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Analytical Method Development and Validation of Azelnipidine and Telmisartan by RP HPLC Method

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Research Article

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ABSTRACT

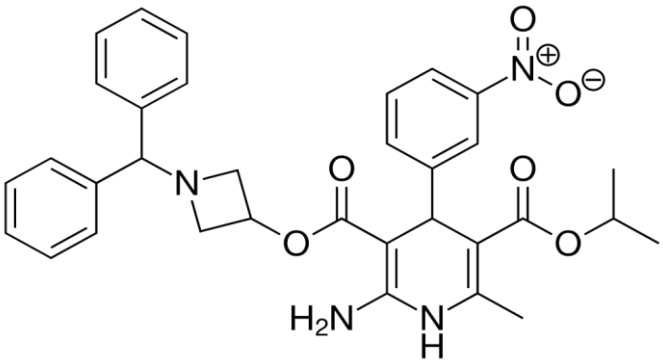
A simple, Accurate, precise method was developed for the simultaneous estimation of the Azelnipidine and Telmisartan in tablet dosage form. Chromatogram was run through standard symmetry C18 (4.6 x 150 mm, 5 mm). Mobile phase containing Buffer 0.01 N KH_2PO: Acetonitrile taken in the ratio 45:55% v/v was pumped through column at a flow rate of 1 ml/min. Optimized wavelength selected was 290 nm. Retention time of Azelnipidine and Telmisartan were found to be 2.131 min and 2.593 min. %RSD of the Azelnipidine and Telmisartan were and found to be 0.8 and 0.6 respectively. %Recovery was obtained as 99.59% and 99.94% for Azelnipidine and Telmisartan respectively. LOD, LOQ values obtained from regression equations of Azelnipidine and Telmisartan were 0.86, 2.60 and 0.09, 0.29 respectively. Regression equation of Azelnidipine is y=12533 x +10387 and y=9061 x +183.8 of Telmisartan. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

INTRODUCTION

Azelnidipine is a compound has a place with the class of natural mixes known as amino benzene sulfonamides. These are natural mixes containing a benzene sulfonamide moiety with an amine gathering joined to the benzene ring (Figure 1) Telmisartan, sold under the exchange name Norvir, is an antiretroviral prescription utilized alongside other medications to treat HIV/AIDS. This blend treatment is known as very dynamic antiretroviral treatment. Regularly a low portion is utilized with other protease inhibitors (Figure 2).

The present study was designed to develop a simple, precise, and rapid analytical RP-HPLC procedure, which can be used for the analytical method was developed for the simultaneous estimation of Azelnidipine and Telmisartan as there was only individual methods reported for both drugs. The combination of these two drugs is not official in any pharmacopoeia, hence no official method is available for the simultaneous estimation of these two drugs in their combined dosage forms. Literature survey of Azelnidipine and Telmisartan revealed several methods for detecting these drugs individually but there is only one method for their simultaneous estimation using RP-HPLC [1].

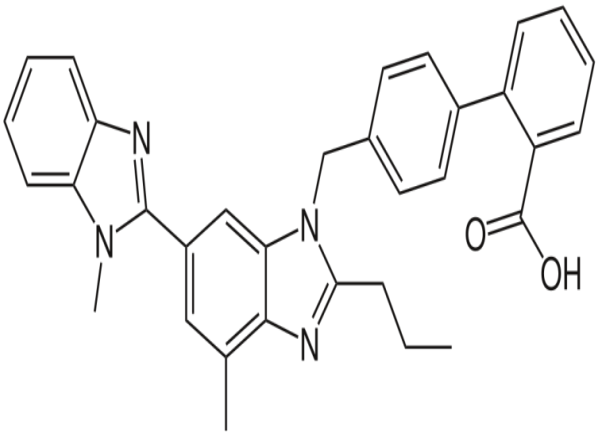
Figure 1. Chemical structure of Azelnidipine.



Objective

Following are the objectives of the present work. To develop a new stability indicating HPLC method for the simultaneous estimation of Azelnidipine and Telmisartan and to develop the validated method according to ICH guidelines. To apply the validated method for the simultaneous estimation of Azelnidipine and Telmisartan in pharmaceutical formulation [2].

Figure 2. Chemical structure of Telmisartan.



MATERIALS AND METHODS

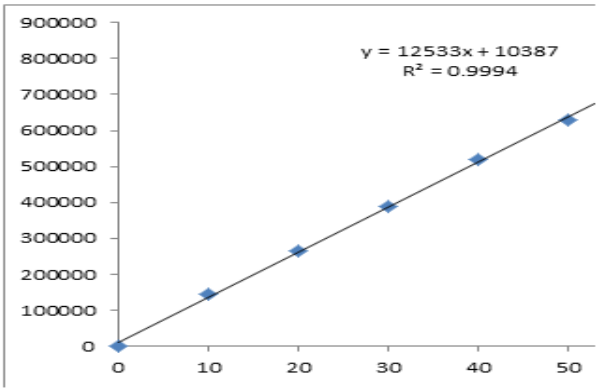
Chemicals and reagents

Azelnidipine and Telmisartan pure drugs (API) were from Rankem and combination of Azelnidipine and Telmisartan tablets (durart-r), Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer and Ortho- phosphoric acid, were purchased from Rankem, Mumbai.

Apparatus and chromatographic condition

Electronics Balance-Denver, PH meter -BVK enterprises, India, Ultrasonicator-BVK enterprises, WATERS HPLC 2695 system equipped with binary pumps, photo diode array detector and Auto sampler integrated with Empower 2 Software (Figure 3). Lab India UV double beam spectrophotometer with UV-win 5 software was used for measuring absorbances of Azelnidipine and Telmisartan [3].

Figure 3. Calibration curve of Azelnidipine and Telmisartan.



The mobile phase was prepared freshly, filtered, sonicated before use and delivered at a flow rate of 1 ml/min and the detector wave length was set at 290 nm injection volume was 10 µL. Diluent used was acaetonitrile and water taken in the ratio of 50:50.

Preparation of standard and sample solutions standard solution

Accurately weighed 20 mg of Azelnidipine, 2.5 mg of Telmisartan and transferred to 50 ml and 100 ml individual volumetric flask and 3/4th of diluents was added to these flask and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution (400 µg/ml of Azelnidipin and 50 µg/ml of Telmisartan). 1 ml from each stock solution was pipetted out and taken into a 10 ml volumetric flask and made up with diluent. (40 µg/ml of Azelnidipine and 5 µg/ml of Telmisartan)[4].

Sample stock solution

The 20 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to one tablet was transferred into a 100 ml volumetric flask. The 25 ml of diluent was added, sonicated for 50 min, further the volume made up with diluent and filtered (4000 µg/ml of Azelnidipine and 500 µg/ml of Telmisartan). It was centrifuged for 20 min. Then the supernatant was collected and filtered using 0.45 µm filters using (Millipore,

Milford, PVDF) 2 ml from sample stock solution was pipetted out and taken into a 10 ml volumetric flask and made up with diluent. (40 µg/ml of Azelnidipine and 5 µg/ml of Telmisartan) [5].

Procedure

Inject 10 µL of the standard and sample solution separately into the chromatographic system and measure the peak areas for Azelnidipine and Telmisartan and calculate the % assay value.

RESULTS AND DISCUSSION

All of the analytical validation parameters for this proposed method were determined according to ICH guidelines. Obtained validation parameters are presented in Table 1.

Table 1. Analytical validation parameters (system suitability and linearity).

Parameter	Azelnidipine	Telmisartan
Linearity (µg/ml)	10-60 µg/ml	12.25-7.5 µg/ml
Slope(m)	12533	9061
Intercept(c)	10387	183.8
Regression equation (Y=mx+c)	y=12533x + 10387	y=9061x + 183.8
Regression coefficient	0.999	0.999
System precision %RSD	0.7	0.8
Method precision %RSD	0.8	0.6
LOD	0.86	0.09
LOQ	2.6	0.29
Retention Time (min)	2.131	2.593

Linearity

The calibration curve was constructed by plotting response factor against respective concentration of Azelnidipine and Telmisartan. The plots of peak area *vs.* Respective concentration of Azelnidipine and Telmisartan were found to be linear in the range of 10-60 µg/mL and 1.25-7.5 µg/mL with coefficient of correlation (r^2) 0.999 for two drugs. The linearity of this method was evaluated by linear regression analysis. The slope and intercept calculated for Azelnidipine and Telmisartan (Table 2) [6].

Accuracy

Three levels of Accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean % Recovery was obtained as 101.14% and 99.49% for Azelnidipine and Telmisartan respectively. The obtained results are presented.

Sensitivity

The Limit of Detection (LOD) was determined as lowest concentration giving response and Limit of Quantification (LOQ) was determined as the lowest concentration analyzed with accuracy of the proposed RP-HPLC method [7]. The

Limit of Detection (LOD) and Limit of Quantification (LOQ) were found to 0.86 µg/ml and 2.60 µg/ml for Azelnidipine and 0.09 µg/ml and 0.29 µg/ml for Telmisartan. The LOD and LOQ showed that the method is sensitive for Azelnidipine and Telmisartan in Table 3.

Table 2. Accuracy table of Azelnidipine and Telmisartan.

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean % Recovery	% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean % Recovery
50%	20	19.88	99.42	99.59%	50%	2.5	2.48	99.15	99.94%
	20	19.85	99.24			2.5	2.5	99.94	
	20	19.92	99.62			2.5	2.5	99.92	
100%	40	40.26	100.64		100%	5	5.04	100.86	
	40	39.84	99.59			5	5.04	100.73	
	40	39.9	99.75			5	5.04	100.74	
150%	60	59.73	99.54		150%	7.5	7.45	99.4	
	60	59.5	99.16			7.5	7.45	99.38	
	60	59.59	99.32			7.5	7.45	99.36	

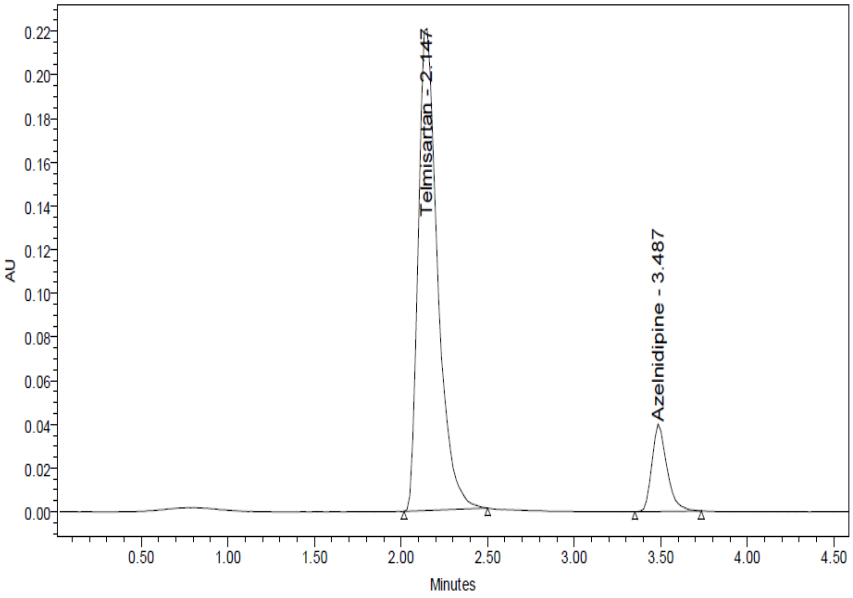
Table 3. Precision of Azelnidipine and Telmisartan.

Drug	Sample Weight (mg)	Inter-day precision		System precision		Repeatability	
		SD	%RSD	SD	%RSD	SD	%RSD
Azelnidipine	20	2916	0.7	3564.3	0.7	4195.2	0.8
Telmisartan	2.5	2916	0.7	285	0.7	244.3	0.6

System suitability test

The specificity of this method was determined by complete separation of Azelnidipine and Telmisartan as shown in Figure 4 with parameters like retention time, resolution and tailing factor. The tailing factor for peaks of Azelnidipine and Telmisartan was less than 2% and resolution was satisfactory [8]. The average retention time for Azelnidipine and Telmisartan were 2.358 min and 3.099 min respectively for five replicates. The peaks obtained for Azelnidipine and Telmisartan were sharp and have clear baseline separation. Analysis was also performed for active Azelnidipine and Telmisartan, placebo sample (All the ingredients except active Azelnidipine and Telmisartan) both at stressed and unstressed condition [9]. After analysis it was found that there is no interference of peak in the placebo and active sample. Hence the developed method was specific for the analysis of this product.

Figure 4. Optimized chromatogram of azelnidipine and telmisartan.



Precision

From a single volumetric flask of working standard solution six injections were given. A study was carried out for intermediate precision with the same analyst on the different day for six sample preparations of marketed formulations. Robustness of the method was determined by small deliberate changes in flow rate, temperature and mobile phase ratio [10]. The content of the drug was not adversely affected by these changes as evident from the low value of relative standard deviation indicating that the method was rugged and robust. The assay results of tablet dosage formulation by the proposed method are presented in Table 4.

Table 4. Sensitivity table of Azelnidipine and Telmisartan.

Molecule	LOD	LOQ
Azelnidipine	0.86	2.6
Telmisartan	0.09	0.29

Stability

In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 24 hr at room temperature. The results show that for both solutions, the retention time and peak area of Azelnidipine and Telmisartan remained almost similar (% R.S.D. less than 2.0) and no significant degradation within the indicated period, thus indicated that both solutions were stable for at least 24 hr, which was sufficient to complete the whole analytical process. Further forced degradation studies were conducted indicating the stability of the method developed. The results of the degradation studies are presented in Tables 5 and 6.

Table 5. Robustness data for Azelnidipine and Telmisartan.

S.no	Condition	%RSD of Azelnidipine	%RSD of Telmisartan
1	Flow rate (-) 0.9 ml/min	0.8	0.7
2	Flow rate (+) 1.1 ml/min	0.7	0.8
3	Mobile phase (-) 44:56 solvent	0.8	1
4	Mobile phase (+) 46:54 solvent	0.9	1

Table 6. Assay result of Pharmaceutical dosage formulation.

Drug	Label strength (mg)	% Assay
Azelnidipine	400	99.96%
Telmisartan	50	100.45%

Assay sample

The contents of Azelnidipine and Telmisartan (40 and 5 mcg each) were collected in 50 ml volumetric flask. Then 20 ml acetonitrile was added, sonicated for 25 min and made up to mark to yield 1110 µg/ml and 500 µg/ml. It was centrifuged for 20 min. Then the supernatant was collected and filtered using 0.45 µm filters using (Millipore, Milford, PVDF). 2 ml from sample stock solution was pipetted out and taken into a 10 ml volumetric flask and made up with diluent. (40 µg/ml of Azelnidipine and 5 µg/ml of Telmisartan) (Tables 7 and 8).

Table 7. Degradation studies of Azelnidipine.

Type of degradation	Azelnidipine		
	%Degraded	Purity angle	Purity threshold
Acid	3.19	0.21	0.402
Base	4.49	0.11	0.402
Peroxide	2.66	0.21	0.402
Thermal	2	0.126	0.421
Uv	2.28	0.208	0.419
Water	6.27	0.218	0.423

Table 8. Degradation studies of Telmisartan

Type of degradation	Telmisartan		
	%Degraded	Purity angle	Purity threshold
Acid	4.23	1.267	1.661
Base	2.27	1.662	2.136
Peroxide	2.88	1.501	1.862
Thermal	4.68	1.244	1.657
Uv	3.27	1.326	1.639
Water	3.24	1.465	1.742

Acid degradation sample

The 1 ml of stock s solution Azelnidipine and Telmisartan, 1 ml of 2 N Hydrochloric acid was added and refluxed for 30 mins at 60°C.The resultant solution was diluted to obtain 40 µg/ml and 5 µg/ml solution and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample. The typical chromatogram of acid degradation was given in Figure 5.

Base degradation sample

The 1 ml of stock solution Azelnidipine and Telmisartan, 1 ml of 2 N sodium hydroxide was added and refluxed for 30 mins at 60°C. The resultant solution was diluted to obtain 40 µg/ml and 5 µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample. The typical chromatogram of acid degradation was given in Figure 6.

Figure 5. Acid Chromatogram of Azelnidipine and Telmisartan.

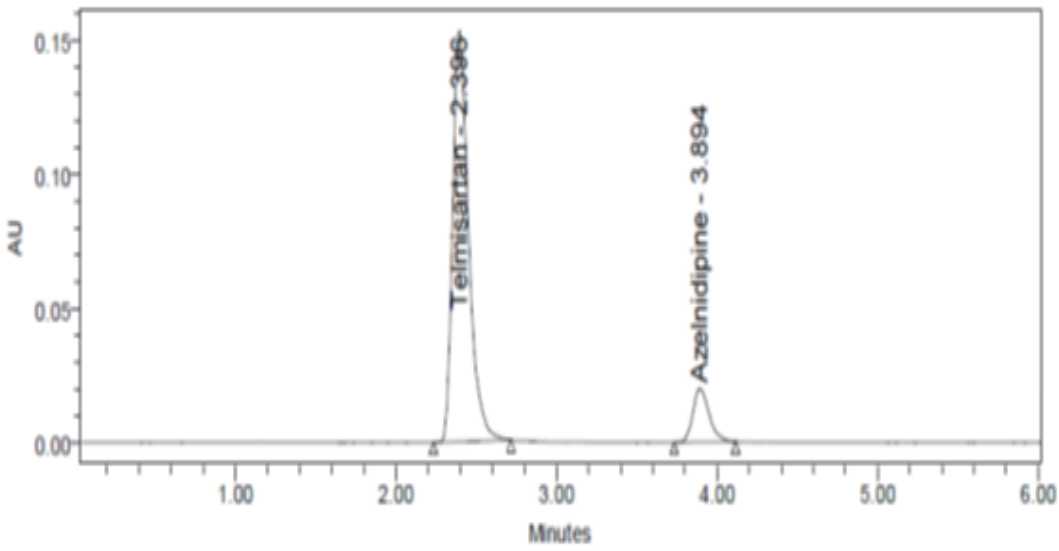
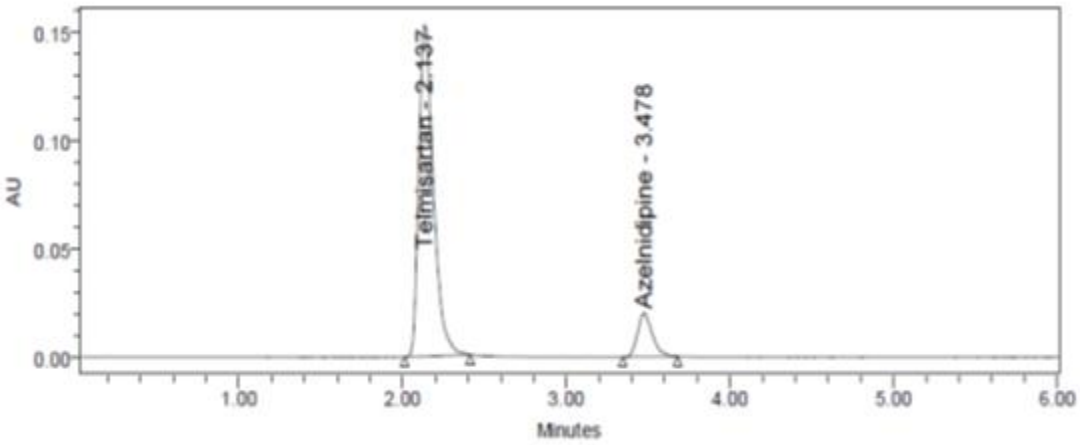


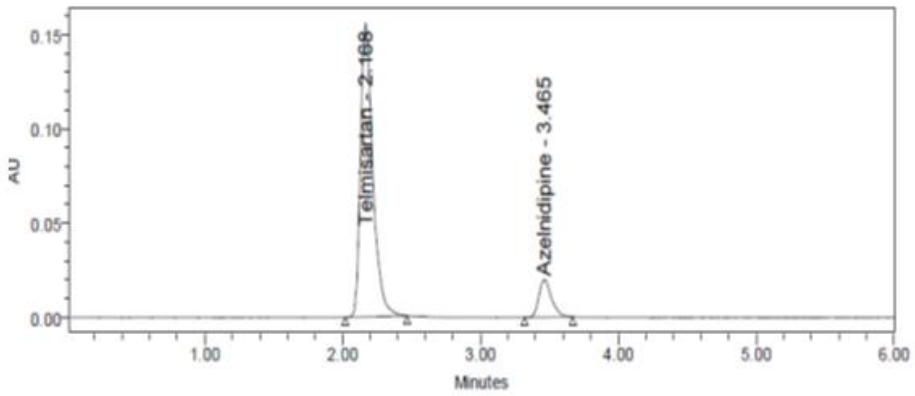
Figure 6. Base Chromatogram of working sample solution.



Oxidation degradation sample

The 1 ml of stock solution of Azelnidipine and Telmisartan, 1 ml of 20% hydrogen peroxide (H_2O_2) was added separately [11]. The solutions were kept for 30 min at 60°C. For HPLC study, the resultant solution was diluted to obtain 40 µg/ml and 5 µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample. The typical chromatogram of oxidative degradation was given in Figure 7.

Figure 7. Peroxide chromatogram of Azelnidipine and Telmisartan.



Neutral degradation studies

Stress testing under neutral conditions was studied by refluxing the drug in water for 1 hours at a temperature of 60ºC. For HPLC study, the resultant solution was diluted to 40 µg/ml and 5 µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample. The typical chromatogram of oxidative degradation was given in Figure 8.

Figure 8. Thermal chromatogram of Azelnidipine and Telmisartan.

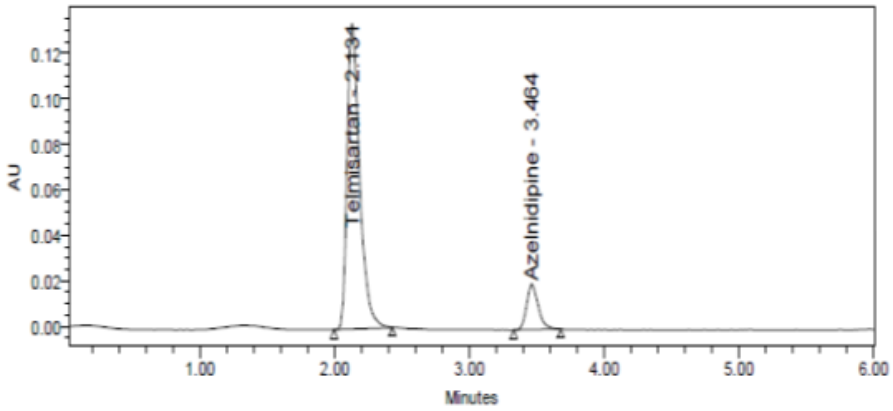
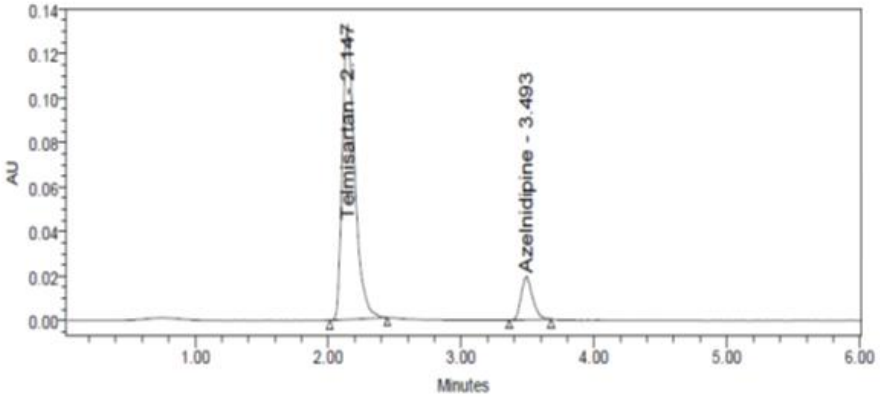


Photo stability studies

The photochemical stability of the drug was also studied by exposing the 250 µg/ml and 625 µg/ml solution to UV Light by keeping the beaker in UV Chamber for 1 day or 200 Watt hours/m^2 in photo stability chamber [12]. For HPLC study, the resultant solution was diluted to obtain 40 µg/ml and 5 µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample. The typical chromatogram of thermal degradation was given in Figure 9.

Figure 9. UV degradation chromatogram of Azelnidipine and Telmisartan.



CONCLUSION

A suitable chromatographic method was developed through optimization by changing various parameters such as the mobile phase, injection volume, flow rate etc. In the present method a standard symmetry C18 (4.6 × 150 mm, 5 µm) column has been used For Azelnidipine and Telmisartan respectively. Mobile phase used was Buffer 0.01 N KH_2PO_4: Acetonitrile (45:55% v/v) for Azelnidipine and Telmisartan respectively, Retention of Azelnidipine and Telmisartan has more dependence on the mobile phase. The separation of the two peaks was also dependent on the buffer and the percentage of mobile phases. Darunavr and Telmisartan were eluted at acceptable retention times and got good resolution. Several assay methods has been developed for the determination of Azelnidipine and Telmisartan in pharmaceutical dosage forms and in biological fluids but this method is most economic and accurate so this method is very useful for the determination of Azelnidipine and Telmisartan in bulk and pharmaceutical dosage forms. This method was validated as per ICH-Q_2 (R1) guidelines and met the regulatory

requirements for selectivity, accuracy and stability. Considering the obtained data, it was possible to affirm that the proposed method was fast, simple and suitable for the accurate determination of Azelnidipine and Telmisartan

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