations (50%, 100%, 150%). Percentage recovery of Telmisartan and Azelnidipine was calculated.

5.6. Robustness

The robustness results were obtained by varying the flow rate and mobile phase composition. Small variation in flow rate and mobile phase composition did not significantly impact the results, proving the robustness of the method.

5.6. Limits of detection (LOD) and limit of quantitation (LOQ) were determined from the signal-to-noise ratio. The detection limit was referred to as the lowest concentration level resulting in a peak area of three times the baseline noise. The quantitation limit was referred to as the lowest concentration level that provided a peak area with a signal-to-noise ratio higher than ten.

LOD = 3:3 δ/S, LOQ = 10 δ/S.

6. RESULTS AND DISCUSSION

6.1. Method development

The selection of each chromatographic parameter for the assessment of Telmisartan and Azelnidipine in the present method was based on systematic experimentation and is scientifically justified as follows.

Mobile Phase Optimization: Among several combinations of organic modifiers and aqueous buffers screened (Table 1), Methanol: Phosphate Buffer (37:63, v/v, pH 4.2) yielded symmetric, well-resolved peaks for both analytes. Methanol was preferred over acetonitrile for its lower cost, lower toxicity, and comparable eluting strength at this composition. The pH of 4.2 was critical as it suppresses the ionization of the carboxylate group of Telmisartan (pKa ~4.1), ensuring adequate retention on the C18 phase and preventing peak broadening caused by the partially-ionized form. Phosphate buffer was selected over other buffers (acetate, formate) owing to its excellent buffering capacity in the pH range 3.0–5.0.

Column Selection: A Phenomenex Luna C18 column (250 mm × 4.6 mm, 5 µm) was selected on the basis of its retentive characteristics for hydrophobic compounds such as TEL and AZL. The C18 stationary phase provides strong hydrophobic interaction and adequate retention for both analytes. The 250 mm column length ensures sufficient theoretical plates (USP plate count >5600 for AZL and >8600 for TEL) for efficient peak resolution.

Flow Rate and Column Temperature: A flow rate of 1.0 mL/min and column temperature of 35ºC were found to produce the best balance between analysis speed, peak symmetry, and back-pressure. Higher flow rates reduced tailing factor. Optimized Chromatogram was shown in Figure-3 and Table-2.

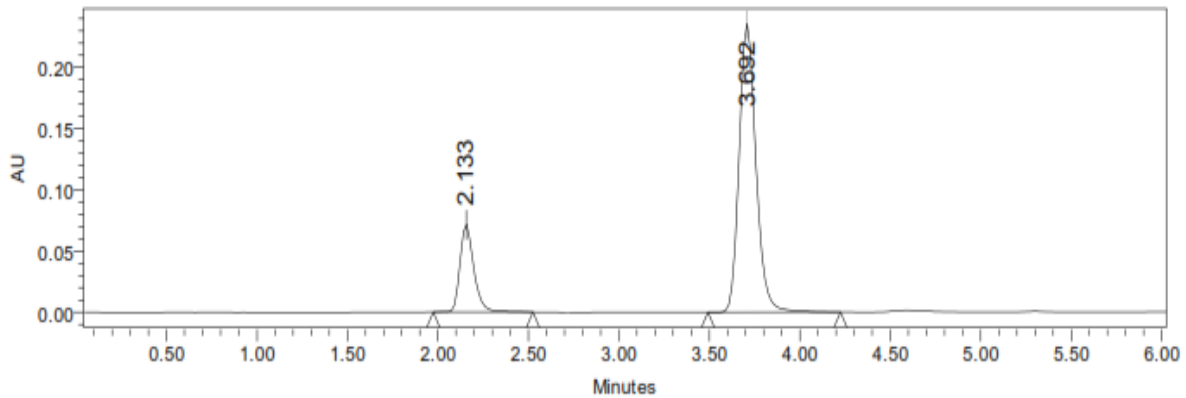

Figure-3: Optimized Chromatogram (Standard).

Table-2: Optimized Chromatogram values (Standard)

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count	Resolution
1	Azelnidipine	2.133	526389	86756	1.56	5679	
2	Telmisartan	3.692	1687285	367532	1.79	8685	9.8

6.2. Assay

Replicate injections were injected and % RSD, Theoretical plate and Tailing factor were found to be within the limits. The % purity of Azelnidipine and Telmisartan in pharmaceutical dosage form was found to be 99.89%. Results for assay of Azelnidipine and Telmisartan are reported in Table-3 &4. The chromatogram of sample is shown in figure 4.

Table-3: Peak results for Assay sample of Azelnidipine.

S. No	RT	Area	Height	USP Tailing	USP Plate Count
1	2.152	536859	87584	1.58	5789
2	2.150	532654	87965	1.59	5784
3	2.187	532685	87465	1.58	5769

Table-4: Peak results for Assay sample of Telmisartan.

S. No	RT	Area	Height	USP Tailing	USP Plate Count
1	3.646	1698568	378562	1.81	8759
2	3.651	1698574	375847	1.80	8795
3	3.601	1698547	376584	1.81	8745

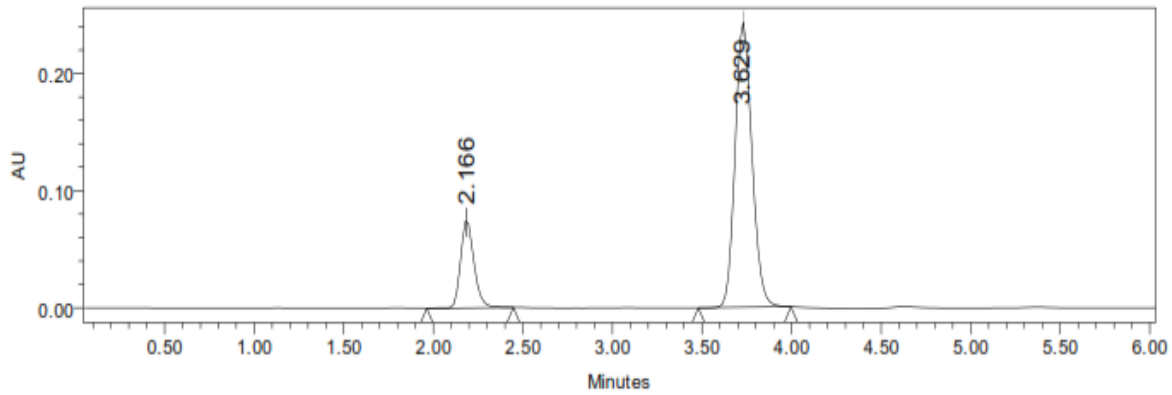

Figure-4: Optimized Chromatogram (Sample).

6.3. Validation of the optimized method

Validation of analytical procedures was performed for Telmisartan and Azelnidipine using the following parameters as per ICH guidelines.

6.3.1. Specificity: Prepared Blank, placebo, standard and sample all had been injected. The blank and placebo did not interfere with the retention time of Telmisartan and Azelnidipine.

6.3.2. System suitability: 5 replicates were injected and %RSD, tailing factor and Theoretical plates were found to be within limits. Results are reported in Table-5 and 6.

Table-5: Results of system suitability for Azelnidipine.

S.No.	RT	Area (µV*sec)	USP Plate Count	USP Tailing
1	2.152	526358	5695	1.56
2	2.157	526548	5652	1.57
3	2.141	526854	5627	1.56
4	2.133	526598	5692	1.57
5	2.166	524874	5641	1.56
Mean		526246.4		
Std. Dev.		787.353		
% RSD		0.149617		

Table-6: Results of system suitability for Telmisartan.

	RT	Area (µV*sec)	USP Plate Count	USP Tailing	Resolution
1	3.674	1682821	8659	1.56	9.8
2	3.631	1682726	8675	1.57	9.9
3	3.625	1687361	8692	1.56	9.8
4	3.692	1682811	8642	1.57	9.8
5	3.629	1683816	8635	1.58	9.8
Mean		1683907			
Std. Dev.		1982.03			
% RSD		0.117704			

6.3.3. Linearity

Linearity was assessed by plotting a calibration curve correlating peak response with their corresponding concentrations. A concentration range of 20-60ppm and 10- 30ppm of Telmisartan and Azelnidipine was used and linearity curves Linearity results are reported in Table-7 and 8 and calibration curves are represented in Figure-5 and 6.

Table-7: Results of Linearity Studies for Azelnidipine.

Concentration µg/ml	Average Peak Area
20	272897
30	402986
40	526389
50	649785
60	769287

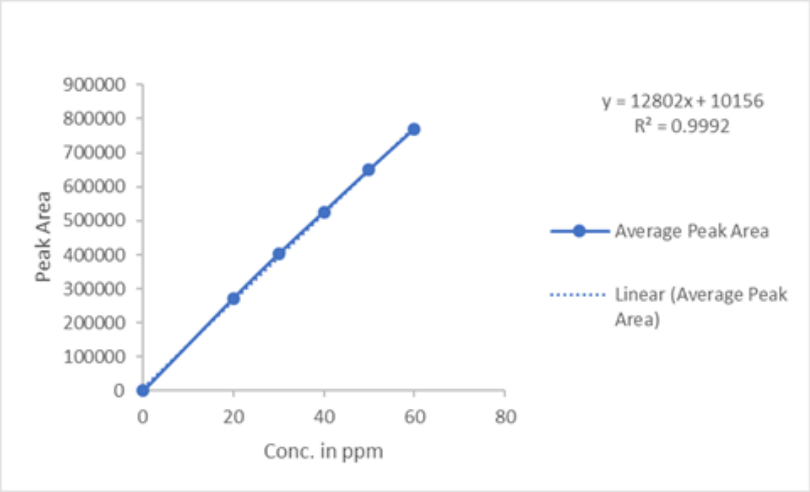

Figure-5: Calibration Curve of Azelnidipine.

Table-8: Results of Linearity Studies for Telmisartan.

Concentration µg/ml	Average Peak Area
10	1000237
15	1448768
20	1887285
25	2365897
30	2826845

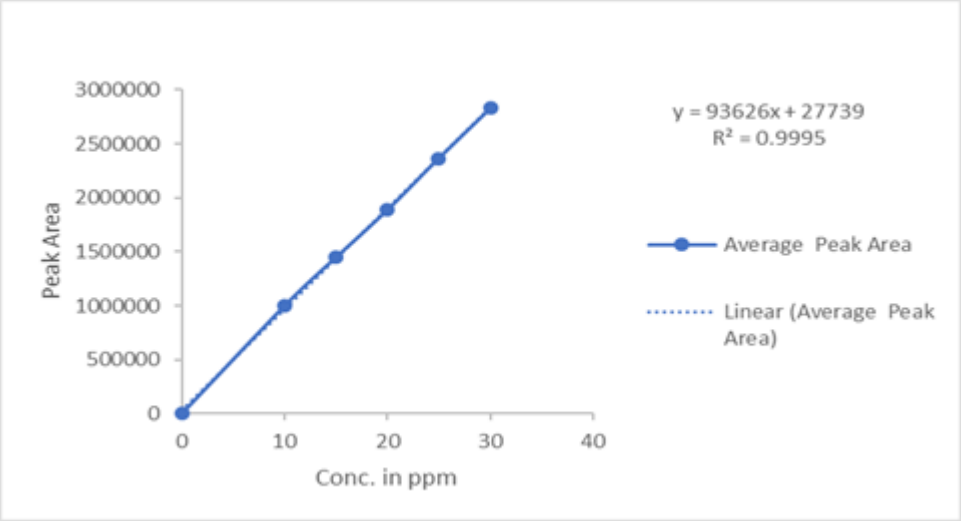

Figure-6: Calibration Curve of Telmisartan.

6.3.4. Precision

Test results for Telmisartan and Azelnidipine indicate that the %RSD results are within limits. Results are reported in Table-9-12.

Table-9: Results of Repeatability for Azelnidipine.

S. No.	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	2.157	526358	86598	5689	1.56
2	2.159	524856	86542	5687	1.57
3	2.186	526985	86578	5684	1.56
4	2.160	528654	86354	5689	1.56
5	2.170	528457	86958	5639	1.56
Mean		527062			
Std.dev		1569.114			
%RSD		0.297709			

Table-10: Results of Repeatability for Telmisartan.

S. No.	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	3.603	1687589	367859	8659	1.79
2	3.608	1685987	368547	8679	1.80
3	3.600	1685987	367985	8645	1.80
4	3.696	1685754	365874	8695	1.79
5	3.629	1685985	364589	8625	1.79
Mean		1686260			
Std.Dev		749.493			
%RSD		0.044447			

Table-11: Results of Intermediate Precision for Azelnidipine.

S. No.	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	2.198	546585	87589	5898	1.58
2	2.196	548758	87985	5879	1.59
3	2.160	549854	87452	5868	1.58
4	2.160	548798	87421	5847	1.59
5	2.160	542659	87963	5896	1.58
6	2.186	548754	87254	5874	1.59ss
Mean		547568			

Std.dev		2631.576			
%RSD		0.480593			

Table-12: Results of Intermediate Precision for Telmisartan.

S. No.	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	3.623	1698587	385482	8789	1.81
2	3.611	1698574	385698	8759	1.80
3	3.696	1698532	385748	8754	1.81
4	3.696	1698574	386958	8754	1.81
5	3.629	1698532	385755	5798	1.80
6	3.642	1698547	386558	8762	1.80
Mean		1698558			
Std.Dev		23.77113			
%RSD		0.001399			

6.3.5. Accuracy

Prepared three independent sample preparations at each level of 50%, 100% and 150% using the same batch of API of the target analytical weight (100%) [Total of 9 samples]. (50%, 100%, 150%). The Test results for Telmisartan and Azelnidipine indicate that the %RSD of results are within limits. Results are reported in Table-13 and 14.

Table-13: Accuracy results for Azelnidipine.

%Concentration (at specification Level)	Peak Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	267011.3	20	20.063	100.315%	100.28%
100%	523752.3	40	40.118	100.295%	
150%	778457.3	60	60.133	100.221%	

Table-14: Accuracy results for Telmisartan.

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	972876.3	10	10.094	100.94%	100.48%
100%	1900122	20	19.998	99.99%	
150%	2851152	30	30.156	100.52%	

6.3.6. Robustness

The tailing factor was determined to be within the limits for Telmisartan and Azelnidipine.

In order to demonstrate the robustness of the method, the optimized conditions (flow rate of mobile phase) were slightly varied. Robustness results are reported in Table-15 and 16.

Table-15: Robustness results for Azelnidipine.

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	526389	2.133	5679	1.56
Less Flow rate of 0.9 mL/min	542685	2.210	5264	1.54
More Flow rate of 1.1 mL/min	526483	2.184	5426	1.52
Less organic phase	516854	2.200	5163	1.57
More Organic phase	506898	2.172	5098	1.51

Table-16: Robustness results for Telmisartan.

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	1687285	3.692	8685	1.79
Less Flow rate of 0.9 mL/min	1725468	4.498	8265	1.68
More Flow rate of 1.1 mL/min	1652847	3.505	8415	1.59
Less organic phase	1687485	4.504	8326	1.62
More organic phase	1674524	3.512	8415	1.63

6.3.7. Limit of detection: LOD for Azelnidipine was found to be 1.04µg/ml.

Limit of detection (LOD) for Telmisartan was found to be 3.12µg/ml.

6.3.8. Limit of quantification (LOQ): **LOQ** for Azelnidipine was found to be 2.1µg/ml.
Limit of quantification (LOQ) for Telmisartan was found to be 6.3µg/ml.

7. CONCLUSION

The present method offers advantages like use of methanol as a cost-effective, less toxic organic modifier, isocratic elution mode for operational simplicity, detection at 260 nm for better specificity and reduced background interference, excellent chromatographic performance with resolution Rs = 9.8, USP plate counts exceeding 5600 and 8600 for AZL and TEL respectively, and tailing factors within acceptable limits, full ICH Q2(R1) validation including accuracy, precision, linearity, specificity, LOD, LOQ, and robustness .This shows that the method developed is simple, rapid, precise and accurate & validated for the simultaneous quantification of Azelnidipine and telmisartan in pharmaceutical dosage form. All the validation parameters were found to be within the acceptance criteria. The developed RP-HPLC method was applied in the analysis of commercial pharmaceutical products containing AZL and TEL.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exists.

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