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ESTIMATION OF AZELNIDIPINE BY UV SPECTROPHOTOMETRIC METHOD AND RP-HPLC METHOD

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Abstract: The assay of Azelnidipine from tablet formulation was established using a straight forward and reliable method. To increase the sensitivity of the suggested UV spectrophotometry method, the solvent system and wavelength of detection were created. The present research is aimed to develop analytical procedures for Azelnidipine to analyze them by development of accurate, precise and robust in UV Spectroscopy and Reversed Phase High Performance Liquid Chromatography method. The quantitative determination of Azelnidipine using the latest UV Spectrophotometric approach was found to be accurate, sensitive, exact, and well-applied. In the RP-HPLC method, the mobile phase had a methanol to water ratio of 75:25, 0.1% glacial acetic acid, and a wave length of 257 nm. The injection volume was 20 µl. Azelnidipine was shown to have a retention time of 6.130 minutes. The statistical validation of the procedure revealed that the %RSD was < 2, indicating huge degree of accuracy and precision. With a mean correlation coefficient of 0.999, Azelnidipine exhibits a rise in absorbance at 257 nm and linearity in the concentration range of 2–12 µg/ml. This UV Spectrophotometric technique was successfully developed and validated for the assay of Azelnidipine in tablet dosage form. The approach was shown to be precise, accurate, linear, and specific. The sample retake in tablet form was in good agreement with the label claim, indicating the method's validity.

Key words: Azelnidipine, UV spectrophotometry, HPLC method, chromatogram, validation, linearity.

INTRODUCTION

Azelnidipine is dihydropyridine derivative and chemically 3-[1-(Benzyldrylazetidin-3-yl] 5-isopropyl- 2- amino-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5dicarboxylate. Azelnidipine classified under the category of Dihydropyridine and is a calcium channel blocker (Imdad Husen Mukeri *et al*, 2021; Rele R. V. and Patil S. P., 2010). Azelnidipine inhibits trans- membrane Ca2+ influx through the voltage dependent channels of smooth muscles in vascular walls (Kunti D Raskapur *et al*, 2012; Punugupati Roja *et al*, 2022; D. Prabhakar *et al*, 2017; Pallavi Suthar and Rajashree Mashru, 2023; Aejaz Ahmed *et al*, 2022).

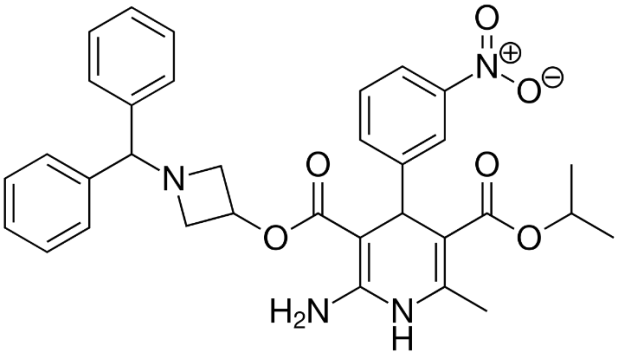

Figure 1. Structure of Azelnidipine

Azelnidipine official method for Liquid Chromatography are available in JP17th edition. A literature survey revealed that several methods have been used for estimation of Azelnidipine alone and also combined with Olmesartan Medoxomil which includes High Performance Liquid Chromatography (HPLC), Ultra-Fast Liquid Chromatography (UFLC), liquid chromatography electrospray ionization tandem mass spectrometry (LC/ESI-MS/MS) (Jayvadan K. Patel and Nilam K. Patel, 2014; P. Ashok *et al*, 2019; Rameshwar Gholve *et al*, 2021; Vangallu Spandana and Siddartha, 2022).

The literature survey reveals that only few works are carried out so far for the estimation of selected drug hence in the present study an attempt is made to analyze the selected compound by RP-HPLC which is a relatively a new technique with high sensitivity and resolution.

EXPERIMENTAL

Estimation of Azelnidipine by UV Spectrophotometric method:

Chemicals and reagents:

Pure drug Azelnidipine gifted by was kindly gifted by M/s Amoli Organics Private Limited, Mumbai. Methanol and Distilled water were used as solvent for drugs. The dosage form of the selected drug is purchased from local market.

Processing procedure for Spectrophotometric method:

When choosing an analytical wavelength, the standard Azelnidipine solution was scanned in spectrum mode from 400 nm to 200 nm. The 257nm max of Azelnidipine was used for procedure 1. The first derivative spectrum was created next. In method 2, the Azelnidipine amplitude peaked at 242.6 nm.

Procedure for Standard stock solution:

Azelnidipine stock solution (1000 µg/ml): Azelnidipine was carefully weighed in an amount of 100 mg, transferred to a 100 mL volumetric flask, and then dissolved in a solvent to obtain a solution of 1000 µg/ml Azelnidipine solution.

Procedure for working standard stock solution of Azelnidipine (100µg/ml):

To obtain a working standard solution of 100 µg/ml, the original solution of 10 ml is drained and transferred to a 100 ml volumetric flask.

Diluent:

The diluent used is Methanol and Water (80:20 V/V).

Calibration Curve of Azelnidipine:

Corresponding amounts of Azelnidipine of 2, 4, 6, 8, and 10 ml were transferred to different volumetric flasks with a capacity of 10 ml from the working stock solutions of Azelnidipine. With the help of a solvent, the volume was adjusted to the required level to obtain a concentration of 2-12 µg/ml. The absorbance of the solutions was measured using the first-order derivative at 242.6 nm and the zero order at 257 nm.

Estimation of Azelnidipine by RP-HPLC method:

Chemicals and reagents:

M/s Amoli Organics Pvt., Ltd., a Mumbai-based company, gave away a sample of the medicine Azelnidipine pure. As a solvent for pharmaceuticals, distilled grade water and methanol were both used. Sigma Aldrich chemicals Pvt. Ltd. provided HPLC grade glacial acetic acid (GAA), methanol, and water.

HPLC Method:

Instruments and Chromatographic Conditions:

To determine the maximum amount (λ max) of Azelnidipine that might be absorbed, the Shimadzu UV-1800 model was employed. Shimadzu HPLC model with an SPD-10A UV-Detector and LC10AD Pump. The column and HPLC were kept at room temperature. A reverse phase analytical inspireC18 column of 250 mm x 4.5 mm and 5 µm was utilised. The mobile phase used was 75:25 methanol: water with 0.1% GAA.

Procedure for Standard solution:

Azelnidipine 50 mg was dissolved in 50 ml of mobile phase to give a concentration of 1 mg/ml, and the primary stock solution was created. It was then kept at 8^{o}C until use.

RESULTS

UV Spectroscopy method:

Linearity:

Analysis of six distinct concentration levels between 2 and 12 µg/ml revealed the linearity of Azelnidipine response in terms of the calibration curve's intercept, slope, and correlation coefficient values.

Precision:

When the above procedures were repeated at different time intervals (morning, afternoon, and evening), on the same day (intra-day precision), and over three consecutive days, all the two methods were expected to be consistent (intra-day precision).

Accuracy:

Precision was achieved using recovery tests involving the injection of various concentrations of pure drug into 4 µg/ml of a pre-analyzed sample solution. The solution was then brought up to the mark with a solution which was at different levels, viz. 80%, 100% and 120%. The proposed method was used for decision analysis. Average percent recovery of peak area was calculated.

Limit of Detection (LOD):

A set of five calibration curves used to determine the linearity of the method was used to calculate the LOD. After calculating the SD of the intercept and repeating the calibration graph six times, the LOD was computed as follows: Using the equation LOD= (3.3 x SD)/slope.

Where, SD= standard deviation of y-intercept of 5 calibration curves. Slope= the mean slope of the 5 calibration curves.

Limit of Quantification (LOQ):

A set of five calibration curves used to determine the linearity of the method was used to calculate the LOQ. The formula for the LOQ is LOQ = 10 × (σ/S). Where, σ = Standard deviation of Y-intercepts of five calibration curves. S = Mean slope of five calibration curves.

Optimization by UV Spectroscopy method:

Linearity:

Analysis of six independent concentration levels in the range of 2–12 µg/mL was performed to determine the linearity of the response to Azelnidipine in terms of the slope of the calibration curve, intercept values, and correlation coefficient.

Precision:

By limiting the aforementioned approaches to specific time intervals (morning, afternoon, and evening) on the same day (intra-day precision) as well as over three subsequent days, the consistency of all these two methods was measured (inter-day precision).

Table 1. Precision data

Method	Conc (µg/ml)	Intraday (%RSD) (n=3)	Interday (%RSD) (n=3)
1.	2	1.93	1.34
	6	0.25	1.80
	12	0.91	1.86
	Mean	1.02	1.67
2.	2	1.32	1.88
	6	0.36	0.90
	12	0.16	1.48
	Mean	0.53	1.48

Accuracy:

Precision was assessed by achieving improvement tests by adding different concentrations of pure drug to a previously tested 4 µg/mL sample solution. The proposed method was used for decision analysis. Determine the average percent recovery from the peak areas.

Table 2. Accuracy data

Method	Drug %	Drug Conc (µg/ml)	Total Conc (µg/ml)	Total Conc Found (µg/ml)	Recovered Amount (µg/ml)	% Recovery	% RSD
1	80	3.1	7.1	7.43	3.21	101.32	0.68
	100	4.1	8.3	8.5	4.34	100.67	0.40
	120	4.7	8.8	9.16	4.94	101.79	0.019
2	80	3.4	7.4	7.20	3.16	100.46	1.38
	100	4.2	8.4	8.7	3.92	100.20	0.59
	120	4.9	8.7	8.78	4.85	96.69	0.69

Limit of Detection (LOD):

Five calibration curves were used to study the LOD and determine the linearity method. After completing the calibration curve six times and calculating the SD of the intercept, the LOD was calculated as follows: Using the equation LOD= (3.3*SD)/slope.

Where, SD= standard deviation of y-intercept of 5 calibration curves. Slope= mean slope of 5 calibration curves.

Limit of Quantification (LOQ):

A set of five calibration curves used to determine the linearity method was used to determine the LOQ.

The LOQ may be calculated as LOQ = 10 × (σ/S).

Where,

Y = intercepts of the five calibration curves.

S = Mean slope of the five calibration curves.

σ = Standard deviation

Table 3. LOD and LOQ

Parameters	Method 1	Method 2
SD of Intercept	0.019	0.0035
Slope	0.044	0.0124
LOD (µg/ml)	0.78	0.89
LOQ (µg/ml)	2.18	2.53

Parameter (µg/ml)	Azelnidipine
LOD	0.193584
LOQ	0.586641

RP-HPLC Method:

Validation procedure:

The suggested method was validated by parameters viz. limit of detection (LOD), limit of quantification (LOQ), linearity range, accuracy, selectivity, precision, robustness, specificity.

Specificity and Selectivity:

The endogenous compounds of chromatographic interference compared with AZP samples.

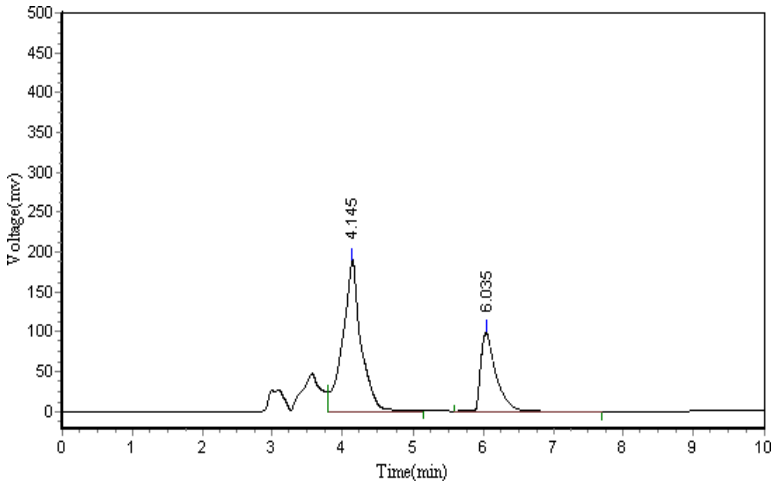

Figure 2. Chromatogram of Azelnidipine

Sensitivity:

The lower limit of quantification (LLOQ), which had a relative standard deviation of <±10%, was set as the lowest concentration that could be accurately and precisely quantified. The quantity that could be detected with a signal to noise ratio of 4 was known as the lowest limit of detection (LLOD).

Linearity:

Six different concentrations of Azelnidipine ranging from 1.0 to 50 µg/mL were plotted on the calibration curve. To ensure the absence of interference, blank samples were examined. Y-axis peak areas and X-axis concentrations were used to construct the Azelnidipine calibration curve.

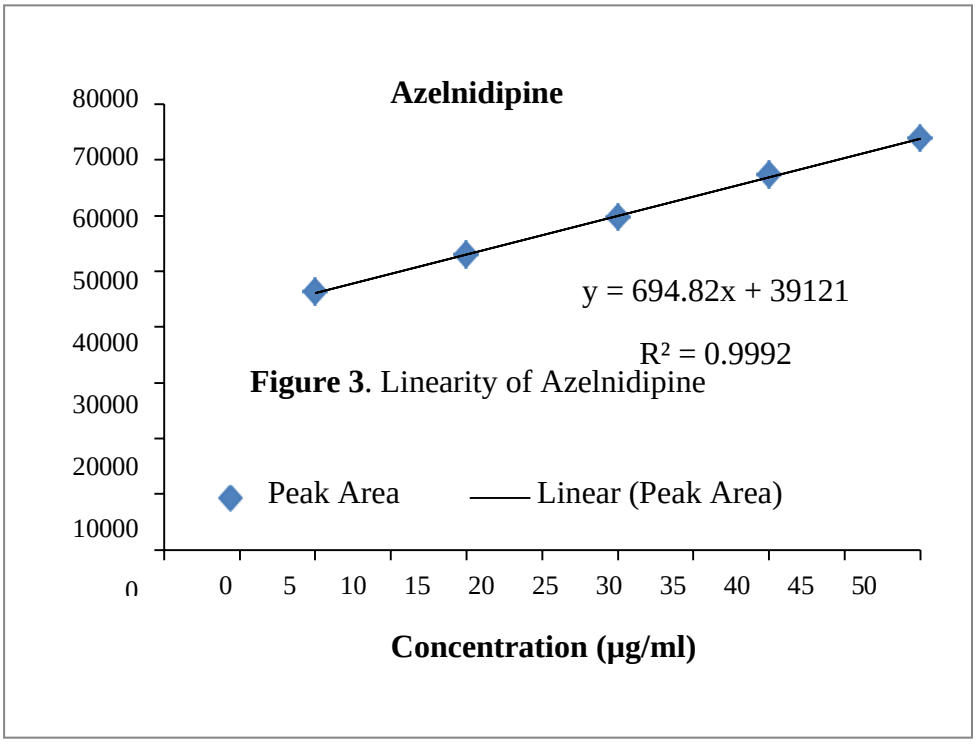

Figure 3. Linearity of Azelnidipine

Precision and Accuracy:

Azelnidipine samples were prepared at low, medium, and high concentrations as previously mentioned to determine the intra- and interday precision and accuracy of the procedure. Accuracy and precision are approved when the coefficient of variation for each concentration is less than ± 10%

and the mean values exceed 95% of the true concentration, except for the LLOQ where the limit exceeds 92%.

Range:

The range between the upper and lower concentration of the analyte in the sample is an analytical procedure. When applied to samples with analyte concentrations within or at the extremes of the specified range of the analytical method, the analytical procedure provides an acceptable level of precision, linearity, and precision.

Robustness:

The analytical technique's robustness is an indicator of its consistency over the course of typical usage and a measurement of its ability to remain unaffected by minute but deliberate adjustments in method parameters. The investigation of robustness involved varying the wavelength and flow velocity.

Table 4. Robustness influencing flow rate and wavelength

Flow rate	Drug	Theoretical Concentration (µg/ml)	Mean (Peak area)	Rt value	%RSD
0.9ml/min	**Azelnidipine**	10	46593.647	5.94	0.99
		30	59765.757	5.92	1.16
1.1ml/min	**Azelnidipine**	10	46581.617	6.03	1.28
		30	59662.518	6.07	0.99
Wavelength	**Drug**	**Theoretical Concentration(µg/ml)**	**Mean (Peak area)**	**Rt value**	**%RSD**
246 nm	**Azelnidipine**	10	46562.029	5.96	0.98
		30	59656.699	5.96	0.93
236 nm	**Azelnidipine**	10	46532.576	5.86	1.29
		30	59593.176	5.89	1.39

Ruggedness:

In this case, a standard solution with in a matrix should be systematically analyzed different condition of operation. The condition reviewed by different instruments change reactant source and solvent, change new column.

Solution Stability Study:

Azelnidipine plasma stability was examined at both the low and high QC sample concentrations. The plasma extraction method was used to check the storage stability of the sample up to 24 hours in short term (6, 12 and 24 hours) at room temperature. Less than 10% of the initial concentration is lost due to stability.

System Suitability Test:

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

Table 5. System Suitability data for Azelnidipine

Parameters	Azelnidipine	Acceptance Limits
Theoretical plates	3969	> 2000
Tailing factor	1.29	< 2.0
Rt	6.2	-
Asymmetry factor	1.29	< 2.0

DISCUSSION

Azelnidipine was absorbed by the UV-VIS method on a Shimadzu UV spectrophotometer (UV 1800), with the highest absorption observed at 254 nm in phosphate buffer. In this procedure, the HPLC-UV detection wavelength is 254 nm. The mobile phase employed for the procedure was straightforward and had ideal Azelnidipine separation without interference from other components. 1 ml/min has been chosen as the flow rate. Selected and particular Azelnidipine chromatogram is indicated in Figure. It had a retention time (Rt) of 6.1 minutes and no endogenous peaks interfered with it. The breakdown in accuracy and precision between and within days. As a result, the LLOQ's intra- and inter-day accuracy (% variation) was within < ± 10%. These findings suggest that the approach adopted has a high degree of precision and accuracy, as demonstrated in tables 2 and 3. Table 4 displays the robustness results. The relative standard deviation (RSD), which was determined to be less than 2%, demonstrates the robustness of the procedure. Azelnidipine is not currently authoritative in any pharmacopoeia, according to a literature review. Azelnidipine can be determined using only one or two analytical techniques, including HPLC-MS-MS, UV spectroscopy, HPLC, LC-ESI-MS, and LC-MS.

CONCLUSION

This UV Spectrophotometric technique was successfully developed and validated for the assay of Azelnidipine in tablet dosage form. The approach was shown to be precise, accurate, linear, and specific. The sample retake in tablet form was in good agreement with the label claim, indicating the method's validity. This page provides information about Azelnidipine improved activity compared to other medications. The provided article offers details on the numerous approaches used to determine Azelnidipine that are documented in the literature. Divergent analytical techniques, such as UV spectroscopy, HPTLC, LC-MS, and HPLC, are presented for both the individual and other combinations. This page also includes information on RP-HPLC and UV Spectroscopy of Azelnidipine.

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