AQbD-Guided Development and Validation of RP-HPLC Method for Quantitative Estimation of Azelnidipine

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Abstract

The new method was developed and validated by quality by design for Azelnidipine in bulk and tablet forms in a simple, sensitive, appropriate, precise, reliable, and robust way using reverse-phase high-performance liquid chromatography techniques. To optimize chromatographic conditions, a Central Composite Design approach was applied. Analytical separation of Azelnidipine was performed using the HPLC system with Chromeleon 7.3 Software on Agilent's Zorbax C18 column. Analysis of separated samples was accomplished using UV/visible detection at 218 nm. For the new RP-HPLC method, the mobile phase consisted of a 70:30 (v/v) mixture of Phosphate Buffer: Acetonitrile and a flow rate of 1.0 mL/min. The retention time for the target compound was 3.6 min. The new method was valid due to its linearity, accuracy, precision, specificity, robustness, and stability. The linear model fitted (r2 = 0.998), accuracy (-2.0% RSD) and recovery (100.28%) results indicated the proposed approach had good reliability and predictability. A fast, precise, strong, and efficient QbD -empowered RP-HPLC technique of the estimation of Azalenidipine had been effectively created and validated.

Keywords: RP-HPLC, QbD, Azalenidipine, Method Development, Validation.

1. Introduction:

Azelnidipine is a 3rd generation dihydropyridine calcium channel antagonist used to treat elevated blood pressure levels (hypertension). Azelnidipine lowers your systolic and diastolic blood pressure more consistently than previous calcium channel antagonists (e.g., amlodipine) without inducing greater reflex elevations in your heart rate. Furthermore, Azelnidipine exhibits additional pharmacological properties which provide benefits beyond controlling high blood pressure and provide organs with potential protection (e.g., anti-atherosclerotic properties and antioxidant properties).[1-2]

Azelnidipine works by binding to the voltage-dependent L-type calcium channel located on the vascular smooth muscle cell surface to block calcium entry into the vascular smooth muscle cell, causing vascular smooth muscle cell relaxation (i.e., vasodilation). In vitro studies demonstrated that Azelnidipine blocks voltage-dependent barium (Ba^{2+}) currents in a dose-dependent manner, with a significantly longer duration of action compared to other calcium channel antagonists also blocking voltage-dependent Ba^{2+} currents.[3-4] Central composite design is an effective feature in optimization of important factors. CCD proposes the best variables value providing the best and desired response and establishing process conditions that are resilient to purposeful changes in factor settings. It is also associated with implying a mathematical model between the response and the critical variables therefore enabling the prediction of response with slight error passed to the response.[5-9]

The literature review was very encouraging as determination was done on drug using HPLC. [10-17]

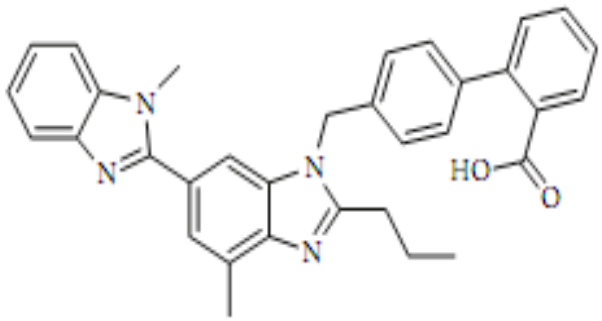

Fig 1 : Chemical structure of Azelnidipine

2. Experimental Method:

2.1 Materials:

The Arni Analytical Laboratory (Nashik) used Azelnidipine for analysis and extraction using HPLC-grade Acetonitrile, Methanol and the Thermo UltimateTM 3000 HPLC System.

The instruments used to develop, validate and analyse the entire project included a Thermo Ultimate 3000 HPLC System, the Chromeleon 7.3 software program and an Agilent Zorbax C18 column (150 x 4 mm, 5 μm). The Software Design Expert version 11.0 was used to create the CCD model.

The next step was to prepare the mobile phase.

2.2 Preparation of Buffer:

To create a 0.01 M solution of Ammonium Dihydrogen Orthophosphate, the following steps need to be followed:

1. Prepare the mobile phase by combining acetonitrile and buffer in a ratio of 30% buffer and 70% acetonitrile.

2. To prepare the analytical test samples use the following method:

2) Add enough of the mobile phase to fill the flask to 100 ml.

3) From the first solution you created above, take 5 ml and add it to another volumetric flask containing 50 ml of mobile phase containing acetonitrile and buffer in a 70:30 ratio to prepare a second solution.

4) Obtain an average weight for 10 tablets by weighing 10 tablets together and dividing their total weight by 10. # Once the average weight is determined, break the tablets into a fine powder and weigh out enough powder so that it will equal the average weight of the tablets in the volumetric flask (e.g., if the average weight of tablet = 500 mg, weigh out 500 mg of tablet powder).

1. Add mobile phase (same as for previous solution) to volumetric flask (60 ml), sonicate for 10 minutes, dilute to 100 ml with mobile phase.

2. Filter using Whatman filter paper, No. 41.

3. Dilute 5 ml filtered sample to 50 ml with mobile phase.

2.3 Review of Preliminary Experiments:

The scouting of the method began by designing a minimal amount of trial runs to try to improve the separation by first defining acceptable conditions for mobile phase system to achieve good resolution of peaks for each of the drugs tested.

At this point, it was discovered that buffer and acetonitrile gave almost equally good resolution for both drugs; therefore the mobile phase for the current study was set as a 70:30 Buffer/Acetonitrile.

Once the composition of the mobile phase was established, the two factors (percentage of acetonitrile and flow rate) that could potentially affect the analyzed response variables were then evaluated.

2.4 Optimization of the design:

The Central Composite Design is being used for its high efficiency in reducing the number of experiments needed to be conducted. For k factors being optimized with a CCD, factorial runs, (2k) axial experiments spaced symmetrically (+, -) along each of the variable axes, as well as at least one centre point (5), would be performed. A CCD for 3 factors as a rotatable CCD ($\alpha = 1.68$) was created in order to find the best level of desired responses at 5 different levels ($-\alpha$, -1, 0, +1, and $+\alpha$) for a total of approximately 13 random experiments (5 of which were center points). An important goal of the analysis of data generated by an HPLC system is to allow for fast analysis and the ability to separate peaks satisfactorily. After identifying which independent or chromatographic factors affect the response of the HPLC system; CCD will allow for further optimization of the independent test variables (i.e., factors). CCD will have the additional or star points for 2-factor experiments with a configuration of factorial rotations or orthogonality that can approximate a 2nd order polynomial equation using the techniques developed by Box and Wilson. Therefore it will generate coefficients for a quadratic regression equation enabling the prediction of on-linear responses for. Variables that is associated with the tests that were conducted. The design of the CCD was completed in accordance with Design Stat-Ease Inc., out of Minneapolis, MN, USA (Expert version 11). A total of thirteen trials were conducted according to the previously described CCD matrix. Please refer to Table 1 for the independent and response variables that were used. For reference, Table 2 contains the levels of each independent variable.

Table 1: Central composite design showing 13 randomized trail runs

Run	% Acetonitrile	Flow rate	Retention time Azelnidipine
1	70	1	3.694
2	70	1	3.688
3	70	1.2	3.476
4	70	0.7	4.046
5	65	0.8	4.686

6	75	1.2	3.243
7	70	1	3.683
8	62	1	4.046
9	70	1	3.696
10	70	1	3.694
11	77	1	3.243
12	75	0.8	3.663
13	65	1.2	3.711

Table 2 : Experimental factors and levels used in central composite design

Name	**Low (-1)**	**Medium (0)**	**High (+1)**
Independent factor			
A : Acetonitrile %	65	70	75
C :Flow rate	0.8	1	1.2
Dependent factor			
R1	Retention time of Azelnidipine		

Table 3:Experimental results and optimized conditions

Parameters	**Description**
Program	Isocratic
Mobile phase	Phosphate buffer: Acetonotrile (70:30 v/v)
Wavelength	218 nm
Inoculation volume	20 µL
Flow rate	1 mL/min

2.5 Method validation:

This procedure passed validation according to ICH (Q2R2) standards and included demonstrations of System suitability, Linearity, LOD, LOQ, Accuracy, Precision and Robustness.[18]

1. Any expected interfering substances
2. Such interfering substances in matrixes typical of the testing of interest.

a.System Suitability:

The chromatographic parameters used for the system suitability test were the relative standard deviation (RSD) values for the peak regions, tailing factors, theoretical plates, and retention durations.

b.Specificity:

The API's specificity was demonstrated via the study of both commercial formulations (as blended) and pure API samples analyzed via HPLC vs their respective baseline or blank ("placebo") HPLC…is there anything else to add, if you have this information, please include it so you can properly justify there is indeed some real-world interest in these products (i.e. Isol, Pure, Actuals, etc).

c.Precision:

When measuring precision, you need to consider how reliably an analytical method is going to produce similar results if repeated with the same control sample. To assess this reliability, you'll want to calculate the relative standard deviation of the area under the curve and retention times (RT) from the control sample's AUC/RT data. The first step in determining how precise an analytical process is to assess how uniform the data is (uniformity) from AUC/RT characteristics when testing/analysing a sample of material for multiple batches processed under identical conditions.

d.Intermediate Precision:

In contrast, intermediate precision refers to the variation observed between different laboratories that have performed an identical test method and sample under identical conditions for a minimum of one week. In general, intermediate precision is defined very much the same way as repeatability, in that they demonstrate the same analysis was conducted multiple times by various laboratories using the same sample and condition and over a brief time period.

e. Accurateness of Test Method:

The precision of a test method indicates how closely test results from the same test method are to the true value for that particular test method. The accuracy is expressed statistically and is calculated as a percentage of the amount of the sample test method that is returned back to the original known amount. In the test method studied, the accuracy is calculated based on percentage basis to show a 100% accuracy by also using three (3) different average test samples, which were tested to: 80%; 100%; and 120%.

f. Test Method's Linearity:

Linear correlation between the five (5) concentration levels (for the test method is equal to 80% and 120% linearity – all data points in relation will fall onto a linear progression as explained below). A linear regression analysis will be used to determine if regression lines (calibration curves) will contact or intersect with each data point if test result data is plotted on x and y axes of the regression line.

g. Quantitative and Qualitative Thresholds:

Qualitative threshold is equal to the lowest observed concentration that the chromatographic technique can detect, whereas Quantitative threshold is equal to the lowest quantity (volume of liquid) that can be detected with the test method.

h. Solution Stability Testing:

Stability testing of the stock standard solution of Azelnidipine included a 24 hours expiration test of Azelnidipine assay method vs. testing Azelnidipine 24 hours after preparation of stock standard solution at room temperature in completely sealed volumetric flasks.

3. Results and Discussions:

3.1 Design and Optimization of Test Method Design:

At last, two factors were evaluated, (the flow rate and the composition of the mobile phase) that affect these two responses through the selection of acetonitrile; an examination of the spectrum of the drug at (200 nm - 400nm) indicated that the maximum wavelength at which Azalenidipine is detected is (218 nm); therefore a detection wavelength for the chromatographic assay was set at 218 nm. Chromatographic separation using Agilent Zorbax C18 column (250 mm x 4.6mm; 5m); a 250mm x 4.6mm (5um) column supported by a Phosphate buffer/Acetonitrile mix (70:30 v/v) at a flow rate of 1.0 mL/min yielded chromatograms with two variables produced the highest precision for the responses defined above; but other factors were fixed throughout all experiments. A central composite design was used to identify the relationships between the two independent factors evaluated by five levels and 13 total experimental runs included in the analysis. Table 3 shows a quadratic regression model developed by the Software of Design Experts; includes 2 independent variable, with 2 dependent variables and results of an Analysis of Variance determining whether the independent factors give a significant result to the dependent variable; based on the ($p < 0.0001$) level of significance and/or (F-value) and supporting evidence..

Figure 2 illustrates the interaction effect of critical factors on retention time for the compound Azelnidipine utilizing. In this study, three distinct variables were evaluated to ascertain if there is a correlation between them; therefore, results from the analysis are presented below using the same method. It is apparent from the data that an increase in flow rate or an increase in the concentration of acetonitrile within the mobile stage consequences in a corresponding reductions in the holding time.

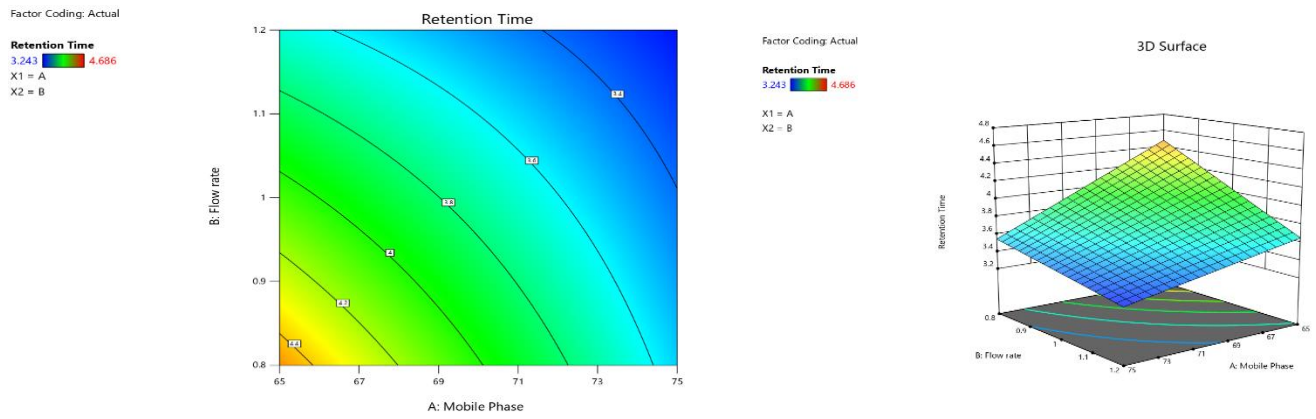

Fig 2 : 2D & 3 D plot for the interaction effect of critical factors on retention time of Azelnidipine

In ***Fig 2*** the 2D & 3D plots exhibit relationship between each of the dependent variable with the two independent variables. The plots clearly suggested that decreases retention time with increase in flow rate and mobile phase acetonitrile concentration.

Table 4: Statistical parameter obtained from ANOVA

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1.54	3	0.5150	38.76	< 0.0001	significant
A-Mobile Phase	0.8624	1	0.8624	64.90	< 0.0001	
B-Flow rate	0.6056	1	0.6056	45.57	< 0.0001	
AB	0.0770	1	0.0770	5.80	0.0394	
Residual	0.1196	9	0.0133			
Lack of Fit	0.1195	5	0.0239	823.98	< 0.0001	significant
Pure Error	0.0001	4	0.0000			
Cor Total	1.66	12				

Table 4 shows the model's significance because the F=38.76 means there is less than 0.01% chance of getting an F=38.76 or more from a random draw. If the p-value is less than 0.0500 for any term in the model, it is evidence of that term's validity.

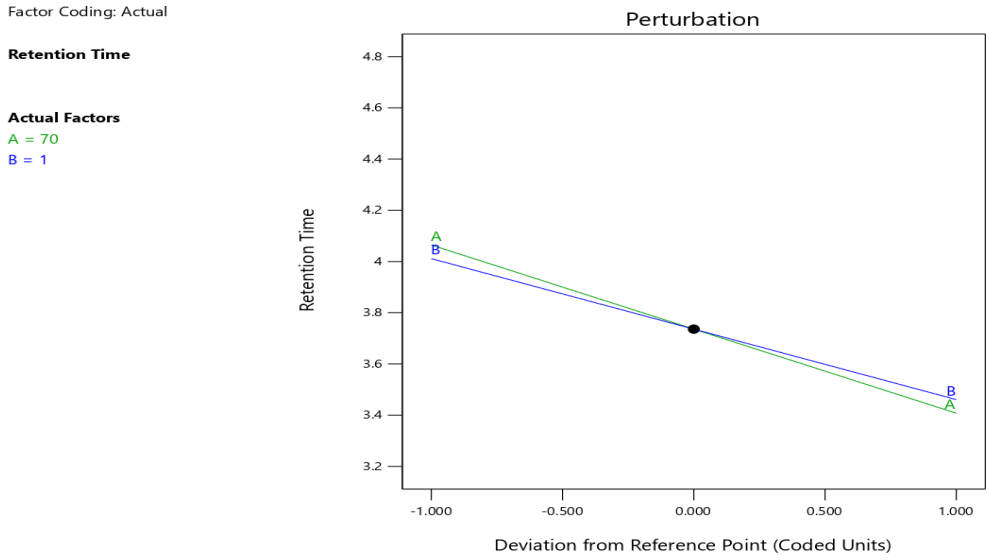

Fig 3: Perturbation plot show effect of factor on responses

Figure 3 shows that both the amount of % acetonitrile (ACN) present in the mobile phase and flow rates correlate negatively with regard to retention time of Azelnidipine. Consequently, both flow rates or mobile phase compositions of _('ACN')/ will be inversely related to the retention times of Azelnidipine, therefore exhibiting a negatively correlated relationship. acetonitrile increases, retention times decrease.

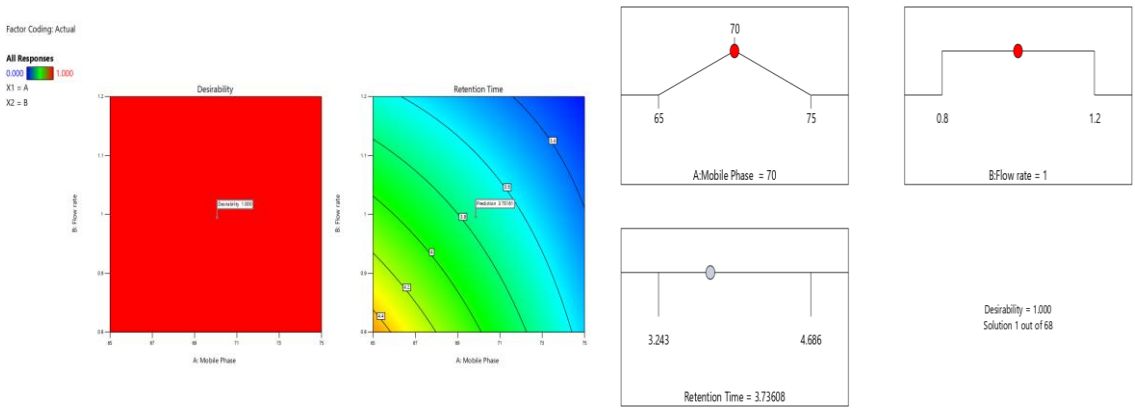

Fig 4.Graphical representation of constraints accepted for determination of global desirability and obtained optimal condition

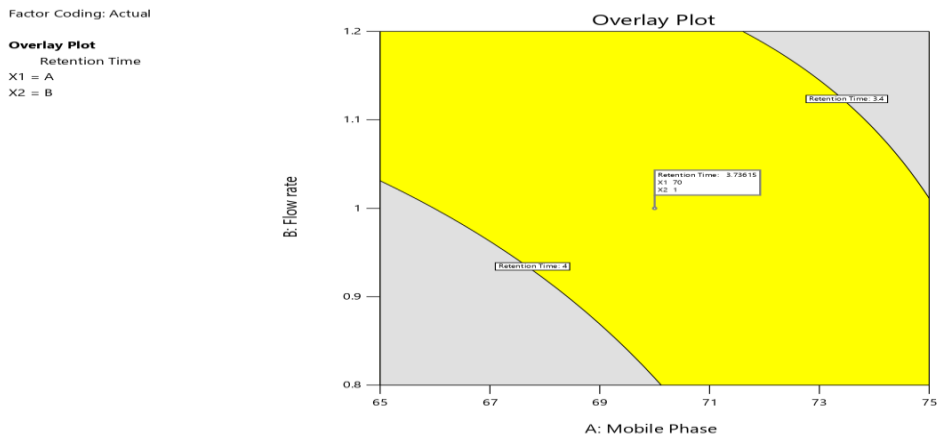

Fig 5. Overlay plot showing the sweet spot where the desired response meet

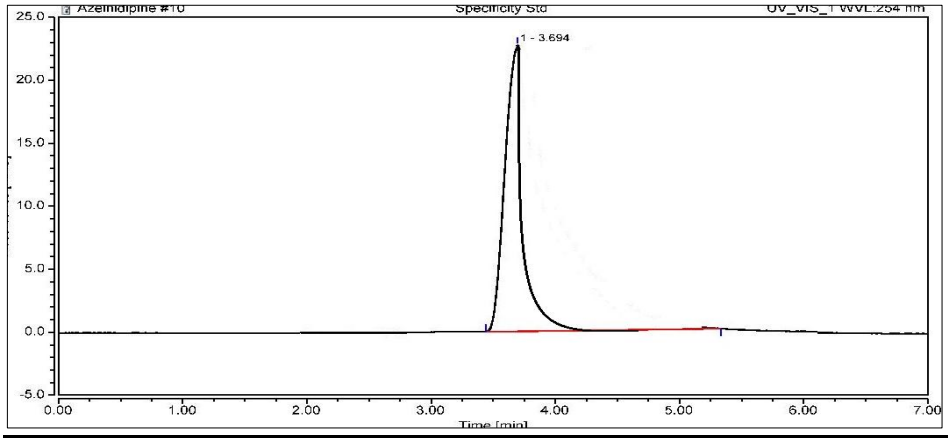

Fig 6.Optimized chromatogram of Azelnidipine

3.2 Validation Parameter:

a) System Suitability:

According to the analysis of the system suitability analysis conducted on the standard deviation using the maximum area, the result for all sample analyses (individually) was less than 2.0; all samples had an average number of theoretical plates of greater than 2000 for each method used in this study. The trailing factor was less than 2, while the resolution of samples was less than 2.0. Therefore, the data obtained from the chromatographic method used in this study indicated that sufficient performance exists to permit for quantitative analysis based upon the determination of system suitability parameters through validation parameters (system suitability validation)**.** was suggested in Table 5.

Table 5 : System suitability result for Azelnidipine.

Sr. No.	AreaArea (mAU·s)	Retention Time (min)	Tailing Factor (Tf)	Theoretical Plate (Tp)
1	10.448	3.703	1.4	5500
2	10.347	3.701	1.42	5520
3	10.551	3.703	1.41	5480
4	10.439	3.694	1.43	5510
5	10.308	3.699	1.39	5490
6	10.408	3.696	1.41	5530
Average	**10.417**	**3.699**	**1.410**	**5505.000**
STDEV	**0.085**	**0.004**	**0.014**	**18.708**
RSD	**0.82**	**0.10**	**1.00**	**0.34**
Limit	**NMT 2.0%**	**NMT 1.0%**	**NMT 2.0%**	**NLT 2000**

b) Specificity

There were no extra or co-eluting peaks noticed in the chromatograms at observed retention time of the analytes. In this way, the mountains were regarded as pure, and this is the reason why the approach is specific. No interference was observed in Standard as well as Sample by Blank.

Table 6 : Specificity result for Azelnidipine

Sr.No.	Azelnidipine	Retention Time
1	Blank	0.000
2	Standard Solution	3.694
3	Sample Solution	3.688

c) Precision

The specificity is given as Relative Standard deviation (RSD) at the range of quantification of one experiment where the standards are assayed in replicate (Intraday) and a series of experiments where standards are assayed in many experiments (Interday). The data of intraday and interday is pointed out in Table 7 and 8 respectively.

Table 7: Intraday Precision Result for Azelnidipine.

Standard Solution	Area		
	5 ppm	10 ppm	15 ppm
No. Of Inj.	Azelnidipine	Azelnidipine	Azelnidipine
1	3.481	6.684	9.384
2	3.467	6.629	9.365
3	3.419	6.648	9.328
4	3.485	6.638	9.348
5	3.426	6.622	9.365
6	3.451	6.649	9.355
Average	**3.455**	**6.645**	**9.358**
STDEV	**0.028**	**0.022**	**0.019**
RSD	**0.81**	**0.33**	**0.20**

Table 8: Interday Precision Result for Azelnidipine.

Standard Solution	Area		
	5 ppm	10 ppm	15 ppm
No. Of Inj.	Azelnidipine	Azelnidipine	Azelnidipine
1	3.219	6.418	9.612
2	3.242	6.427	9.627
3	3.275	6.437	9.674
4	3.236	6.495	9.647
5	3.245	6.482	9.621
6	3.216	6.412	9.639
Average	**3.239**	**6.445**	**9.637**
STDEV	**0.021**	**0.035**	**0.022**
RSD	**0.66**	**0.54**	**0.23**

d) Accuracy

The estimated percentage recovery of the Azelnidipine following each injection of each concentration is 99.98, 100.19, 100.68 and the average recovery of 100.10 which falls within the acceptance criteria is 100 thus the methodology used to determine the percentage assay of Azelnidipine is correct.

Table 9: Accuracy result for Azelnidipine

Sr.No.	Level	mg of drug spiked	Area	mg of drug Recovered	% Recovery
1	80%	0.040000	8.728	0.040749	99.98
2	100%	0.050000	10.933	0.050182	100.19
3	120%	0.060000	13.184	0.060154	100.68
Average					100.10
Std.dev					0.91
%RSD					0.91

e) Linearity

Calibration curves created from five injections demonstrated linearity at 80 to 120 percent. Results obtain for each of the five injections fall within this range and the standard curve's correlation coefficient is 0.998, indicating that the method is linear across the specified range.

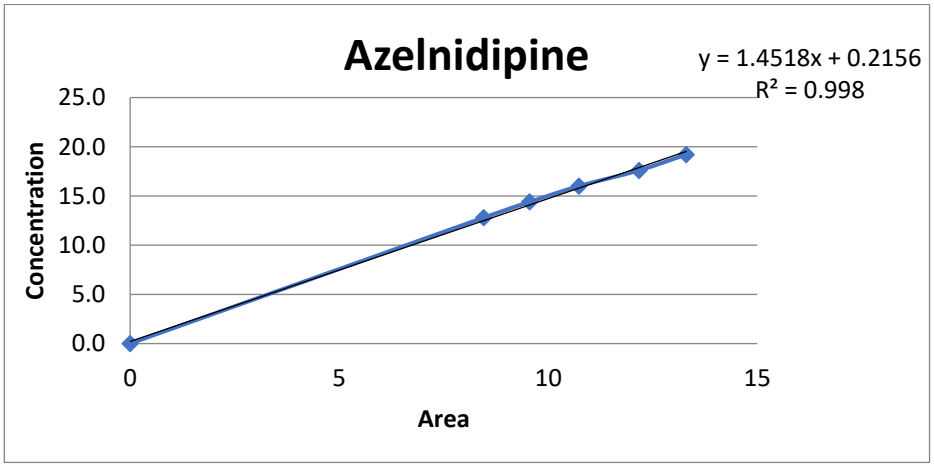

Fig 4 : Calibration curve for Azelnidipine

Table 10 : Linearity result for Azelnidipine

Sr.No.	Concentration in ppm	Concentration of Solution(%)	Diluted to ml	Area
1	12.8	80	50	8. 456
2	14.4	90	50	9.554
3	16.0	100	50	10.733
4	17.6	110	50	12.171
5	19.2	120	50	13.300

f) Detection limit, Quantitation limit and Solution Stability.

Quantifiable values were as 0.15 mg/ml for detection limits and as 0.46 mg/ml for quantifying limit. % relative standard deviation was < 2, therefore stability at rt ≤ 4 hours for Azelnidipine 16 mg solution sample.

Table 11: Limit of Detection Calculation.

Parameter	Value
Formula	(3.3 × STDEV) / Slope
Standard Deviation (STDEV)	0.104
Slope (S)	2.2684
Calculation	(3.3 × 0.104) / 2.2684
LOD Result	**0.15 ppm**

Table 12: Limit of Quantification Calculation.

Parameter	Value
Formula	(10 × STDEV) / Slope
Standard Deviation (STDEV)	0.104
Slope (S)	2.2684
Calculation	(10 × 0.104) / 2.2684
LOQ Result	**0.46 ppm**

Table 13: Solution stability result for Azelnidipine

Sr.No.	Hours	Area	% Assay	%Assay Difference
1	Initial	7.000	100.34	-----
2	8 Hour	5.199	73.30	27.04
3	24 Hour	5.154	72.99	27.36

g) Robustness:

The stability of the method created was tested to evaluate robustness by changing the minor intentional flow rate, ±0.5 mL/min, and the configuration of mobile phase, ±2%, throughout the study. There were no significant differences in either peak area or retention time and the percent RSD's are deemed acceptable. Thus, the results confirm that the method is robust and the robustness data is included in Table 12.

Table 14: Robustness results of Azelnidipine

Sr.No	Precision	Flow rate	Composition -1	Composition -2	
		0.9ml/min	1.1 ml/min		
Std	10.462	10.624	10.248	10.595	10.157
Sample	10.485	10.618	10.234	10.597	10.172
% Assay	100.22	99.94	99.86	100.02	100.15
Difference	-	0.27	0.35	0.20	0.07

Table 15: Validation results of Azelnidipine

Parameters	Azelnidipine
Specificity	There was no interference observed in Standard & Sample due to Blank
Linearity	
Range (µg/mL)	80-120 %
Y-Intercept	0.2156

Slope	2.2684
Correlation coefficient	0.998
Accuracy	
% recovery	100.28
Precision	
System Precision	RSD - 0.98 %
Method Precision	% Assay - 100.48
Intermediate precision	% Assay – 98.92
LOD (ug/ml)	0.15
LOQ (ug/ml)	0.46
Stability of solution	RSD < 2%

Conclusion:

After introducing the developer of the HPLC method for Azelnidipine, a detailed description of how this analytical technique works is provided in the following paragraph. This methodology includes the combination of multivariate regression analysis and chromatography. For instance, a central composite design (CCRD) was utilized for optimizing chromatographic conditions (CCs) and determining if the effects were linear or quadratic. Azelnidipine's method description will help us understand the process that can validate .

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Informed consent : None

Data availability : None

References :

1. Wellington K, Scott LJ. Azelnidipine. Drugs. 2003;63(23):2613-2621; discussion 2623-2624. doi:10.2165/00003495-200363230-00004. PMID:14636080.
2. Shewale VU, Aher SS, Saudagar RB. Azelnidipine: a review on therapeutic role in hypertension. J Drug Deliv Ther. 2019 Jun;9(3).
3. Tomoda T, Aishima M, Ito Y, Teramoto N. The actions of azelnidipine, a dihydropyridine-derivative Ca antagonist, on voltage-dependent Ba^{2+} currents in guinea-pig vascular smooth muscle. Br J Pharmacol. 2006;149(6):786-796. doi:10.1038/sj.bjp.0706919. PMID:17016499; PMCID:PMC2014663.
4. Sada T, Saito H. Pharmacological profiles and clinical effects of azelnidipine, a long-acting calcium channel blocker. Folia Pharmacol Jpn. 2003;121:253-262
5. Kavitha D, Sahoo SK, Prasad VR, Nagamani M, Ch B. Quality by design development and validation of RP-HPLC method for determination of metformin and sitagliptin in bulk and pharmaceutical dosage form. Int J Pharm Sci Rev Res. 2017;5:34-39.
6. Sebaiy MM, El-Adl SM, Baraka MM, Hassan AA, El-Sayed HM. Quality by design approach for development and validation of an RP-HPLC method for simultaneous estimation of xipamide and valsartan in human plasma. BMC Chem. 2022;16:1-13.
7. Jatte KP, Masne DD, Khachane MA, Charde MS. Quality by design approach in analytical method development: a review. Int J Pharm Pharm Res. 2021;21:1-19.
8. Nadpara NP, Thumar RV, Kalola VN, Patel PB. Quality by design (QbD): a complete review. Int J Pharm Sci Rev Res. 2012;17:20-28.
9. International Conference on Harmonisation. Pharmaceutical development Q8(R2). ICH harmonised guideline. 2009. p. 1-28.
10. Sahoo S, Meyyanathan SN, Gowtham GP, Balaji R, Vinay B, Reethu Sree C, et al. Development and validation for the estimation of azelnidipine in its formulation by HPLC. J Pharm Negative Results. 2022;13
11. Spandana KVL, Subhashini NJP. Telmisartan and azelnidipine quantification employing HPLC stratagem; stability investigation on telmisartan and azelnidipine. Int J Appl Pharm. 2022;14(1). doi:10.22159/ijap.2022v14i1.43321.

12. Goyal P, Jaimini M. Development and validation of stability-indicating RP-HPLC method for quantitative estimation of metoprolol succinate and azelnidipine from synthetic mixture. J Pharm Negative Results. 2023;14(3):772-779. doi:10.47750/pnr.2023.14.03.101.
13. Raimalani J, Kotadiya R. Gradient reversed-phase HPLC method for the quantitation of azelnidipine and chlorthalidone in a fixed-dose synthetic mixture. J Chem Metrol. 2023;2:238-248. doi:10.25135/jcm.100.2311.2977.
14. Sahoo S, Meyyanathan SN, Gowtham GP, Balaji R, Vinay B, Reethu Sree C, et al. Development and validation for the estimation of azelnidipine in its formulation by HPLC. J Pharm Negative Results. 2022;13
15. Ivanescu D, et al. Validated stability-indicating RP-HPLC method for the simultaneous determination of azelnidipine and olmesartan in their combined dosage form. Sci Pharm. 2014;82(3):541-554. doi:10.3797/scipharm.1312-14.
16. Puchhakayala Y, Sammaiah G. Method development and validation for the estimation of azelnidipine in bulk form and marketed pharmaceutical dosage form by using RP-HPLC. Int J Allied Med Sci Clin Res. 2023;11(2):181-191. doi:10.61096/ijamscr.v11.iss2.181-191.
17. Patil SD, Bachhav RS, Udavant PB, Ahirrao S, Bhambere DS. Development of a stability-indicating RP-HPLC method for azelnidipine. NVEO J. 2024;8(5).
18. International Council for Harmonisation. Validation of analytical procedures Q2(R2). ICH harmonised guideline. 2022. p. 1-38.