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Research Article

Stability Indicating RP-HPLC Method Development and Validation for simultaneous estimation of Azelnidipine and Telmisartan in Bulk and Pharmaceutical Dosage Form

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ABSTRACT

A simple, Accurate, Precise method was developed for the simultaneous estimation of Azelnidipine and Telmisartan in bulk and pharmaceutical dosage form by RP- HPLC technique. Chromatogram was run through Std Denali C18 150mm x 4.6 mm, 5µ. Mobile phase 0.1% OPA: Acetonitrile in the ratio of 60:40 and flow rate were maintained at 1.0 ml/min. Buffer used in this method was 0.1%OPA. Column temperature was set to 30^{o}C.Optimised wavelength selected was 242.0nm. Retention time of Azelnidipine and Telmisartan were found to be 2.116 min & 3.188mins. %RSD of the Azelnidipine and Telmisartan System were found to be 1.6% and 1.0% respectively. %Recovery was obtained as 100.15% and 100.20% for Azelnidipine and Telmisartan respectively. LOD and LOQ values obtained from regression equations of Azelnidipine and Telmisartan were 0.04,0.13 and 0.38,1.14 respectively. Regression equation of Azelnidipine and Telmisartan is Y= 36260x+2218 and Y=30420x+8163.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 tests in industries.

Key Words: Azelnidipine, Telmisartan, RP-HPLC

INTRODUCTION

Azelnidipine is a dihydro calcium channel blocker. It has a gradual onset of action and produces a long-lasting decrease in blood pressure, with only a small increase in heart rate, unlike some other calcium channel blockers. It is currently being studied for post-ischemic stroke management. Telmisartan is an angiotensin 2 receptor antagonist (ARBs). Recent studies suggest that it have PPAR gamma agonistic properties that could potentially confer beneficial metabolic effects. Both drugs are used in the management of Hypertension. Literature survey revealed that there were few analytical methods reported for Azelnidipine & Telmisartan in RP-HPLC. However, an extensive literature search didn't reveal any estimation

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method for Azelnidipine & telmisartan in API & Pharmaceutical dosage form. Therefore, an attempt has been made to develop and validate simple, precise, accurate economical RP-HPLC method as per ICH guidelines for the estimation of Azelnidipine& telmisartan in Bulk and Pharmaceutical dosage form.

MATERIALS AND METHODS

Chemicals and Reagents: Acetonitrile (HPLC grade), orthophosphoric acid (HPLC grade), water (HPLC grade) was purchased from Mark (India) Ltd, Worli, Mumbai, India. All active pharmaceutical ingredients (APIs) of Azelnidipine and Telmisartan reference standards were procured from Spectrum Pharma labs, Hyderabad, India.

Instruments and Chromatographic Conditions Electronics Balance-Denver, P^Hmeter -BVK enterprises, India, Ultrasonicator-BVK enterprises, WATERS HPLC 2695 system equipped with quaternary pumps, photo diode array detector and Auto sampler integrated with Empower 2 Software. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz cells integrated with UV-win 6 Software was used for measuring absorbances of Azelnidipine and Telmisartan solutions. The mobile phase used was 0.1%OPA: Acetonitrile (60:40) at a flow rate of 1.0ml/min, samples were analyzed at 242 nm detector wavelength and at an injection volume of 10µL using Denali C18(150 x 4.6mm,5µm) with run time of 6mins.

Methods

Diluent: Based up on the solubility of the drugs, diluent was selected, Acetonitrile and Water taken in the ratio of 50:50.

Buffer: 0.1%OPA Buffer (1ml of Ortho phosphoric acid was diluted to1000 ml with HPLC grade water.)

Standard stock solution Preparation: Accurately weighed 20mg of Telmisartan, 4mg of azelnidipine transferred to 50ml volumetric flasks and 3/4th of diluents was added to these flasks and sonicated for 10 mins. Flasks were made up with diluents and labeled as standard stock solution.(400µg/ml of telmisartan and 80µg/ml of azelnidipine).

Standard working solution Preparation: 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (40µg/ml of telmisartan and 8µg/ml of azelnidipine).

Sample stock solution Preparation: 10 tablets were accurately weighed and the average weight equivalent to 1 tablet was transferred into a 100 ml volumetric flask, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters. (400µg/ml of telmisartan and 80µg/ml of azelnidipine).

Sample working solution preparation:1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (40µg/ml of telmisartan and 8µg/ml of azelnidipine).

Method Validation

As per ICH guidelines the method was validated and the parameters like Linearity, Specificity, Accuracy, Precision, Limit of Detection (LOD) and Limit of Quantitation (LOQ) were assessed.

Specificity: Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

Linearity: Stock solutions of Azelnidipine and Telmisartan is taken into 6 different volumetric flasks and diluted to 10ml with diluents. Linearity solutions are prepared such that 0.25, 0.5, 0.75, 1, 1.25, 1.5ml.

Accuracy: Preparation of Standard stock solutions: Accurately weighed 20mg of Telmisartan, 4mg of Azelnidipine transferred to two separately 50ml and volumetric flasks, 3/4 th of diluents was added and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution. (400µg/ml of telmisartan and 80µg/ml of azelnidipine).

Preparation of 50% Spiked Solution: 0.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 100% Spiked Solution: 1.0ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 150% Spiked Solution: 1.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Robustness: Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines.
Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much effected and all the parameters were passed. %RSD was within thelimit.

LOD sample Preparation: 0.25m each from two Standard stock solutions was pipetted out and transferred to two 10ml volumetric flasks and made up with diluents. From the above solution 0.1ml each of Telmisartan Azelnidipine solutions, were transferred to 10ml volumetric flasks and made up with the samediluents

LOQ sample Preparation: 0.25ml each from two Standard stock solution was pipetted out and transferred to two 10ml volumetric flasks and made up with diluents. From the above solution 0.3ml each of Telmisartan and Azelnidipine solutions, were transferred to 10ml volumetric flasks and made up with the samediluent.

System suitability parameters: The system suitability parameters were determined by preparing standard solutions of Telmisartan (40ppm) and Azelnidipine (8ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined. The % RSD for the area of six standard injections results should not be more than 2%.

Assay: Assay of the marketed formulation was carried out by injecting sample corresponding to equivalent weight into HPLC system

RESULTS & DISCUSSIONS

Optimization of Chromatographic Conditions: To develop and establish a suitable RP-HPLC method for estimation of Azelnidipine and Telmisartan in bulk and tablet dosage forms, different preliminary tests were performed and different chromatographic conditions were tested and optimized chromatographic conditions were developed which were given in Table-1.The final analysis was performed by using 0.1% Ortho phosphoric acid: Acetonitrile (60:40) at a flow rate of 1.0ml/min, samples were analyzed at 242 nm detector wave length and at an injection volume of 10µL using Std Denali C18 4.6 x 150mm, 5µmwith run time of 6min. The proposed method was optimized to give sharp peak with good resolution and minimum tailing effect for Azelnidipine and Telmisartan, the optimized chromatogram was obtained as shown in (Figure- 3).

Validation: Linearity was established for Telmisartan (10-60µg/ml) and Azelnidipine (2-12µg/ml) at six different concentrations each were injected in a duplicates and average areas were determined and linearity equations were obtained as y = 36260x +2218 for Azelnidipine and y=30240x+8163 for Telmisartan. correlation coefficient (R^2) was determined as 0.999. The Linearity calibration curves were plotted as shown in (Figure-4,5). Retention times of Telmisartan and Azelnidipine were 3.188 and 2.116min where no interfering peaks in blank and placebo were found in this method. So, this method holds its specificity. Three levels of Accuracy samples 50%, 100%, 150% were prepared and triplicates of injections were given for each level of accuracy and mean% Recovery was obtained as 100.15% and 100.20% for azelnidipine and telmisartan. % RSD was calculated from the corresponding peaks obtained by injecting six times a known concentration of Abiraterone was obtained as 0.20% and the % RSD for Repeatability was obtained as 0.6% and 1.2% for azelnidipine and telmisartan. Low % RSD values indicates that the method developed was precise as shown in table. The LOD and LOQ values were evaluated based on Relative standard deviation of response and slope of the calibration curve of the two drugs. The detection limit value was obtained as0.04, 0.38 for azelnidipine and telmisartan. Quantitation limit was found to be 0.13 &1.14 for azelnidipine and telmisartan as given in (Table-4). Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus (55:45), mobile phase plus (65:35), temperature minus (25°C) and temperature plus (35°C) were maintained and samples were injected in duplicate manner (Table-5). System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit (Table -6). Azelnidipine and Telmisartan pure drug(API) was obtained from Spectrum Pharma research solutions, combination dosage form Telma Az label claim(40/8 mg).Assay was performed with the above formulation. Average % Assay obtained for Telmisartan and Azelnidipine was 100.22% and 100.13% and the chromatogram of standard drugs and pharmaceutical dosage forms were shown in (Figure-6,7) respectively.

Degradation Studies: Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation (Table 8).

CONCLUSION

Chromatographic conditions used are stationary phase Std Denali C18 (150mmx 4.6mm 5µ) Mobile phase 0.1 % OPA: Acetonitrile in the ratio of 60:40 and flow rate were maintained at 1.0ml/min, detection wave length was 242 nm, column temperature was set to 30ºC. Conditions were finalized as optimized method. System suitability parameters were studied by injecting the standard six times and results were well under the acceptance criteria. Linearity study was carried out between 25% to150 % levels, R^2 value was found to be for Telmisartan 0.9998 and Azelnidipine0.9997. Precision was found to be 1.6 and 1.0 for Azelnidipine and Telmisartan. LOD and LOQ are0.04,0.38 and 0.13,1.14 for Azelnidipine and Telmisartan respectively. By using above method assay of marketed formulation was carried out 100.22% and 100.13% for Telmisartan respectively. Degradation studies of Azelnidipine and Telmisartan were done, in all condition's purity threshold was more than purity angle and within the acceptable range. Full length method was not performed; if it is done this method can be used for routine analysis of Azelnidipine and Telmisartan. Retention times are 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.

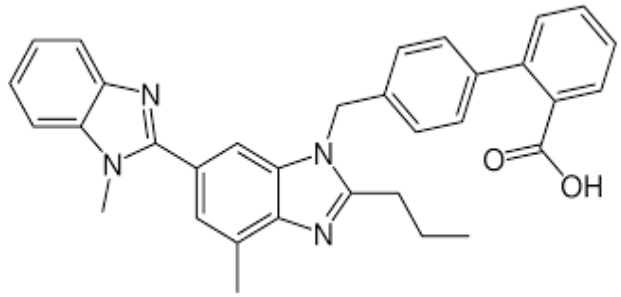

Figure-1: Chemical Structure of Telmisartan

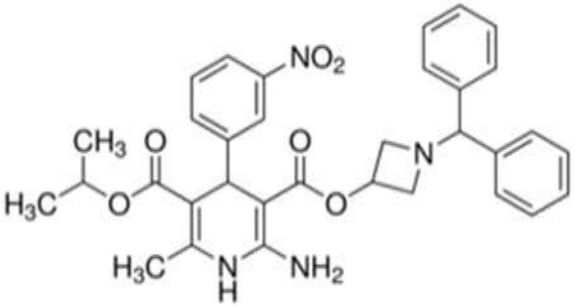

Figure-2: Chemical Structure of Azelnidipine

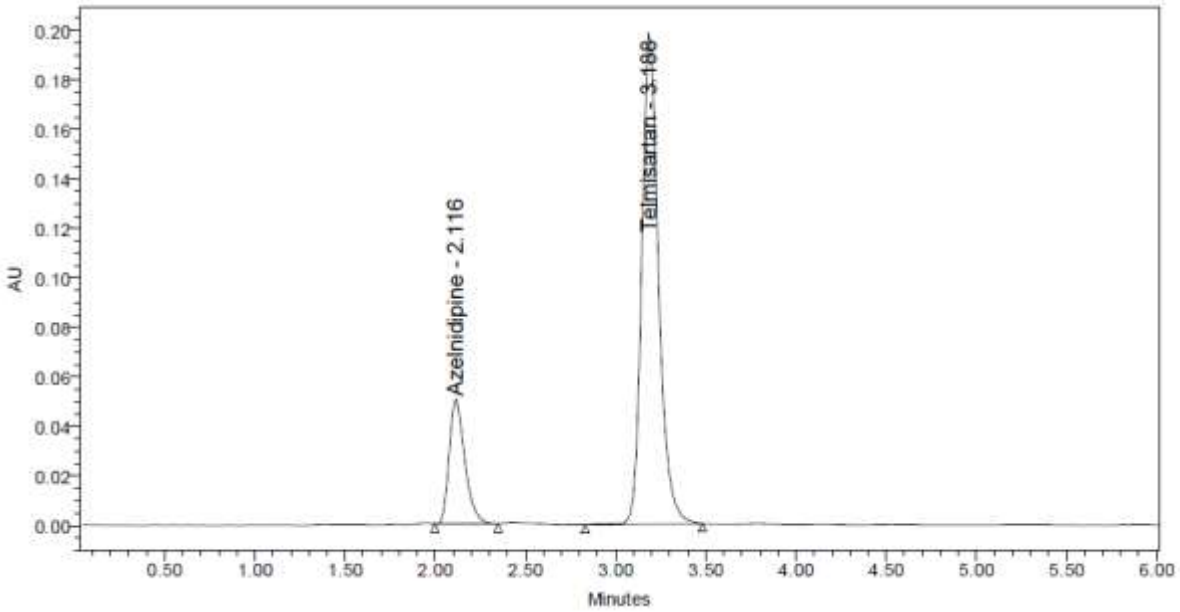

Figure-3: Optimized Chromatogram of Azelnidipine and Telmisartan

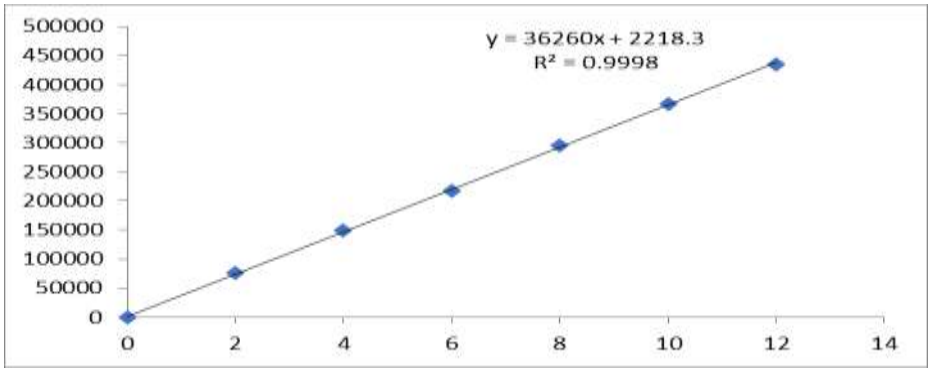

Figure -4: Linearity curve of Azelnidipine

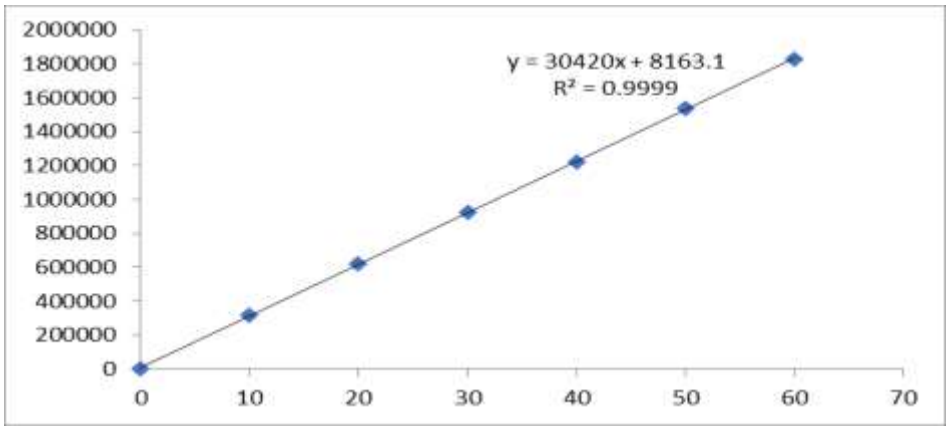

Figure -5: Linearity curve of Telmisartan

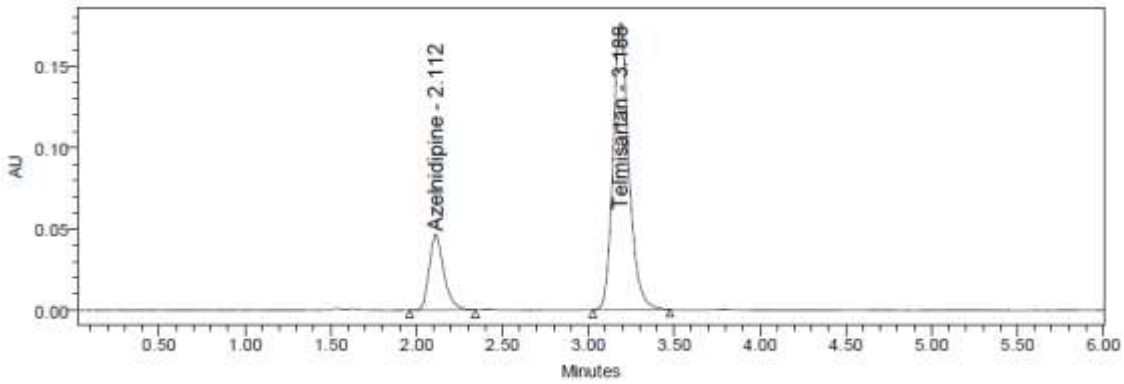

Figure -6: Standard Chromatogram of Azelnidipine and Telmisartan

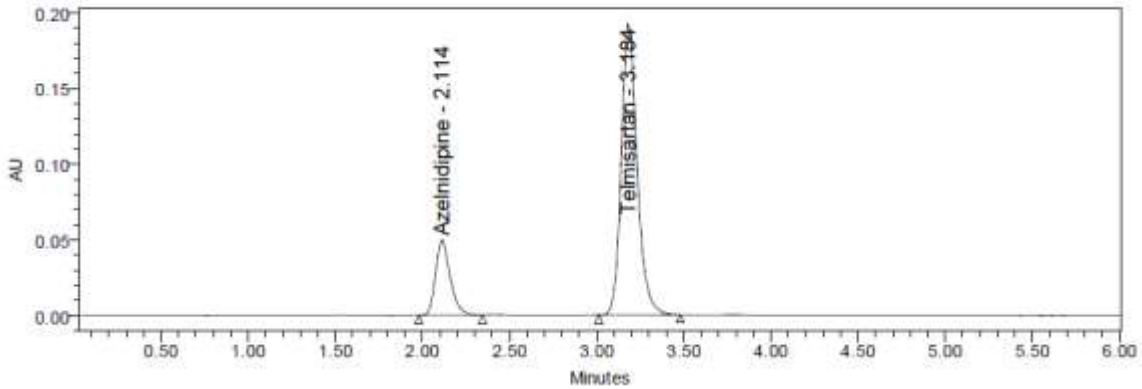

Figure -7: Sample Chromatogram of Azelnidipine and Telmisartan

Table 1: Optimized Chromatographic Conditions

Parameter	Conditions
RP-HPLC	WATERS HPLC SYSTEM equipped with quaternary pumps with PDA detector
Mobile Phase	0.1% OPA :Acetonitrile (60:40)
Flow rate	1.0ml/min
Column	Denali C18(4.6x150mm,5µm)
Injection Volume	10 µl
Run Time	6 mins
Diluent	Water and Acetonitrile in ratiob 50:50
Retention Time	Azelnidipine-2.116 mins, Telmisartan – 3.188mins
Theoretical Plates	Azelnidipine- 2740, Telmisartan- 5651

Table-2: Precision Results of Azelnidipine and Telmisartan

s.no	Repeatability		Intermediate Precision	
	Azelnidipine	**Telmisartan**	**Azelnidipine**	**Telmisartan**
1	294833	1249380	282667	1205161
2	291094	1214505	290967	1196312
3	290866	1218032	290137	1201359
4	292949	1225126	290743	1202242
5	290448	1245002	284755	1215203
6	293720	1223169	283107	1215252
Mean	292318	1229202	287063	1205922
S.D	1777.2	14495.3	3963.2	7752.2
%RSD	0.6	1.2	1.4	0.6

Table-3: Accuracy results of Azelnidipine (Drug 1) and Telmisartan(Drug 2):

%Level	Amount Spiked(µg/ml)		Amount Recovery (µg/ml)		% Recover		Mean % Recovery	
	Drug 1	**Drug 2**	**Drug 1**	**Drug 2**	**Drug 1**	**Drug 2**	**Drug 1**	**Drug 2**
50%	4	20	4.11	19.81	101.0	99.05	100.15%	100.20%
	4	20	3.98	19.90	98.8	99.51		
	4	20	3.99	20.25	99.1	101.24		
100%	8	40	7.99	40.26	99.9	100.64		
	8	40	8.1	39.94	100.9	99.85		
	8	40	8.0	40.45	100.1	101.13		
150%	12	60	12.01	59.75	100.2	99.58		
	12	60	12.1	60.18	100.9	100.30		
	12	60	12.03	60.31	100.4	100.52		

Table-4: LOD and LOQ values of Azelnidipine and Telmisartan

Molecule	LOD	LOQ
Azelnidipine	0.04	0.13
Telmisartan	0.38	1.14

Table-5 Robustness Data of Azelnidipine and Telmisartan

S.no	Condition	%RSD of Azelnidipine	% RSD of Telmisartan
1	Flow rate (-) 0.9ml/min	0.5	0.4
2	Flow rate (+) 1.1ml/min	0.6	0.4
3	Mobile phase (-) 65B:35A	0.2	0.3
4	Mobile phase (+) 55B:45A	0.4	0.3
5	Temperature (-) 25°C	0.5	0.5
6	Temperature (+) 35°C	0.5	0.2

Table-6 System Suitability Parameters for Azelnidipine and Telmisartan

S.no	Azelnidipine			Telmisartan			
Inj	**RT(min)**	**USP Plate Count**	**Tailing**	**RT(min)**	**USP Plate Count**	**Tailing**	**Resolution**
1	2.111	2877	1.29	3.186	5830	1.23	6.6
2	2.112	2879	1.29	3.188	5695	1.20	6.6
3	2.112	2897	1.27	3.188	5943	1.21	6.5
4	2.113	3120	1.28	3.188	6184	1.21	6.6
5	2.113	3154	1.29	3.189	6291	1.21	6.6
6	2.114	3014	1.27	3.189	6087	1.22	6.6

Table-7 Assay Results ofAzelnidipine and Telmisartan

S.no	% Assay Azelnidipine	%Assay Telmisartan
1	101.08	101.77
2	99.80	98.93
3	99.72	99.22
4	100.43	99.80
5	99.57	101.41
6	100.70	99.64
Avg	100.22	100.13
Stdev	0.61	1.18
%RSD	0.6	1.18

Table-8 Degradation Data forAzelnidipine and Telmisartan

Type of Degradation	Azelnidipine		Telmisartan	
	% Degraded	**% Recorded**	**% Degraded**	**% Recorded**
Acid	6.41	93.59	6.76	93.24
Alkali	5.01	94.99	5.33	94.67
Oxidation	3.67	96.33	3.07	96.93
Thermal	2.49	97.51	2.24	97.76
UV	1.79	98.21	1.80	98.20
Water	0.59	99.41	0.65	99.35

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