IJBPAS, November, 2025, 14(11): 5908-5919

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

UV SPECTROPHOTOMETRIC METHOD FOR THE QUANTIFICATION OF AZELNIDIPINE IN BULK DRUG AND PHARMACEUTICAL FORMULATION BY MULTI-VARIATE CALIBRATION TECHNIQUE

PRIYANKA BH, KOKILAMBIGAI K. S* AND MANIKANDAN K

Department of Pharmaceutical Analysis, SRM College of Pharmacy, SRM Institute of Science and Technology, Kattankulathur - 603203, Chengalpattu District, Tamil Nadu, India

***Corresponding Author: Dr. Kokilambigai K S: E Mail: kokilams@srmist.edu.in; kokilampharm@gmail.com**

Received 15th July 2024; Revised 20th Sept. 2024; Accepted 1st Nov. 2024; Available online 1st Nov. 2025

https://doi.org/10.31032/IJBPAS/2025/14.11.9555

ABSTRACT

This research aims to establish a UV-visible spectroscopic technique for Azelnidipine by applying a multivariate regression equation that is the precise, sensible, and reproducible method. The proposed technique depends on the equation of the linear regression performed by taking absorbance at five distinct wavelengths. Azelnidipine maximum absorbance was obtained at 254 nm using distilled water as the solvent. The graph obtained from concentration 5-15 µg mL^{-1} resulted in a linear curve and the regression coefficient was obtained as 0.999 % RSD values for intra-day, as well as inter-day precision, were obtained as 0.5453 and 0.4680. The assay value determined was between 98.40-101.67% w/w.

Keywords: Azelnidipine, UV-visible spectroscopy, Assay, ICH guidelines, Multivariate calibration

INTRODUCTION

The molecular formula of Azelnidipine (AZEL), (±)-3-(1-diphenylmethylazetidin-3-yl) 5-isopropy12-amino-1, 4-dihydro-6-methyl-4-(3-nitrophenyl) 3,5-pyridine dicarboxylic acid ($C_{33}H_{34}N_4O_6$) is yellow powder, slightly soluble in methanol and ethanol sparingly soluble in water. This kind of calcium channel blocker (CCB), 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+ channel 6, which is used to treat hypertension, is dihydropyridine (DHP) based.The asymmetric carbon at the

DHP ring's fourth position gives rise to AZEL's two enantiomers **[1]**. The (R)-enantiomer of AZEL is where its pharmacological activity is found. In stark contrast, the biological activity 1 of other CCBs is attributed to the (S)-enantiomers. The distinct pharmacological properties of AZEL, which are not shared by other DHPs, including its long-lasting blood pressure lowering, heart rate reduction, and antiatherosclerosis activity, may be connected to its unusual three-dimensional structure. AZEL also exhibits a diuretic effect by reducing ion retention and raising urine output **[2-7]**.

In most cases, smooth muscle contraction brought on by calcium contributes to hypertension. The vascular smooth muscle does not contract when calcium channels are closed, which causes the vascular smooth muscle walls to relax and lower blood pressure. Two methyl groups are found in the structure at the di-hydro pyridine ring's 2-and 6-positions, while an amino group in the Azelnidipine molecule structures the other methyl group at the 2-position. When treating hypertensive patients, Azelnidipine is administered orally in doses of 8–16 mg once daily **[8-11]**.

There is no approved pharmacopeia for this medication. Only HPLC, spectrophotometric, and methodologies have been published for drug validation in the literature survey. Here, a straightforward, accurate, and repeatable UV spectrophotometric technique has been created for the measurement of Azelnidipine in pharmaceutical formulations and bulk drugs. The created technique will be helpful for regular analysis in research institutions and the pharmaceutical industry **[12–15]**.

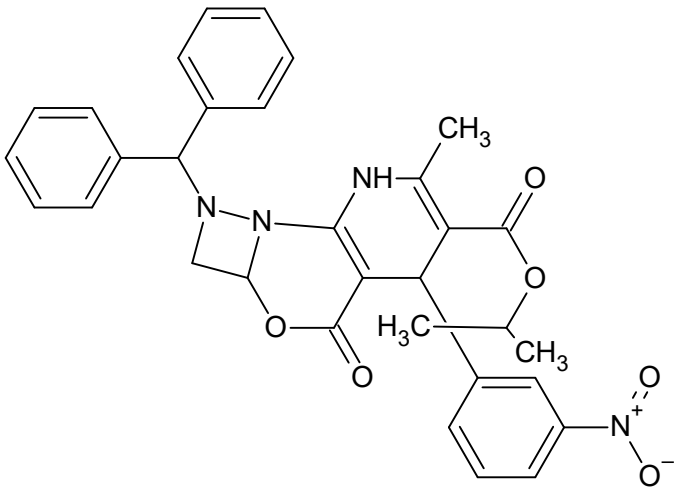

Figure 1: The chemical structure of Azelnidipine

The suggested method directly assesses Azelnidipine and has been verified with higher accuracy and precision than a traditional UV-visible assay, results can be trusted more. This method can be utilized for bulk pharmaceuticals and many dosage forms and is more direct, quick, and affordable than previous approaches **[16–18]**. A multivariate standardization procedure produced a particular outcome, and the conversion of the outcome yielded a dependent variable, "m." This technique offers excellent sensitivity, resolving power, expediency, and economic analytical efficiency for a determined quantification of AZE. AZE (X) refers to the absorbance of an analyte. It requires scanning five distinct concentrations (λ = 248,251,254,257, and 260 nm); for any desired wavelength, the following formula can then be used **[19, 20]**.

$A_{\lambda 248} = a\ X\ C_x + \ k_1$ ---------- (1)

$A_{\lambda 251} = b\ X\ C_x + \ k_2$ ---------- (2)

$A_{\lambda 254} = c\ X\ C_x + \ k_3$---------- (3)

$A_{\lambda 257} = d\ X\ C_x + \ k_4$---------- (4)

$A_{\lambda 260} = e\ X\ C_x + \ k_5$---------- (5)

Whereas absorbance of the analyte is denoted as A_{λ}, the analyte's slope of the linear regression functions are a, b, c, d, and e; The analyte's concentration is indicated as Cx, and the k1, k2, k3, k4, and k5 indicates the intercepts at the specific wavelengths.

The selected five wavelengths equation (1-5) listed above summarised in the following

$A_T = a\ X\ C_x + \ b\ X\ C_x + \ c\ X\ C_x + d\ X\ C_x + \ e\ X\ C_x + \ K_T$---------- (6)

The aforementioned equation can be further simplified to

$A_T = C_x\ (a + b + c + d + e) + \ K_T$--- (7)

The sums of the intercepts from regression equations at a chosen five wavelengths are denoted by A_T and K_T, respectively **[21, 22]**. The concentration of the analyte X is calculated using the formula given below [1], [2], [3], [4], [5], [6], [7].

$$C_x = \frac{A_T - K_T}{(a+b+c+d+e)} \quad \text{---------- (8)}$$

MATERIALS AND METHODS

Chemicals and reagents

- Methanol.
- Azelnidipine API was ex-gratis from Ideal Analytical Laboratory, Puducherry
- The marketed tablet formulation used was Torrent Pharmaceuticals Ltd., purchased from a nearby market.

Instrumentation

- Double beam UV spectrophotometer (LAB INDIA 3092)
- The Ultra Sonicator
- Microbalance
- The Micropipette

Analytical method development

Solvent selection:

Azelnidipine is readily soluble in Methanol. Therefore, both the standard and the sample were further diluted using Methanol.

Standard stock solution

A 25 Ml volumetric flask containing 25 mg of the standard medication is diluted with methanol to create the stock solution of Azelnidipine. Aliquots of this solution with concentrations, (5 – 15 µg Ml^{-1}) were prepared and utilized for further analysis.

Determination of λ_{max}

Azelnidipine maximum absorbance is established using a solution made by dissolving the standard stock solution in methanol to a concentration of 10 µg Ml^{1}. The prepared solution was scanned in the UV-visible region between 200 and 400 nm. The obtained graph plot between the concentrations against absorbance gives a linear curve**.** The outcomes are examined around the spectrum range 254 nm, i.e., 248, 251, 254, 257, and 260 nm, for improving correlation and diminishing the oscillations of the instrument.

Sample solution preparation

Preparation of sample solution is done by taking Azelnidipine tablets, precisely weighed and powdered. A 25 Ml standard flask was filled with the weight equivalent to 25 mg, the sample was dissolved, and methanol was added to make up the volume. After being filtered, this solution was utilized to further analyze the data.

Method Validation

The ICH guidelines have been followed in the validation of this method's sensitivity, precision, accuracy, and linearity **[23]**.

Linearity

Standard stock solutions were used to prepare a range of concentrations, from 5-15 µg Ml^{-1}. These solutions underwent evaluation across a range of wavelengths to reduce instrumental fluctuations and improve the correlation: 248, 251, 254, 257, and 260 nm **(Figure 3, Table 1)**.

Table 1: UV Calibration data at five distinct wavelengths

Concentration (µg mL^{-1})	Absorbance*				
	248 nm	251 nm	254 nm	257 nm	260 nm
5	0.271	0.279	0.281	0.279	0.273
7.5	0.385	0.401	0.573	0.401	0.396
10	0.544	0.563	0.573	0.565	0.549
12.5	0.672	0.701	0.710	0.703	0.690
15	0.802	0.839	0.857	0.849	0.833

[#]**Average of 5 determinations; UV= Ultraviolet**

The graph is plotted as concentration against absorbance and standardizations were achieved. By utilizing the following formula to determine the limit of detection and quantification, the sensitivity of the method was determined.

LOD = 3.3 σ/S ……... (8)

LOQ = 10 σ /S..... (9)

Hereby, the lowest concentration of standard deviation (SD) and the standard curve of the slope is denoted as S.

Precision

To determine the intra-day and inter-day precision, a 10-µg mL^{-1} was taken, and was

scanned six times. Six different days were used to measure the intra-day precision and within the same day to measure the inter-day precision.

Accuracy

For the suggested method, the recovery study was determined as 80%, 100%, 120%, by the standard addition technique, and using this % recovery was calculated. The solutions for the recovery study were prepared from both standard and sample stock solutions.

Assay

By measuring the absorbance at 254 nm from the extracted tablet solution, the amount of Azelnidipine present in the tablet was determined.

RESULTS AND DISCUSSION

As seen in **Figure 2**, the Azelnidipine maximum absorbance was measured at 254 nm using water as the solvent.

Within the concentration range between 5 - 15 µg $mL^{-1,}$ this technique was found to be linear. An excellent linear correlation is obtained from the calibration plots with R^2 - 0.9991- 0.9999. The % relative standard deviation for precision was obtained as 0.5453 and 0.4680. The detection and quantification limits were at 0.4569 and 1.3846 µg mL^{1}, respectively. Hence the values come under validation parameters following the ICH guidelines limitations.

Linearity

The linearity spectra are depicted in **Figure 3** and the corresponding calibration curves are shown from **Figure 4 to 8.** The approach is deemed accurate and dependable for every wavelength, as indicated by the low values of the relative standard deviation. The calculation of detection and quantification of limit has been done and the results are depicted in **Table 2**.

Precision

The suggested technique is distinct, dependable, and accurate, as evidenced from the low standard deviation values. The intra-day precision and inter-day precision values are 0.5453 and 0.468095, respectively. It is within tolerances of less than 2% at every wavelength. (**Figure 9, 10**).

Recovery

The Azelnidipine % recovery was found between 97% to 103% W/V, according to the ICH guidelines. The acceptable range of % recovery was from 98.40-101.67% W/V **(Figure 11, Table 3)**.

Assay:

The Azelnidipine maximum absorbance was measured at 254 nm for the tablet formulation by UV-visible spectroscopy. The amount and assay percentages were obtained as 16.08 mg and 100.33 % w/v, further % Relative standard deviation value is depicted in **Table 4**.

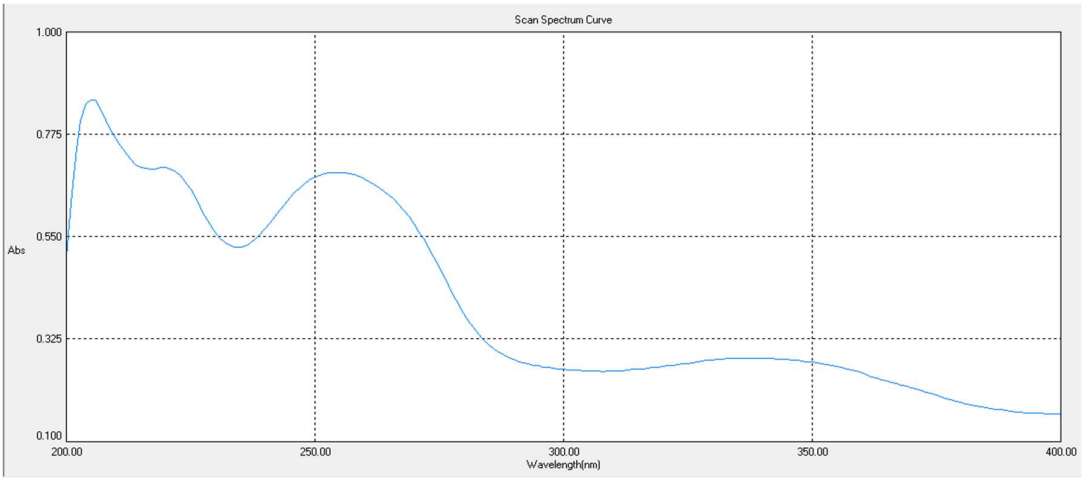

Figure 2: UV spectrum of Azelnidipine (10 µg mL $^{-1}$), λ_{max} at 254 nm

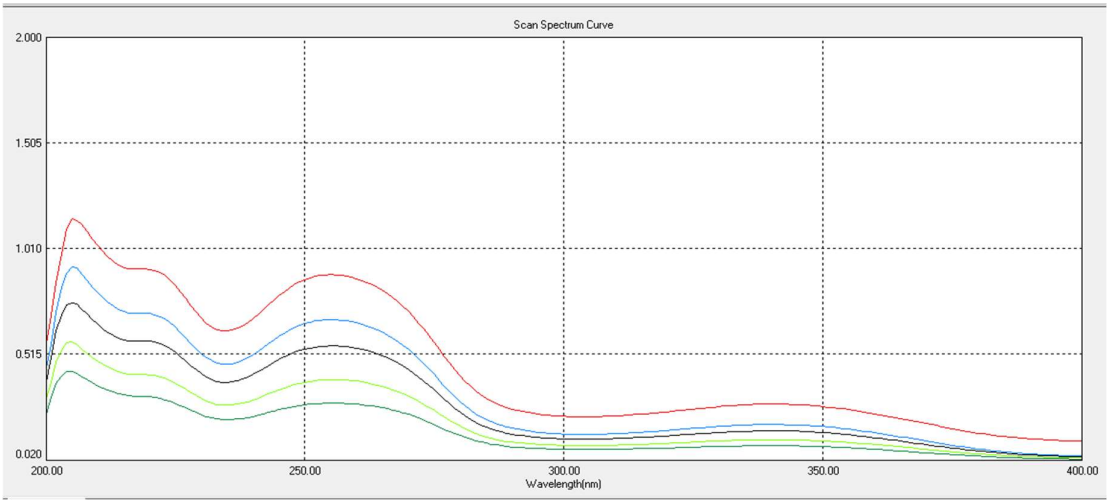

Figure 3: UV Spectrum of Azelnidipine showing linearity at 254 nm

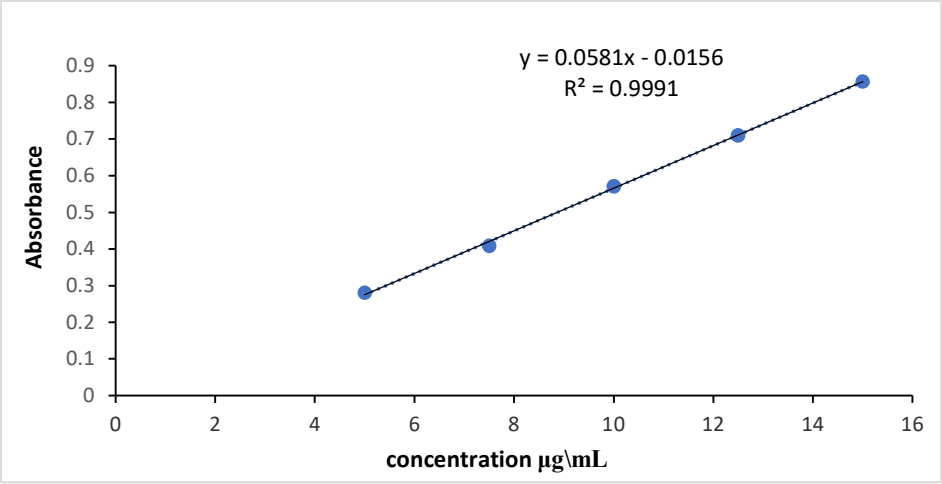

Figure 4: Calibration curve at 248 nm

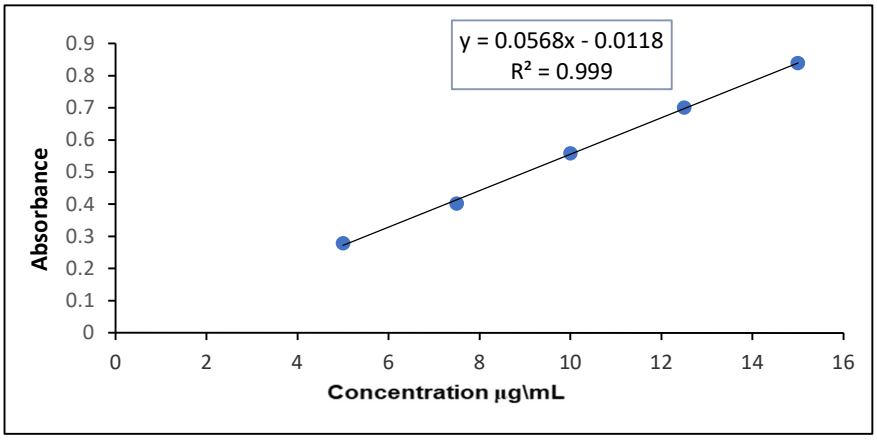

Figure 5: Calibration curve at 251 nm

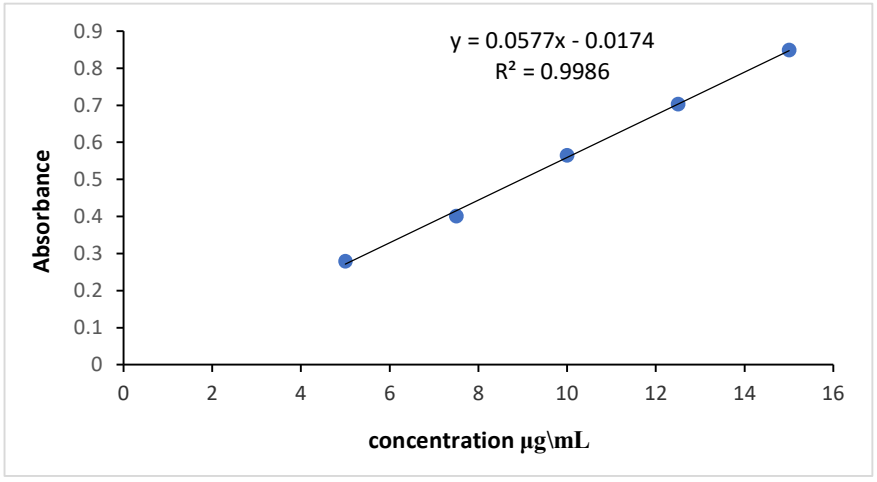

Figure 6: Calibration curve at 254 nm

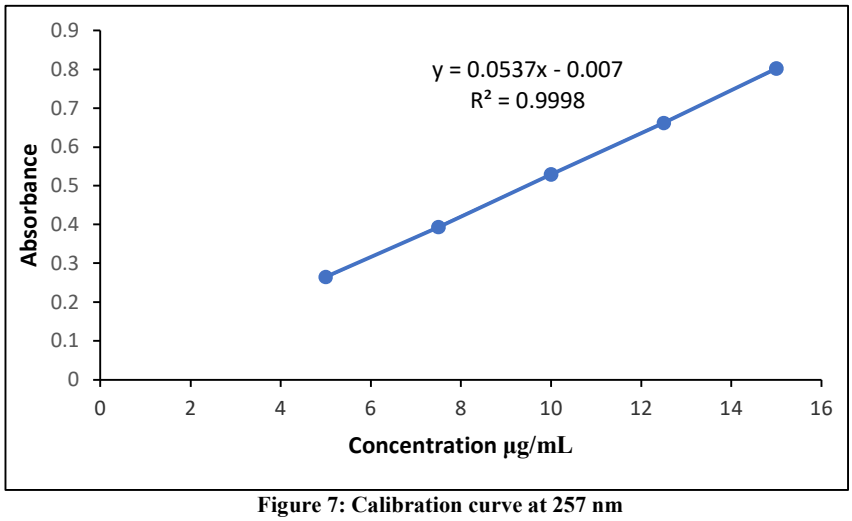

Figure 7: Calibration curve at 257 nm

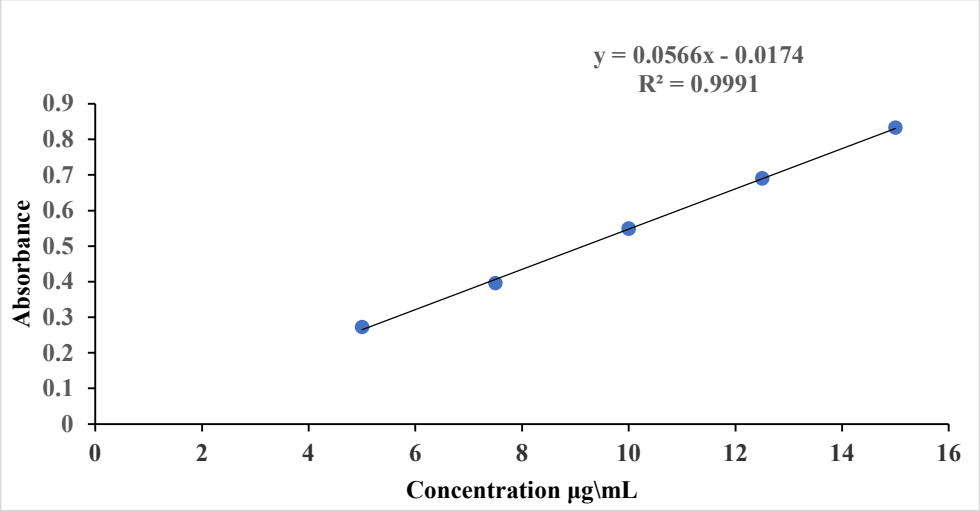

Figure 8: Calibration curve at 260 nm

Table 2: Linearity data with LOD and LOQ at selected five wavelengths

Wavelength (nm)	Regression equation	R^2	LOD (µg mL ^{1})	LOQ (µg mL ^{1})	% RSD
248	y = 0.0537x - 0.007	0.9998	0.2183	0.6616	0.6703
251	y = 0.0568x - 0.0118	0.999	0.4825	1.4624	1.4934
254	y = 0.0581x - 0.0156	0.9991	0.4569	1.3846	1.4228
257	y =0.0577x - 0.0174	0.9986	0.5709	1.7302	1.7840
260	y = 0.0566x - 0.0174	0.9991	0.4509	1.8431	1.3664

*nm = nanometre; µg mL 1= Microgram per millilitre

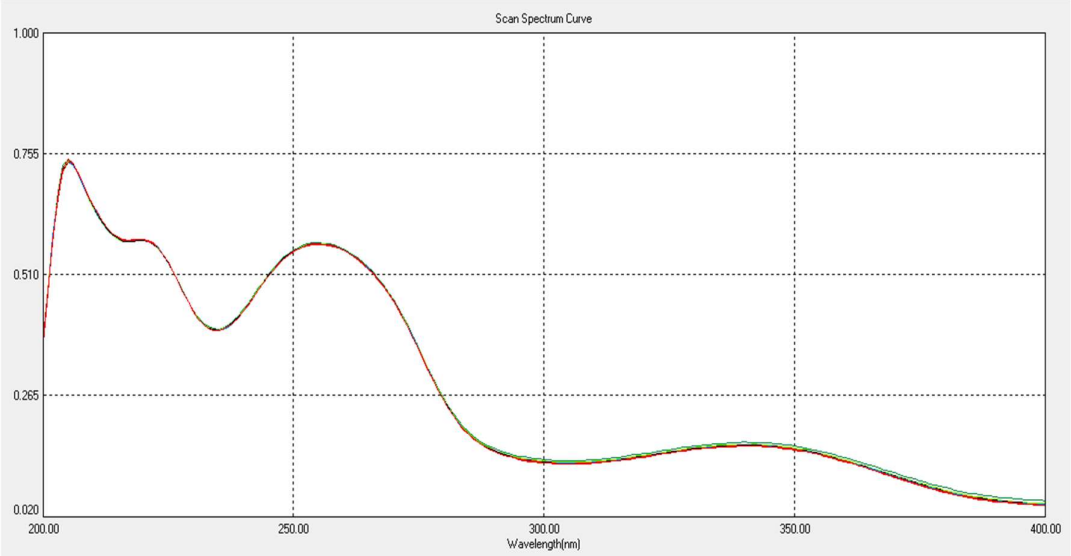

Figure 9: UV spectra showing intraday precision

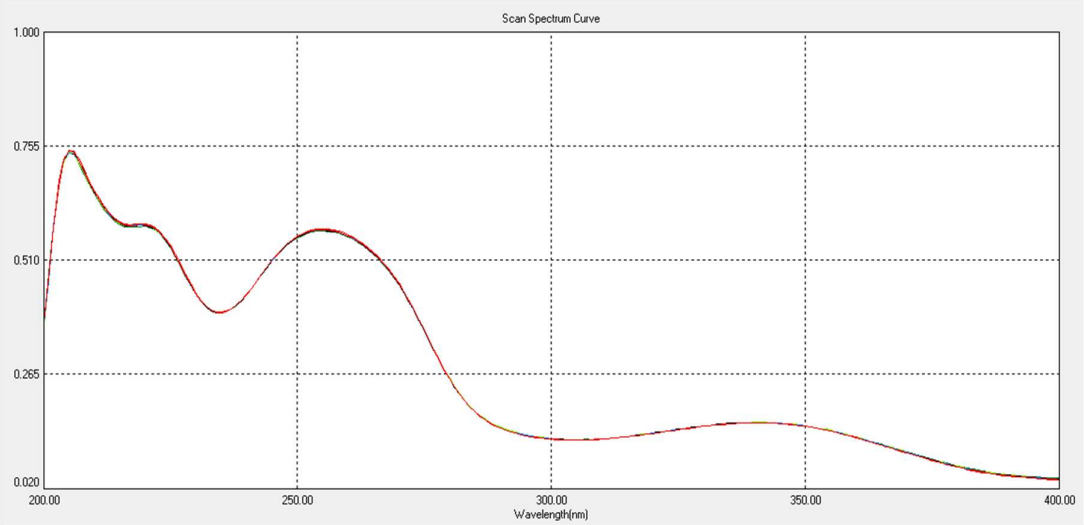

Figure 10: UV spectra showing interday precision

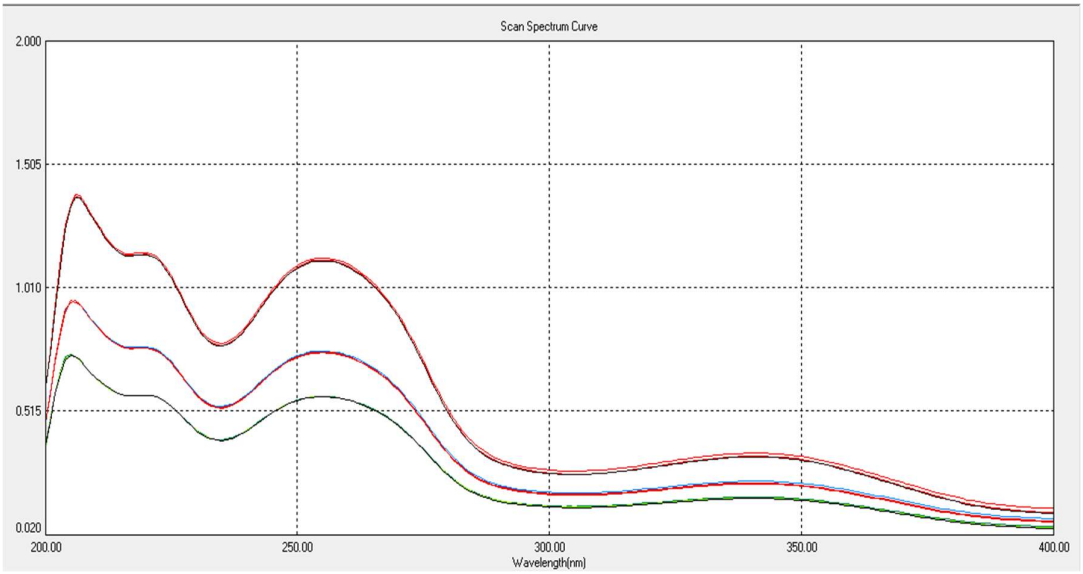

Figure 11: UV Spectrum showing the accuracy of Azelnidipine.

Table 3: Recovery Studies

Wavelength (nm)	Amount present (µg mL^{-1})	Amount added (µg mL^{-1})	Absorbance	Amount recovered (µg mL^{-1})	% Recovery
248 nm	5	3	0.423	8.06	100.75
		5	0.544	10.12	101.20
		7	0.62	12.1	100.83
251 nm	5	3	0.44	8.06	100.75
		5	0.563	10.4	104.00
		7	0.64	11.91	99.25
254 nm	5	3	0.447	8.01	100.13
		5	0.573	10.2	102.00
		7	0.675	12.2	101.67
257 nm	5	3	0.442	8.05	100.63
		5	0.565	9.98	99.80
		7	0.646	11.92	99.33
260 nm	5	3	0.425	8.05	100.63
		5	0.549	10.06	100.60
		7	0.659	12.02	100.17

Table 4: Assay of Azelnidipine

Label claim (mg)	Amount obtained (mg)	% Assay
16	15.98	99.88
16	16.01	100.06
16	16.17	101.06
Average	16.05	100.33
SD		0.6384
% RSD		0.6363

CONCLUSION:

Comparing the suggested method against the traditional UV-visible spectrophotometry for Azelnidipine assay, it is more exact, accurate, repeatable, and economical. Azelnidipine standard drug and tablet dosage form of Azelnidipine quantified by the multivariate regression equation. According to the Quality Guidelines of ICH, this method has been validated and they are inside the range of the limits. This method was found simpler and more economical than complicated HPLC and HPTLC methods and it is used for the analysis of the sample of Azelnidipine bulk drugs and pharmaceutical dosage forms.

ETHICAL STATEMENT

This study does not involve experiments on animals or human subjects.

ACKNOWLEDGMENTS

The authors are thankful to the Chancellor, SRM Institute of Science and Technology, and the management of SRM College of Pharmacy, SRM Institute of Science and Technology, Kattankulathur for providing various reprographic sources for carrying out this work.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article exists.

FUNDING SOURCES

There is no funding to report.

REFERENCES:

[1] Azelnidipine: Uses, Interactions, Mechanism of Action DrugBank: https://go.drugbank.com/drugs/DB09230

[2] Attimarad M, Chohan MS, Katharigatta Narayanaswamy V, Nair AB, Sreeharsha N, Shafi S, et al. mathematically processed uv spectroscopic method for quantification of chlorthalidone and azelnidipine in bulk and formulation: Evaluation of Greenness and Whiteness. Journal of Spectroscopy. 2022; 2022.

[3] Kawade DP, Ikhankar MA, Chaple DR, Asnani AJ, Lokhande AS, Raut SM, et al. UV spectroscopic method for the estimation of azelnidipine-a review. World Journal of Pharmaceutical Research 2015; 11(2).

[4] Paul Richards M, Madankumar, Nandankumar, N Sharath Gowda, K Khairat, Prajwal R Shankar. Method development and validation of

azelnidipine drug by ultraviolet visible double beam spectrophotometer. RGUHS J Pharm Sci. 2022; 12(1):18–22.

[5] Suma R, Preeti Karwa, V Kusum Devi. Development and validation of UV spectrophotometric method for determination of tacrolimus in bulk and capsule dosage form. RGUHS J Pharm Sci. 2022; 12(3):21–5.

[6] Ubale S, Kalshetti MS, Habib B, Mittha J, Adlinge S. UV spectrophotometric method development and validation of azelnidipine in bulk and dosage form. International journal of creative research thoughts (IJCRT) Vol. 9. (7).2021(1).

[7] Mashru RC, Mali PP. Suthar and Mashru , Advanced UV spectrophotometric method development and validation online first indian j pharm drug studies 1 advanced UV spectrophotometric method development Vol 2022:(121-127).

[8] Eswarudu MM, Roja P, Sankar PR, Babu PS. An updated review on analytical methods for estimation of azelnidipine and telmisartan. Asian journal of pharmaceutical research and development. 2022 Apr 15; 10(2):59–76.

[9] Pharma D, Patel KB, Barot R V. ISSN 0975-413X CODEN (USA): PCHHAX .A Review on analytical methods for estimation of azelnidipine and chlorthalidone in pharmaceutical dosage form. 2022(9):6–11.

[10] Noolvi MN, Upasna K. A review on analytical methods for estimation of azelnidipine and chlorthalidione in pharmaceutical dosage form. Certified Journal 533 Upasna et al World Journal of Pharmacy and Pharmaceutical Sciences. 2023; 12.

[11] Mistry R, Nitin Chauhan N, Mandale D, Chauhan N. An analytical approach of azelnidipine: a review. Certified Journal 682 Mandale et al World Journal of Pharmacy and Pharmaceutical Sciences. Vol 10(3) 2021; 10.

[12] Rele Rajan V. Spectrophometric estimation of azelnidipine in bulk and pharmceutical dosage form by second order derivative method. J Chem Pharm Res. 2014; 6(8).

[13] Jeeva A S Fathimath Shareena P. K, Nivedhya P. P Sheeja. Method development and validation of azelnidipine in bulk and dosage form by visible spectroscopy in NED reagent. Journal of emerging technology and innovative research. 2023; 10(6).

[14] Kunti.D Mrunali M, Anandkumari D, Captain. UV spectrophotometric method development and development and validation for determination of azelnidpine in pharmaceutical dosage form. Int J Pharm Pharm Sci. 2012; 4(1):2–4.

[15] Mukeri IH, Kushwaha AK, Neupane NP, Kumar A. Analytical method development and validation of azelnidipine by uv-visible spectroscopy. Article in World Journal of Pharmaceutical Research 2021(Aug 10): 858-872, D0I: 10.20959/wjpr202110-21174.

[16] Kokilambigai K S, Logesh M, Anusha Reddy C, Seetharaman R, Kavitha J, Lakshmi. K. S. Multivariate calibration technique for spectrophotometric quantification of ondansetron in bulk drug and pharmaceutical formulations. Journal of medical pharmaceutical and allied sciences. 2022 Aug 30; 11(4):5050–6.

[17] Vimal Ravi, Kokilambigai K S, Kavitha J, Seetharaman R, Lakshmi K S. Multivariate UV spectrophotometric quantification of Cilnidipine in bulk drug and pharmaceutical formulations. Journal of medical pharmaceutical and allied sciences. 2022 Mar 30; 11(2):4672–8.

[18] Mani Aravinth R, Kokilambigai K. S, Anusha Reddy C, Seetharaman R, Kavitha J, Lakshmi. K. S, 2021. Spectrophotometric quantification of granisetron in bulk drug and pharmaceutical formulations employing multivariate calibration technique. Journal of Medical pharmaceutical and allied sciences. 2021 Oct 15; 10(4):3435–9.

[19] Rani DN, Kokilambigai KS, Lakshmi KS. Multivariate Calibration Technique for the Spectrophotometric Quantification of Rasagiline in Bulk drug and Pharmaceutical Formulations. Res J Pharm Technol. 2020; 13(2):843.

[20] Durgadevi P, Kokilambigai KS, Lakshmi KS. First Order Derivative Spectrophotometric Method for the Quantification of Telmisartan employing Multivariate Calibration Technique. Res J Pharm Technol. 2020; 13(2):774.

[21] Madhan S, Kavitha J*, Lakshmi KS. Multivariate calibration technique for the spectrophotometric quantification of ivermectin in pharmaceutical formulation. Asian Journal of Pharmaceutical and Clinical Research. 2019 Jan 31; 444–51.

[22] Susmitha AS, Durgadevi P, Kokilambigai KS, Lakshmi KS. Spectrophotometric Quantification of Telmisartan Employing Multivariate Calibration Technique in Bulk and Pharmaceutical Formulations. Res J Pharm Technol. 2019; 12(4):1799.

[23] ICH. Harmonised tripartite guideline validation of analytical procedures: text and methodology Q2 (R1). Geneva, Switzerland, 2005.