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Development And Validation Of RP-HPLC Method For Simultaneous Quantification Of Telmisartan And Azelnidipine In Pharmaceutical Formulations

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Abstract

A sensitive, selective, and cost-effective RP-HPLC method was developed and validated for simultaneous quantification of Telmisartan (TEL) and Azelnidipine (AZL) in pharmaceutical formulations using Azilsartan as internal standard (IS), in accordance with ICH guidelines. Chromatographic separation was achieved on a Symmetry C_{18} column (150 × 4.6 mm, 3.5 µm) with a mobile phase of acetonitrile:10 mM Ammonium Formate containing 0.1% formic acid (40:60, v/v) at 1.0 mL/min and ambient temperature. Analysis employed a Waters Alliance 2695 HPLC system coupled with UV Detector and data was acquiesced with Empower-2 software. The retention times of TEL, AZL and IS were found to be 3.47 min (TEL), 4.86 min (AZL), and 7.73 min (IS) respectively. The developed method was found to be precise, accurate, sensitive, robust and linear ($r^2 > 0.999$) over 4–80 ng/mL (TEL) and 0.8–16 ng/mL (AZL), with LLOQ values of 4 ng/mL and 0.8 ng/mL, respectively. The validated method was successfully applied to the assay of pharmaceutical formulations and the stability of the drugs was studied under variety of stability conditions.

Keywords: Telmisartan, Azelnidipine, Azilsartan, Assay, Degradation studies, RP-HPLC, Validation, ICH guidelines

1. Introduction

1.1 Profile of the selected Drugs

Telmisartan (TEL) Profile: Telmisartan [1-6] (Micardis®), an angiotensin II receptor blocker (ARB), treats hypertension and cardiovascular risk reduction by selectively antagonizing AT_1 receptors (3000-fold higher affinity than AT_2), inhibiting vasoconstriction and aldosterone release. It presents as a white to off-white crystalline powder; IUPAC name: 4'-[(4-methyl-6-(1-methyl-1H-benzimidazol-2-yl)-2-propyl-1H-benzimidazol-1-yl)methyl] biphenyl-2-carboxylic acid; formula $C_{33}H_{30}N_4O_2$; MW 514.6 g/mol. Solubility: insoluble in water, sparingly soluble in dichloromethane and organic solvents, soluble in strong base and methanol Pharmacokinetics: Rapid oral absorption (bioavailability ~50%, unaffected by food), >99.5% plasma protein binding, minimal metabolism (<3% hepatic glucuronidation), long half-life (~24 h), large Vd (~500 L), and 97% fecal excretion unchanged. Cautions: Risk of hyperkalemia with potassium-sparing agents; acute kidney injury with NSAIDs in renal impairment. Chemical structure of TEL is shown in Fig. 1

Azelnidipine (AZL) Profile: Azelnidipine [7-8] (CalBlock®), a third-generation dihydropyridine calcium channel blocker, manages hypertension by dual blockade of L-type and T-type channels in vascular smooth muscle, reducing calcium influx, vasoconstriction, and peripheral resistance with gradual onset and minimal reflex tachycardia. Light yellow to yellow crystalline powder; IUPAC name: 3-[1-(benzhydrylazetidin-3-yl)] 5-isopropyl 2-amino-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate; formula $C_{33}H_{34}N_4O_6$; MW 582.65 g/mol. Solubility: insoluble in water, slightly soluble in methanol, soluble in ethyl acetate/acetone/acetic acid. Marketed in Japan by Daiichi Sankyo and in India as Azusa® (Ajanta Pharma, 2020). Chemical structure of AZL is shown in Fig.2.

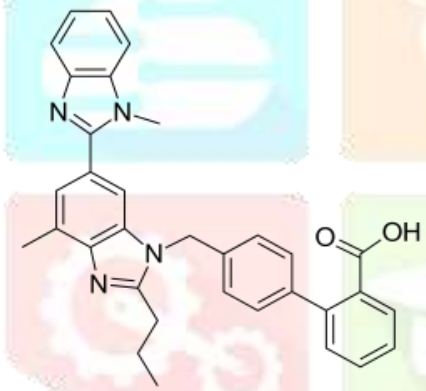

Fig.1: The chemical structure of TEL

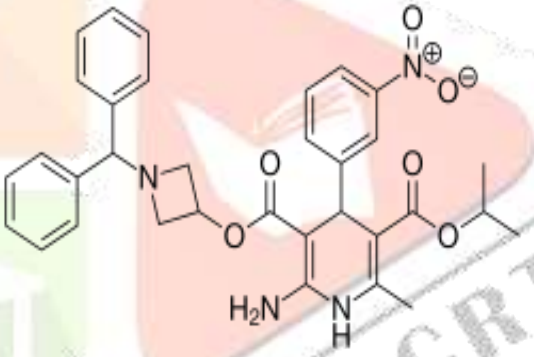

Fig.2: The chemical structure of AZL

1.2 Literature Review

An extensive literature survey was carried out and found various analytical methods for individual quantification of telmisartan (TEL) like UV spectrophotometry [9-13], HPLC [14-24], and HPTLC [25]. Azelnidipine (AZL) has been determined by UV [6-28], HPLC [29-34], and LC-MS/MS [35-37] in formulations or plasma Simultaneous TEL-AZL methods include UV spectrophotometry [38-39], RP-HPLC [40-46], primarily in tablets, with limited bioanalytical reports in plasma (one HPLC method; RP-HPLC in human plasma).

1.3 Aim and Scope of the work

The aim of the present investigation is to develop simple, cost effective and rapid alternative RP-HPLC method. The objective of the work is to set up initial chromatographic conditions for RP-HPLC method development for simultaneous estimation of TEL and AZL, followed by method validation as per ICH-guidelines, stability studies under various conditions and quantify TEL and AZL in formulations using the validated method

2. Materials and Methods

2.1 Instrumentation

Chromatographic analysis was performed using a Waters Alliance 2695 HPLC system (high-speed autosampler, column oven, and degasser) coupled to UV Detector. Data acquisition and processing were performed using Empower-2 software (Waters).

2.2 Reagents and Chemicals

Reference standards of telmisartan (TEL), azelnidipine (AZL), and Azilsartan (internal standard) were obtained from Glenmark Pharmaceuticals (Mumbai, India). LC-MS grade acetonitrile and methanol, and analytical-grade reagents were purchased from Merck (Mumbai, India). The combination product of telmisartan and amlodipine label claim (telmisartan 40 mg and amlodipine 5 mg). Telma-AM, tablets (Sun Pharmaceutical, Mumbai, India) were purchased from the local market HPLC water was generated using a Milli-Q purification system.

2.3 Preparation of solutions

Stock solutions (50 µg/mL) of telmisartan (TEL) and azelnidipine (AZL) were prepared by transferring accurately weighed quantities (5 mg TEL or 8 mg AZL) into separate 100 mL volumetric flasks, dissolving in diluent (50:50 v/v acetonitrile: water), and diluting to volume. Working standard solutions (~160 ng/mL) were obtained by diluting 0.32 mL TEL stock or 0.04 mL AZL stock to 10 mL with diluent. A combined working solution was prepared by transferring 1.0 mL each of TEL and AZL working solutions into a 10 mL volumetric flask and diluting to volume with diluents. Azilsartan stock solution (50 µg/mL) was prepared by dissolving 5 mg in a 100 mL volumetric flask with diluent. IS solution The working (160 ng/mL) was obtained by diluting 0.32 mL of stock to 10 mL, followed by further dilution of 1.0 mL to 10 mL with diluents. Ammonium Formate buffer (10 mM, pH 3.0) was prepared by dissolving 0.63 g in 1 L Milli-Q water (HPLC grade), adjusting pH with 0.1% formic acid, sonicating (15 min), and filtering through a 0.45 µm PVDF membrane. The mobile phase (40:60 v/v acetonitrile: buffer) was mixed, sonicated (15 min), and filtered through a 0.45 µm membrane prior to use

2.4 Method Development

The selected drugs are polar and soluble in the polar solvent such as methanol. The selection of stationary phase and mobile phase in chromatographic separation depends upon the nature of the drug molecules to be separated and quantified. As the components are polar, a reversed phase HPLC mode of separation is chosen, in which bonded phase non polar columns and polar mobile phases are chosen in trials. The absorption spectra of selected drugs are obtained by scanning in the UV region from 200 to 400 nm and it is observed that wavelength 234 nm is an isosbestic point, therefore, wavelength 234 nm is selected as the wavelength of detection of the components. The proposed method is optimized by changing one of the chromatographic parameters at a time while keeping the others constant, and chromatograms are obtained under a set of chromatographic conditions. The optimized chromatographic conditions, Symmetry C_{18} column (150 × 4.6 mm, 3.5 µm) with a mobile phase of acetonitrile:10 mM Ammonium Formate containing 0.1% formic acid (40:60, v/v) at 1.0 mL/min and ambient temperature, injections 10 µL, ambient temperature of the column, run time for 10 minutes. Analysis employed a Waters Alliance 2695 HPLC system coupled with UV Detector and data was acquiesced with Empower-2 software. The system suitable parameters are evaluated by the software. Blank, calibration standards (4–80 ng/mL TEL; 0.8–16 ng/mL AZL), quality control samples, and samples were injected (10 µL) into the HPLC. Chromatograms were recorded using Empower-2 software at a wavelength of 234 nm Peak areas of TEL, AZL, and internal standard (Azilsartan) were integrated automatically using the apex algorithm with baseline correction. Analyte-to-IS peak area ratios were calculated and plotted against nominal concentrations to generate weighted ($1/x^2$) calibration curves ($r^2 > 0.99$). Unknown sample concentrations were back-calculated from the calibration curve equation via least-squares regression. System suitability was confirmed by retention time reproducibility (±2%), resolution (>2.0), and theoretical plates (>5000)

3. Method Validation

The developed method is validated as per the ICH Guidelines

3.1 System suitability:

System suitability was assessed by six replicate injections (10 µL) of working standard solution containing telmisartan (TEL; 40 ng/mL), azelnidipine (AZL; 8 ng/mL), and internal standard Azilsartan (160 ng/mL) onto a Symmetry C18 column (150 × 4.6 mm, 3.5 µm). The mobile phase (acetonitrile:10 mM Ammonium Formate pH 3.0 [40:60 v/v]) was delivered at 1.0 mL/min over 10 min runs. Representative chromatograms (blank and system suitability) are shown in Fig.3–4 respectively

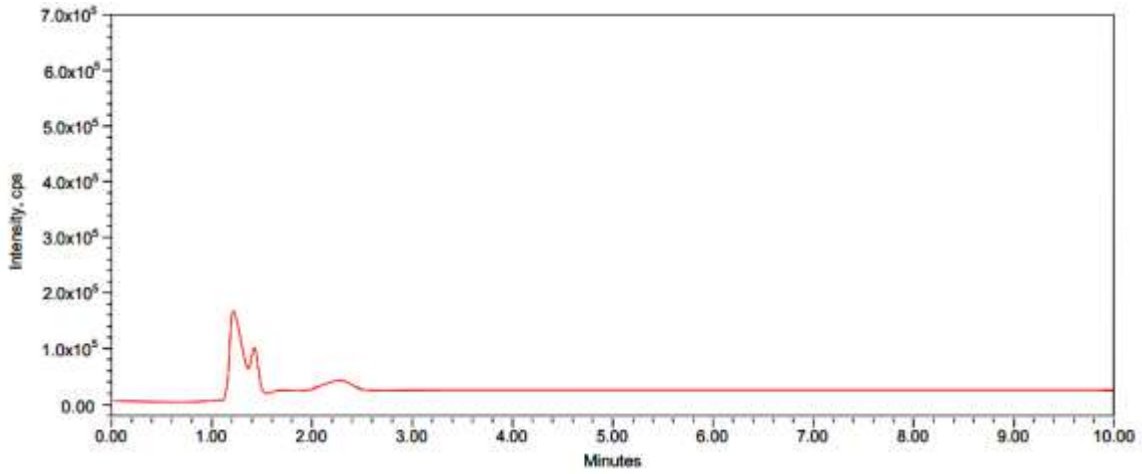

Fig-3: Chromatogram of Blank solution

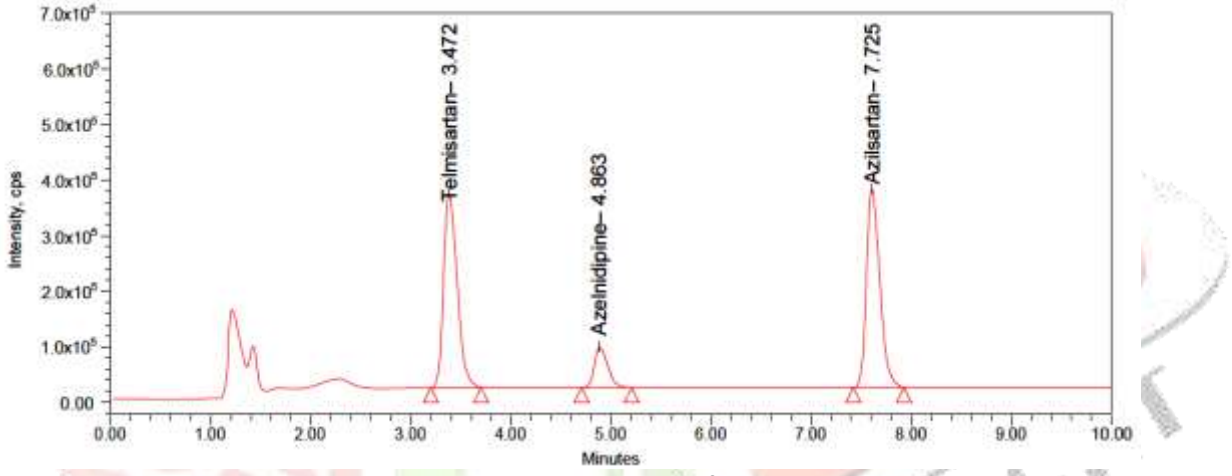

Fig-4: Chromatogram of System suitability

3.2 Specificity:

The mobile phase was filtered before use through a 0.45micron membrane filter and pumped from the respective solvent reservoirs into the column at a flow rate of 1ml/min. Prior to injection of the standard or sample solutions; the column is equilibrated for at least 30min with the mobile phase flowing through the system. The response of the detector is recorded at 234 nm. It is observed that the base line is parallel to x-axis and no peaks are found in the chromatogram. Chromatograms are obtained for standard and sample solutions under the optimized chromatographic conditions, and then the blank chromatogram is compared with the standard and sample chromatograms given by Fig.5.

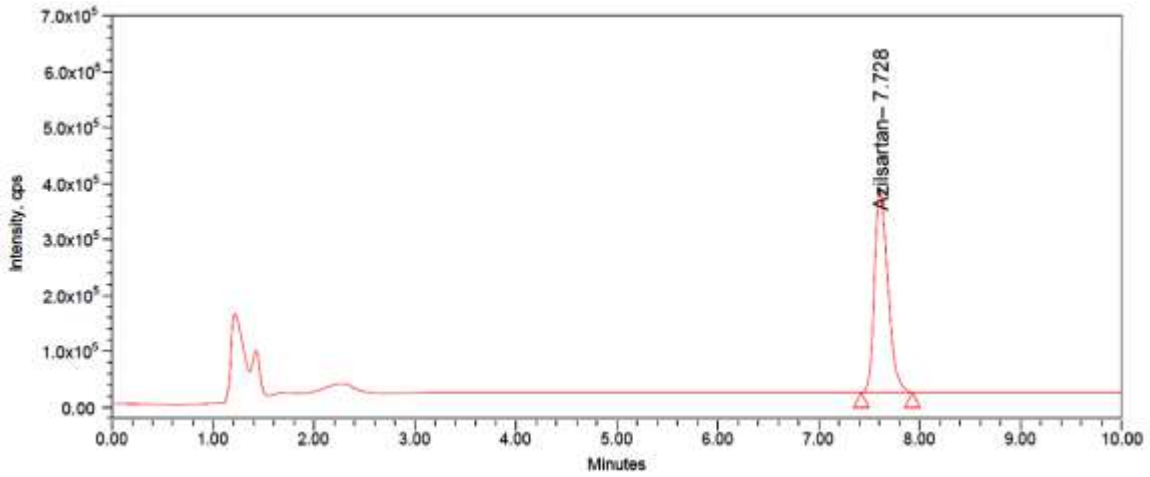

Fig.-5 Specificity Chromatogram of Internal Standard

3.3 Linearity:

Calibration standards (TEL: 4–80 ng/mL; AZL: 0.8–16 ng/mL) were prepared and analyzed in duplicate across five independent runs (n=10). Analyte-to-internal standard (Azilsartan) peak area ratios were plotted against nominal concentrations using weighted linear least-squares regression and representative linear plots were shown in Fig.6–7 respectively.

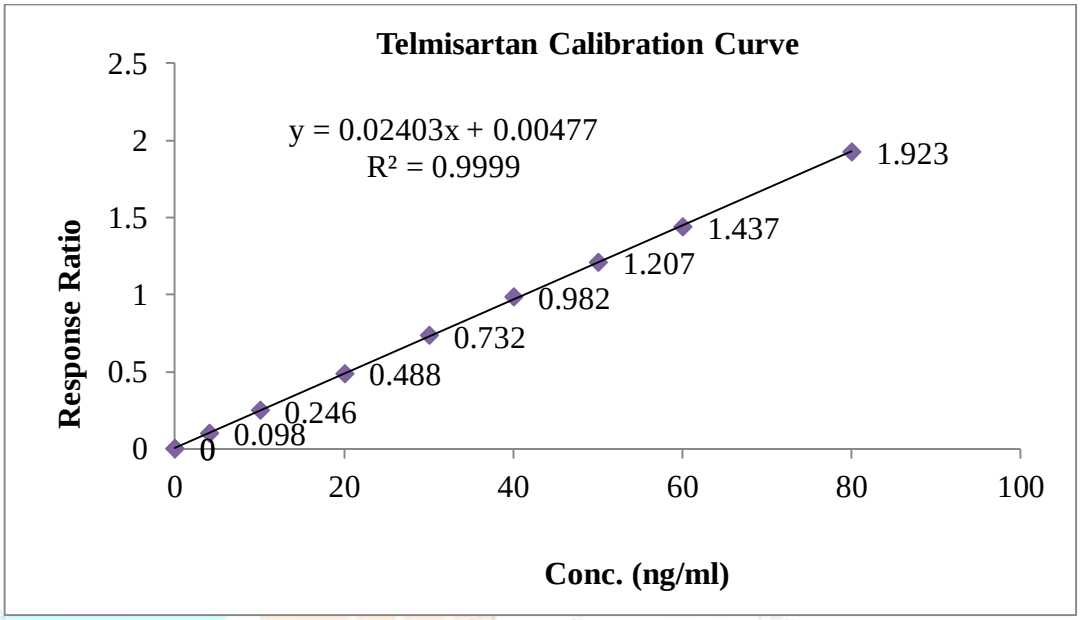

Fig.-6: Calibration plot for concentration v/s Area ratio of TEL

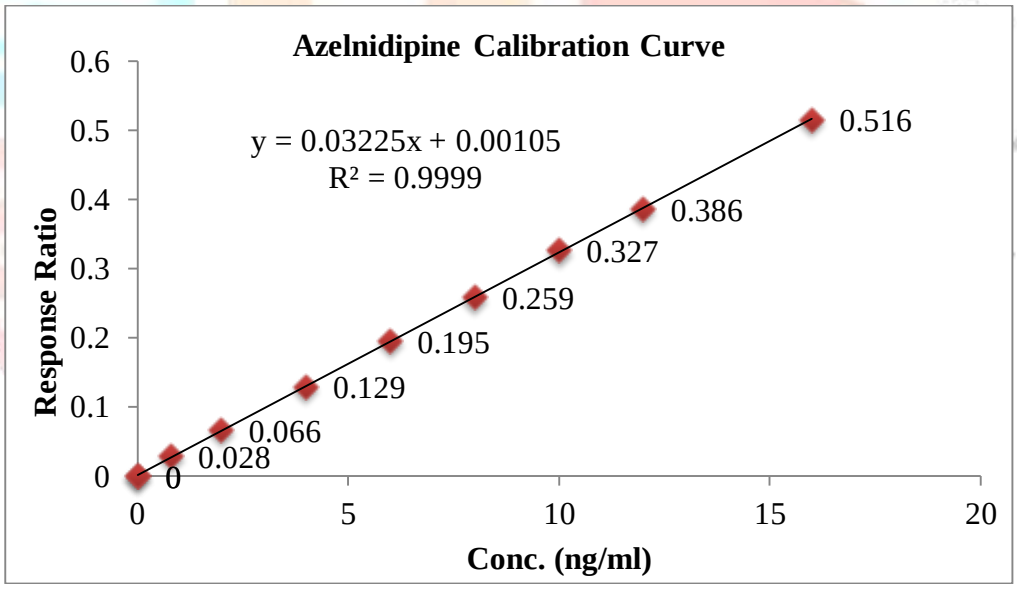

Fig.-7: Calibration plot for concentration v/s Area ratio of AZL

3.4 Sensitivity

Method sensitivity was established at the LLOQ concentrations of 4 ng/mL (TEL) and 0.8 ng/mL (AZL), determined as the lowest concentrations yielding signal-to-noise ratios >10:1 with acceptable precision and accuracy. Six replicate injections of LLOQ standards demonstrated mean calculated concentrations of 99.37% (TEL; SD 0.0061 ng/mL, %CV 1.66%) and 99.22% (AZL; SD 0.00585 ng/mL, %CV 5.76%) of nominal values, meeting FDA/ICH criteria (%CV ≤20%, accuracy 80–120%). A sample chromatogram of study of specificity is given in Fig.-8.

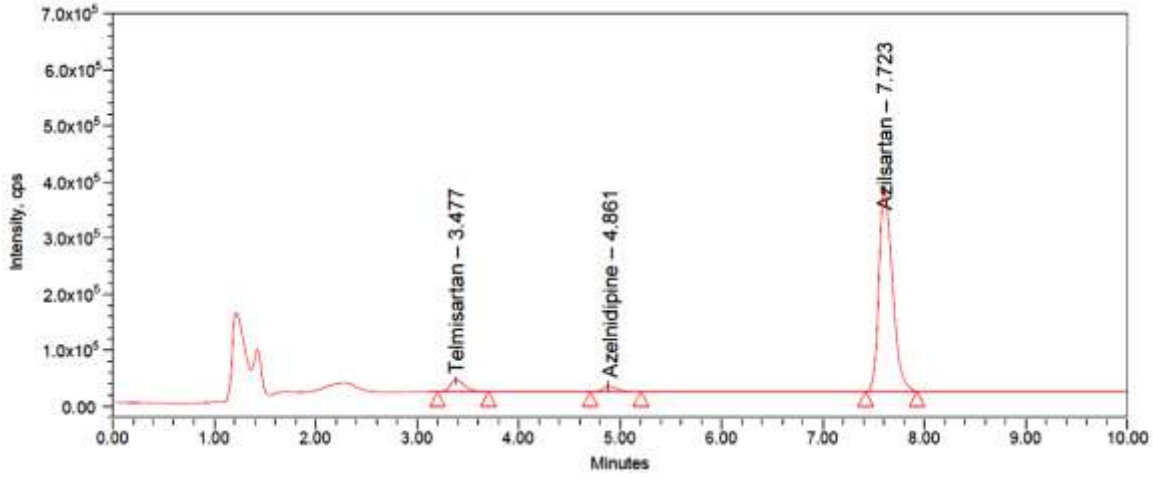

Fig.-8: Sensitivity Chromatogram of LLOQ

3.5 Precision and Accuracy

Accuracy of calibration standards was determined across five independent runs (n=5 concentrations × 2 replicates). Back-calculated concentrations for all standards excluding LLOQ were within 85–115% of nominal values, while LLOQ accuracy met 80–120% criteria per FDA/ICH guidelines. Intra-assay precision and accuracy were determined by analyzing six replicates at four QC levels (LLOQ QC, LQC, MQC, HQC) within a single run. Inter-assay precision was evaluated across four independent runs (n=24 per level). Representative chromatograms (HQC, MQC, LQC) are shown in Fig. 9–11 respectively.

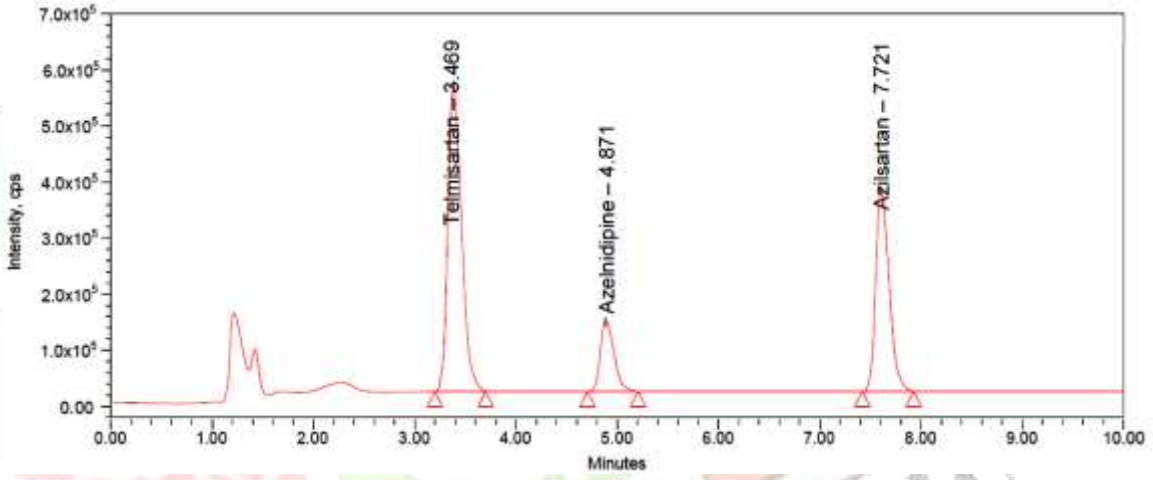

Fig.-9: Chromatogram of Precision and accuracy of HQC

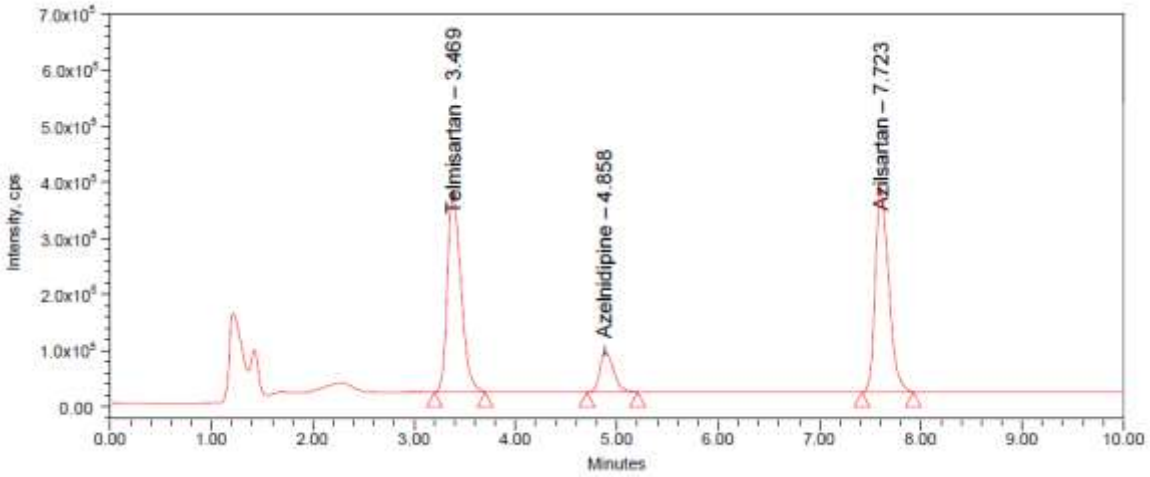

Fig.-10: Chromatogram of Precision and accuracy of MQC

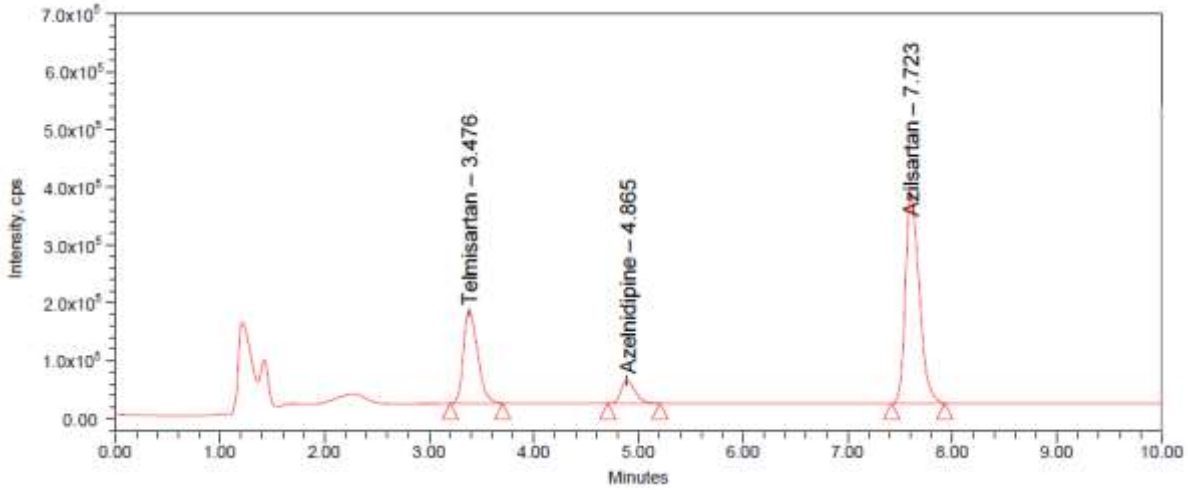

Fig.-11: Chromatogram of Precision and accuracy of LQC

3.6 Recovery

Absolute recovery of telmisartan (TEL), azelnidipine (AZL), and internal standard (Azilsartan) was determined at three QC levels (LQC, MQC, HQC; n=6 each; TEL 60, 40 and 20 ng/ml and AZL 12, 8 and 4 ng/ml)) by comparing mean peak areas samples. Representative chromatograms (LQC, MQC, HQC) shown in Fig.12–14 respectively.

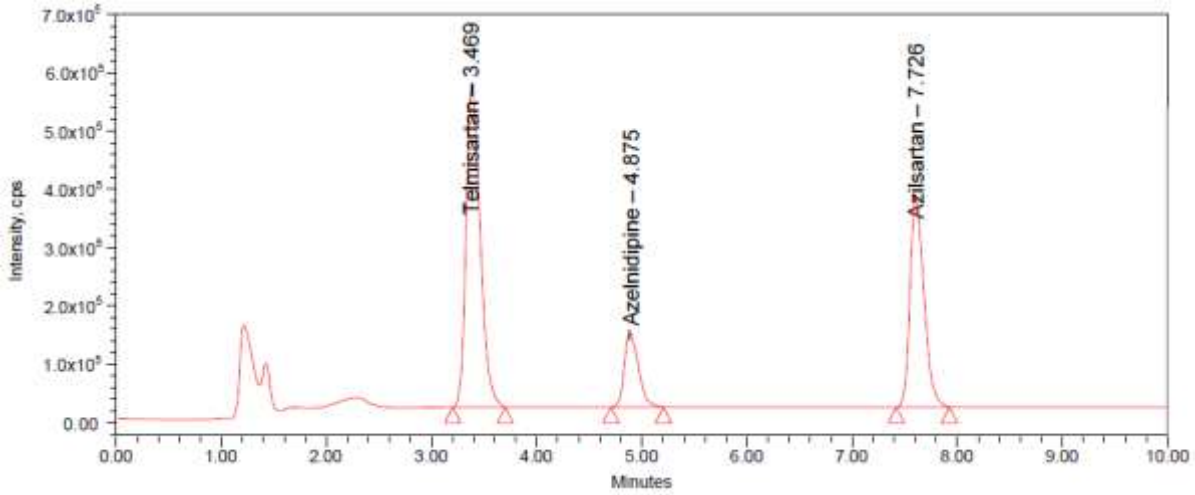

Fig.12: Chromatogram for recovery studies HQC

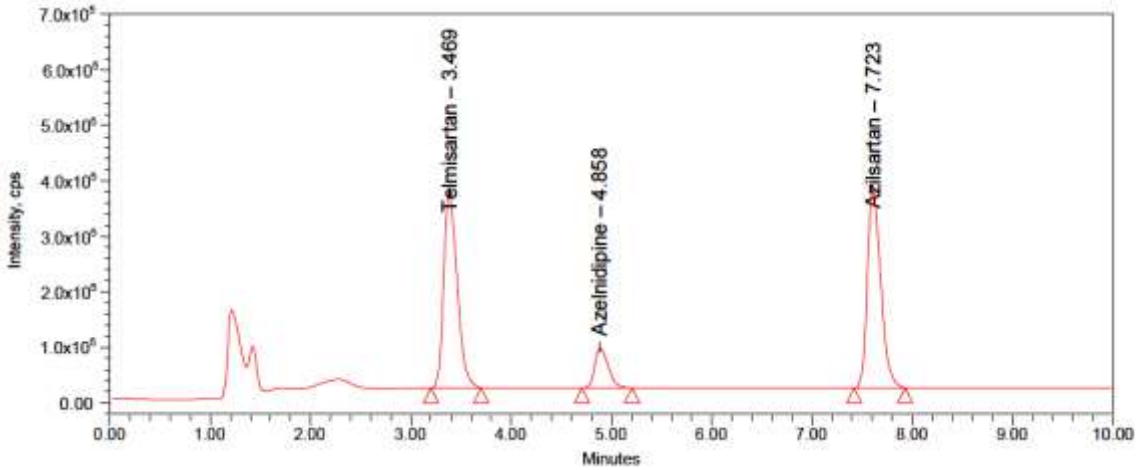

Fig.13: Chromatogram for recovery studies MQC

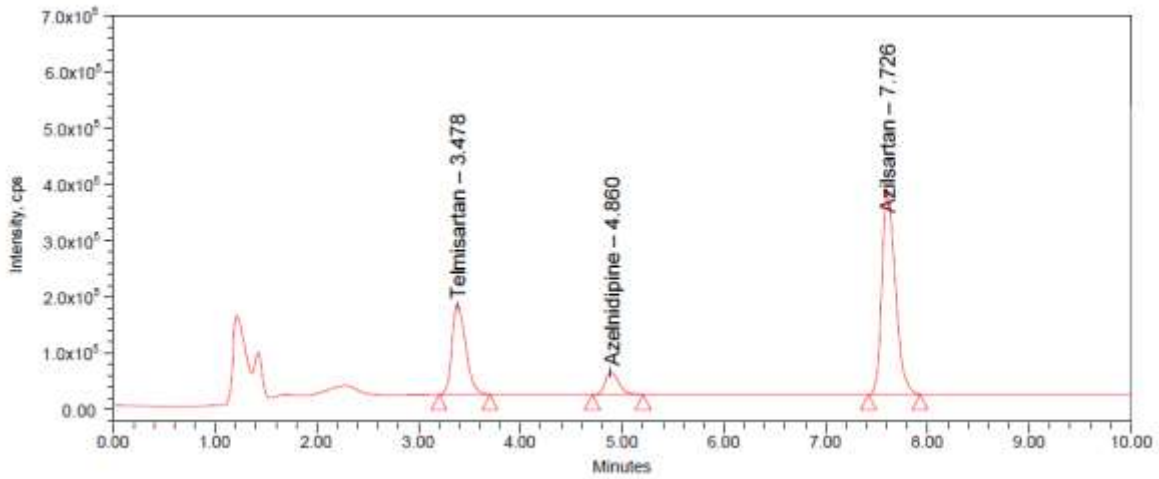

Fig.14: Chromatogram for recovery studies LQC

3.7 Robustness

Robustness is the study of the effect of variation of optimized parameters such as composition of the mobile phase, flow rate and detection wavelength on system suitability. A standard solution is prepared as per the test method.

Robustness of the method was determined by injecting the system suitability standard in five replicates by making slight deliberate changes in chromatographic conditions, such as; Wavelength ±5 nm, Flow rate ± 10%, PH of Mobile Phase ± 0.2 units, Mobile Phase composition ± 2% Organic solvent

3.8 Ruggedness

A study of ruggedness is a study of the variation between of results between two analysts and two systems. Six sample solutions were prepared as per the test method, and each analyzed by both analysts as per test method. System to system variation: System to system variability study was conducted on different HPLC systems, under similar conditions at different times. A Comparison of both the results obtained on two different HPLC systems, shows that the assay test method is passes for ruggedness for system-to-system variability.

3.9 Stability Studies

Stress degradation (forced degradation) is crucial in analytical method validation, especially for stability-indicating methods**,** where it involves intentionally breaking down a drug under harsh conditions to generate impurities, prove the method's specificity understand degradation pathways, and ensure it can detect changes, all per ICH guidelines. This "stress testing" confirms the method's ability to accurately quantify the intact drug and impurities, making it reliable for long-term stability. Stability was evaluated at three QC levels (LQC, MQC, HQC; n=6 each) under FDA/ICH-recommended conditions: bench-top (24h, room temp), processed sample stability (auto sampler, 4°C, 12/18 h), freeze-thaw (3 cycles, -80°C to RT), short-term (-20°C, 24 h), long-term (-80°C, 28 days), and wet stability (12/18 h) were investigated.

4. Results and Discussion

4.1 System suitability:

From the study of system suitability, the results obtained were summarized as follows. Retention times were 3.473 min (TEL), 4.866 min (AZL), and 7.723 min (IS), with mean peak areas of 3.824×10^5 cps (TEL), 1.028×10^5 cps (AZL), and 3.974×10^5 cps (IS). Mean retention time %CV was ≤1.0% (acceptance: ≤2.0%), and analyte-to-IS peak area ratio %CV was ≤2.0% (acceptance: ≤5.0%), confirming chromatographic performance, resolution (>2.0), and theoretical plates (>5000). The results were presented in Table-1

Table-1: System suitability Results of TEL and AZL

MQC (40ng/ml)	TEL Area (cps)	TEL RT (min)	ISTD Area (40ng/ml)	ISTD RT (min)	Area Ratio
Mean	$3.824x10^5$	3.473	$3.974x10^5$	7.723	0.9622
SD	0.00725	0.00303	0.00611	0.00314	0.00267
%CV	0.19	0.09	0.15	0.04	0.28

*Statistical evaluation on six replicate measurements

MQC (8ng/ml)	AZL Area (cps)	AZL RT (min)	ISTD Area (40ng/ml)	ISTD RT (min)	Area Ratio
Mean	$1.028x10^5$	4.866	$3.974x10^5$	7.723	0.2586
SD	0.00777	0.00378	0.00611	0.00314	0.00178
%CV	0.76	0.08	0.15	0.04	0.69

*Statistical evaluation on six replicate measurements

4.2 Specificity

Specificity was evaluated using six individual blanks processed identically to samples. No endogenous interferences were observed at retention times of telmisartan (3.47 min), azelnidipine (4.86 min), or internal standard Azilsartan (7.73 min). In blank, peak responses at analyte retention times were ≤20% of LLOQ signal (TEL: 4 ng/mL; AZL: 0.8 ng/mL), and IS channel interference was ≤5% of LLOQ IS response. All six matrix lots (100%) met FDA/EMA acceptance criteria, confirming method selectivity. Carryover was assessed by injecting six replicates of upper limit of quantification (ULOQ) standard (TEL 80 ng/mL, AZL 16 ng/mL) Analyte peak areas in blank chromatograms were <20% of LLOQ response (TEL 4 ng/mL; AZL 0.8 ng/mL) and ≤0.25% of ULOQ response, meeting FDA/ICH acceptance criteria. No carryover was observed in subsequent calibration standards or QC samples.

4.3 Linearity:

The results of linearity studies were summarized and presented in Table-2. Calibration curves exhibited excellent linearity (mean r^2 = 0.9999 ± 0.0001) over the validated ranges, regression parameters (slope, intercept, r^2) for TEL and AZL confirming robust quantitative performance per ICH Q2(R1)/FDA guidelines. Back-calculated concentrations of all standards fell within ±15% of nominal values

Table-2: Linearity Results of TEL and AZL

Conc. in ng/ml	Response of TEL	Area response ratio	Conc. in ng/ml	Response of AZL	Area response ratio
0	0	0.0	0	0	0.0
4.00	0.389	0.098	0.80	0.112	3.972
10.00	0.976	0.246	2.00	0.261	3.967
20.00	1.942	0.488	4.00	0.515	3.982
30.00	2.898	0.732	6.00	0.773	3.959
40.00	3.878	0.982	8.00	1.022	3.948
50.00	4.761	1.207	10.00	1.289	3.944
60.00	5.713	1.437	12.00	1.535	3.976
80.00	7.622	1.923	16.00	2.046	3.963
Slope	0.0240		Slope	0.0322	
Intercept	0.00477		Intercept	0.00519	
r^2 Value	0.9999		R^2 Value	0.9999	

4.4 Sensitivity

Method sensitivity was established at the LLOQ concentrations of 4 ng/mL (TEL) and 0.8 ng/mL (AZL), determined as the lowest concentrations yielding signal-to-noise ratios >10:1 with acceptable precision and accuracy. Six replicate injections of LLOQ standards demonstrated mean calculated concentrations of 99.37% (TEL; SD 0.0061 ng/mL, %CV 1.66%) and 99.22% (AZL; SD 0.00585 ng/mL, %CV 5.76%) of nominal values, meeting FDA/ICH criteria (%CV ≤20%, accuracy 80–120%). Acceptance Criteria: At least 67 % (4 out of 6) of samples should be within 80.00-120.00 %. Percent of mean accuracy should be within 80.00-120.00 %. % CV accuracy should be ≤ 20.00 %.

Table-3 Results of Sensitivity of Telmisartan and Azelnidipine

Replicate Number	LLOQ Concentration of TEL (4ng/ml)	LLOQ Concentration of AZL (0.8ng/ml)
	Peak area	Peak area
1	$0.385x10^5$	$0.105x10^5$
2	$0.382x10^5$	$0.099x10^5$
3	$0.389x10^5$	$0.094x10^5$
4	$0.375x10^5$	$0.106x10^5$
5	$0.372x10^5$	$0.109x10^5$
6	$0.379x10^5$	$0.097x10^5$
n	6	6
Mean	$0.380x10^5$	$0.102x10^5$
SD	0.00631	0.00585
% CV	1.66	5.76
% Mean Accuracy	99.37%	99.22%

4.5 Precision and Accuracy

In accuracy and precision studies at least 75% (including LLOQ and ULOQ) of calibration standards complied with acceptance criteria across all runs. In blank and zero standard chromatograms, interfering peak areas at analyte retention times were ≤20% of LLOQ response, and ≤5% of LLOQ IS response at IS retention time, confirming selectivity. Detailed accuracy data presented in Table-4&5. Mean accuracy was 85–115% (LQC, MQC, HQC) and 80–120% (LLOQ QC), with precision (%CV) ≤15% (≤20% at LLOQ), meeting FDA/ICH M10 criteria.

Table-4: Precision and accuracy Results of TEL

Concentration (ng/ml)	HQC	MQC	LQC	LLQC
	60	40	20	4
Mean	$5.710x10^5$	$3.819x10^5$	$1.905x10^5$	$0.382x10^5$
SD	0.00585	0. 00325	0.00407	0.00606
% CV	0.10	0.09	0.21	1.59
% Mean Accuracy	99.55%	99.87%	99.63%	99.90%

**Statistical analysis on six replicate measurements*

Table-5: Precision and accuracy Results of AZL

Concentration (ng/ml)	HQC	MQC	LQC	LLQC
	12	8	4	0.8
Mean	$1.525x10^5$	$1.025x10^5$	$0.508x10^5$	$0.101x10^5$
SD	0.00214	0.00579	0.00502	0.00339
% CV	0.14	0.56	0.99	3.34
% Mean Accuracy	98.90%	99.71%	99.41%	98.25%

**Statistical analysis on six replicate measurements*

4.6 Recovery

Absolute recovery of telmisartan (TEL), azelnidipine (AZL), and internal standard (Azilsartan) was determined at three QC levels (LQC, MQC, HQC; n=6 each; TEL 60, 40 and 20 ng/ml and AZL 12, 8 and 4 ng/ml)) by comparing mean peak areas samples Mean recoveries were consistently 98–99% across all levels for both analytes and IS, with acceptable precision (%CV <5%).

4.7 Ruggedness

The %CV for TEL, and AZL was passed the Ruggedness on precision accuracy and the results were presented in Table 6-7.

Table-6: Ruggedness on precision accuracy of results of TEL

Replicate No.	HQC	LQC	MQC
	Nominal Concentration(ng/ml)		
	60	20	40
	Analyte peak area		
n	6	6	6
Mean	$5.715x10^5$	$1.901x10^5$	$3.819x10^5$
SD	0.00509	0.00531	0.00454
%CV	0.09	0.28	0.12
% Mean Accuracy	99.63%	99.42%	99.87%

**Statistical analysis on six replicate measurements*

Table-7: Ruggedness on precision accuracy of results of AZL

Replicate No.	HQC	LQC	MQC
	Nominal Concentration(ng/ml)		
	12	4	8
	Analyte peak area		
n	6	6	6
Mean	$1.523x10^5$	$0.511x10^5$	$1.020x10^5$
SD	0.00505	0.00631	0.00587
%CV	0.33	1.23	0.58
% Mean Accuracy	98.77%	99.42%	99.22%

**Statistical analysis on six replicate measurements*

4.8 Stability Studies

The stability under different conditions like bench-top (24h, room temp), processed sample stability (auto sampler, 4°C, 12/18 h), freeze-thaw (3 cycles, -80°C to RT), short-term (-20°C, 24 h) and long-term (-80°C, 28 days) at three different concentrations of HQC, LQC and MQC were studied, and Mean, SD, %CV, Mean accuracy for Telmisartan, Azelnidipine were found to be within the limits. Acceptance Criteria: At least 67 % (8 out of 12) of total QC samples and 50 % (3 out of 6) at each level should be within 85.00-115.00 %. The % mean accuracy of LQC and HQC should be within 85.00-115.00 %. The % CV of LQC and HQC samples should be ≤ 15.00 %. Mean % recovery ranged 84.0–99.9% for TEL and AZL, with precision (%CV ≤15%). The results were presented in Table 8-12respectively.

Table-8: Bench Top Stability of TEL and AZL

	TEL			AZL		
	HQC	LQC	MQC	HQC	LQC	MQC
Con. ng/ml	60	20	40	12	4	8
Mean	$5.713x10^5$	$1.899x10^5$	$3.819x10^5$	$1.530x10^5$	$0.510x10^5$	$1.023x10^5$
SD	0.00589	0.00931	0.00826	0.00554	0.00627	0.00517
%CV	0.10	0.49	0.22	0.36	1.23	0.51
% Mean Accuracy	99.60%	99.32%	99.87%	99.22%	99.22%	99.51%

**Statistical evaluation on six replicates*

Table- 9: Auto Sampler Stability of TEL and AZL

	TEL			AZL		
	HQC	LQC	MQC	HQC	LQC	MQC
Con. ng/ml	60	20	40	12	4	8
Mean	$5.700x10^5$	$3.802x10^5$	$1.900x10^5$	$1.514x10^5$	$1.021x10^5$	$0.512x10^5$
SD	0.01003	0.01067	0.01055	0.00769	0.00659	0.00724
%CV	0.18	0.28	0.56	0.51	0.65	1.41
% Mean Accuracy	99.06%	99.42%	99.37%	98.18%	99.32%	99.61%

**Statistical data on 24 replicate measurements*

Table-10: Freeze Thaw Stability of TEL and AZL

	TEL			AZL		
	HQC	LQC	MQC	HQC	LQC	MQC
Con. ng/ml	60	20	40	12	4	8
Mean	$5.709x10^5$	$1.909x10^5$	$3.808x10^5$	$1.516x10^5$	$0.508x10^5$	$1.019x10^5$
SD	0.00599	0.00573	0.00607	0.00611	0.00585	0.00374
%CV	0.10	0.30	0.16	0.40	1.15	0.37
% Mean Accuracy	99.53%	99.84%	99.58%	99.31%	98.83%	99.12%

**Statistical data on six replicate measurements*

Table-11: Short term Stability of TEL and AZL

	TEL			AZL		
	HQC	LQC	MQC	HQC	LQC	MQC
Con. ng/ml	60	20	40	12	4	8
Mean	$5.489x10^5$	$1.820x10^5$	$3.660x10^5$	$1.481x10^5$	$0.490x10^5$	$0.987x10^5$
SD	0.00629	0.00585	0.00585	0.00539	0.00335	0.00611
%CV	0.11	0.32	0.16	0.36	0.68	0.62
% Mean Accuracy	95.69%	95.19%	95.71%	96.04%	95.33%	95.43%

**Statistical data on six replicate measurements*

Table-12: Long term Stability (28 days) of TEL and AZL

	TEL			AZL		
	HQC	LQC	MQC	HQC	LQC	MQC
Con. ng/ml	60	20	40	12	4	8
Mean	$4.980x10^5$	$1.631x10^5$	$3.318x10^5$	$1.321x10^5$	$0.432x10^5$	$0.890x10^5$
SD	0.00779	0.00556	0.00797	0.00602	0.00471	0.00374
%CV	0.16	0.34	0.24	0.46	1.09	0.42
% Mean Accuracy	86.82%	85.30%	86.77%	85.67%	84.05%	86.58%

**Statistical data on six replicate measurements*

5. Conclusions

A sensitive, selective, economic and rapid stability RP-HPLC method was successfully developed and fully validated for simultaneous quantification of telmisartan (TEL) and azelnidipine (AZL) in pharmaceutical formulations as per guidelines. The method demonstrated excellent linearity ($r^2 > 0.999$) over 4–80 ng/mL (TEL) and 0.8–16 ng/mL (AZL), LLOQ sensitivity, precision (%CV ≤15%), accuracy (85–115%), recovery (98–99%), and stability across all tested conditions. The developed method can be used as an alternative method for routine analysis in any pharmaceutical industries.

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7. Conflict of Interests

The authors declare that there is no conflict of interest.

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All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCIDs, given below:

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