Scienxt Journal of Pharmacy and Drug Research (SJPDR)

Method development and validation for the estimation of azelnidipine in bulk form and marketed pharmaceutical dosage form by using rp-hplc

Yamuna Puchhakayala[1], Gade Sammaiah*[2]

[1,2] Department of Pharmaceutical Chemistry, University College of Pharmaceutical Sciences (UCPSC) Kakatiya University, Hanamkonda, Warangal, Telangana

Corresponding Author: Gade Sammaiah

ABSTRACT

The present work includes a simple, economic, rapid, accurate and precise isocratic RP-HPLC method development for estimation of Azelnidipine in bulk form and its marketed formulation. Estimation was done at 286nm which was found to be λmax of Azelnidipine. The simple, selective, isocratic RP-HPLC method for Azelnidipine was developed on Phenomenex Luna (C_{18}) RP Column; 250 mm x 4.6 mm, 5µm with a mobile phase of Phosphate Buffer (pH-4.6) and Methanol were taken in the ratio of 65:35% v/v at a flow rate of 1.0 ml/min and detection wavelength 286nm. The developed method was validated successfully according to ICH Q2 (R1) guidelines. The chromatographic methods showed a good linear response with r2 values of 0.9995. The percentage relative standard deviation for method was found to be less than two, indicating that the methods were precise. The mean percentage recovery was for RP-HPLC method was 100.437%. From the results it could be concluded that both the developed method was specific, selective and robust. The method could be successfully applied for analysis of Bulk form and Marketed formulation of Azelnidipine.

Keywords: Azelnidipine, RP-HPLC, Method Development, Validation, ICH Guidelines.

INTRODUCTION

Azelnidipine is a dihydropyridine calcium channel blocker. It ismarketed 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. Azelnidipine[1] is a vasodilator that induces a gradual decrease in blood pressure in hypertensive patients. Unlike other membersof its drug class, Azelnidipine does not induce reflex tachycardia due to vasodilation. This is likely due to the fact that it elicits a gradual fall in blood pressure. It also exhibits a prolonged hypotensive effect and has been shown to have a strong anti-arteriosclerotic action in vessels due to its high
affinity for vascular tissue and antioxidative activity. Clinical studies have demonstrated that Azelnidipine[2] markedly reduced heart rate and proteinuria in hypertensive patients by inhibiting sympathetic nerve activity. Azelnidipine has also been confirmed to have cardio-protective, neuroprotective, and anti- atherosclerotic properties, and has also been found to prevent insulin resistance. Azelnidipine[3] 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 Ca2+ 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[4]. The IUPAC Name of Azelnidipine is 3-O-(1-benzhydrylazetidin-3-yl) 5-O-propan-2-yl 2-amino-6-methyl-4-(3-nitro phenyl)-1, 4-dihydro pyridine-3, 5-dicarboxylate. The Chemical Structure of Azelnidipine as shown in following Literature survey[34-38] revealed that Azelnidipine was determined in bulk form and pharmaceutical dosage forms by RP-HPLC as well asin biological fluids using liquid chromatography and liquid chromatography mass spectrometric methods.
In the present work the authorshave developed a simple, rapid, precise, accurate and robust stability indicating liquid chromatographic method for the determination ofAzelnidipine in bulk and pharmaceutical dosage forms as per ICH guidelines.

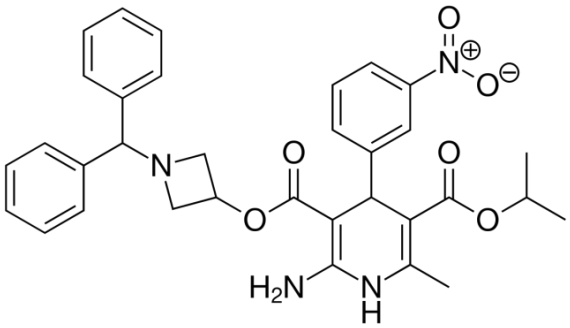

Fig 1: Chemical Structure of Azelnidipine

Experimental

Table 1: List of Instrument used

S. No.	Instruments/Equipment/Apparatus
1.	HPLC with Empower2 Software with Isocratic with UV-Visible Detector (Waters).
2.	ELICO SL-159 UV – Vis spectrophotometer
3.	Electronic Balance (SHIMADZU ATY224)
4.	Ultra Sonicator (Wensar wuc-2L)
5.	Thermal Oven
6.	Symmetry ODS RP C18,5µm, 15mm x 4.6mm i.d.
7.	PH Analyzer (ELICO)
8.	Vacuum filtration kit (BOROSIL)

Chemicals / reagents used

Table 2: List of Chemicals used

S.No.	Name	Specifications		Manufacturer/Supplier
		Purity	Grade	
1.	Doubled distilled water	99.9%	HPLC	Sd fine-Chem ltd; Mumbai
2.	HPLC Grade Water	99.9%	HPLC	Sd fine-Chem ltd; Mumbai
3.	Methanol	99.9%	HPLC	Loba Chem; Mumbai.
4.	Hydrochloric Acid	99.9	A.R.	Sd fine-Chem ltd; Mumbai
5.	Acetonitrile	99.9%	HPLC	Loba Chem; Mumbai.
6.	Sodium Hydroxide	99.9	A.R.	Sd fine-Chem ltd; Mumbai
7.	Ethanol	99.9	A.R.	Sd fine-Chem ltd; Mumbai
8.	Octanol	99.9	A.R.	Sd fine-Chem ltd; Mumbai

Method Development and its Validation for Azelnidipine By RP-HPLC

Selection of Wavelength

The standard & sample stock solutions[5] were prepared separately by dissolving standard & sample in a solvent in mobile phase diluting with the same solvent. (After optimization of all conditions) for UV analysis. It scanned in the UV spectrum in the range of 200 to 400nm. This has been performed to know the maxima of Azelnidipine, so that the same wave number can be utilized in HPLC UV detector for

Further dilution was done by transferring 1ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase.

Optimization of Chromatographic Conditions

The chromatographic conditions[7] were optimized by different means. (Using different column, different mobile phase,

different flow rate, different detection wavelength & different diluents for sample preparation etc.

Table 3: Summary of Process Optimization

Column Used	Mobile Phase	Flow Rate	Wave length	Observation	Result
Xterra ODS (C_{18}) RP Column, 150 mm x 4.6 mm, 5µm	Methanol : Acetonitrile: Water = 40:30:30	0.8ml/min	286nm	Very Low response	Method rejected
Zorbax ODS (C_{18}) RP Column, 250 mm x 4.6 mm, 5µm	Methanol : Acetonitrile = 70 : 30	0.9ml/min	286nm	Low response	Method rejected
Develosil ODS (C_{18}) RP Column, 150 mm x 4.6 mm, 5µm	Acetonitrile: Methanol = 60 : 40	1.0ml/min	286nm	Tailing peaks	Method rejected
Phenomenex Luna (C_{18}) RP Column, 250 mm x 4.6 mm, 5µm	Phosphate Buffer (pH-5.2) : Methanol = 80:20	1.0ml/min	286nm	Resolution was not good	Method rejected
Phenomenex Luna (C_{18}) RP Column, 250 mm x 4.6 mm, 5µm	Phosphate Buffer (pH-3.8) : Methanol = 55:45	1.0ml/min	286nm	Tailing peak	Method rejected
Phenomenex Luna (C_{18}) RP Column, 250 mm x 4.6 mm, 5µm	Phosphate Buffer (pH-4.6) : Methanol = 65:35	1.0ml/min	286nm	Nice peak	Method accepted

Preparation of Mobile Phase

650ml of prepared phosphate buffer and 350ml of HPLC Grade Methanol were mixed well and degassed in ultrasonic water bath for 15 minutes. The solution was filtered through 0.45 µm filter under vacuum filtration.

Analytical Method Validation

Technique validation[8] can be characterized according to ICH[33] as," Establishing recorded confirmation, which gives a high level of affirmation that a particular action will reliably create a coveted outcome or item meeting its foreordained details and quality attributes".

Specificity/Selectivity

The terms selectivity and specificity[9] are frequently utilized conversely. As indicated by ICH, the term particular for the most part alludes to a strategy that delivers a reaction for a solitary analyte just while the term specific alludes to a technique which gives reactions to various substance elements that might be recognized from one another. In the event that the reaction is recognized from every other reaction, the technique is said to be specific. Since there are not very many techniques that react to just a single analyte, the term selectivity is typically more suitable.

Linearity

Linearity[10] of an investigative technique is its capacity (inside an offered extend) to get test results which are specifically relative the focus (sum) of analyte in the example. A straight relationship ought to be assessed over the scope of the explanatory system. It might be exhibited straightforwardly on the medication substance (by weakening of a standard stock arrangement) as well as partitioned weighing of engineered blends of the medication item segments, utilizing the proposed strategy.

Range

Range[11] is the interim between the upper and lower centralization of the analyte in the example for which it has a reasonable level of exactness, precision and linearity.

Accuracy

Precision ought to be evaluated on tests (tranquilize substance/medicate item) spiked with known measures of polluting influences. In situations where it is difficult to get tests of specific polluting influences as well as debasement items, it is viewed as worthy to look at results gotten by a free strategy. The reaction factor of the medication substance can be utilized. It ought to be clear how the individual or aggregate polluting influences are to be resolved e.g., weight/weight or zone percent, in all cases as for the major analyte. Exactness[12] ought to be surveyed utilizing at least 9 judgments over at least 3 fixation levels covering the predefined run (e.g. 3 fixations/3 reproduces every one of the aggregate scientific technique).

Precision

Precision[13] is the proportion of how shut the information esteems are to one another for a progression of estimations under the same explanatory conditions acquired from various inspecting of the same homogeneous example.

Limit of Detection

Breaking point[14] of identification is the least convergence of analyte in an example which can be recognized, yet not really quantitated, as a correct an incentive under the expressed trial conditions.

A. In view of Visual Evaluation

B. In view of Signal-to-Noise

C. In view of the Standard Deviation of the Response and the Slope

As far as possible (DL) might be communicated as:

$$DL = \frac{3.3\ \sigma}{S}$$

Where,
σ = the standard deviation of the reaction
S = the slant of the adjustment bend

Limit of Quantification

Breaking point of measurement is the most reduced centralization of analyte in an example which can be quantitatively decided with satisfactory exactness and precision under the expressed trial conditions. A few methodologies for deciding as far as possible will be conceivable, contingent upon whether the technique is a non-instrumental or instrumental. Methodologies other than those recorded beneath might be adequate.
A. In light of Visual Evaluation
B. In light of Signal-to-Noise Approach
C. In light-weight of the quality Deviation of the Response and therefore the Slope
As way as doable (QL[15]) may well be communicated as:

$$LOQ = \frac{10\sigma}{S}$$

Where,
σ = the quality deviation of the reaction
S = the slope of the standardization curve
The slope S is also calculable from the standardization curve of the analyte.

Ruggedness

Ruggedness[16] isn't tended to in the ICH archives[32]. Its definition has been supplanted by reproducibility, which has indistinguishable importance from roughness, characterized by the USP as the level of reproducibility of results acquired under an assortment of conditions, for example, unique research facilities, examiners, instruments, ecological conditions, administrators and materials. Roughness is a proportion of reproducibility[17] of test results under typical, expected operational conditions from research center to lab and from examiner to expert. Toughness is controlled by the examination of aliquots from homogeneous parts in various labs.

Robustness

ICH characterizes strength as a proportion of the technique's ability to remain unaffected by very little, but assume varieties in strategy parameters and offers a symptom of its unwavering quality amid typical utilization. Sincerity will be incompletely bonded with nice framework appropriateness[18] determinations. The assessment of strength need to be thought-about amid the development stage and depends upon the type of system below investigation.

RESULTS AND DISCUSSION

Development of a Method
Selection of Wavelength

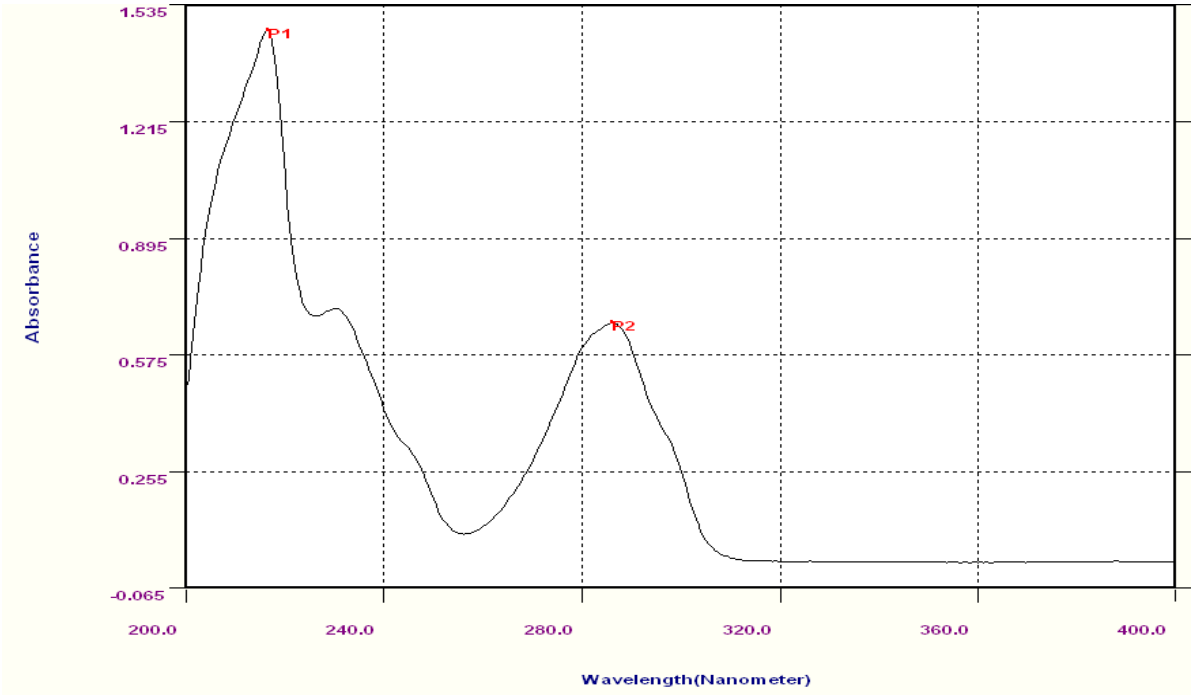

Fig 2: UV Spectrum for Azelnidipine

While scanning the Azelnidipine solution we observed the maxima at 286nm. The UV spectrum has been recorded on ELICO SL-159 make UV – Vis spectrophotometer model UV-2450.

Summary of Optimized Chromatographic Conditions

The Optimum Chromatographic conditions[19] obtained from experiments can be summarized as below:

Table 4: Summary of optimized Chromatographic Conditions

Mobile phase	Phosphate Buffer (pH-4.6) : Methanol = 65:35%
Column	Phenomenex Luna (C_{18}) RP Column, 250 mm x 4.6 mm, 5µm
Column Temperature	Ambient
Detection Wavelength	286 nm
Flow rate	1.0 ml/ min.
Run time	10 min.
Temperature of Auto sampler	Ambient
Diluent	Mobile Phase
Injection Volume	20µl
Type of Elution	Isocratic
Retention time	4.778 minutes

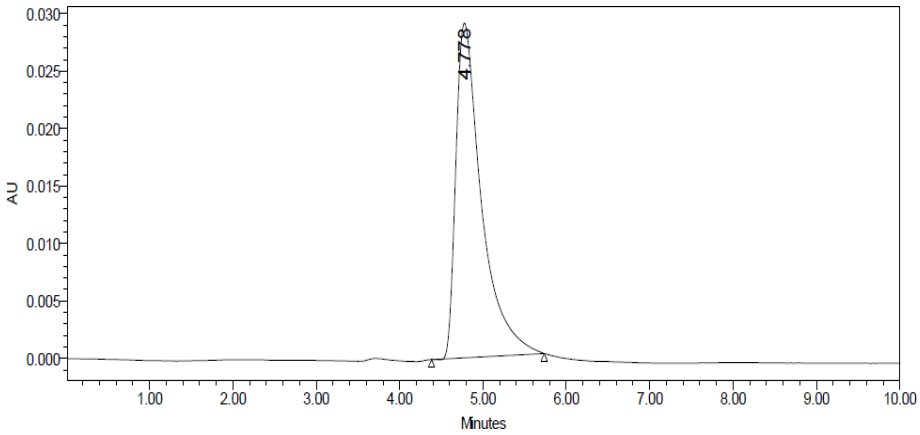

Fig 3: Chromatogram of Azelnidipine in Optimized Condition

The selected and optimized mobile phase was Phosphate Buffer (pH-4.6): Methanol = 65:35% and conditions optimized were flow rate (1.0 ml/minute), wavelength (286nm), Run time was 10 mins. Here the peaks were separated and showed better resolution, theoretical plate count and symmetry. The proposed chromatographic conditions were found appropriate for the quantitative determination of the drug.

Method Validation

Once the chromatographic and the experimental conditions were established, the method was validated by the determination of the following parameters such as specificity, system suitability, linearity, precision, accuracy, robustness, limit of detection (LOD) and limit of quantitation (LOQ) as per ICH Q2 (R1) guidelines[31].

1. System Suitability Test

System suitability testing[20] is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analysed constitute an integral system that can be evaluated as such. Following system suitability test parameters were established. The data are shown in Table 5 & 6.

Table 5: Data of System Suitability Test

S.No.	Injection No.	RT	Area	USP Plate Count	USP Tailing
1	Injection 1	4.817	745236	6986	1.39
2	Injection 2	4.783	743652	6857	1.37
3	Injection 3	4.840	742587	6856	1.36
4	Injection 4	4.783	742946	6847	1.39
5	Injection 5	4.817	743654	6896	1.38
6	Injection 6	4.778	741698	6874	1.37
Mean			**743295.5**	**6886**	**1.37666**
S.D			**1199.773604**		
%RSD			**0.161412736**		

Table 6: Data of System Suitability Parameter

S.No.	Parameter	Limit	Result
1	Retention Time	RT > 2	Azelnidipine= 4.778
2	Asymmetry	T ≤ 2	Azelnidipine= 1.35
3	Theoretical plate	N > 2000	Azelnidipine= 6859
4	Tailing Factor	T<2	Azelnidipine= 1.37

2. Linearity

To evaluate the linearity, serial dilution of analyte were prepared from the stock solution was diluted with mobile phase to get a series of concentration ranging from 60-140µg/ml. The prepared solutions were sonicated. From these solutions, 10µl injections of each concentration were injected into the HPLC system and chromatographed under the optimized conditions. Calibration curve[21] was constructed by plotting the mean peak area (Y-axis) against the concentration (X-axis).

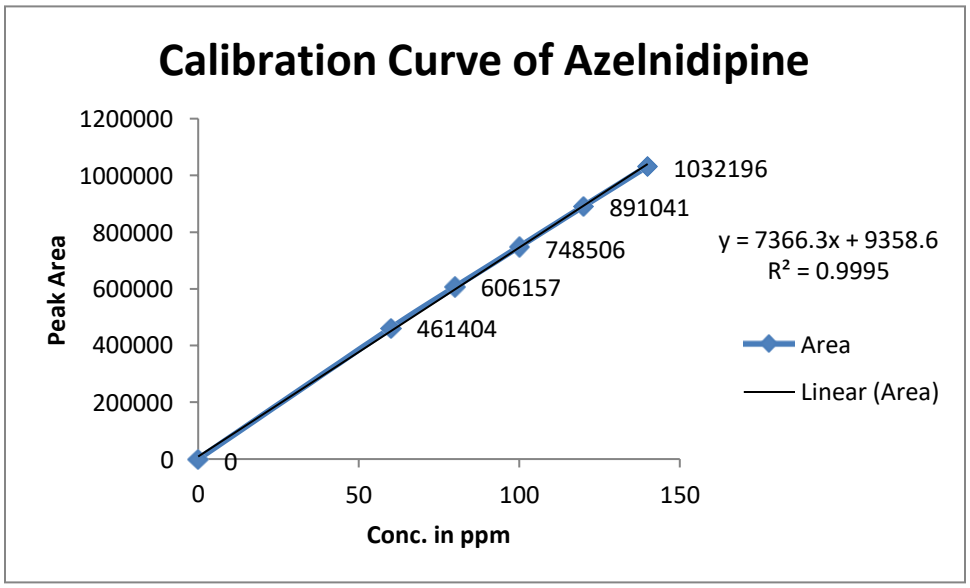

Fig 4: Calibration Curve of Azelnidipine

Table 7: Linearity Data for Azelnidipine

Conc. (µg/ml)	Area
0	0
60	461404
80	606157
100	748506
120	891041
140	1032196

Accuracy

The accuracy of the method was determined by recovery studies and the percentage recovery[22] was calculated. The recoveries of Azelnidipine were found to be in the range of 99-102%. The proposed Liquid Chromatographic method was applied to the determination of Azelnidipine. The results for Azelnidipine comparable with the corresponding labeled amounts.

Table 8: Shown Accuracy Observation of Azelnidipine

Accuracy	Amount Added	Amount Recovered	Peak Area	% Recovery	Mean Recovery
80%	80	80.798	604517	100.997	100.437%
	80	80.673	603598	100.841	
	80	80.756	604213	100.945	
100%	100	99.933	745471	99.933	
	100	100.083	746574	100.083	
	100	100.365	748652	100.365	
120%	120	120.290	895415	100.241	
	120	120.201	894762	100.167	
	120	120.442	896541	100.368	

Precision

Repeatability

The precision of each method was ascertained separately from the peak areas & retention times obtained by actual determination of six replicates of a fixed amount of drug. Azelnidipine (API). The percent relative standard deviation[23] was calculated for Azelnidipine are presented in the table 9.

Table 9: Repeatability Data for Azelnidipine

S. No.	INJECTION	PEAK AREA
1	Injection 1	743826
2	Injection 2	745277
3	Injection 3	742506
4	Injection 4	747576
5	Injection 5	746715
6	Injection 6	741278
7	**Average**	**744529.6667**
8	**SD**	**2440.4116**
9	**% RSD**	**0.32777**

Intermediate Precision

The Intermediate Precision[24] consists of two methods:-

Intra Day: In Intra Day process, the 80%, 100% and 120% concentration are injected at different intervals of time in same day.

Inter Day: In Inter Day process, the 80%, 100% and 120% concentration are injected at same intervals of time in different days.

Table 10: Results of intra-assay & inter-assay

Conc. of Azelnidipine (API) (µg/ml)	Observed Conc. of Azelnidipine (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Mean (n=6)	% RSD	Mean (n=6)	% RSD
80	80.096	0.487	79.685	0.688
100	100.074	0.968	100.057	0.789
120	120.056	0.847	120.016	0.698

The intra & inter day variation of the method was carried out for standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Azelnidipine revealed that the proposed method is precise.

Specificity

Specificity[25] can be determined by comparing the chromatograms obtained from the drugs with the chromatogram obtained from the blank solution. Blank solution was prepared by mixing the excipients in the mobile phase without drug. Drug solutions were prepared individually and the sample containing one drug was also prepared. Now these mixtures were filtered by passing through 0.45 µ membrane filter before the analysis. In this observation no excipient peaks were obtained near the drug in the study run time. This indicates that the proposed method was specific.

The chromatograms representing the peaks of blank, Azelnidipine and the sample containing the one drug was shown in following figures respectively.

In this test method blank, standard solutions were analyzed individually to examine the interference. The above chromatograms show that the active ingredient was well separated from blank and their excipients and there was no interference of blank with the principal peak. Hence the method is specific.

Method Robustness

Influence of small changes in chromatographic conditions[26] such as change in flow rate 1.0 ml (± 0.1ml/min), Wavelength of detection 286 (±2nm) & organic phase content in mobile phase (±5%) studied to determine the robustness of the method are also in favour of (Table-11, % RSD < 2%) the developed RP-HPLC method[27] for the analysis of Azelnidipine (API).

Table 11: Results for Robustness

Parameter Used for Sample Analysis	Peak Area	Retention Time	Theoretical Plates	Tailing Factor
Actual Flow rate of 1.0 mL/min	742946	4.778	1.37	2896
Less Flow rate of 0.9 mL/min	698965	4.783	1.39	2986
More Flow rate of 1.1 mL/min	786598	4.817	1.42	2985
Less organic phase	732642	4.842	1.29	3102
More organic phase	702546	4.773	1.37	3247

Estimation of Azelnidipine in Pharmaceutical Dosage Form

Each tablet contains: 16mg

Twenty Tablets were taken and the I.P. method was followed to determine the average weight. Above weighed tablets were finally powdered and triturated well. A quantity of powder equivalent to 25 mg of drugs were transferred to 25 ml volumetric flask, make and solution was sonicated for 15 minutes, there after volume was made up to 25 ml with same solvent. Then 10 ml of the above solution was diluted to 100 ml with mobile phase. The solution was filtered through a membrane filter (0.45 µm) and sonicated to degas. The solution prepared was injected in five replicates into the HPLC system[28] and the observations were recorded. The data are shown in Table 12.

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \text{Avg Wt.} = \text{mg}$$

Where:

AT = Peak Area of drug obtained with test preparation
AS = Peak Area of drug obtained with standard preparation
WS = Weight of working standard taken in mg
WT = Weight of sample taken in mg
DS = Dilution of Standard solution
DT = Dilution of sample solution
P = Percentage purity of working standard

Table 12: Recovery Data for estimation Azelnidipine in Uniaz 16 Tablets

Brand name of Azelnidipine	Labelled amount of Drug (mg)	Mean (± SD) amount (mg) found by the proposed method (n=6)	Assay % (± SD)
Uniaz 16 Tablets (Torrent Pharmaceuticals Limited.)	16mg	15.558 (± 0.468)	99.825 (± 0.418)

The amount of drug in Uniaz 16 Tablets was found to be 15.558 (± 0.468) mg/tab for Azelnidipine & % Purity[29] was 99.825 %.

Stability studies

The API (Azelnidipine) was subjected to worry conditions in numerous ways that to look at the speed and extent of degradation that's seemingly to occur within the course of storage and/or when administration to body. This is often one style of accelerated stability studies that helps United States deciding the fate of the drug that's seemingly to happen when on time storage, at intervals an awfully short time as compare to the important time or future stability testing. The various degradation pathways studied are acid chemical reaction, basic chemical reaction, thermal degradation, and photolytic degradation and Oxidation degradation.

Results of degradation studies: The results of the strain studies indicated the specificity of the tactic that has been developed. Azelnidipine was stable in oxidation and thermal stress conditions. The results of forced degradation studies[30] are given in the following table-13.

Table-13: Results of Forced Degradation Studies of Azelnidipine API

Stress Condition	Time in hrs	Assay of active substance	Assay of degraded products	Mass Balance (%)
Acid Hydrolysis (0.1 M HCl)	24Hrs.	92.406	7.594	100.0
Basic Hydrolysis (0.1 M NaOH)	24Hrs.	95.314	4.686	100.0
Wet heat	24Hrs.	93.241	6.759	100.0
UV (254nm)	24Hrs.	89.342	10.658	100.0
3 % Hydrogen peroxide	24Hrs.	90.355	9.645	100.0

SUMMARY

To develop a precise, linear, specific & suitable stability indicating RP-HPLC method for analysis of Azelnidipine, different chromatographic conditions were applied & the results observed are presented in previous chapters. Isocratic elution is simple, requires only one pump & flat baseline separation for easy and reproducible results. So, it was preferred for the current study over gradient elution.

In case of RP-HPLC various columns are available, but here Phenomenex Luna (C18) RP Column, 250 mm x 4.6 mm, 5µm Column was preferred because using this column peak shape, resolution and absorbance were good. Mobile phase & diluent for preparation of various samples were finalized after studying the solubility of API in different solvents of our disposal

(methanol, Acetonitrile, water, 0.1N NaOH, 0.1NHCl). Detection wavelength was selected after scanning the standard solution of drug over 200 to 400nm. From the U.V spectrum of Azelnidipine it is evident that most of the HPLC work can be accomplished in the wavelength range of 286 nm conveniently. Further, a flow rate of 1.0 ml/min & an injection volume of 20µl were found to be the best analysis. The result shows the developed method is yet another suitable method for assay and stability related impurity studies which can help in the analysis of Azelnidipine in different formulations.

CONCLUSION

A sensitive & selective stability indicting RP-HPLC method has been developed & validated for the analysis of Azelnidipine in bulk and pharmaceutical dosage form. Based on peak purity results, obtained from the analysis of samples using described method, it can be concluded that the absence of co-eluting peak along with the main peak of Azelnidipine indicated that the developed method is specific for the simultaneous estimation of Azelnidipine in the bulk and pharmaceutical dosage forms. Further the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility. The specific Retention time for Azelnidipine are found to be 4.778min. The tailing factor was found to be 1.37 with theoretical plates 6859 for Azelnidipine. The %Recoveries was determined as 100.437% for Azelnidipine in Accuracy. The %RSD in Repeatability is 0.327 with Intermediate Precision (Intra & Inter Day) are 0.767 & 0.725 for Azelnidipine in Precision respectively. In Linearity, the correlation coefficient was found to be 0.9995 for Azelnidipine. The LOD for Azelnidipine was 1.469 and LOQ for Azelnidipine are 4.454 respectively.

REFERENCES

1. Available from: https://go.drugbank.com/drugs/DB09230.
2. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Azelnidipine.
3. Available from: https://en.wikipedia.org/wiki/Azelnidipine. Wikipedia.
4. Available from: https://medicaldialogues.in/generics/azelnidipine-2722518.
5. Skoog, Holler and Nieman, Chapter 28. Principles of instrumental analysis. 5th ed, Harcourt Publishes Int Company. p. 726-66.
6. Neue D, Wiley VC. HPLC columns. Theor Technol Pract Uwe. https://www.wiley.com/en-gl/HPLC+Columns:+Theory,+Technology,+and+Practice-p-9780471190370
7. Handbook of HPLC. Vol. 78, by Elena Katz et al. Marcel Dekker Inc. 1999. https://www.semanticscholar.org/paper/Handbook-of-HPLC-%E2%80%93-Chromatographic-Science-Series-Lloyd-Kennedy/5446ebc6108a8594de1657929583a2e785841b53
8. Ewing GW. Instrumental methods of chemical analysis. 5th ed. McGraw-Hill Book Company; 1988.
9. HPLC in pharmaceutical industry. Fong and long, Marcel Dekker Series. 1991. http://libraryopac.bits-hyderabad.ac.in/cgi-bin/koha/opac-detail.pl?biblionumber=15742
10. Instrumental Method of Chemical Analysis. Chatwal Anand, Himalaya Publishing House, p. no. 615-623.
11. Dr. Kealey, Haines PJ. Analytical chemistry. 1st ed. Bios Publisher; 2002. p. 1-7.
12. Practical pharmaceutical chemistry. 4th ed. part 2, by Beckett and Stenlake, CBS Publishers and Distributors, 2005; P.No.157-174. Available from: https://www.jpsr.pharmainfo.in/Documents/Volumes/vol11issue11/jpsr11111910.pdf
13. Govt of India, Ministry of Health and Family Welfare. Delhi: publication by controller of publication. Indian Pharmacopoeia. 2007;2:484-554.
14. British Pharmacopoeia. Published on the recommendation of the medicines commissions pursuant to Medicines Act 1968. international ed. Vol. 1; 1993. p. 429, 483.
15. United States Pharmacopoeia 29 NF;24, Published on the Recommendation of the Medicines Commissions Pursuant to Medicines, page no. 587.
16. Skoog, West, Holler, Crouch. Fundamentals of analytical chemistry. 8th ed. New Delhi: Cengage learning India Pvt ltd, Page no. 271-280; 2009 (Indian edition).
17. Kasture AV, Mahadik KR, Wadodkar SG, More HN. A textbook of pharmaceutical analysis, Instrumental methods. Nirali Prakashan. 9th ed, page no. 5-7;2:28-30.
18. Settle FA. Handbook of instrumental techniques for analytical chemistry. 1st ed. Singapore: Pearson Education Inc; 2004.
19. Willard HH, Dean AJ. Instrumental methods of analysis. CBS Publishers and distributors. 7th ed; 1986. p. 513-5.
20. Connors AK. In: Wiley A, editor. A text book of pharmaceutical analysis. 3rd ed. Interscience Publication; 2005. p. 373-400.
21. In AS. High pressure liquid chromatography of comprehensive analytical chemistry. Elsevier Publications; 2006.
22. Principles and methods. In: Amesham biosciences of reversed phase chromatography. 1999. p. 6-8. Available from: https://books.google.com/books/about/Reversed_Phase_Chromatography.html?id=4Q3eQgAACAAJ
23. Synder LR, Kirkland JJ, Glajch JL. Practical HPLC method development. 2nd ed. Canada: John Wiley & Sons Inc; 1997.
24. Mohammad T et al. HPLC method development and validation for pharmaceutical. Int Pharm Sci. 2012;2(3):14 Analysis-A [review].
25. Snyder LR, Kirkland JJ, Glajch JL. Practical HPLC method development. 2nd ed; 2001.
26. Vibha G et al. Development and validation of HPLC method – a review. Int Res J Pharm Appl Sci. 2012;2(4):22-3.
27. Bliesner DM. Validating chromatographic methods. John Wiley & sons Inc; 2006. p. 88-92.
28. Validation of analytical procedures: methodology. ICH-guidelines Q2B. Geneva. 1996, 11. (CPMP/ICH/281/95).

29. Gupta V et al. Development and validation of HPLC method – a review. Int Res J Pharm Appl Sci. 2012;2(4):17-25.
30. A review: HPLC method development and validation. Santosh Kumar Bhardwaj et al. International Journal of Analytical and Bioanalytical Chemistry, accepted 20 November 2015.
31. Method Development: A Guide to Basics Quantitative & Qualitative HPLC, LC, GC Chromacademy. Available from: https://www.waters.com/webassets/cms/support/docs/715003355_dev_meth.pdf
32. Sonawane LV. Bioanalytical method validation and its pharmaceutical application- A review pharmaceutica analytical. Acta. 2014;5:3 Center for Drug Evaluation and Research (CDER) Reviewer Guidance. https://www.researchgate.net/publication/269538705_Bioanalytical_Method_Validation_and_Its_Pharmaceutical_Application-_A_Review
33. ICH, Topic Q. 2 (R1) validation of analytical procedures: text and methodology. Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/ich-q-2-r1-validation-analytical-procedures-text-methodology-step-5_en.pdf
34. Patil SD, Bachhav RS, Udavant PB, Ahirrao SP. Deepak S.bhambere4, Development and Validation of Stability Indicating RP-HPLC Method for Azelnidipine for bulk drug. Natl Volatiles Essent Oils. 2021;8(5):11151-7.
35. Gore MG, Pratap S. Dabhade, RP-HPLC method development and validation of azelnidipine, Gore and Dabhade, IJPSR. Vol. 7(12); 2016. p. 5111-4.
36. Prabhakar D, Sreekanth J, Jayaveera KN. Method development and validation of azelnidipine by RP-HPLC. Int J ChemTech Res. 2017;10(10):418-23.
37. Ubale S, Dr. Kalshetti MS, Habib B, Mittha J, Adlinge S. Development and validation of RP-HPLC method for quantification of azelnidipine in tablet. Int J Creat Res Thoughts (IJCRT). 2021;9 (7, July): 797-802.
38. Jadhav, Lokhande, Narwade, Mahananda V. Ghodke, Rutuja. S. Desai and Prerna R. Mote, Method development & validation of stability indicating Rp-HPLC method for simultaneous estimation for azelnidipine & telmisartan in bulk & pharmaceutical dosage form, world. J Pharm Med Res Wjpmr. 2022;8(3):216-22.