IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 5, Issue 8, May 2025
Impact Factor: 7.67

Analytical Method Development & Validation of Azelnidipine By using RP-HPLC

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Abstract: *A precise and reliable high-performance liquid chromatography (HPLC) method was developed and validated for the quantitative determination of Azelnidipine in pharmaceutical formulations. The method employed a reverse-phase chromatographic separation using a C18 column, with a mixture of mobile phase. Detection was performed at a wavelength of 240 nm. This study presents a comprehensive theoretical and practical framework for the development and validation of a high-performance liquid chromatography (HPLC) method for the quantitative analysis of Azelnidipine in pharmaceutical formulations. The theoretical aspects of HPLC, including chromatographic theory, column chemistry, and detector principles, are discussed in relation to the development of a robust and reliable analytical method. The validated method can be readily applied for the routine analysis of Azelnidipine in pharmaceutical products, ensuring the quality and purity of the active pharmaceutical ingredient.*

Keywords: Azelnidipine, HPLC, Method development, Method validation, pharmaceutical analysis, Chromatography, Quantitative analysis

I. INTRODUCTION

Azelnidipine is a third-generation dihydropyridine calcium channel blocker (CCB) developed primarily for the treatment of hypertension (high blood pressure). It is chemically and pharmacologically related to other CCBs like amlodipine and nifedipine, but it has some unique features that make it stand out in its class. Mechanism of Action: Azelnidipine inhibits the influx of calcium ions into vascular smooth muscle cells and cardiac muscle cells, leading to vasodilation and a decrease in blood pressure. Its long-acting properties allow for once-daily dosing. Pharmacological Profile: Azelnidipine has a high lipid solubility, which contributes to its long duration of action. It is extensively metabolized by the liver and has a low potential for drug-drug interactions. Analytical chemistry is a branch of chemistry that deals with the study of the introduction of components (qualitative) and the determination of the number of components (quantitative) of substances or samples, or mixtures.

There are two different forms of analysis:

1. Qualitative analysis: Identification of the mixture or sample's constituents or analytes is made by qualitative analysis.
2. Quantitative analysis: Quantitative analysis involves quantifying a mixture of sample components or analytes. Not only chemistry but also biology, zoology, the arts), space exploration, and medical diagnosis required analytical data.

HPLC CHROMATOGRAPHY: High-Performance Liquid Chromatography (HPLC) is an analytical technique used to separate, identify, and quantify components of a mixture. It's a powerful method that utilizes high pressure to force a mobile phase (solvent) through a column packed with a stationary phase (sorbent).

Separation: separates components in a mixture based on their different interactions with the stationary and mobile phases.

- Identification: The time it takes for each component to elute from the column (retention time) helps identify it.

DOI: 10.48175/IJARSCT-26918

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- Quantification: The area of the peak generated on a detector can be used to quantify the amount of each component.
- High Pressure: The use of high pressure allows for faster analysis and better separation efficiency compared to traditional column chromatography.
- Mobile and Stationary Phases: The mobile phase is a liquid that carries the sample through the column, while the stationary phase is a material packed inside the column that interacts with the components.

Products that support excellent health are in high demand because health is a major priority in our everyday lives. Cookies have a longer shelf life than other food items, are portable, and are always ready to consume. Sugar, butter, and refined wheat flour are typical cookie ingredients. Due of its rheological qualities, wheat is typically used. An edible algae called Spirulina platensis is used as a raw material to make wholesome goods that are beneficial to health. It has adequate levels of single-cell proteins (60–70%) as well as all other necessary elements, including pro-vitamin A, magnesium, zinc, ions, and the important fatty acid gamma-linolenic acid. The plant is characterized by the presence of several secondary metabolites, such as flavonoids, terpenoids, and chemical groups called polyphenols. Among its many therapeutic benefits include the inhibition of digestive enzymes such as lipases, glucosidase, and amylase, as well as anti-inflammatory and anti-arthritic properties. The prevention and treatment of cardiovascular diseases, type 2 diabetes, and their associated co-morbidities, such the metabolic syndrome, may benefit from such advantages.

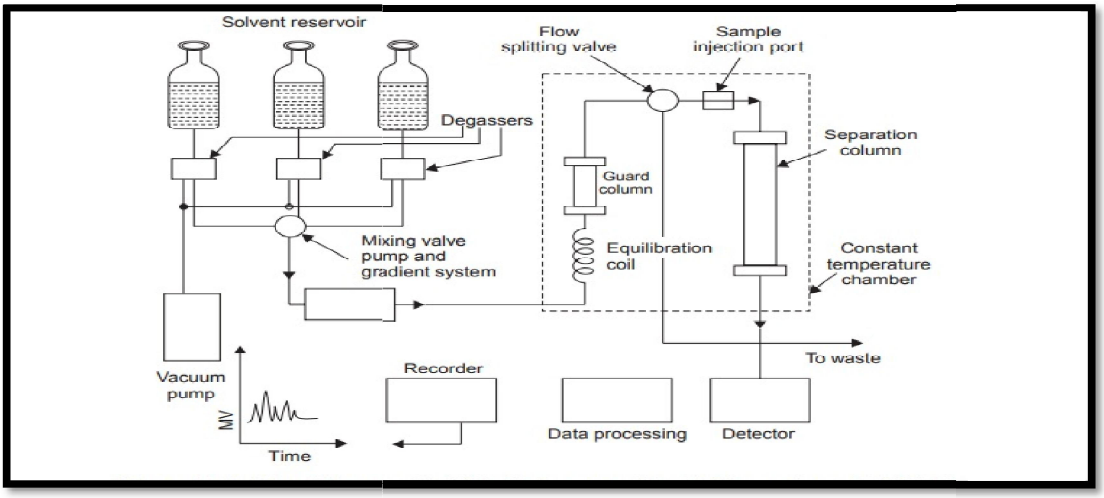
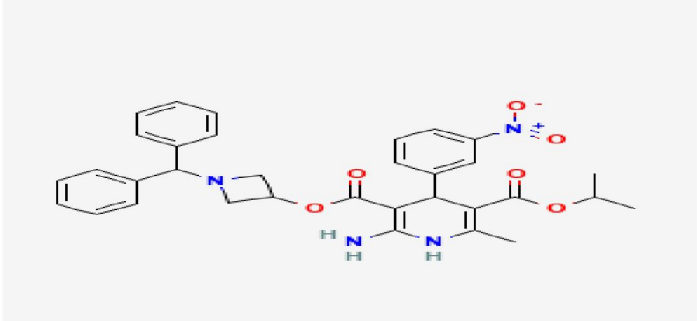


Fig 2. Instrumentatn diet.

DRUG PROFILE

Parameter	Information
Drug Name	Azelnidipine
Brand Name	Calblock
Structure	

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Weight	503.58 g/mol
Chemical formula	$C_{33}H_{34}N_4O_6$
IUPAC Name	3-(1-Benzhydrylazetidin-3-yl) 5-isopropyl 2-amino-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate
BCS Class	Class II (High permeability, Low solubility)
Half life	Approx. 12-24 hours
Pka 1	4.5
Pka2	8.5
Log P	6.5
Particle size	10-30 µm
Hygroscopicity	Hygroscopic (absorbs moisture from the air)
Polymorphic form	Amorphous form
Solid state Stability	Stable in the solid state
Melting Point	162-165°C
T max	2-4 hours
Solubility	Practically insoluble in water, soluble in ethanol, and freely soluble in methanol.

Table 1 Drug Profile

II. MATERIALS & METHODS

Material:

- Chemical and reagents
- Instrumentation and chromatographic conditions.
- Preparation of standard stock solution.
- Analysis of Ketoconazole

Validation parameter:

- Specificity / Selectivity
- Precision
- Accuracy / Recovery
- Linearity and Range
- Limit of Detection (LOD)
- Limit of Quantitation (LOQ)
- Solution Stability
- Intermediate Precision
- Robustness

III. RESULT & DISCUSSION

1) Specificity / Selectivity

The specificity of the method was ascertained by analyzing standard drugs and sample. The retention time (RT) of Azelnidipine was confirmed by comparing the RT with that of the standard. The use of the standard Azelnidipine and interference was observed in the chromatogram of blank.

a) Mix Standard preparation:

Dissolve accurately 8mg of Azelnidipine in 100ml volumetric flask and add 50ml of Acetonitrile sonicate for 10 minutes and makeup the volume with Water. Further dilute 5ml of above solution to 50ml with Acetonitrile.

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b) Sample preparation

Determine average weight. Crush tablets into fine powder. Transfer powder containing 8mg of Azelnidipine (1 Tablet Weight), to a 100 mL dry volumetric flask, add 50 ml of Acetonitrile sonicate for 10 minute with intermittent shaking. and dilute upto the volume with water. Further dilute 5ml of the above solution to 50ml with water

SYSTEM SUITABILITY

No of Inj	Azelnidipine
1	1748371
2	1752617
3	1757934
Average	1752974
STDEV	4791.485
RSD	0.27

Sample ID	RT (minutes) Azelnidipine	Remarks
Blank	Nil	----------
Standard	4.119	No interference
Sample	4.112	No interference

Acceptance Criteria:

Placebo and Blank should not interfere in the main peak of the Azelnidipine.

Conclusion:

There is no interference from of Blank with the main peak of the Azelnidipine in the standard & sample solution

2) Precision

Precision is a measurement of degree of Reproducibility of analytical method and it will be expressed in terms of % relative standard for the area and retention time of Solution prepared. The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

a) Mix Standard preparation:

Dissolve accurately 8mg of Azelnidipine in 100ml volumetric flask and add 50ml of Acetonitrile sonicate for 10 minutes and makeup the volume with Water. Further dilute 5ml of above solution to 50ml with Acetonitrile.

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Determine average weight. Crush tablets into fine powder. Transfer powder containing 8mg of Azelnidipine (1 Tablet Weight), to a 100 mL dry volumetric flask, add 50 ml of Acetonitrile sonicate for 10minute with intermittent shaking. and dilute up to the volume with water. Further dilute 5ml of the above solution to 50ml with water

(for Standard Preparation)

No of Tablet	Standard Area
	Azelnidipine
1	1831717
2	1846449

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3	1844977
Average	1841048
SD	8114.043
% RSD	0.44

Acceptance Criteria:
%RSD should not be more than 2.0 % for area and RT. Theoretical plate should be NLT 2000,
Tailing Factor should NMT 2.0.
(for Sample Preparation)

No of sample	Area	% Assay
	Azelnidipine	Azelnidipine
1	1845435	100.24
2	1833581	99.59
3	1841101	100.00
Average	1840039	99.95
STDEV	5997.9340	0.3258
% RSD	0.33	0.33

6) Limit of Detection: The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.
Calculation:

LOD = (3.3 ×STD.Devation)/Slope
== (3.3 ×5997.9340)/(221247.2589) = 0.09ppm

7) Limit of Quantitation:
The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision
Calculation:

LOQ = (10 ×STD.Devation)/Slope
== (10 ×5997.9340)/(221247.2589) = 0.27ppm

8) Solution Stability:
The solution stability of Azelnidipine in the assay method was carried out by leaving the working standard in tightly capped volumetric flasks at room temperature for 24 hrs. The assay sample is also prepared and kept for stability up to 24 hours.

a) Mix Standard preparation:
Dissolve accurately 8mg of Azelnidipine in 100ml volumetric flask and add 50ml of Acetonitrile sonicate for 10 minutes and makeup the volume with Water. Further dilute 5ml of above solution to 50ml with Acetonitrile.

b) Sample preparation:
Determine average weight. Crush tablets into fine powder. Transfer powder containing 8mg of Azelnidipine (1 Tablet Weight), to a 100 mL dry volumetric flask, add 50 ml of Acetonitrile sonicate for 10minute with intermittent shaking. and dilute upto the volume with water. Further dilute 5ml of the above solution to 50ml with water.
(for Azelnidipine)

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Time (Hrs)	% Assay	Difference
0	99.95	---
8	98.60	1.34
24	98.52	1.43

Acceptance Criteria:

%Assay difference should not be more than 2.0 %

Accuracy / Recovery:

The accuracy of an analytical method is defined as the closeness between the observed values with actual or true value for a specific concentration. Accuracy - closeness to the true value, measured by % recovery of sample spikes or % error in the analysis of a reference sample

Preparation of Solutions:

Standard Solution:

Dissolve accurately 8mg of Azelnidipine in 100ml volumetric flask and add 50ml of Acetonitrile sonicate for 10 minutes and makeup the volume with Water. Further dilute 5ml of above solution to 50ml with Acetonitrile.

Sample Solutions for Accuracy:

1) 6.4ppm Azelnidipine Solution: - Dissolve accurately 6.4mg of Azelnidipine in 100ml volumetric flask and add 50ml of Acetonitrile sonicate for 10 minutes and makeup the volume with Water. Further dilute 5ml of above solution to 50ml with Acetonitrile.

2) 8ppm Azelnidipine Solution: Dissolve accurately 8mg of Azelnidipine in 100ml volumetric flask and add 50ml of Acetonitrile sonicate for 10 minutes and makeup the volume with Water. Further dilute 5ml of above solution to 50ml with Acetonitrile.

3) 9.6ppm Azelnidipine Solution: Dissolve accurately 9.6mg of Azelnidipine in 100ml volumetric flask and add 50ml of Acetonitrile sonicate for 10 minutes and makeup the volume with Water. Further dilute 5ml of above solution to 50ml with Acetonitrile.

(For Azelnidipine)

Level	Prepared Concentration (ppm)	Recovered Concentration (ppm)	% Recovery
80%	0.006400	0.006401	100.02
100%	0.008000	0.008008	100.10
120%	0.009600	0.009634	100.36
Average			100.16
SD			0.18
%RSD			0.18

Acceptance Criteria:

%RSD for %Recovery should not be more than 2.0 %.

%Recovery should be between 98.0% to 102.0% for individual and for all level

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6) Limit of Detection: The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

Calculation:

LOD = (3.3 ×STD.Devation)/Slope

== (3.3 ×5997.9340)/(221247.2589) = 0.09ppm

7) Limit of Quantitation:

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision

Calculation:

LOQ = (10 ×STD.Devation)/Slope

== (10 ×5997.9340)/(221247.2589) = 0.27ppm

8) Solution Stability:

The solution stability of Azelnidipine in the assay method was carried out by leaving the working standard in tightly capped volumetric flasks at room temperature for 24 hrs. The assay sample is also prepared and kept for stability up to 24 hours.

a) Mix Standard preparation:

Dissolve accurately 8mg of Azelnidipine in 100ml volumetric flask and add 50ml of Acetonitrile sonicate for 10 minutes and makeup the volume with Water. Further dilute 5ml of above solution to 50ml with Acetonitrile.

b) Sample preparation:

Determine average weight. Crush tablets into fine powder. Transfer powder containing 8mg of Azelnidipine (1 Tablet Weight), to a 100 mL dry volumetric flask, add 50 ml of Acetonitrile sonicate for 10minute with intermittent shaking. and dilute upto the volume with water. Further dilute 5ml of the above solution to 50ml with water.

(for Azelnidipine)

Time (Hrs)	% Assay	Difference
0	99.95	---
8	98.60	1.34
24	98.52	1.43

Acceptance Criteria:

%Assay difference should not be more than 2.0 %

Accuracy / Recovery:

The accuracy of an analytical method is defined as the closeness between the observed values with actual or true value for a specific concentration. Accuracy - closeness to the true value, measured by % recovery of sample spikes or % error in the analysis of a reference sample

Preparation of Solutions:

Standard Solution:

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Sample Solutions for Accuracy:

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2) 8ppm Azelnidipine Solution: Dissolve accurately 8mg of Azelnidipine in 100ml volumetric flask and add 50ml of Acetonitrile sonicate for 10 minutes and makeup the volume with Water. Further dilute 5ml of above solution to 50ml with Acetonitrile.
3)9.6ppm Azelnidipine Solution: Dissolve accurately 9.6mg of Azelnidipine in 100ml volumetric flask and add 50ml of Acetonitrile sonicate for 10 minutes and makeup the volume with Water. Further dilute 5ml of above solution to 50ml with Acetonitrile.

(For Azelnidipine)

Level	Prepared Concentration (ppm)	Recovered Concentration (ppm)	% Recovery
80%	0.006400	0.006401	100.02
100%	0.008000	0.008008	100.10
120%	0.009600	0.009634	100.36
Average			100.16
SD			0.18
%RSD			0.18

Acceptance Criteria:
%RSD for %Recovery should not be more than 2.0 %.
%Recovery should be between 98.0% to 102.0% for individual and for all level

4) Linearity and Range:
Linearity Sample Stock Solution:
Dissolve accurately 8mg of Azelnidipine in 100ml volumetric flask and add 50ml of Acetonitrile sonicate for 10 minutes and makeup the volume with Water. Further dilute 5ml of above solution to 50ml with Acetonitrile.
Linearity Solutions:

- Linearity Solution (6.4ppm Azelnidipine): Further dilute 4.0ml of standard to 50 ml with Acetonitrile.
- Linearity Solution (7.2ppm Azelnidipine): Further dilute 4.5ml of standard to 50 ml with Acetonitrile.
- Linearity Solution (8.0ppm Azelnidipine): Further dilute 5.0ml of standard to 50 ml with Acetonitrile.
- Linearity Solution (8.8ppm Azelnidipine): Further dilute 5.5ml of standard to 50 ml with Acetonitrile.
- Linearity Solution (9.6ppm Azelnidipine): Further dilute 6.0ml of standard to 50 ml with Acetonitrile

Acceptance Criteria:
The limit for coefficient of correlation for the standard curve must not be less than 0.9900

(For Azelnidipine)

No of Tablet	Concentration (ppm)	Area
1	6.4	1396243
2	7.2	1603166
3	8.0	1773086
4	8.8	1958231
5	9.6	2110729
Coefficient Correlation =		0.9997
Y-Intercept =		-1405.8929
Slope =		3539.9561

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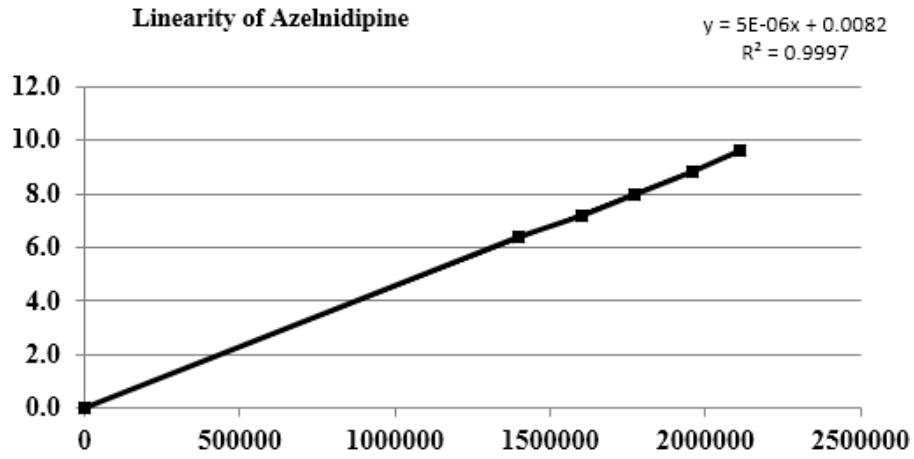

Acceptance Criteria:
Correlation coefficient should not be less than 0.99

6) Limit of Detection: The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.
Calculation:

LOD = (3.3 ×STD.Devation)/Slope
== (3.3 ×5997.9340)/(221247.2589) = 0.09ppm

7) Limit of Quantitation:
The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision
Calculation:

LOQ = (10 ×STD.Devation)/Slope
== (10 ×5997.9340)/(221247.2589) = 0.27ppm

8) Solution Stability:
The solution stability of Azelnidipine in the assay method was carried out by leaving the working standard in tightly capped volumetric flasks at room temperature for 24 hrs. The assay sample is also prepared and kept for stability up to 24 hours.
a) Mix Standard preparation:
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Determine average weight. Crush tablets into fine powder. Transfer powder containing 8mg of Azelnidipine (1 Tablet Weight), to a 100 mL dry volumetric flask, add 50 ml of Acetonitrile sonicate for 10minute with intermittent shaking. and dilute upto the volume with water. Further dilute 5ml of the above solution to 50ml with water.

(for Azelnidipine)

Time (Hrs)	% Assay	Difference
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Acceptance Criteria:

%Assay difference should not be more than 2.0

IV. CONCLUSION

Parameter	Limit	Observation
Specificity	Placebo and Blank should not interfere in the main peak of the Azelnidipine	There is no interference from of Blank with the main peak of the Azelnidipine in the standard & sample solution
Precision	(Method Precision) Azelnidipine content in Tablet should be within limits (NLT 90% to NMT 110%)	% Assay is 99.95% for Azelnidipine,
Accuracy /Recovery	% Recovery should be between 98.0% to 102.0% for individual and for all level.	%Recovery for Azelnidipine is 100.02% for 80%, 100.10% for 100%, 100.36% for 120%, and 100.16% for all level
Linearity and Range	Correlation coefficient should not be less than 0.9900.	Correlation coefficient is 0.9997 for Azelnidipine
Solution Stability	% Assay difference should not be more than 2.0 %.	% Assay difference for Azelnidipine is 1.43, up to 24 hours, which is well acceptable limit; hence the solution is stable up to 24 hours.
Intermediate Precision	Difference of average assay between Precision %Assay & Intermediate Precision % Assay	% Assay analysis for Azelnidipine is 99.84%, Difference of average Assay is, 0.11 for Azelnidipine, between Precision %Assay & Intermediate Precision % Assay
Robustness	Percentage assay difference for each sample should be within 2.0 %	Complies

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