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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR QUANTIFICATION OF AZELNIDIPINE IN TABLET.

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ABSTRACT:
The analytical method was developed and validated for determination of Azelnidipine in bulk and pharmaceutical dosage forms by High performance liquid chromatography. The separation was carried out on Luna C18 (150 x4.6mm,5µ) column. The mobile phase consists of ACN : Water in the ratio 90:10 at flow rate 1 ml/min at 255nm.The column temperature was adjusted at 30º ± 0.5˚C with injection volume 20µl.The retention time of Aelnidipine was 3.5min. The linearity of the calibration curve was linear over the concentration range 10-50µg/ml (r^2=0.999).The validation was carried out as per ICH guidelines. The development of method was easy, rapid, linear, precise, accurate and consistent.

Keywords: Azelnidipine, RP-HPLC, Validation, Chromatogram,Linearity..

1.0 INTRODUCTION:

Hypertension is a condition where blood pressure is elevated to an extent that clinical benefit is obtained from BP lowering. Hypertension is one of the most important risk factor for both coronary artery disease and cardiovascular disease [1]. Azelnidipine (AZEL) (3-[1-(diphenylmethyl)azetidin-3-yl] 5-propan-2-yl 2-amino-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate) is a new dihydropyridine derivative with calcium antagonistic activity. Azelnidipine is inhibits trans membrane Ca+2 influx through the voltage dependent channels of smooth muscle in vascular walls. They enter the cells through cell membrane, lower peripheral vascular resistance and arterial pressure. It is used for treatment of essential hypertension and angina pectoris [2]. Azelnidipine is Ca+2 channel blocker inhibits trans membrane Ca +2 influx through the voltage dependent channels of smooth muscle in vascular walls. They enter the cells through cell membrane, lower peripheral vascular resistance and arterial pressure.Ca+2 channels are classified into various categories including L-type, T-type, N-type, P/Q- type, R-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 BP [3].

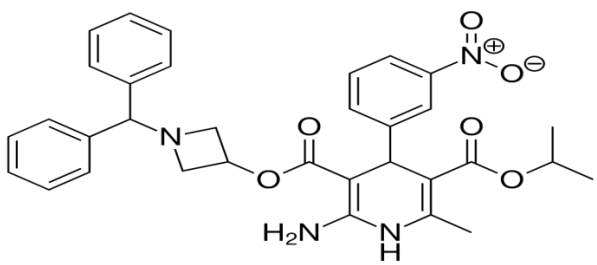

Figure1: Structure of Azelnidipine[4]

The drug is official in Indian Pharmacopiea. A few analytical methods have been reported for the determination of the selected drug. The reported methods for estimation of azelnidipine were UV-Spctrophotometric methods [5,6], HPLC [7,8,9,10,11,12], UFLC [13,14], HPLC-MS/MS [15], LC-ESI-MS [16]. Although various HPLC methods were reported in the literature for determining Azelnidipine and impurities in active pharmaceutical ingredients, Retention time is longer in these methods. The present study was aimed to

develop new HPLC method for estimation of azelnidipine in tablet and validated in accordance with International Council For Harmonization (ICH) guideliness [17]

2.0 MATERIALS AND METHOD

2.1 Chemicals and reagents

The drug Azelnidipine was obtained as gift sample from Pure Chem Pvt Ltd. RO Water and HPLC grade Acetonitrile and Methanol (Merck) Mumbai, India. 0.45 µm Millipore syringe filters(Ultipor®N_{66}®Nylon Membrane) were from PALL Life sciences.

2.2 Instruments

Analytical balance (Shimadzu AY220), HPLC (Younglin Acme 9000), UV-Spectrophotometer (Shimadzu UV-1800), Vortex machine (Remi CM 101 plus), Sonicator (Oscar Microclean 103).

2.3 Chromatographic equipment and conditions

The optimized chromatographic conditions are as follows :

Column used for chromatographic separation was Luna C18 (150 x4.6mm,5µ). Acetonitrile : water (90 :10) was used as mobile phase. Methanol ws used as diluent. Flow rate was set to 1ml/min and injection volume to 20 µl. Detection was carried out at 255 nm in UV detector at 30^0 C. Retention time was 3.5 min and run time was 10 min.

2.4 Preparation of standard stock solution

Initially Prepare a Standard Stock Solution (SSS-I) of Azelnidipine by adding 10mg in 10 ml volumetric flask & add 5 ml diluent and Mix and sonicate for 5 minutes. Make up the volume to 10 ml with diluent. (Conc. = 1000µg/ml)

Pipette out 1.0 ml of SSS-I in 10 ml volumetric flask. Add 5 ml diluent and vortex; make up the volume with diluent. (Conc. = 100 µg ml)

2.5 Preparation of Mobile Phase

a.HPLC grade Acetonitrile and purified RO water used for the preparation of mobile phase.

b. Accurately measured the each component of the mobile phase

(90% ACN and 10% Water).

c. Each component of mobile phase filtered through 0.45µ membrane filter twice.

d. Sonication is performed for 15mins to degas the mobile phase.

3.0 RESULTS AND DISCUSSION

3.1 Method Development

The proposed HPLC method was developed and optimized for a series of trails in the terms of mobile phase selection, composition, wavelength, choice of stationary phase of column, flow rate and column temperature. Azelnidipine showed the absorbance maxima at 255nm. Hence this wavelength was chosen as a working wavelength for the proposed HPLC method.

3.2 Method Validation

1. Specificity:

The retention time was 3.5 min for AZEL in API and in drug product. This indicates that there is no drug - excipient interference and it is specific for AZEL.

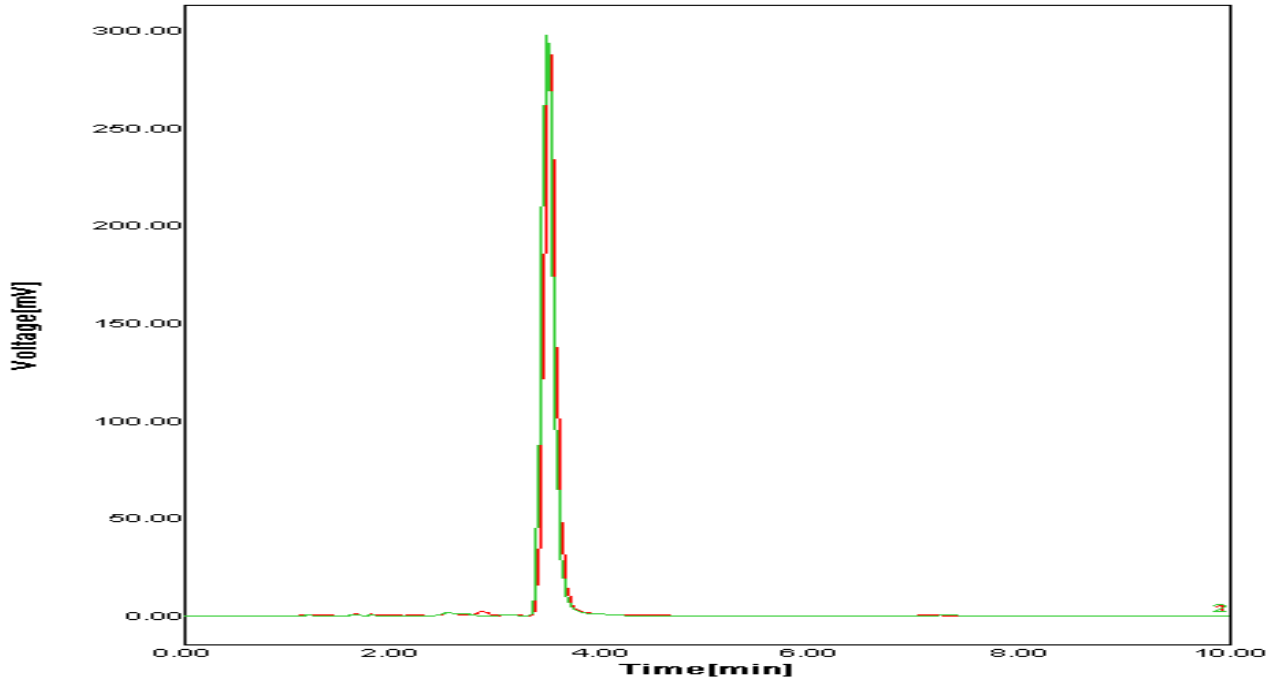

Fig.1: Overlay of 40 µg/ml solution chromatogram (green) & Azovas 16mg tablet chromatogram (red)

2. Linearity:

The peak response is proportional to the concentration and linear in the range of 10-50µg/ml (Table No.1).The correlation coefficient (r^2) is 0.999

TABLE 1: LINEARITY OF AZEL

Sr. no.	Conc. (µg/ml)	Area (mAU)
1	10	620
2	20	1281
3	30	1850
4	40	2418
5	50	3019

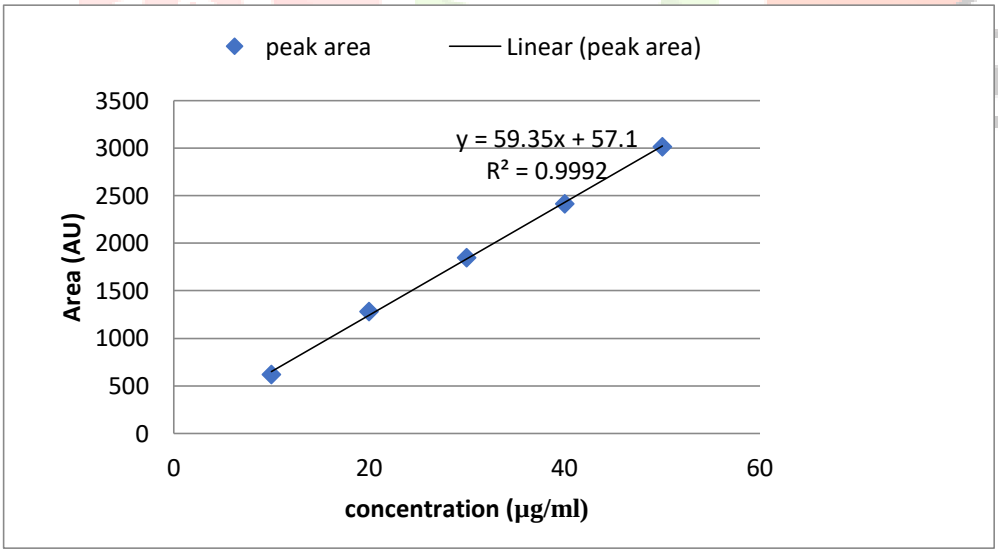

Fig.6: Calibration curve of AZEL

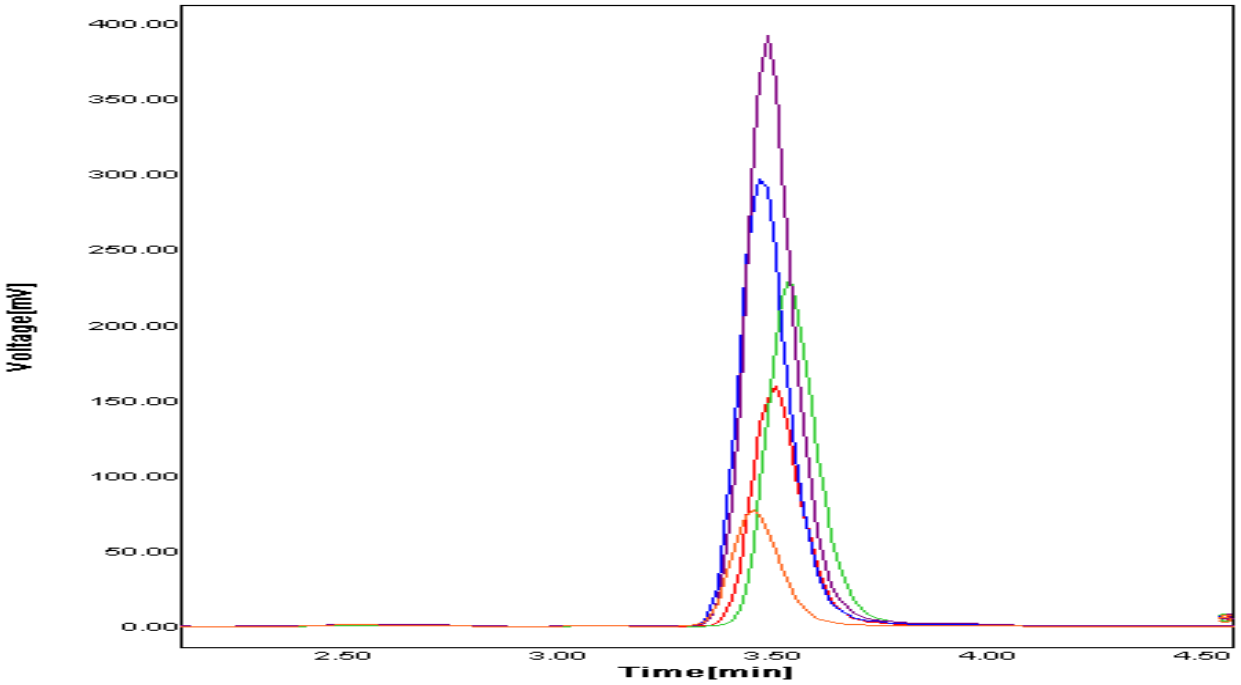

Fig.2: Overlay of Chromatogram of 10-50nµg/ml AZEL

3. Range:

The range is from 10-50 µg/ml.

4. Accuracy:

The percentage recoveries of the results indicate that the method is accurate (Table No.2).

TABLE 2: ACCURACY OF AZEL

	Conc of spiked level	Amount spiked (µg)	Amount recovered (µg)	% Recovery
AZEL	75%	15	15.39	102.6
	100%	20	20.56	102.8
	125%	25	25.61	102.4

5. Precision :

The % RSD < 2 values obtained shows that method developed is precise. (Table No.3)

TABLE 3: RECISION RESULTS OF AZEL

INTRADAY PRECISION		INTERDAY PRECISION	
Sample	**Area**	**Sample**	**Area**
1	1423	1	1433
2	1456	2	1412
3	1465	3	1435
4	1475	4	1423
5	1433	5	1465
6	1412	6	1453
Average	1443.5	**Average**	1436.8
STDEV	24.768	**STDEV**	19.395
%RSD	1.715	**%RSD**	1.349

6. Robustness :

The robustness results were shown in the table 4, the relative standard deviation (RSD) was found to be less than 2%, and low% of RSD value confirms that robustness of method. (Table No.4)

TABLE 4: ROBUSTNESS CHANGING WAVELENGTH AND FLOWRATE.

Wavelength (nm)	Drug	Theoretical Concentration(µg/ml)	Area	Rt value
254	AZEL	30	1937	3.6
256			2117	3.4
Flow Rate(ml/min)	**Drug**	**Theoretical Concentration(µg/ml)**	**Area**	**Rt value**
0.9	AZEL	30	2531	4.4
1.1			2501	3.2

7. System suitability:

Parameters such as retention time , tailing factor and theratical plates calculated from the standard chromatogram to evaluate system suitability as shown in (Table No.5.)

TABLE 5: SYSTEM SUIABILITY FOR AZEL

Parameters	Values
Theoretical plates	6452
Retention time	3.5 min
Asymmetry (Tailing Factor)	1.23

8. LOD:

LOD of AZEL was found to be 1.73µg/ml .

9. LOQ:

LOQ of AZEL was found to be 5.26µg/ml

4.0 Assay:

Amount of drugs present in marketed formulation AZOVAS ® 16 mg Tablet was calculated. Amount of AZEL was found to be 98.4% of label claim. Results complies with Indian Pharmacopoiea[17]. This method can be employed for routine analysis of simultaneous estimation of AZEL in dosage form .

5.0 Summary of Validation Parameters: (Table No.6)

The developed method is validated in terms of linearity, accuracy, precision and robustness. Shorter retention time and use of less solvents as compared to existing methods is observed. Hence this method is suitable for analyzing AZEL in dosage forms.

TABLE 6: SUMMARY OF VALIDATION PARAMETERS

Parameter	AZEL
Retention time (min)	3.5
Theoretical plate count	6452
Linearity range (µg/ml)	10-50
Regression equation	y=59.35x+ 57.1
Slope	59.35
Intercept	57.1
Regression coefficient (r^2)	0.999
Repeatability(n=6) % RSD	1.3
LOD (µg/ml)	1.73
LOQ (µg/ml)	5.26

6.0 Acknowledgement:

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7.0 Conclusion: An analytical HPLC method was developed & validated thoroughly for quantitative determination of Azelnidipine in bulk drug and formulation. The presented method was found to be simple, precise, accurate, rugged, reproducible and gives an acceptable recovery of the analyte, which can be directly easily applied to the analysis of pharmaceutical formulation of Azelnidipine.

8.0 References:

1. Walker R and Whittlesea C. Clinical Pharmacy and Therapeutics; 5thEdn; Elsevier (2007): 295-307.
2. Chen B., et al. "Clinical use of azelnidipine in the treatment of hypertension in Chinese patients". Therapeutics and Clinical Risk Management 11 (2015): 309-318.
3. Tamargo J and Ruilope LM. "Investigational calcium channel blockers for the treatment of hypertension". Expert Opinion on Investigational Drug (2016): 1-51.
4. Azelnidipine image [Internet]. wikipidia [cited 2020 Nov 6]. Available from: https://en.wikipedia.org/wiki/Azelnidipine#/media/File:Azelnidipine_structure.svg
5. Rajan R. Spectrophotometric estimation of azelnidipine in bulk and pharmaceutical dosage form by second order derivative methods. J Chem Pharm Res. 2014;6(8):198-202.
6. Kunti R, Mrunali P, Anandkumari C. Uv-Spectrophotometric Method Development and Validation for Determination of Azelnidipine in Pharmaceutical Dosage Form. Int J Pharm Pharm Sci. 2012;4(1):238-240.
7. Jenisha M, Shivangi P, Namrata P, Shreya S, Prasanna P, Upadhayay U. Stability indicating analytical method development and validation for estimation of azelnidipine. World J Pharm Res. 2016;5(2):831-847.
8. Prabhakar D, Sreekanth J, Jayaveera K. Method Development and validation of Azelnidipine by RP-HPLC. Int J ChemTech Res. 2017;10(10):418-423.
9. Megha G, Pratap D. RP-HPLC Method Development and Validation of Azelnidipine. Int J Pharm Sci Res. 2016;7(12):5111-5114.
10. Selvadurai M, Subramaniy P, Vijayan V. SIMPLE VALIDATION OF AZELNIDIPINE BY RP-HPLC METHOD. Rapports De Pharmacie. 2015;1(1):43-45.
11. Raveendra G, Jayachandra R, Himabindu V, Rampraksah. STABILITY INDICATING LIQUID CHROMATOGRAPHIC METHOD FOR THE SIMULTANEOUS DETERMINATION OF OLMESARTAN MEDOXOMIL AND AZELNIDIPINE IN COMBINED TABLET DOSAGE FORM. Indian J Pharm Sci Res. 2014;5(6).
12. Jayvadan P, Nilam P. Validated Stability-Indicating RP-HPLC Method for the Simultaneous Determination of Azelnidipine and Olmesartan in Their Combined Dosage Form. Sci Pharm. 2014; 82: 541–554.
13. Amin et al. Simultaneous Determination of Azelnidipine and Olmesartan Medoxomil in Pharmaceutical Dosage Forms by UFLC Method. J of PharmaSciTech. 2016;6(2).
14. Amin et al. Stability Indicating Ultra-Fast Liquid Chromatography Coupled with Photodiode Array Method for Simultaneous Estimation of Azelnidipine and Olmesartan Medoxomil in Pharmaceutical Formulations. J of PharmaSciTech. 2019;9.
15. Ramesh, et al. A novel method for the simultaneous determination of Azelnidipine and Olmesartan in Human plasma by using liquid chromatography-electro spray Ionization tandem mass spectrometry and application to a pharmacokinetic study. Int J of Pharm. 2017;7(3):111-124.
16. L. Ding et al. Determination of Azelnidipine in human plasma by liquid chromatography-electrospray ionization-mass spectrometry. J of Pharm and Biomedical Analysis.2007; 575–579.
17. International Conference on Harmonization (ICH), Validation of Analytical Procedures: Text on Validation of Analytical Procedures Q2A, 1994.