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Impurity profiling of Azelnidipine and Telmisartan in Fixed Dose Combination using Gradient RP-HPLC Method

Manish Kumar[1*], Umesh Chandra[1], Arun Garg[1] , Pankaj Gupta[1]

[1,*] Department of Pharmacy, School of Medical and Allied Sciences, K.R. Mangalam University, Sohna Road, Gurugram, Haryana-122003.

E mail: manish.krmu2018@gmail.com

ABSTRACT

The objective is to develop and validate, stability indicating RP-HPLC method used for the determination and quantification of possible degradants & impurities present in Azelnidipine and Telmisartan in 8mg/40mg fixed-dose combination (FDC) tablets. The method was developed using RP-HPLC, Inertsil C-18 Column with 150×4.6 mm×5 µm, flow rate 1.5 mL/min, Injection volume10 µL, column oven temperature 40°C and run time 40.0 minutes at 254 nm using Acetonitrile and buffer as mobile phase in gradient mode. The developed method was accurate, repeatable, and detectable towards determination of Impurties present in Azelnidipine and Telmisartan combination without any unwanted interference. When evaluated on various parameters like system suitability, precision, accuracy, linearity, force degradation study, solution stability, it can be concluded that the method is efficient in separating drugs from their Impurties and can be utilized for analyzing the samples of Azelnidipine and Telmisartan.

Keywords: Azelnidipine, Telmisartan, RP-HPLC, Method Validation, Stability, Impurties.

1. INTRODUCTION:

Various regulatory guidelines like International Conference on Harmonization (ICH), European Medicines Agency (EMA) and United States Food and Drug Administration (USFDA) have specification limits for impurities present in API and Finished formulations.

Impurties means presence of any foreign matter or substance which differs from the drug substance in terms of its structure, pharmacological, and toxicological effects and any component in drug product i.e. not Active Pharmaceutical Ingredients (API) or excipient. Impurties may be organic, inorganic, process related, formulation related and residual solvents.

Description of known and unknown impurities in drug substance and drug product is called impurity profiling **[1-4].**

From the extensive literature search, it can be inferred that UV-Spectroscopy, HPLC, LC-MS/MS **[5-12]** are the only techniques used for estimating Azelnidipine and Telmisartan either alone or in combination with other drugs. To the best of our knowledge that, no report has been documented so far for determination of impurities of Azelnidipine and Telmisartan in FDC.

2. MATERIAL AND METHODS:

2.1. Chemicals and reagents:

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The working standard of Azelnidipine, Telmisartan were received as gift samples from M/s. Synokem Pharmaceutical Limited, Haridwar, Uttrakhand and FDC tablets of Azelnidipine and Telmisartan were prepared with label claim of 8 mg and 40 mg respectively. Azelnidipine impurties viz AZE-4, AZE IMP A, AZE INB Acetoacetate and Telmisartan impurties viz TEL IMP A, TEL IMP B and Other reagents including Acetonitrile, Methanol (HPLC grade) and Ammonium dihydrogen orthophosphate, Orthophosphoric acid, Hydrochloric acid, Sodium hydroxide, Hydrogen peroxide (Analytical grade) and Milli-Q water were obtained from M/s. Kimia Biosciences Limited, Gurugram, Haryana.

2.2. HPLC method development:

2.2.1. Chromatographic Conditions and instrument:

For chromatographic analysis, HPLC instrument equipped with Photodiode-Array Detection (PDA) detector was used (Agilent make, model-LC-1210 with empower software). The stationary phase is Inertsil C-18 column 150×4.6 mm×5 µm, HPLC column oven temperature was 40°C, autosampler temperature 10°C, with the flow of 1.5 mL/min. The injection volume was kept at 10 µL with a run time of 40.0 minutes and a wavelength of 254 nm was optimized. The mobile phase in gradient mode as shown in (**Table 1)**. Other instruments used in the validation like analytical balance, ultra sonicator, and pH meter, Hot air oven etc. were calibrated.

2.2.2. Preparation of Buffer solution:

Accurately weighed and transfer about 2.0 gm of Ammonium dihydrogen orthophosphate in suitable flask, add 1000 mL of HPLC grade water, mix well and dissolve. Adjust the pH with dilute Ortho-phosphoric acid to 3.0±0.05. Filter a solution through 0.45 µm millipore membrane filter and sonicate the solution to degas.

2.2.3. Preparation of Diluent:

The buffer and Acetonitrile solution was filtered and degassed in the ratio of (25:75 % v/v) used as diluent and blank solution.

2.2.4. Preparation of 0.1N sodium hydroxide solution:

Accurately weighed and transfer about 4.0 gm pellets of sodium hydroxide, poured in a 1000 mL volumetric flask containing HPLC grade water, dissolved properly and diluted with HPLC grade water to made up the final volume.

2.2.5 Gradient program: Various combinations of Acetonitrile and Buffer (Ammonium dihydrogen orthophosphate pH 3.0 ± 0.05) for mobile phase as described in **(Table 1)**

Table 1:Gradient Program.

Gradient Time	Pump A % (Buffer)	Pump B % (Acetonitrile)
0.01	80	20
5.00	80	20
20.00	30	70

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30.00	30	70
35.00	80	20
40.00	80	20

2.2.5. Preparation of stock and standard solutions:

Accurately weighed and transfer about 14.12 mg Azelnidipine and 149.70 mg Telmisartan in to a 50 mL calibrated volumetric flask. Add 20 mL of diluent sonicate to dissolve and made up volume with diluent. Labelled this solution as stock solution.

From this stock solution, accurately pipette and transferred 5 mL of aliquot into a 25 mL volumetric flask, diluted to final volume with the diluent. All the contents were mixed well to get the final concentration of 56.24 µg/mL Azelnidipine and 596.76 µg/mL of Telmisartan. The resultant solution was filtered using a 0.45 µm PVDF membrane filter and labelled this solution as the standard solution.

2.2.6. Preparation of placebo solution:

Weighed accurately about 1030 mg of the placebo (weight equivalent to 5 tablets) powder in a 100 mL volumetric flask, add 30 mL of diluent, sonicate for 15 minutes with intermittent shaking, cool to room temperature and made up the volume with diluent, mix and filter through 0.45 µm PVDF membrane filter.

2.2.7. Preparation of sample solution:

Weighed and powder 10 tablets of Azelnidipine and Telmisartan and transferred powder weight equivalent to 5 tablets in to a 100 mL volumetric flask. To this mixture around 30 mL of diluent was added and sonicated for 20 minutes and made up with diluent. Filter through a 0.45 µm PVDF filter.

2.2.8 Preparation of Impurity stock solution:

Impurity stock solution was prepared by individually weighed 1mg of each Impurties{AZE 4, Azelnidipine (AZE) IMP A, AZE INB Acetoacetate, Telmisartan (TEL) IMP A, TEL IMP B} and transferred in to 10 mL each seprate volumetric flasks. Add 5 mL diluents and sonicate for 15 minutes with intermittent shaking, cool to room temperature and made up the volume with diluent. Mix and filter through 0.45 µm PVDF membrane filter (each impurity concentration:100 µg/mL).

2.2.9 Preparation of System Suitability Solution:

2 mL standard solution was taken into 20 mL volumetric flask and add 2 mL of each Impurity stock solution and made up the volume with diluent. (Final concentration: Azelnidipine: 5.62 µg/mL, Telmisartan: 59.68 µg/mL, AZE 4: 10 µg/mL, AZE IMP A: 10 µg/mL, AZE INB Acetoacetate: 10 µg/mL, TEL IMP A : 10 µg/mL and TEL IMP B: 10 µg/mL.)

2.2.10 Preparation of Identification Solution:

Prepared individual identification solution of Azelnidipine, Telmisartan and their impurities equal to the concentration as mentioned in system suitability solution.

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3. Result and Discussion:

The developed method proposed in this study was validated for various parameters like specificity, force degradation study, precision (system, method), accuracy, linearity, stability in analytical solution (SIAS).

3.1.System Suitability:

System Suitability acceptance criteria are theoretical plates not less than 3000 and tailing factor should not be more than 2.0. Resolution between any two peaks in system suitability solution not less than 2.0 **(Figure 2)**. % RSD obtained from 6 replicate injections of system suitability solution for peak area should be NMT 5.0%. Details of system suitability parameters of Azelnidipine and Telmisartan and their impurities are mentioned in **(Table 2 and 3)**.

Table 2: System Suitability Parameters:

Compound name	RT	Area	USP Resolution	USP Plate Count	USP Tailing	Purity Angle	Purity Thershold
AZE 4	4.092	10339	-	7256	1.2	0.436	0.701
AZE IMP A	9.750	148393	34.16	85460	1.2	0.297	0.396
TELMISARTAN	14.078	389558	30.54	150791	1.1	0.100	0.272
TEL IMP B	15.764	290650	11.46	189798	1.0	0.152	0.328
AZE INB Acetoacetate	16.748	73375	6.50	190911	1.0	0.408	0.611
AZELNIDIPINE	18.054	112094	7.83	175153	0.8	0.587	0.829
TEL IMP A	25.813	222794	30.41	99260	1.0	0.153	0.339

Table 3: System Suitability with Peak Area Counts:

Compound name	AZE 4	AZE IMP A	AZE INB ACETOACETATE	AZELNIDIPINE	TEL IMP A	TEL IMP B	TELMISARTAN
	10339	148393	73375	112094	222794	290650	389558

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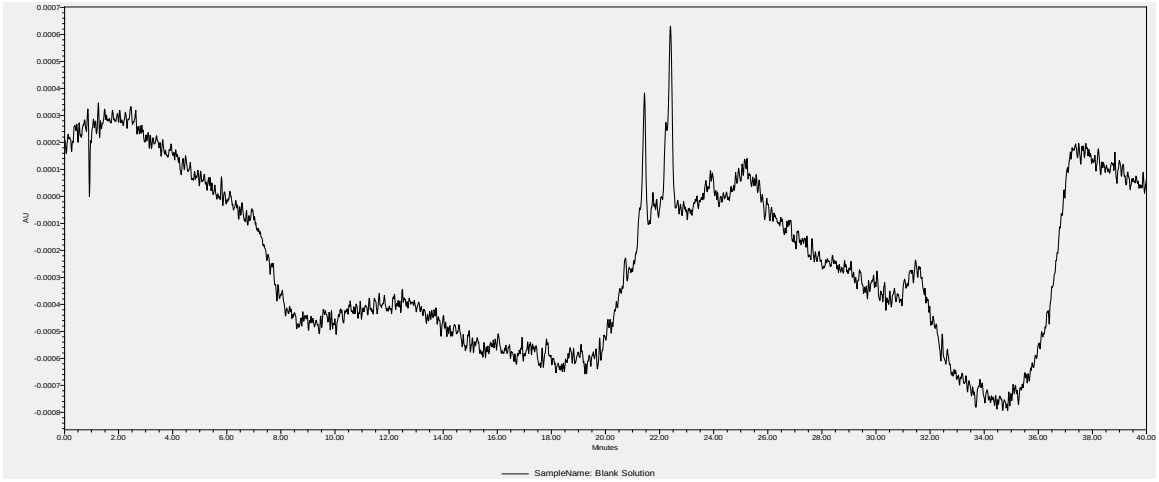
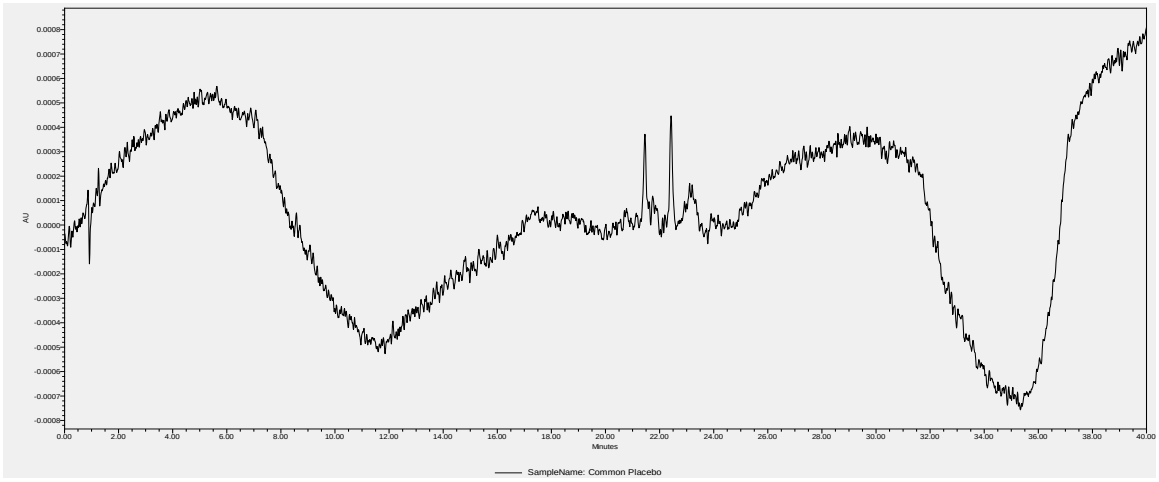
Peak Area	10370	145360	73447	111970	221924	298148	389243
	10056	143645	73023	112077	222476	298884	390248
	9998	142435	67482	110819	221394	299128	390151
	10433	140908	73949	109504	222765	299391	390175
	9841	139073	73752	109500	222327	298021	390123
Mean	10172.83	143302.33	72504.67	110994.00	222280.00	297370.33	389916.33
SD	240.209	3305.712	2481.260	1250.458	538.847	3336.306	413.877
% RSD	2.36	2.31	3.42	1.13	0.24	1.12	0.11





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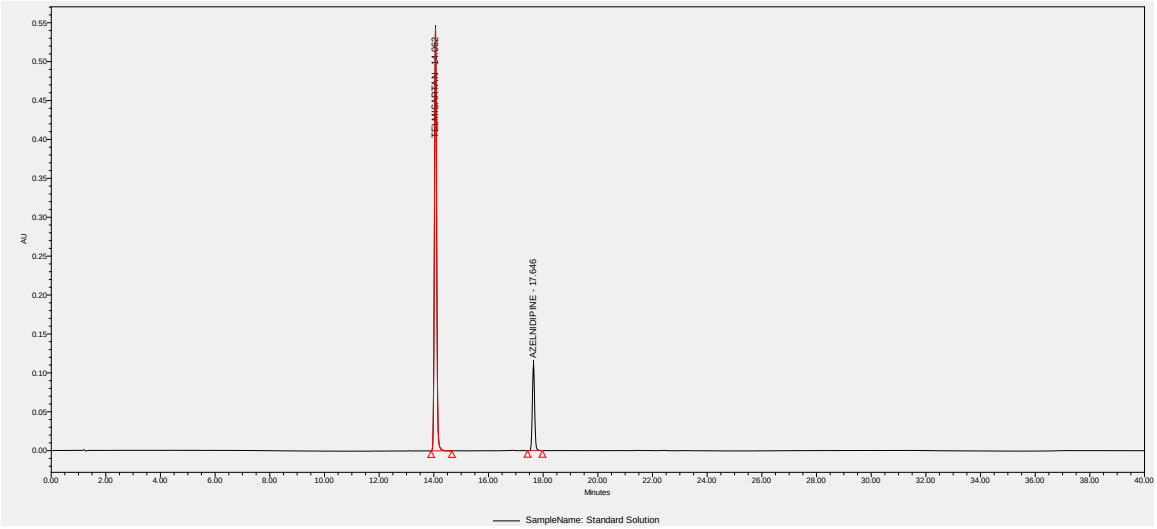

Fig. 1: The chromatogram of (A) Blank, (B) common placebo, (C) standard solution under optimized chromatographic conditions.

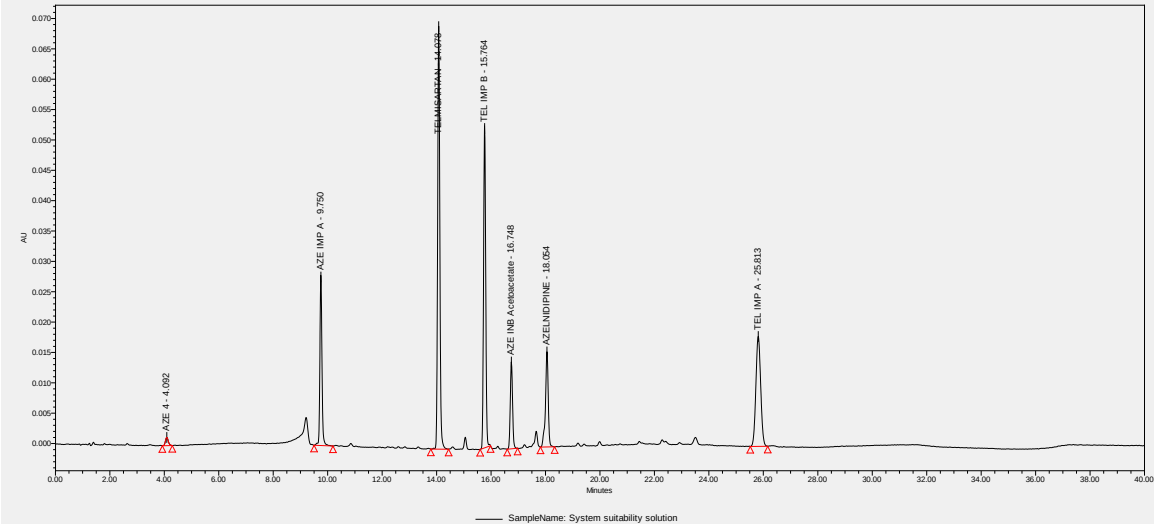

Fig. 2: The chromatogram of system suitabilty under optimized chromatographic conditions.

3.2. Specificity:

The specificity is determined by injecting single run of Blank, common placebo, Standard solution and spiked sample solution. No interference was observed at the retention time (Rt) of Azelnidipine, Telmisartan and their impurities **(Figure 1).** The purity angle of Azelnidipine and Telmisartan peak is less than the purity threshold in the chromatogram of the sample solution **(Table 2)**.

3.3. Identification of Drugs and their Impurties:

Single injection of Blank, common placebo and individual identification solution of Azelnidipine, Telmisartan and their impurities was injected. The recorded well resolved chromatograms were proved that developed method is suitable for impurity profiling of Azelnidipine and Telmisartan in FDC dosage form **(Figure 3)**.

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The retention time (Rt) of Telmisartan and Azelnidipine was 14.07 and 18.05 respectively. Azelnidipine impurities i.e. AZE 4 , Azelnidipine IMP A, Azelnidipine INB Acetoacetate were well seprated at Rt 4.09, 9.75 and 16.74 respectively, whereas Telmisartan impurities i.e. Telmisartan Imp B and Telmisartan Imp A were well seprated at 15.76 and 25.81**(Table 2)**.

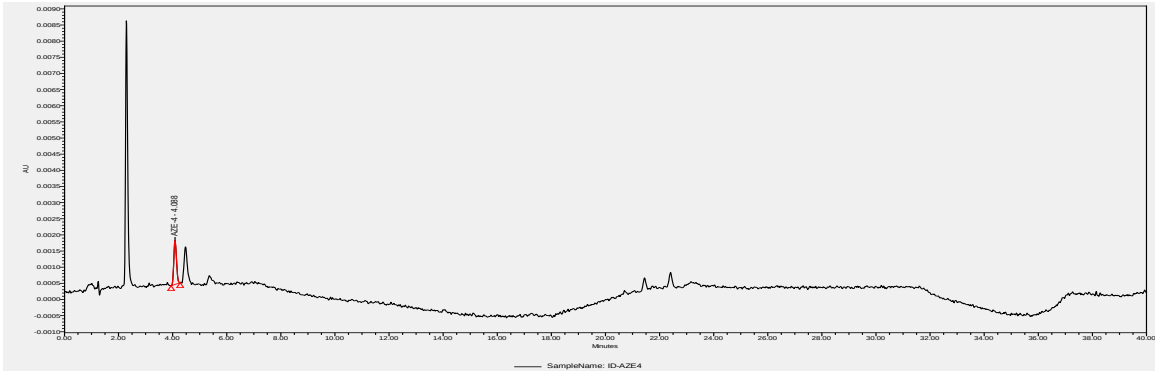
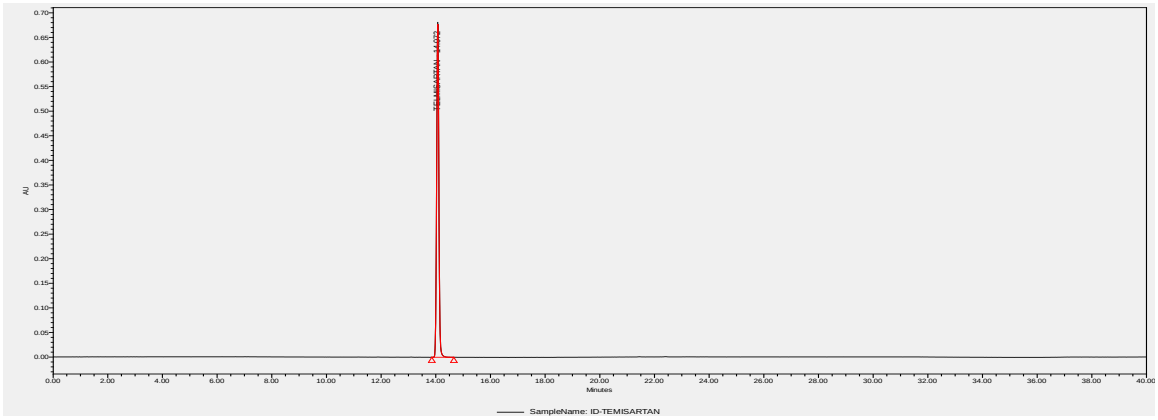
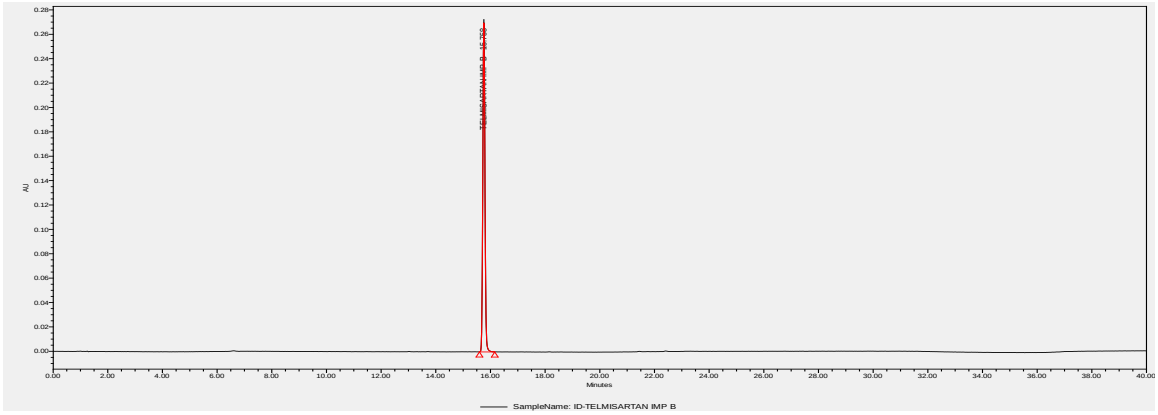


Fig 3 (A)Chromatogram of Impurity AZE 4 (Rt 4.088)



3 (B)Chromatogram of Telmisartan (Rt 14.072)



3 (C)Chromatogram of Telmisartan Impurity B (Rt 15.758)

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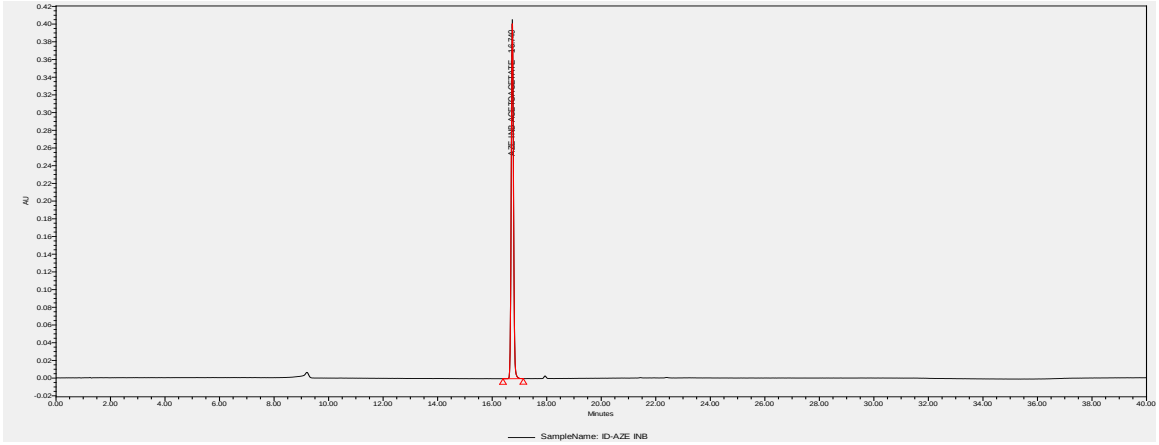
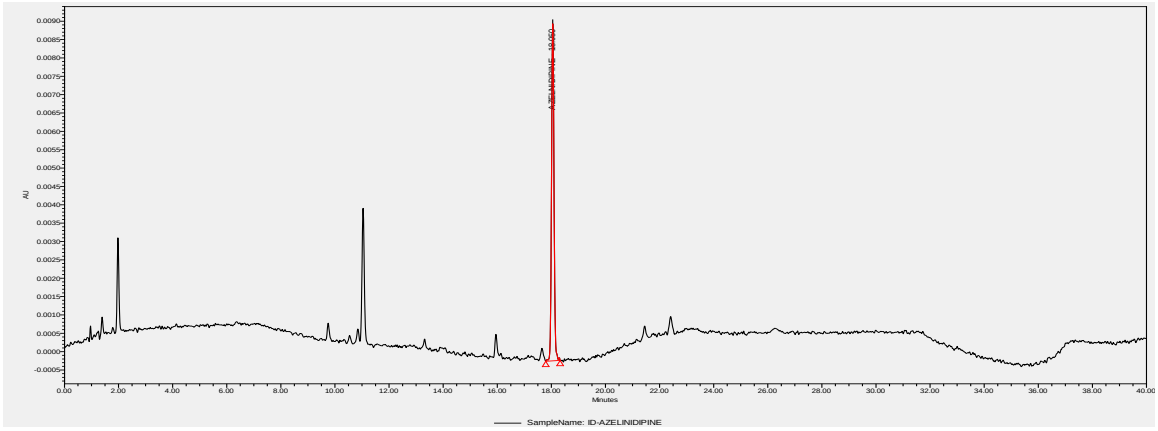
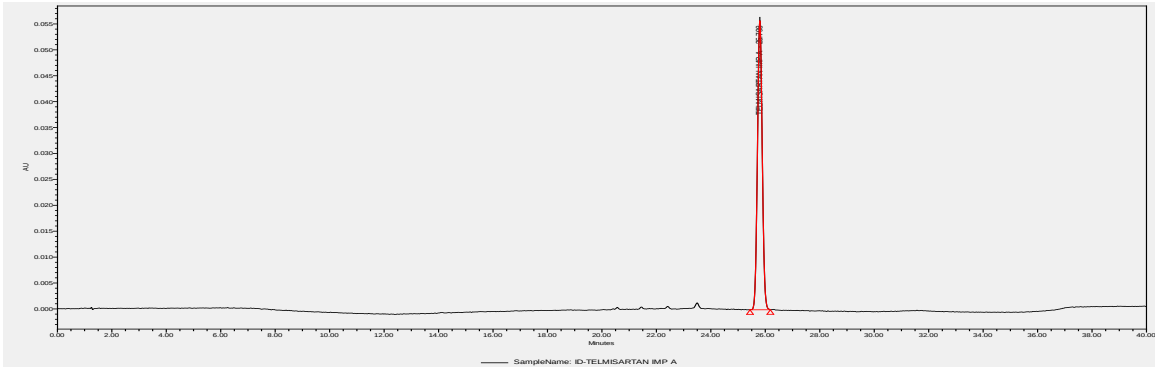


3 (D)Chromatogram of Impurity AZE INB Acetoacetate (Rt 16.740)



3 (E)Chromatogram of Azelnidipine (Rt 18.050)



3 (F)chromatogram of Telmisartan Impurity A (Rt 25.700)

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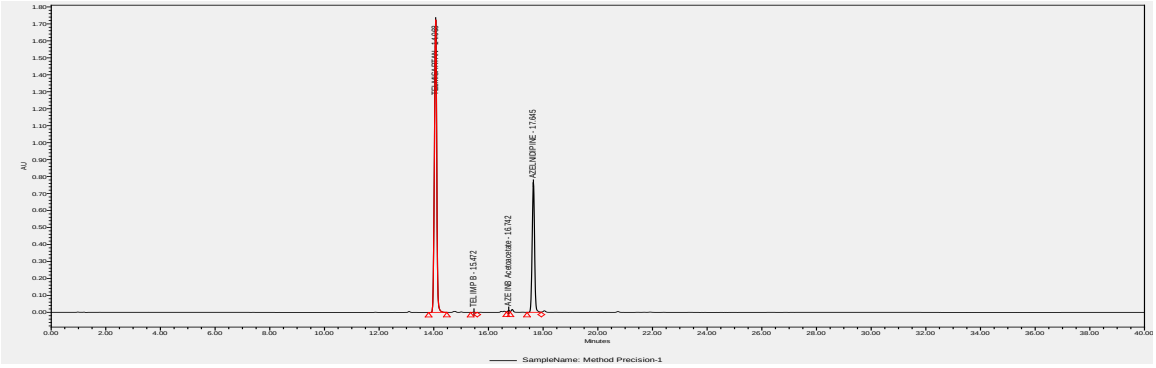

Fig. 4: The chromatogram of method precision under optimized chromatographic conditions.

3.4. Precision:

System precision was calculated by injecting six replicate injection of standard solution and % RSD was observed as 0.1% and 0.1% for Azelnidipine and Telmisartan respectively.

Method precision was performed by injecting 6 independently prepared sample solution and % RSD of peak response of six replicate injections was NMT 5%(**Table 4).**

In method precision sample peak of Telmisartan, Telmisartan Imp B, Azelnidipine INB Acetoacetate (Impurity) and Azelnidipine was found at the Rt of 14.08, 15.47, 16.74 and 17.64 respectively.

Table 4: Peak Area counts of Method Precission Sample:

Name of Drug and their Impurties	**AZE INB ACETOACETATE**	**AELNIDIPINE**	**TEL IMP B**	**TELMISARTAN**
Peak Area	50816	4669918	7407	10176936
	50727	4691509	7142	10106446
	50727	4633170	7421	10215136
	51456	4621257	7691	10127028
	53984	4597788	7972	9975701
	55287	4562495	7932	10187472
Mean	52166.17	4629356.17	7594.17	10131453.17
SD	1975.541	47022.002	327.334	86154.852
% RSD	3.79	1.02	4.31	0.85

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3.5. Accuracy (Recovery):

To demonstrate the accuracy, prepared accuracy samples by taking known quantity of working standard at level 80%, 100%, and 120% in triplicate. Mean % Recovery should not be less than 98% and not more than 102%. Result of Mean % recovery mentioned in (**Table 5**).

3.6. Linearity:

Prepare solutions corresponding to 50%, 80%,100%,120% and 150% in diluent by using stock solution.

Linearity graph shall be plotted between area response and concentration. Regression coefficient, Correlation coefficient, Slope and Y-intercept shall be calculated (**Table 5**). The calibration curve as shown in **Figure 5 (A, B, C and D)**.

Table 5: Summary of Linearity and Recovery parameters.

Name	**AZELNIDIPINE**	**TELMISARTAN**	**AZE INB ACETOACETATE**	**TEL IMP B**
Linearity range (µg/mL)	22.50-67.49	238.71-716.12	4.05-12.15	23.40-70.19
Slope	15537.1	6369.7	6832.6	197.7
Y-intercept	-65599.7	11003	240.3	-2355
Regression Cofficent (R^2)	0.99674	0.99911	0.99880	0.99835
Limit of Detection (µg/mL)	16.41	15.34	0.79	0.06
Limit of Quantitation (µg/mL)	49.73	46.49	2.38	0.19

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% Recovery	99.8-101.2	99.1-99.7	99.1-100.9	97.9-101.4

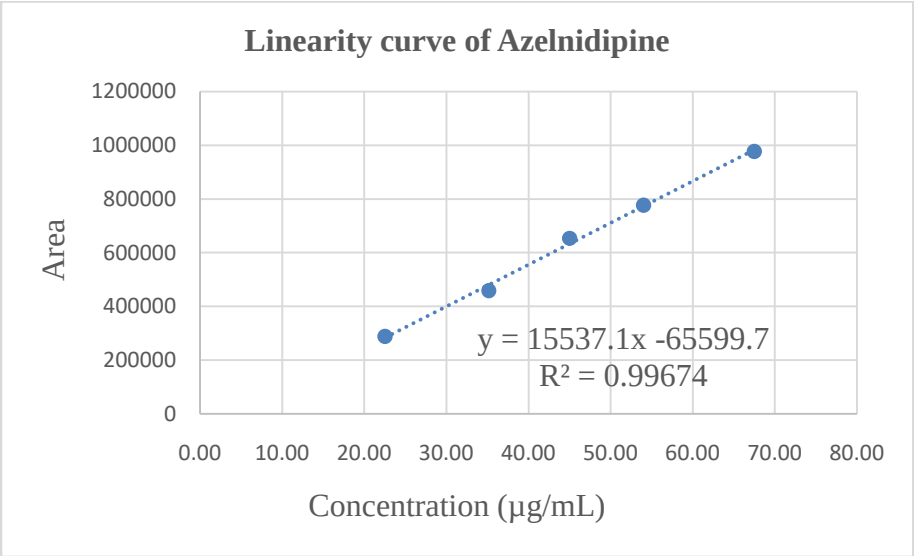

Figure 5 (A): Linearity graph of Azelnidipine

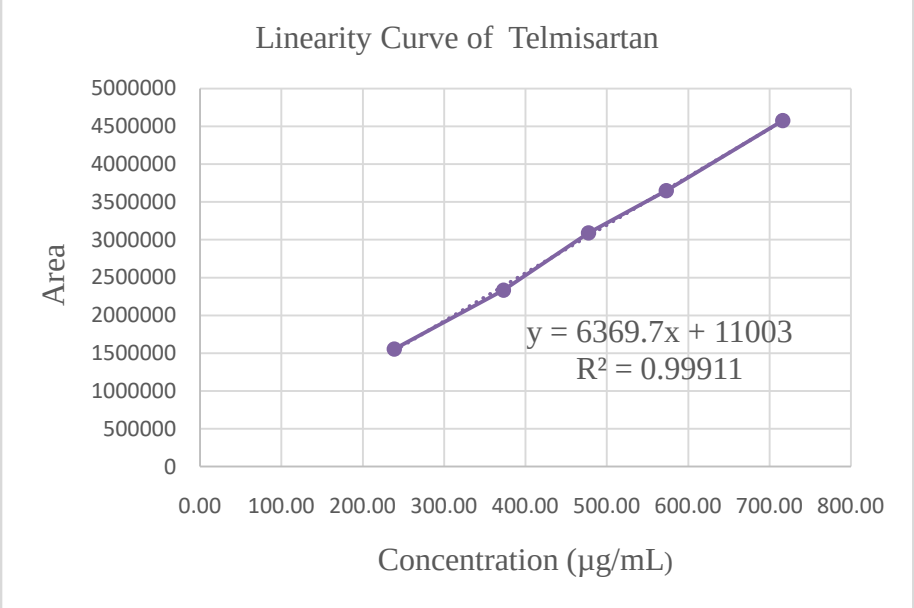

Figure 5 (B): Linearity graph of Telmisartan

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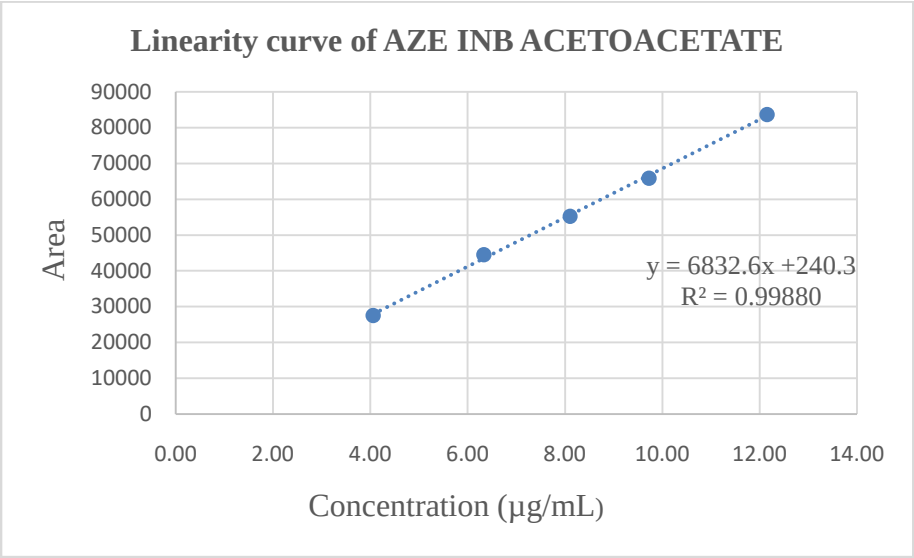

Figure 5 (C): Linearity graph of AZE INB acetoacetate

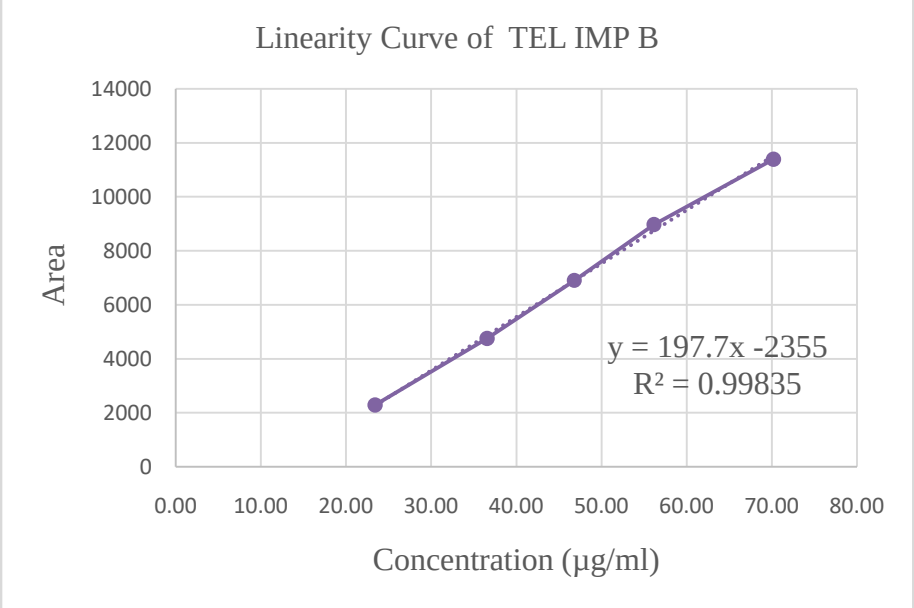

Figure 5 (D): Linearity graph of TEL IMP B

3.7. Forced Degradation Study

3.7.1. Acid hydrolysis:

For this analysis samples of drug products as well as placebo were treated with 5 mL of 5N HCl , Heat at 80°C for 1 hr. and then neutralized with 5mL of 5N NaOH (**Figure 6 A)**.

3.7.2. Base Hydrolysis:

Here the drug product and placebo were treated with 5 mL of 5N NaOH, Heat at 80°C for 1 hr. and then neutralized with 5mL of 5N HCl **(Figure 6 B)**.

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3.7.3. Oxidative degradation:

The drug product and placebo were treated 5 mL of Hydrogen peroxide (30%), Heat at 80°C for 1 hr **(Figure 6 C)**.

3.7.4. Thermal degradation:

The test, as well as placebo, were kept inside the oven for 48 hr. at 80ºC **(Figure 6 D)**.

3.7.5. Photolytic degradation:

The samples of drug product and placebo were placed under ultraviolet at 254 nm (short wavelength) in an ultraviolet region chamber for 48 hr **(Figure 6 E)**.

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3.7.6. Humidity exposure:

The sample of the drug product and placebo was exposed for 78 hr above 75% RH **(Figure 6 F)**.

Details of Force degradation parameters are mentioned in (**Table 6, 7**)

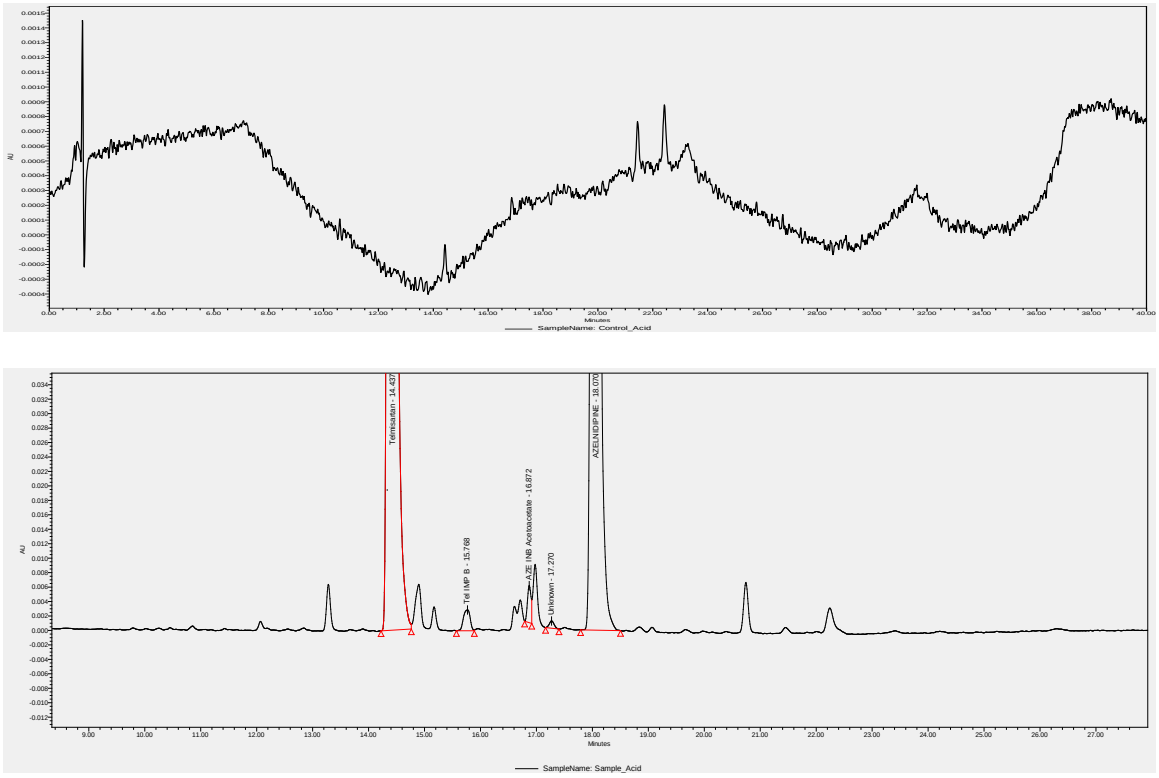

Fig. 6 (A): Acid degradation of the sample as well as placebo in acidic conditions.

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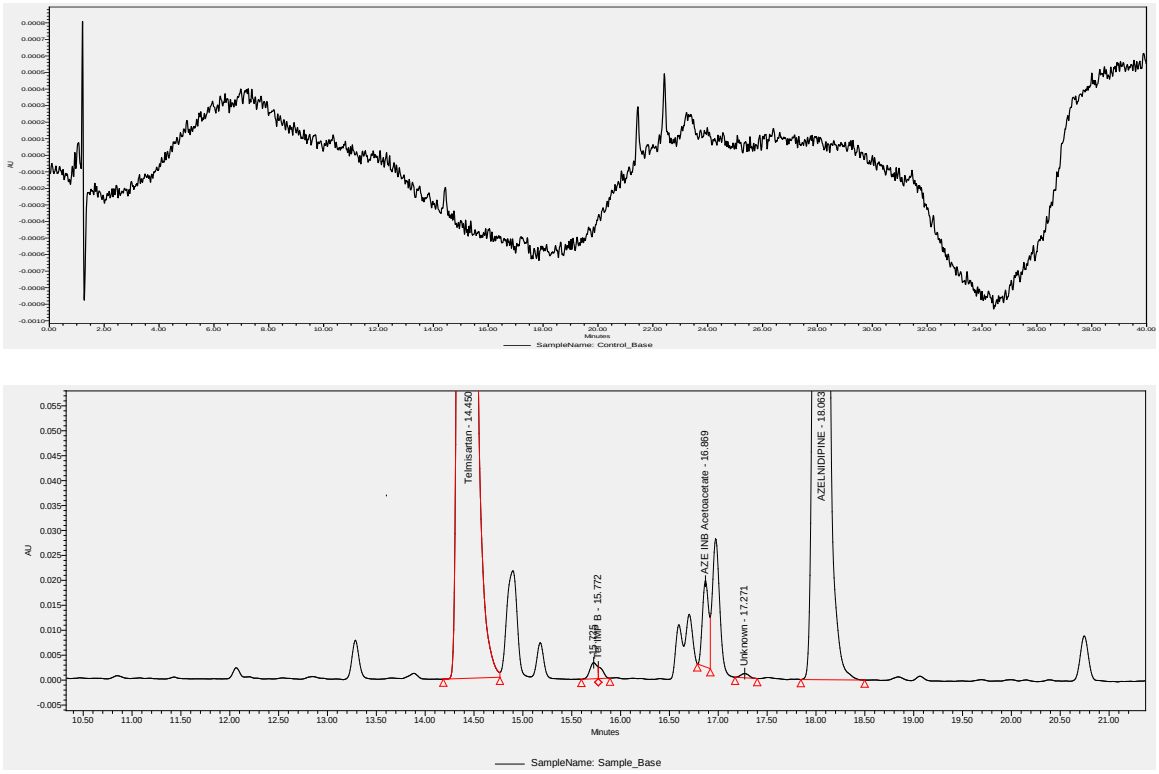

Fig. 6 (B): Force degradation of Azelnidipine and Telmisartan in basic environmental conditions.

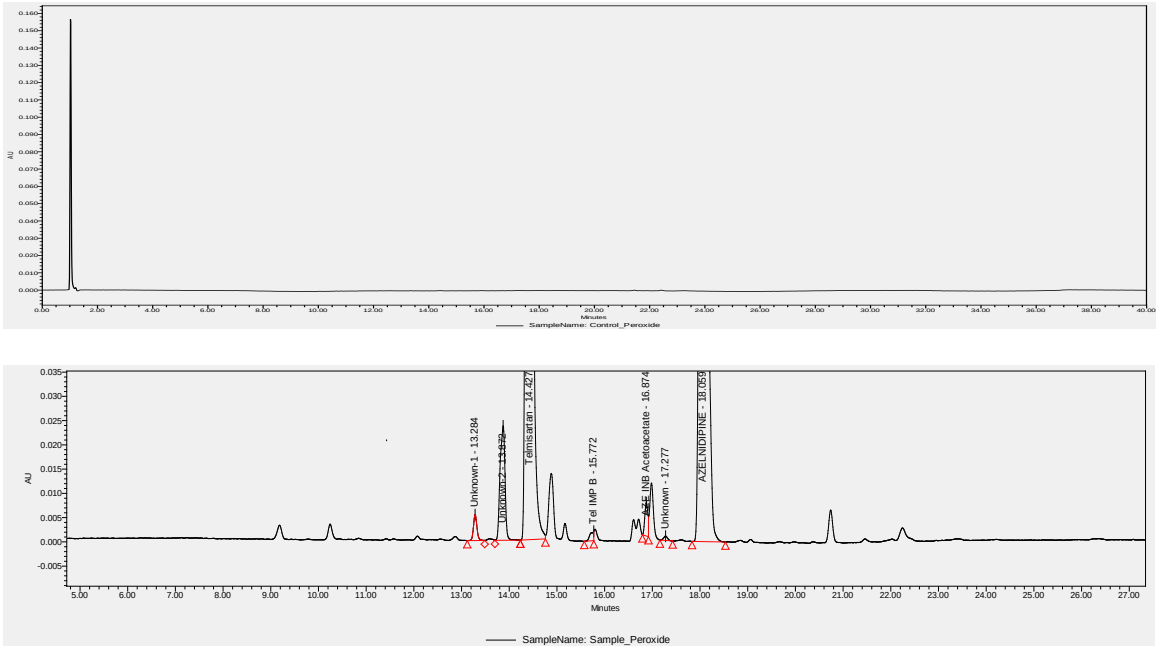

Fig. 6 (C): Chromatograms of Placebo and sample solution showing peroxide degradation pattern.

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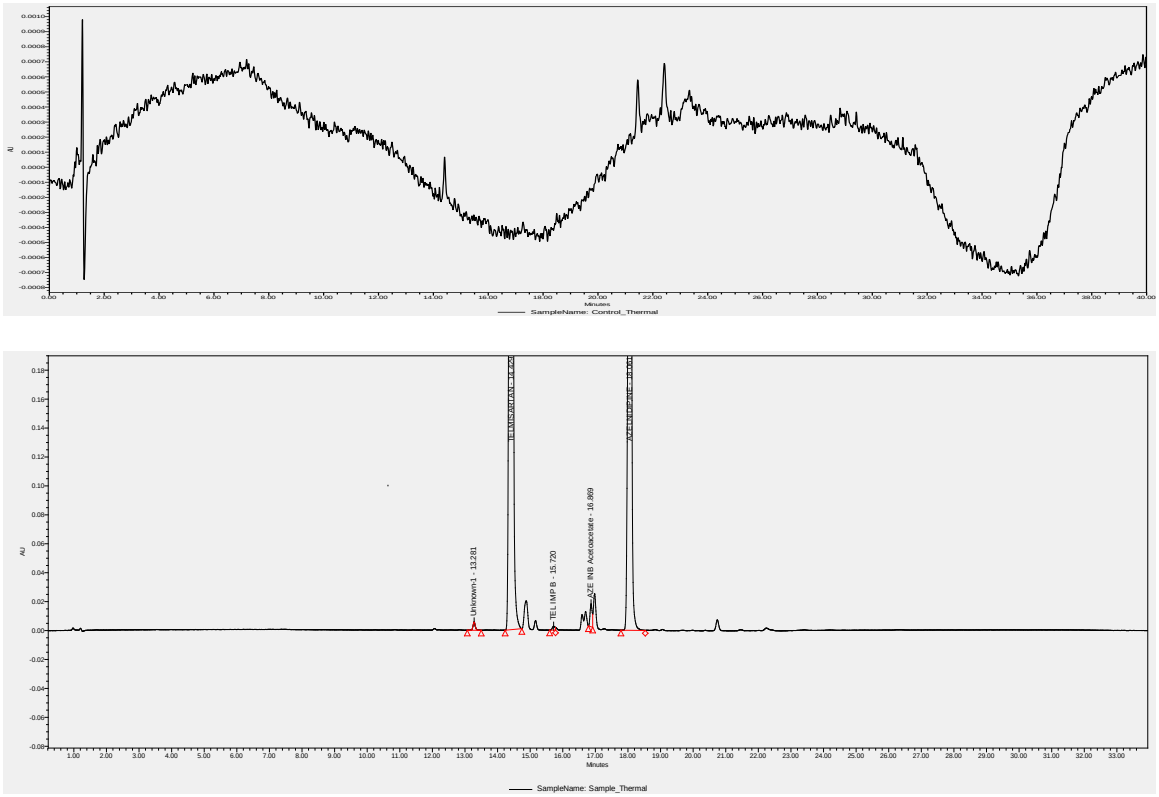

Fig. 6 (D): Chromatogram showing thermal degradation of Placebo and sample solution

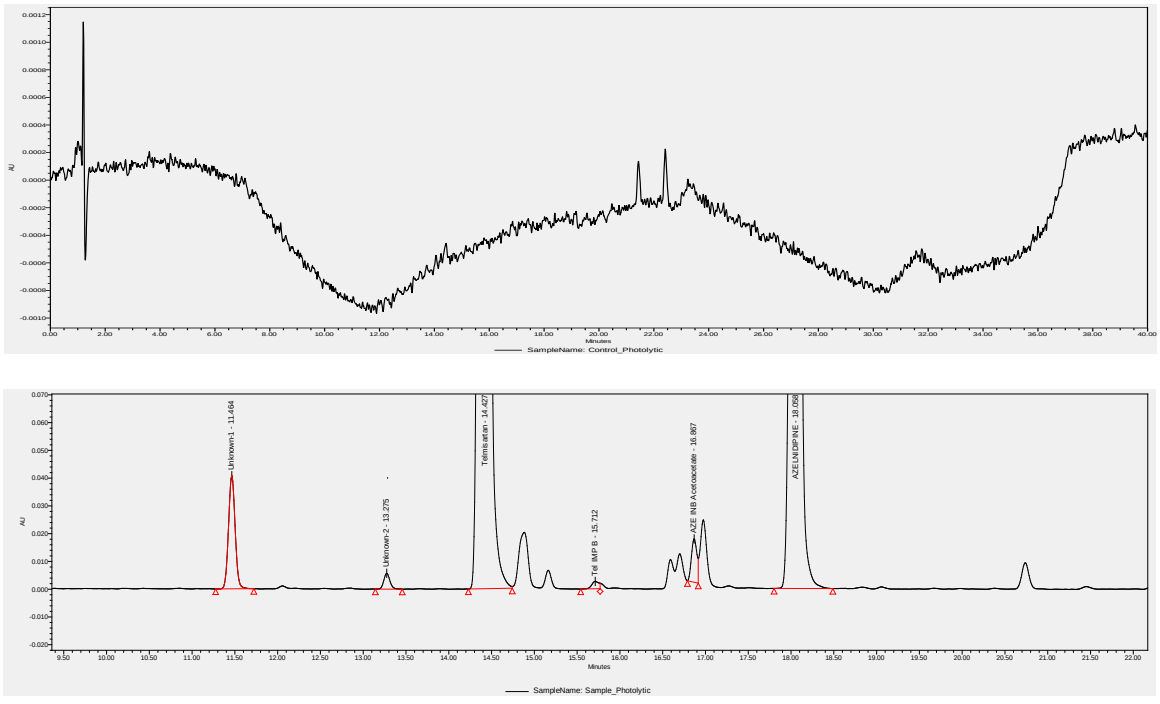

Fig. 6 (E): The chromatographic representation of photolytic degradation of Placebo and sample solution

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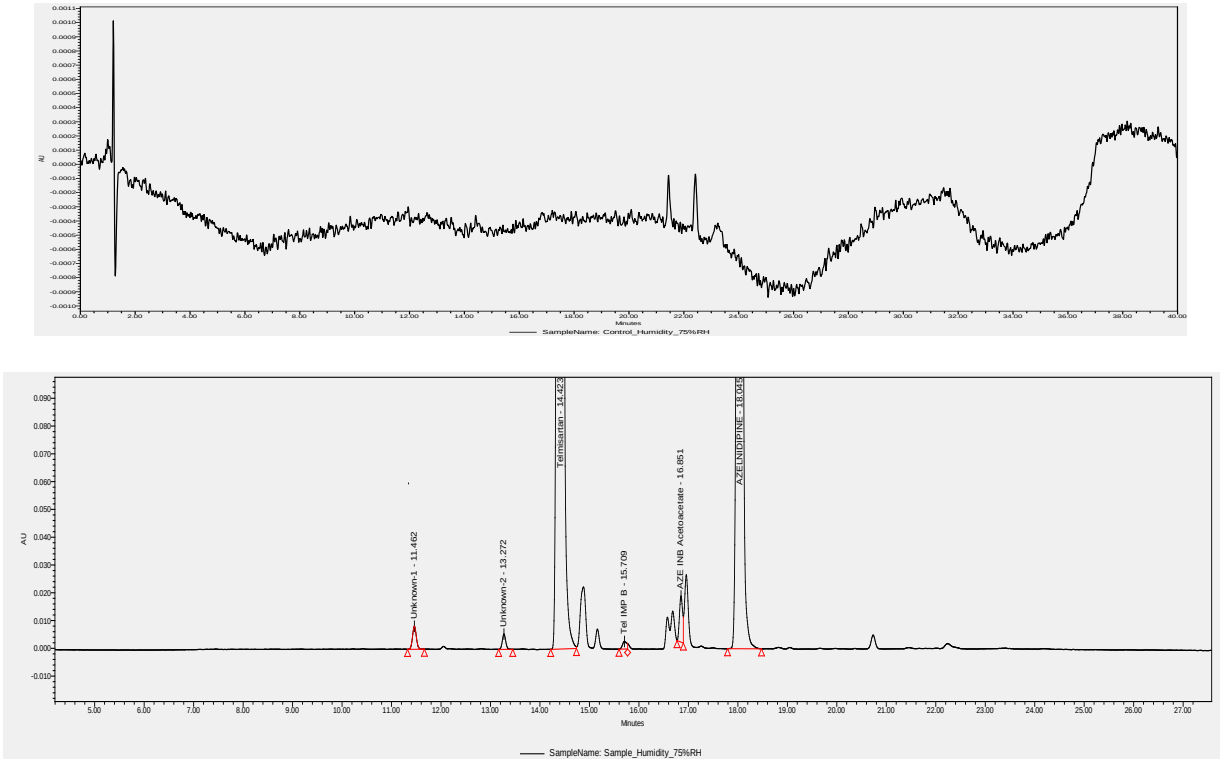

Fig. 6 (F): The chromatographic representation of humidity at 75%RH of Placebo and sample solution

Table 6: Focre degradation conditions:

Sample Name	Telmisartan (Rt 14.437)		Tel IMP B (Rt 15.768)		AZE INB Acetoacetate (Rt 16.872)		Azelnidipine (Rt 18.070)	
	Purity Angle	Purity Threshold	Purity Angle	Purity Threshold	Purity Angle	Purity Threshold	Purity Angle	Purity Threshold
Acid Degradation	3.761	3.917	6.466	90.000	1.469	90.000	0.493	1.176
Base Degradation	3.000	4.234	4.162	39.485	0.801	8.077	0.557	0.705
Peroxide Degradation	0.401	2.379	-	-	1.203	45.727	1.071	1.154
Thermal degradation	0.865	3.05	3.041	6.051	0.439	1.192	0.185	0.376
UV Degradation	2.253	3.026	5.128	6.872	0.647	1.423	0.245	0.376
Humidity degradation	2.156	3.19	4.135	5.207	0.54	1.287	0.307	0.364

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Table 7: Summary of degradation of Azelnidipine and Telmisartan

Degradation Condition	% Assay		% Degradation	
	Telmisartan	Azelnidipine	Telmisartan	Azelnidipine
Acidic hydrolysis	95.9	96.2	4.7	3.7
Basic hydrolysis	96.3	98.5	4.3	1.4
Oxidation	94.8	96.7	5.8	3.2
Thermal	96.1	99.5	4.5	0.4
Photolytic	97.2	99.5	3.4	0.4
Humidity	97.3	99.8	3.3	0.1

3.8. Solution stability (SIAS):

The stability of the analytical solution was performed by injecting sample solution (Method precision sample) initially and at specific time intervals and no significant change observed in concerning initial Rt, area, and resolution showing stability in aqueous solution over 25 hrs. and no significant change was observed in system suitability parameters.

CONFLICT OF INTEREST:

The author declares no conflict of interest for the present manuscript.

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