Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 10 [2] January 2021 : 19-27

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

Method Development and Validation for the Simultaneous Estimation of Azelnidipine and Telmisartan in Pharmaceutical Formulation by High Performance Liquid Chromatography

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ABSTRACT

A Precise, Specific, Linear, Accurate and Robust method was developed for the simultaneous estimation of the Azelnidipine and Telmisartan in tablet dosage form and Validated as per ICH Validation guidelines. Method was optimized byWaters Xbridge C18 (150 × 4.6 mm, 5µm) column at a flow rate of 1.0ml/min, Mobile phase waspH 4.0 Phosphate buffer, Acetonitrile(70:30 respectively). The Column Oven temperature was maintained at 25°C and working wave length was selected at 254nm. The retention times of Telmisartan and Azelnidipine were found to be 2.327min and 3.540min. % RSD of the Azelnidipine and Telmisartanwere and found to be 0.2% and 0.9% respectively. In Method precision Parameter, % Assay were found 95.0 to 105.0% and % Recovery were obtained as 99.8% and 99.9% for Telmisartan and Azelnidipine respectively. Linearity was obtained as 0.999and 0.999 for Telmisartan and Azelnidipine.Analytical Range was found from the linearity and accuracy for Azelnidipine was 4µg/mL to 12µg/mL andTelmisartanwas 40µg/mL to 80µg/mL.

Key Words: *Azelnidipine,Telmisartan and Waters Xbridge C18 Column*

Received 21.10.2020 Revised 26.11.2020 Accepted 20.01.2021

INTRODUCTION

Azelnidipine is a dihydropyridine calcium channel blocker. It is marketed by Daiichi-Sankyo pharmaceuticals, Inc. in Japan. 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 [1]. Azelnidipine structure is shown in figure 1.

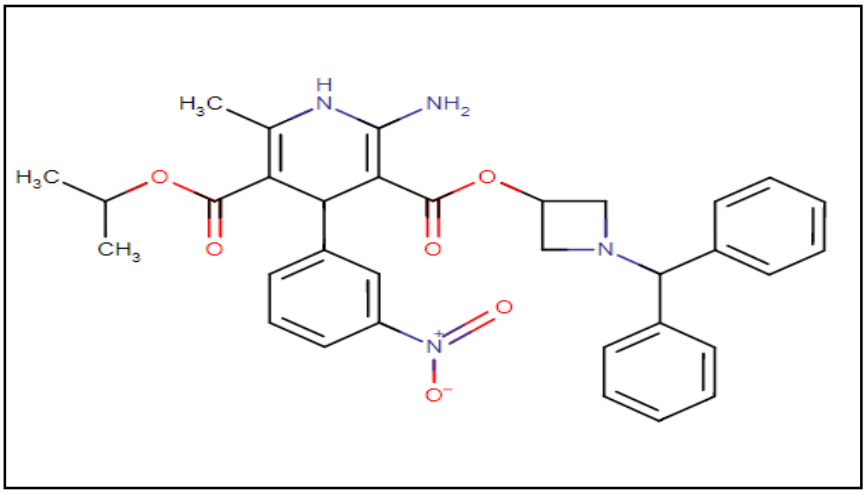

Fig1. Structure of Azelnidipine [1]

Azelnidipine inhibits trans-membrane Ca2+ influx through the voltage-dependent channels of smooth muscles in vascular walls. Ca2+ channels are classified into various categories, including L-type, T-type, N-type, P/Q-type, and R-type Ca2+ channels. The L-type Ca^{2+} channels. Normally, calcium induces smooth muscle contraction, contributing to hypertension. When calcium channels are blocked, the vascular smooth muscle does not contract, resulting in relaxation of vascular smooth muscle walls and decreased blood pressure [1].

Telmisartan is an Angiotensin II receptor antagonist (ARB) used in the management of hypertension. Generally, Angiotensin II receptor blockers (ARBs) such as Telmisartan bind to the Angiotensin II type 1 (AT1) receptors with high affinity, causing inhibition of the action of Angiotensin II on vascular smooth muscle, ultimately leading to a reduction in arterial blood pressure. Recent studies suggest that Telmisartan may also have PPAR-gamma agonistic properties that could potentially confer beneficial metabolic effects [2].Telmisartan structure is shown in figure 2.

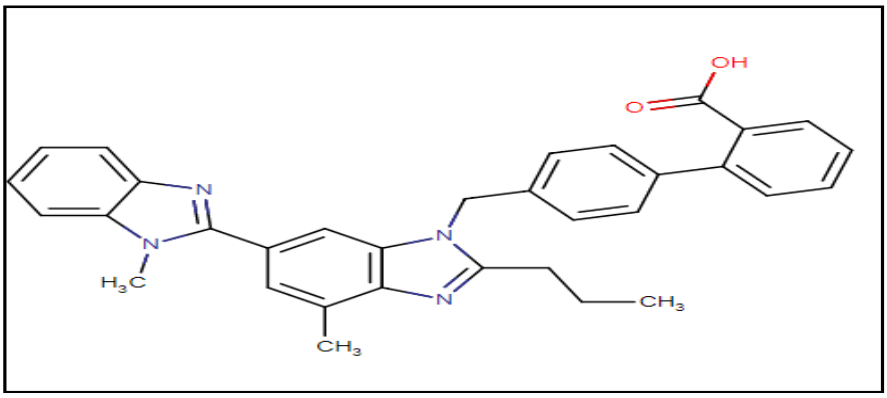

Fig2. Structure of Telmisartan [2]

Telmisartan interferes with the binding of Angiotensin II to the Angiotensin II AT_1-receptor by binding reversibly and selectively to the receptors in vascular smooth muscle and the adrenal gland. As Angiotensin II is a vasoconstrictor, which also stimulates the synthesis and release of Aldosterone, blockage of its effects results in decreases in systemic vascular resistance. Telmisartan does not inhibit the Angiotensin converting enzyme, other hormone receptors, or ion channels. Studies also suggest that Telmisartan is a partial agonist of PPARγ, which is an established target for Antidiabetic drugs. This suggests that Telmisartan can improve carbohydrate and lipid metabolism, as well as control insulin resistance without causing the side effects that are associated with full PPARγ activators [2].

There were no methods to estimate simultaneously for Azelnidipine and Telmisartan by HPLC, There were only individual methods for the estimation of Azelnidipine and Telmisartan. This method could be a first method to estimate simultaneously for Azelnidipine and Telmisartan in pharmaceutical formulations by RP-HPLC [5-9].

MATERIAL AND METHODS

Instruments was Used: Shimadzu Balances, Thermo pH Meter, Agilent 1290 HPLC with PDA equipped with quaternary pump and Auto sampler integrated with Open labsoftware, Shimadzu UV-Visible spectrophotometer with Lab solution software, Ultasonicator.

Drug Samples: Azelnidipine and Telmisartan Active Pharma ingredients and Marketed samples of Azelnidipine and Telmisartan Tablet.

Chemicals and Reagents: Potassium di hydrogen ortho phosphate (Make: Merck and Grade: Empata ACS), Orthophosphoric acid (Make:Merck and Grade: Emparta ACS), Acetonitrile (Make :Merckand Grade: HPLC)

Methodology of Analysis

Working wavelength optimization:

Preparation of Standard solutions:

Weighed accurately 5mg of each Azelnidipine in to 50mL volumetric flask and added30mL of Methanol by sonication for dissolving the drug substances and Volume made up with Methanol and mixed well. Taken 5mL above stock solution and further diluted to 50mL this solution with methanol(10µg/mL of Azelnidipine)

Prepared 10µg/mL of Telmisartan solution in above preparation manner

Above standard solutions of Azelnidipine and Telmisartan were scanned 200 to 400nm and graphs were shown in Fig3 and Fig 4.

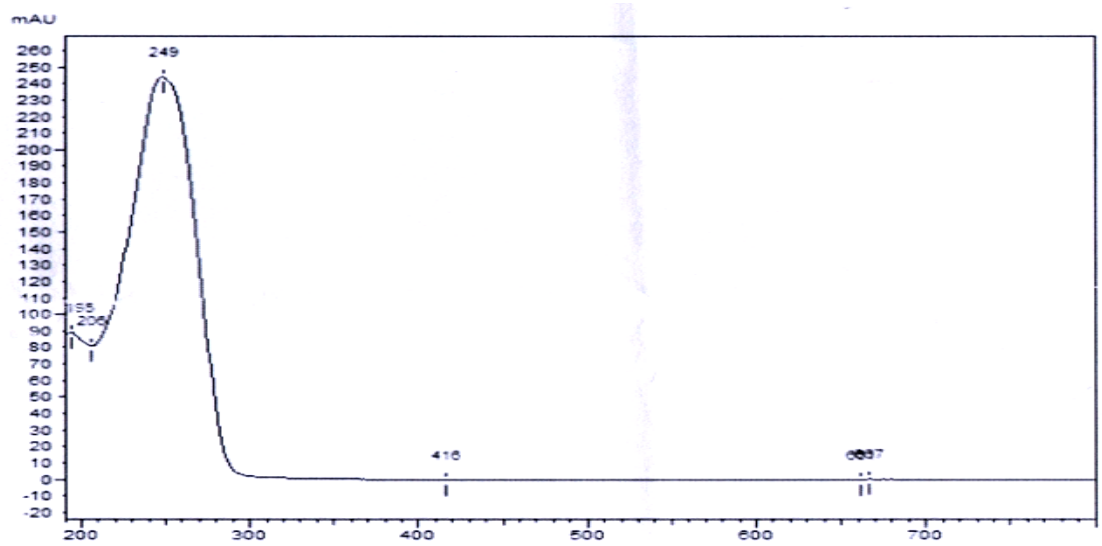

Fig3. UV graph of Azelnidipine

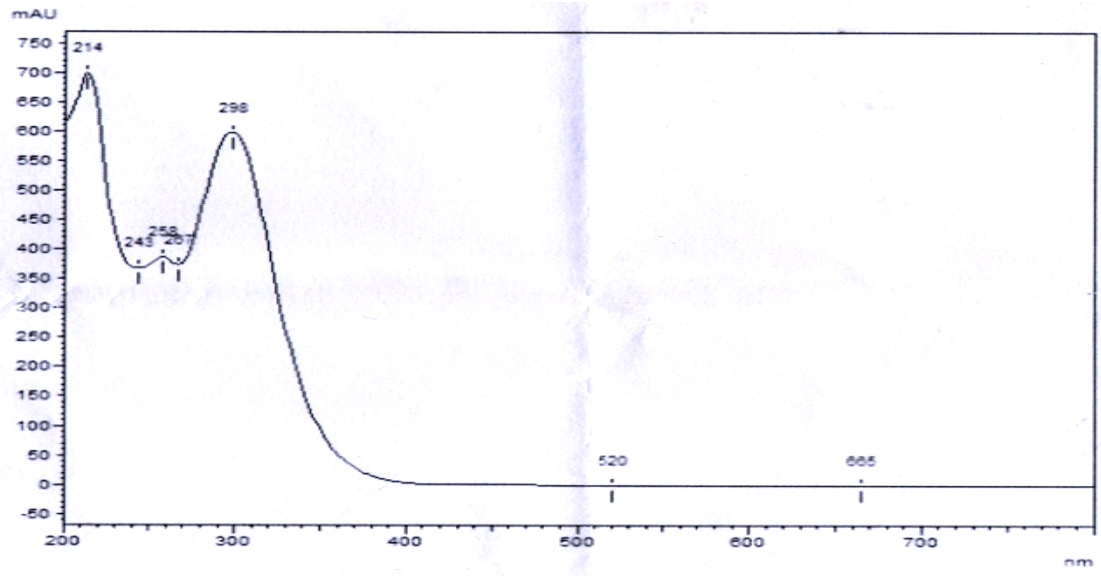

Fig4. UV graph of Telmisartan

Azelnidipine shown maximum absorbance at 249nm and Telmisartan shown maximum absorbance at 293nm, however Telmisartan shown maximum at 298, Selected 254nm as a working wavelength because of Azelnidipine has low intensity at the of 298nm

Method Development: After Several trials by changing the column, Buffers and Mobile phase Ratios, below mentioned conditions were optimized, In Optimized condition, Blank and Placebo interference were checked and %Assay also checked. Finally these diluents, mobile phase and Chromatographic conditions were taken as a optimized.

Diluted Ortho phosphoric acid Preparation:

Diluted 5mL of ortho phosphoric acid 50mL with water and mixed well.

Preparation of pH 4.0 Phosphate Buffer:

Accurately weighed1.36g Potassium dihydrogen ortho phosphate and Transferred in to 1000mL of water, mixed well and adjusted pH 4.0 with diluted Ortho phosphoric acid. Filtered through 0.45µm PVDF membrane filter

Preparation of Mobile Phase

Mixed Accurately 700mL of pH 4.0 Phosphate Buffer, 300mL of Acetonitrile. Degassed by sonication for 10min.

Diluent:
Mixed pH 4.0Potassium dihydrogen ortho phosphate Buffer and Acetonitrile in the ratio of 70:30.
Preparation of Standard stock:
Weighed Accurately and transferred 16mg of Azelnidipine and 160mg of Telmisartan in to 200mL Volumetric flask, added 150mL of Diluent then kept for sonication up to dissolved, Final volume was made up to mark with diluent and mixed well. (80µg/mL of Azelnidipine,800µg/mL of Telmisartan)
Preparation of Standard working solution:
Taken 5mL of above standard stock solution in to 50mL volumetric flask then diluted up to mark with diluents and mixed well. (8µg/mL of Azelnidipine, 80µg/mL of Telmisartan)
Preparation of Sample stock solution
Taken accurately 20Tablets and weighed, taken average weight from the twenty tablets and crushed in to fine powder by using the mortar and pestle. Weighed accurately crushed powder equivalent to 16mg of Azelnidipine, 160mg of Telmisartan in to 200mL Volumetric flask, added 150mL of Diluent then kept on sonicator up to 30min with intermittent shaking by maintain sonicator temperature 23±2°C, After sonication kept flask on bench top to attain room temperature, Volume made up to mark with diluent and mixed well. This solution centrifuged at 5000RPM for 10min.
Preparation of Sample solution
Accurately Taken 5mL of Clear supernatant of sample stock solution and transferred in to 50mL volumetric flask, diluted up to mark with diluents and mixed well. Filtered these solution through 0.45µ PVDF Syringe filter by discarding the 3mL of filtrate.
Optimized Conditions
The optimized chromatographic conditions were obtained with several trail and error methods and the optimum chromatographic conditions were shown in Table 1 and the optimum chromatogram was shown in Fig 5.

Table 1: Optimised Chromatographic conditions:

Column	Waters Xbridge C18 (150 × 4.6 mm, 5µm)
Mobile Phase and Composition	pH 4.0Phosphate Buffer: Acetonitrile(70:30)
Flowrate	1.0mL/min
Column oven Temperature	25°C
Injection volume	20µL
Detection wavelength	254nm
Auto sampler Temperature	25°C
Retention Times	2.327min of Telmisartanand 3.540min of Azelnidipine (Total Run time 5.0min)

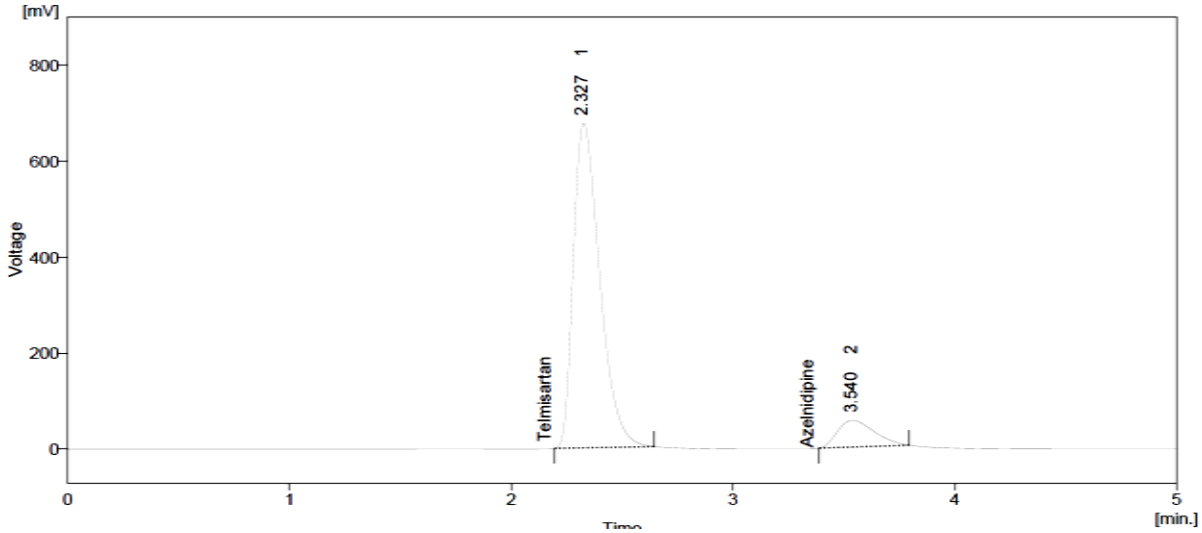

Fig5. Optimized Chromatogram

Method Validation
By using Optimised condition Analytical Method of Assay carried out by ICH Guideline Q2B.[3-4]
The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose.

RESULTS AND DISCUSSION

System Suitability and System Precision

According to ICH guidelines [3, 4] System suitability checking out is an integral a part of many analytical procedures. The tests are based on the idea that the equipment, analytical operations and samples to be analyzed represent an integral system that can be evaluated as such. System suitability check parameters parameters to be established for a particular procedure depend on the type of procedure being validated.

According to ICH Specifications: Theoretical Plates should not be less than 2000, Tailing factor should not be 0.9 to 2.0 and Resolution should not be less than 2.0 between Azelnidipine and Telmisartan

System Precision Specification:%RSD for Area and Retention time for the six replicate injections should not be more than 2.0 of each analyte and system suitability results were shown in table 2 and system precision were summarized in Table3.

Table 2: Results of System suitability

S.No	Telmisartan			Azelnidipine			Resolution
	Rt in min	Plate count	Tailing Factor	Rt in min	Plate count	Tailing Factor	
1	2.320	3755	1.41	3.523	3943	1.39	4.4
2	2.327	3785	1.40	3.520	3955	1.37	4.4
3	2.323	3751	1.42	3.537	4051	1.39	4.5
4	2.327	3740	1.41	3.530	4021	1.39	4.4
5	2.317	3742	1.40	3.520	3942	1.38	4.4
6	2.327	3742	1.43	3.530	3951	1.40	4.4

Observation: Theoretical Plates, Tailing factor and Resolution was within the acceptance criteria.

Table 3:Results of System Precision

S.No	**Telmisartan**		**Azelnidipine**	
	Rt in min	Area	Rt in min	Area
1	2.320	5599.365	3.523	707.341
2	2.327	5626.643	3.520	692.140
3	2.323	5600.893	3.537	687.020
4	2.327	5606.288	3.530	696.712
5	2.317	5593.455	3.520	700.091
6	2.327	5593.358	3.530	695.011
AVG	2.324	5603.334	3.527	696.386
Std dev	0.0	12.4	0.0	6.9
%RSD	0.2	0.2	0.2	0.9

Observation:% RSD (Relative standard deviation) of Six Replications of Area and Retention time for Azelnidipine and Telmisartan was less than 2.0%

SPECIFICITY [3, 4]

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc.

Procedure for Specificity: Blank (Diluent used as a Blank) and Placebo solutions were injected into HPLC system. The blank and placebo chromatograms were shown in Fig.6 and Fig.7 respectively.

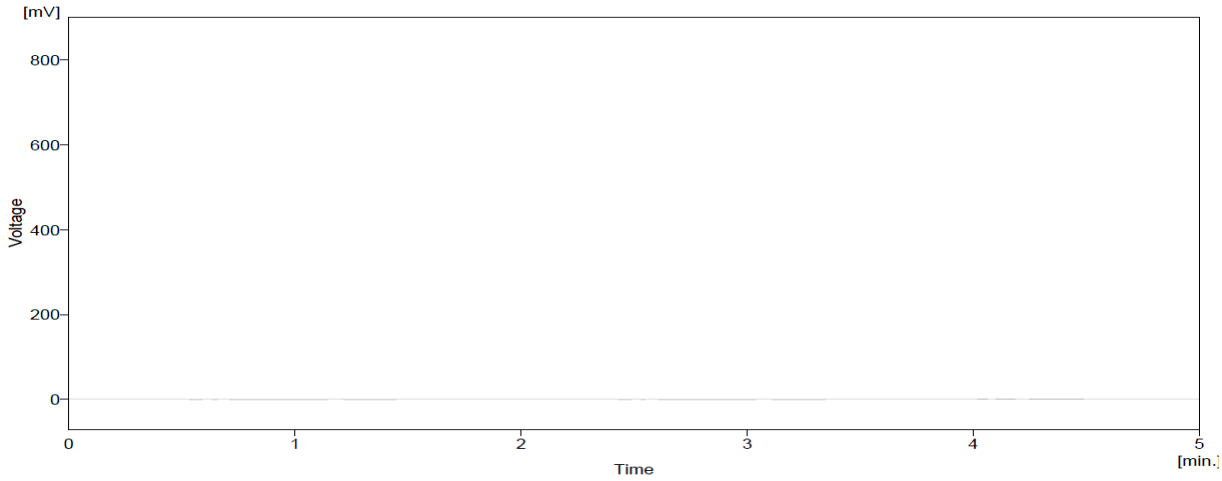

Fig6. Blank Chromatogram

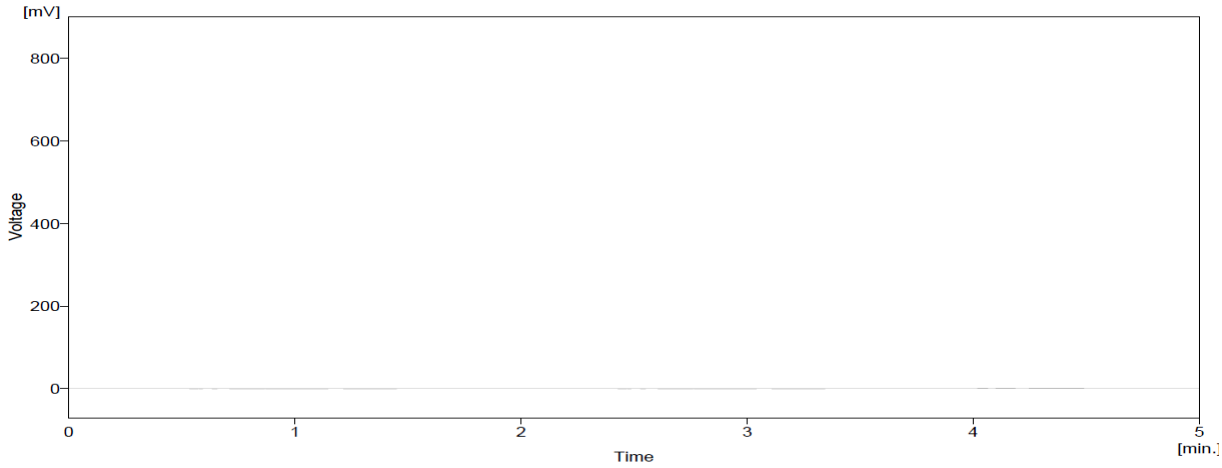

Fig7. Placebo Chromatogram

Acceptance Criteria:
Blank solution and Placebo solution should not be interfered at the retention time of the two main analyte peaks
Observation: No Blank and Placebo interference was observed at theretention times of the two main analyte peaks.

METHOD PRECISION
Closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample [3, 4].
Sample solutions were six prepared individually and each injected in to HPLC System, Calculated %Assayby using average area of Six Standards. The %RSD for % Assay of all peak area and retension time of Telmisartan and Azelnidipine were calculated and summarized in the table 4.

Table 4: Method Precision

Name of the Sample	%Assay of Telmisartan	%Assay of Azelnidipine
Method Precsion-01	99.3	98.1
Method Precsion-02	99.8	98.4
Method Precsion-03	99.8	98.6
Method Precsion-04	99.5	101.2
Method Precsion-05	99.5	98.0
Method Precsion-06	99.7	98.4
AVG	99.6	98.8
Std dev	0.2	1.2
%RSD	0.2	1.2

Observation: Average and individual %Assay was obtained between 95.0 to 105.0% individual preparation and %RSD for %Assay of six replicate preparations was obtained below 2.0

ACCURACY AND RECOVERY STUDIES

Expresses the closeness of agreement between the value which is accepted either as a conventional true value and the value found [3, 4]

Three levels (50%, 100% and 150%) of accuracy sample were prepared in triplicate by Standard API addition method to the Placebo. At each level API taken 50%, 100% and 150% respectively in the presence of Placebo.The accuracy, recovery and %RSD of Telmisartan and Azelnidipine were calculated and summarized in the table-5.

Table5: Details of Accuracy

Name of the Level	**Telmisartan**	**Azelnidipine**
50% Accuracy	99.5	100.4
100% Accuracy	99.7	99.9
150% Accuracy	100.1	99.5
Mean	99.8	99.9
Std dev	0.3	0.5
%RSD	0.3	0.5

Observation: Each level of Accuracy and %Recovery mean was obtained 99.9% for Telmisartan and99.9% for Azelnidipine.

LINEARIT AND RANGE:

Five linearity solutions (50%, 80% 100%, 120% and 150%) were prepared from standard stock solution i.e., 4µg/mL to 12µg/mL for Azelnidipine, 40µg/mL to 120µg/mL for Telmisartan [3, 4].

The linearity of different concentrations of the Telmisartan and Azelnidipine were calculated and summarized in table-6 and graphs were shown in Fig-8-9.

Table 6: Calibration curve details of Telmisartan and Azelnidipine

Telmisartan		Azelnidipine	
Conc. in µg/mL	Area	Conc. in µg/mL	Area
40	3236.788	4	375.912
60	4409.861	6	520.885
80	5560.106	8	647.488
100	6560.326	10	780.529
120	7803.508	12	902.314
Correlation coefficient	0.999	Correlation coefficient	0.999

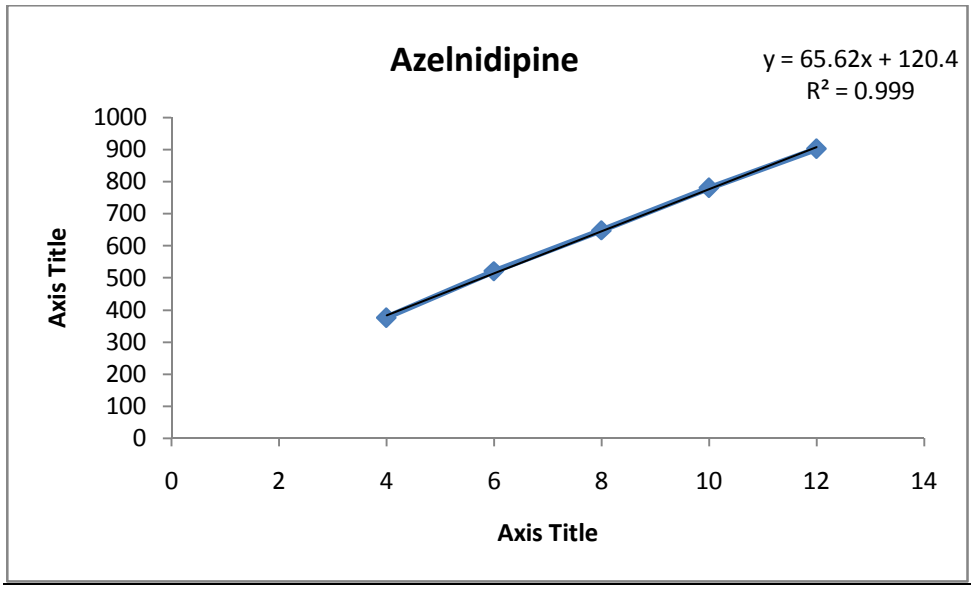

Fig.8 Calibration Curve of Azelnidipine

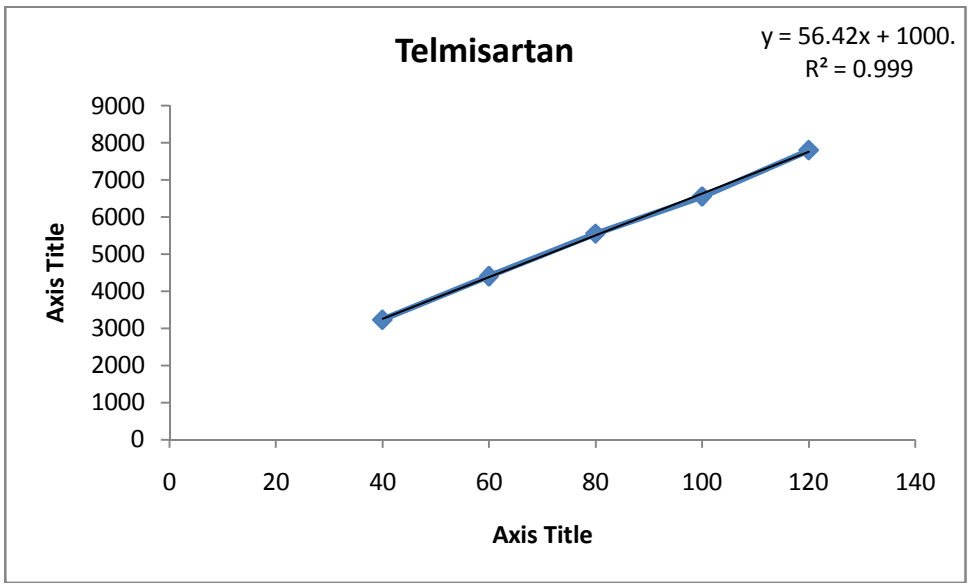

Fig.9 Calibration Curve ofTelmisartan

Observation: The Correlation coefficient was found 0.999 for Azelnidipine and Telmisartan

ROBUSTNESS

Robustness conditions like flow (1.0mL/min±0.2mL) and Wavelength (254nm±5nm) was maintained in the HPLC System and injected six standards replicate injections in each condition. System suitability parameters were within the acceptance criteria, %RSD for Area of six standard injections for within the limit. Robustness of different conditions were conducted, calculated and summarized in the table-7.

Table 7: Robustness

S. No.	Condition	%RSD for Azelnidipine	%RSD for Telmisartan
01	Flow Rate_0.8mL/min	0.52	0.23
02	Flow Rate_1.2mL/min	0.33	0.41
03	Wavelength (249nm)	0.25	0.51
04	Wavelength (259nm)	0.62	0.55

CONCLUSION

A Specific, Accurate, Precise and Robust indicating Assay method was developed for the simultaneous estimation of the, Azelnidipine and Telmisartan in Tablet dosage form, For the optimised conditions of Assay method was Validated by Using ICH Q2B guidelines. Method was shown precise, Specific, accurate, linear and robust results. % RSD of the Telmisartan and Azelnidipine were and found to be 0.2 and 0.9% respectively in system precision. In method precision sample has shown precise %Assay results.That was 95.0 to 105.0%.% Recovery was obtained as 99.8% and 99.9% for Telmisartan and Azelnidipine respectively. Linearity was obtained as 0.999, 0.999 and 0.999 for Telmisartan and Azelnidipine respectively. So this method very use full to Routine analysis like in Quality control to reduce the time and cost.

ACKNOWLEDGEMENT

Authors are very thankful to my guide Dr. Somasekhar Reddy Kanala and the management ofRaghavendra Institute of Pharmaceutical Education and Research, Ananthapuramu, Andhra Pradesh, forproviding the necessary facilities to carry out the research work.

CONFLICT OF INTEREST

Conflict of interest declared none.

REFERENCES

1. https://go.drugbank.com/drugs/DB00966
2. https://go.drugbank.com/drugs/DB09230
3. International Conference on Harmonization Quality guidelines Q2R1 validation of analytical procedures: text and methodology
4. ICH Harmonised Tripartite Guideline,(2011). Stability Testing of New Drug Substances and Products, Q1A (R2).
5. Patel Jayvadan et.al (2014). Validated Stability-Indicating RP-HPLC Method for the Simultaneous Determination of Azelnidipine and Olmesartan in Their Combined Dosage Form.Scientia Pharmaceutica.82(3):541-554. (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4318177)
6. Raveendra Babu Ganduri et.al (2014).Stability Indicating Liquid Chromatographic Method for the Simultaneous Determination of Olmesartan Medoxomil and Azelnidipine in Combined Tablet Dosage Form. International journal of Pharma Sciences and Research, 5(6);275-282. (http://www.ijpsr.info/docs/IJPSR14-05-06-012.pdf)
7. Alaa El Sayed Ahmed Amin et.al (2016). Simultaneous Determination of Azelnidipine and Olmesartan Medoxomil in Pharmaceutical Dosage Forms by UFLC Method. Journal of Pharma Sci Tech,: 6(2);69-74.(http://www.pharmascitech.in/admin/php/uploads/128_pdf.pdf)
8. Megha G. Gore et.al (2016). RP-HPLC Method Development And Validation Of Azelnidipine, International Journal of Pharmaceutical Sciences and Research.7(12):5111-5114.(https://ijpsr.com/bft-article)
9. Dhiraj Kumar et.al (2018). Development Of Stability Indicating Rp-Hplc Method For Simultaneous Estimation Of Amlodipine And Olmesartan In Pure And Pharmaceutical Dosage Form. International Journal of Pharmaceutical Sciences and Drug Research.;10(5):379-385. (https://ijpsdr.com/index.php/ijpsdr/article/view/643)
10. Principles of chromatography(https://www.khanacademy.org/science/class-11-chemistry-india)
11. Introduction to new Chromatography(https://www.britannica.com/science/chromatography).

CITATION OF THIS ARTICLE

K Konam, S R Kanala. Method Development and Validation for the Simultaneous Estimation of Azelnidipine And Telmisartan in Pharmaceutical Formulation by High Performance Liquid Chromatography. Bull. Env. Pharmacol. Life Sci., Vol10[2] January 2021: 19-27