https://doi.org/10.48047/AFJBS.6.11.2024. 423-434

African Journal of Biological Sciences

Advancing Azelnidipine Dosage Form Analysis: A Quality by Design Guided Journey with Green Chemistry Innovations

Kirtimaya Mishra[1], Swagatam Sahoo[2], Purnima Mahato[3], Nilesh Kumar Singh[3], Diptimayee Jena[1*]

[1,1*]Centurion University of Technology and Management, Bhubaneswar, Odisha

[2]DRIEMS University, Tangi, Cuttack, Odisha

[3]ARKA JAIN University, Jamshedpur, Jharkhand

Corresponding Author

Miss Diptimayee Jena
Assistant Professor
School of Pharmacy & Life Sciences
Centurion University of Technology & Management
Bhubaneswar, 752050
Contact No. 7008825597
diptimayee.pharma@gmail.com

Article History
Volume 6, Issue 11, 2024
Received: 02 Jun 2024
Accepted: 15 Jun 2024
doi: ***10.48047/AFJBS.6.11.2024.423-434***

ABSTRACT

AIM: Green chemistry, also known as ecologically sustainable chemistry, is an area of study in chemistry whose major goal is the reduction of the adverse impacts of chemical processes along with products. Objective is supporting the advancement of viable & ecologically therapies by reducing/eliminating the implimentation of noxiuous chemicals, limiting the fabrication of explosive chemicals, along with conserving energy, as well as resources.

MATERIAL/METHOD: This investigation found that the concentration range of azelnidipine (AZDN) was linear, ranging from 02 to 12 µg/mL. Having a sample size of six, the recovery %age from tablet formulation was assessed to be 99.6% with a standard deviation (SD) of ± 0.04. The accuracy study inferred recovery rates that, on average, varied between 99.6 and 100.1%.

RESULTS: Throughout intra-day assessments, resulting %RSD, showed a value that was significantly less than 2%, stipulating a remarkable range of accuracy in the strategic plan. Such recommended processes can be implemented inside the aided quality control laboratories, according to the results of validation and statistical analyses of the technique.

CONCLUSION: The current approach is considered to be appropriate for quantitatively evaluating AZDN in tablet dosage formulations subsequently, it successfully removes any of the possible obstructions among commonly utilized preservative. Therefore, this particular method can be used for routine analytical objectives.

KEYWORDS: Ecologically Sustainable Chemistry, Accuracy, Eco-friendly, Spectrophotometric, Azelnidipine, Quality by design, Toxic Chemicals

INTRODUCTION

In an effort to lessen the harm that chemicals produced and distributed cause to the environment, a comprehensive strategy termed as "green chemistry" is being established. Development of ecologically friendly alternatives to typical chemical-based techniques is the primary emphasis of this inquiry in order to reduce waste, conserve resources, and increase the use of organic products [1]. Because of the increased advancement of science & technology developments, revenue generation is currently occurring all over the world. However, this economic growth also contributes to environmental degradation, as evidenced by problems like ozone holes, climate change, and the accumulation of non-invasive organic contaminants throughout the natural world [2]. Azelnidipine (AZDN), signified by the IUPAC (International Union of Pure and Applied Chemistry) naming, 3-1-Benzhydryl-3-azetidinyl-5-isopropyl-2-amino-6-methyl-4-(m-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate (Fig 1). This drug acts by blocking L-type calcium channels, which are responsible for allowing calcium to pass through vascular smooth muscle cells. This causes vasodilation, which lowers blood pressure [3]. Because of its distinct pharmacokinetic characteristics and established therapeutic advantages, AZDN is an essential addition to the treatment toolkit for hypertension [4].

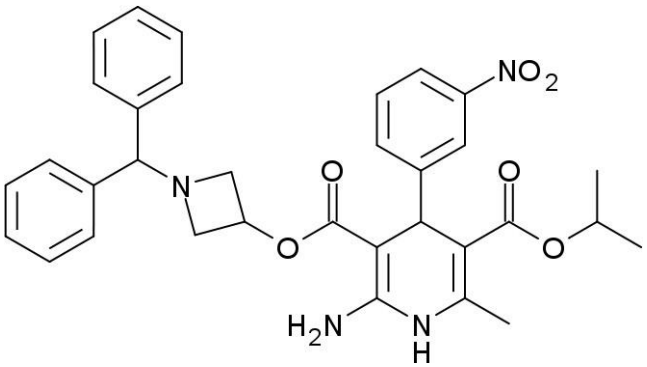


Figure 1 Chemical structure of AZDN

AZDN in tablet dosage forms as well as biological samples is measured using the LC-MS, HPLC, and UV-visible spectroscopy methods [5]. However, the revealed UV spectrophotometric technique has a number of flaws. A restricted linearity range, the absence of Sandell's sensitivity, along with the omission of the molar extinction coefficient are a few of these [6]. In order to further develop a novel and developed UV spectrophotometric approach for

quantifying AZDN through tablet manufacture, Quality-by-Design (QbD) was employed as a result.

An integrated strategy, Quality-by-Design (QbD), which integrates excellence in all areas of the business to ensure that the intended result is realised. International Council for Harmonisation (ICH) regarding technical requirements for pharmaceuticals for human use, predominantly in ICH-Q8-(R2), has established standards for systematic creativity that QbD complies with. This method starts with goals that are well-defined and emphasises understanding and preserving the ultimate product and its organisation [7]. The method used in this plan is founded on sound scientific principles and includes precautions to reduce hazards, ensuring the accuracy and reliability of the outcomes. QbD has been introduced since the FDA released its "Pharmaceutical Current Good Manufacturing Practises (cGMPs) for the 21st Century" in 2002. Implementation of the empirical QbD Perspective along with arriving at a robust, dependable, & high-quality analysis technique involving six steps.

Moreover, applying the QbD methodology shortens the time needed to create a reliable analytical method and takes into account a financially sensible approach to guarantee quality starting from the beginning of method development. DoE is commonly seen as an important technique for QbD, since it plays a key role in identifying the optimal configuration domain for method efficiency. This study is primarily concerned with the application of strict experimental designs; the overall objective is the reduction of amount of fluctuation regarding the spectrophotometric aspects of AZDN. The main goal is to identify the best choices. The study initiated a factor screening analysis with the use of a Fractional Factorial Design (FFD) in line to identify critical technique variables that impacting the performance. The central composite design (CCD) was then put into practice to enhance the process, guaranteeing its durability and achieving predefined goals. This investigation's specific goal was to prosper a novel, rigorous, along with accountable UV spectrophotometric methodology for figuring out how much AZDN was present in tablet formulations.

MATERIALS AND METHODOLOGY

REAGENTS AND STANDARDS

Presenting the AZDN standardised sample was Laksh Finechem Pvt. Ltd. with a purity of over 99.5%. The ethanol (EtOH) that we used to prepare our pharmaceutical & reagent solution was purchased from Merck Ltd. located in Jamshedpur, India. Since, commercially available tablet form of AZDN (Zeblong 16mg) was available, it was acquired and examined in accordance with the protocol.

INSTRUMENTATION AND OPTICAL CHARACTERISTICS

10mm calibrated quartz cuvettes were used in the spectrum research, which was performed using microprocessor controlled single beam system along with LI-285 UV spectrophotometer (produced by Lasany, India). A high-precision analytical device was utilised to guarantee accurate readings of the reagents. The use of ultrasonication (Enertech, India) was used to affect the tablet dosage form's dissolution.

INCORPORATION OF ANALYTICAL TARGET PROFILE

To create an analytical target profile, a comprehensive analysis of the available literary text sources and drug profiles—including their physical and chemical characteristics—was carried out. This profile includes an analytical approach in addition to a brief summary of the quality features. The ultimate goal of this research was to develop a cost-effective, accurate, and efficient analytical technique for calculating the concentration of AZDN in tablet form. Therefore, in keeping with the main goal regarding this investigation, UV spectrophotometric method was utilized for speeding up the analysis of AZDN. UV spectrophotometric method's benefits over more sophisticated analytical techniques, such as its simplicity and efficiency, led to the decision to use it for drug analysis [8].

INCORPORATION OF RISK MANAGEMENT & CAUSE-EFFECT RELATIONSHIP

One of the simplest tools, Ishikawa fish-bone diagram, for observing how various parameters that could impact a method's performance are related. To acquire more skills regarding these variables might alter AZDN's UV spectrophotometric effects, an Ishikawa diagram was created. Based on the Control-Noise-Experimentation (CNX) technique, researchers employed Cause-Effect Risk Assessment Matrixes to determine which variables are most imaginable to impact the analytical aspects of the investigation. Numerous Critical Method Variables (CMVs) those were connected towards increased final reports were discovered by the investigation, suggesting that they are high-risk variables. These CMVs are made up of several combinations of detecting solvent type, intensity of sampling, scan rate, wavelength, sample integrity, as well as sample pH. In order to estimate these critical technique parameters, screening design was also implemented to examine the CMVs. The organisation of response surface optimisation was then carried out using a preferable experimental design.

SCREENING OF CMVS BY FFD

Design Expert 13 software, version 13.0.14, USA, is being used to monitor key parameters in order to discover the variables that carry a high risk. The differentiation in the precision, absorbance, and spectrum design, led to the selection of a number of variables as crucial method variables. To determine the detection wavelength, solvent type, and sample integrity, prioritisation studies were conducted based on existing knowledge and the Ishikawa fish-bone diagram. These factors were verified through firsthand observation. Using design expert software, an FFD experiment with at least five trials (one as a centre point) was carried out for evaluating the parameters regarding methodology such as, EtOH concentration (X1), Sample pH (X2), as well as Sampling interval (X3). Similarly, how parameters were assessed at both higher as well as lower values, the programme was also utilized for identifying CMVs that had an impact on the response variable's absorbance (Y). By analysing the plot of actual values vs anticipated values, the prediction equation, the pareto chart, and the fitting summary plot, significant parameters were found.

METHOD OPTIMIZATION & ROBUSTNESS STUDY IMPLEMENTING CCD

To ensure that the procedure for obtaining the ideal method circumstances was dependable, the CCD was used. Thirteen dummy runs, including the Conc. of EtOH (A) & pH (B), were prepared as a result of the screening investigations. At least five central points were selected and relied upon CCD to obtain the best CMVs. Absorbance at 252 nanometre was used as the response variable to examine the experiment results. A standard AZDN of 10µg/mL was utilized for each test.

The designed Expert software is employed to fix experimental details using Multiple Linear Regression Analysis (MLRA) into a mathematically represented model. Main and interaction effects might be investigated with this model. For assessment of the model, only significant coefficients (p value<0.05) were used for parameter analysis and polynomial equation framing, such as R^2, adjusted R^2, and the Predicted Residual Sum of Squares (PRESS), respectively. These analyses were conducted using ANOVA. Feasibility of the replica was assessed using a variety of profilers, including three-dimensional response surface profilers, projection profilers, and interaction profilers. By finding a balance between the several elements to be considered, we were able to discover the ideal solution using a numerical desirability function. This was then used to demarcate the region of the design.

METHOD CONTROL STRATEGY

The DoE technique created a design space that was utilized to guide the advancement of control strategies regarding the method. This space allowed the methodology to maintain its flexibility even when slight modifications in its performance were made.

PREPARATION OF STANDARD STOCK SOLUTION

10 mg of AZDN was mixed in 10 mL of EtOH to create the AZDN standard stock solution (1000µg/mL). Five millilitres from the prepared stock solution were shifted to a 50 millilitre volumetric flask, and contents were labelled with water to generate 50 millilitres, in order to create 100 g/mL standard solutions.

TABLET DOSAGE FORM ANALYSIS

The label of Zeblong 16/Laksh Finechem Pvt. Ltd. states that the formulation of its AZDN tablets contains 16 mg. 10 millilitres of EtOH should be used to dilute 10 mg equivalent weight of AZDN for the stock solution. Once the solution was prepared, 5 millilitres from it were added into a 50 millilitre volumetric flask & labelled to 50 millilitre level using water. The material was ultrasonically treated for thirty minutes. To further filter this solution for particulates, Whattmann filter paper was used. The filtered solution was further diluted with water in order to examine it. We calculated the amount of medication in the sample solution using a calibration curve based on the conc. of standard AZDN.

METHOD VALIDATION

THE SPECIFICITY

The presence of the medicine in the formulation excipients was used to evaluate the specificity of the UV spectrophotometric approach. In order to determine whether the excipients could have caused any potential interference, the spectrums were analysed in line.

LINEARITY

At the conclusion of the process, several aliquots were taken with the AZDN working standard solution and put into different 10 mL volumetric flasks. These were then diluted with H_2O to produce a measurement of concentrations ranging from 2 to 12 g/mL. At particular wavelength of 252 nanometre, UV absorption was measured. To determine the outcomes such as linear, calibration curve was plotted by placing the concentration (in g/mL) & the absorbance on X & Y axes, respectively.

PRECISION & ACCURACY

Three different levels were utilised in the investigation, using the conventional dilution approach, to evaluate the precision and recovery of the methodology: 80%, 100%, and 120% of the AZDN (10µg/mL) test solution. For each degree, recovery studies were conducted in duplicate. Plotting a calibration curve was used to calculate the standard medication, AZDN, combined with the recovery solution. To assess intra-day precision, six replicates of a constant

conc. of AZDN (10µg/mL) examined in single day, and the percentage RSD values were then calculated [9].

RESULTS

The current study devised the Ultraviolet Spectrophotometric (UV Spectrophotometry) method to ascertain the quantity of AZDN in a tablet formulation. The QbD approach was implemented to determine the variable key factors that would be required to build the final spectrophotometric settings. A traditional Ishikawa fish-bone diagram was created in order to identify the technique variables. The physical assessment of the procedure's variables was completed. It was found that neither acetone nor ether caused the medicine to disintegrate. In water, AZDN was soluble. The wavelength of 252 nm was selected as the detecting wavelength because it is the wavelength at which the std. AZDN solution meets its λmax, maximum absorption in water (Fig. 2).

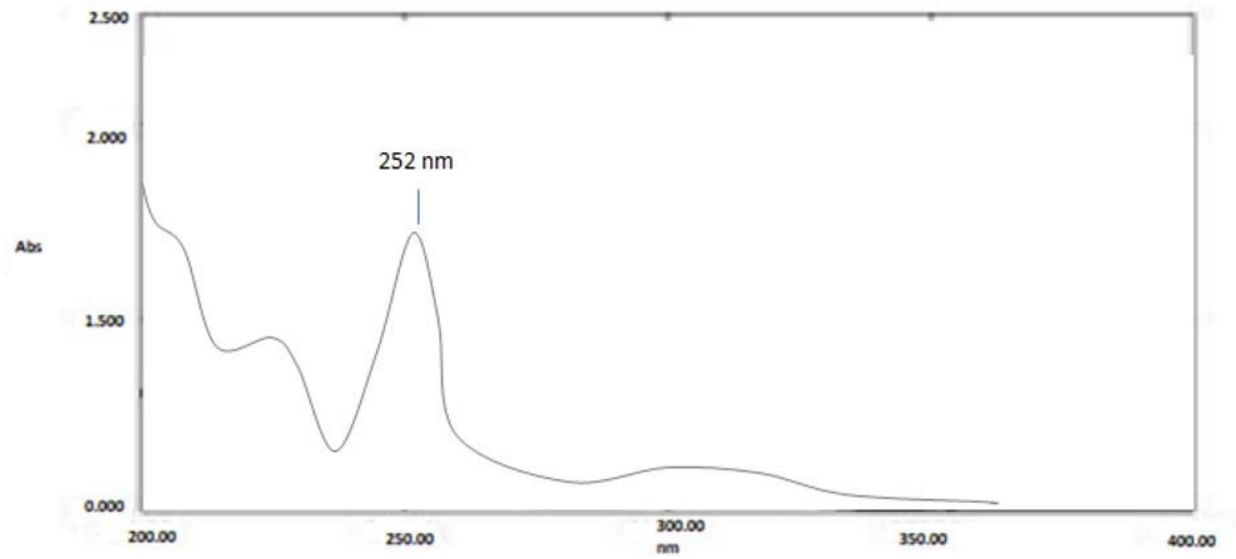


Fig 2 Typical UV absorption spectrum of AZDN

Good sample integrity shown by the melting point test. Sampling interval, Sample PH, scan speed, and however, are necessary regarding a comprehensive investigation to evaluate their responses about procedure robustness. Using FFD makes it simpler to screen CMVs based on sample PH, conc. of EtOH, and scan speed. The model's fitness was displayed in actual vs. forecasted charts. The appropriateness of the model was demonstrated by the p-value (0.0004), R_2 (0.9399), and RMSE (Root Mean Square Error) (0.0270). The fit summary displayed the adjusted R2 (0.8970) and the expected R2 (0.5729).

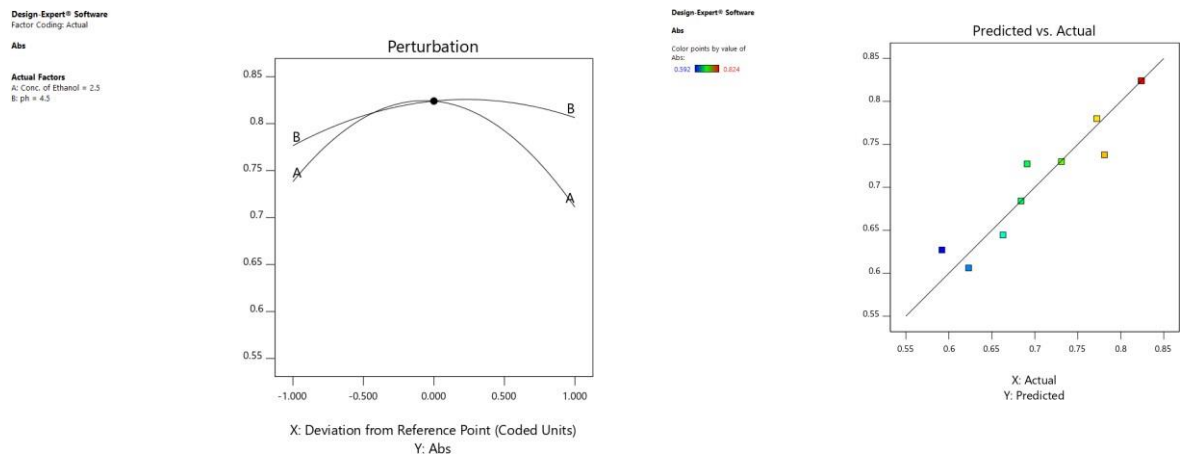
In order to verify how the CMVs altered the reaction absorbance, the CCD was put into use. Thirteen trials were conducted in a random order using a UV spectrophotometer in order to obtain a bias-free result with a minimum of five centre points. The spectrophotometric range examined and the observations from each experiment are displayed in Table 1.

Table 1. Experimental-design matrix displaying spectrophotometric range studied for robustness study and obtained responses

Run No.	Conc. of EtOH in ml(A)	pH (B)	Absorbance (Y)
1	2.5	4.5	0.824
2	1	6	0.684
3	2.5	4.5	0.824
4	1	3	0.691
5	2.5	6.62132	0.772
6	2.5	4.5	0.824
7	2.5	4.5	0.824
8	4	3	0.592
9	4.62	4.5	0.623
10	4	6	0.731
11	2.5	4.5	0.824
12	0.37868	4.5	0.663
13	2.5	2.37868	0.781
Range	Low	High	
EtOH	1	4	
pH	3	6	

Depending on a preset significant range of 0.05 regarding p-value, the null hypothesis (H0) was accepted. To arrive at significant findings, a thorough analysis of the CCD model was carried out using a variety of statistical analytic methods, including ANOVA, parameter estimations, and prediction profilers.

Plots of perturbations show the expected models and the effects of independent factors on a particular response in Figure 3(A), with all other parameters fixed at a reference point. A slope or curve's degree of steepness reveals how sensitive an impact is to a particular element. According to the analysis shown in Fig. 3(A), factor B, the sampling interval had the second-greatest significant effect regarding absorbance, after sample pH. Baseline model is displayed in the actual vs predicted graphic (blue dots), and it can be seen, the line derived with respect to the experiment data precipitates smoothly regarding the accuracy interval parameters (Fig. 3(B)). Due to the observed outcome's strong resemblance regarding the expected data, null hypothesis is refuted & model's ability to adequately describe data variation is demonstrated.



A **B**

Fig 3(A) Pertubation Plot, **3(B)** Predicted vs. Actual Plot

Response surface examples are plotted against pH and EtOH concentrations in (Fig 4) (The pH is used to represent the concentration of EtOH). Analysing perturbation and response plots and optimising models revealed that factors significantly affected the analytes' absorbance [10].

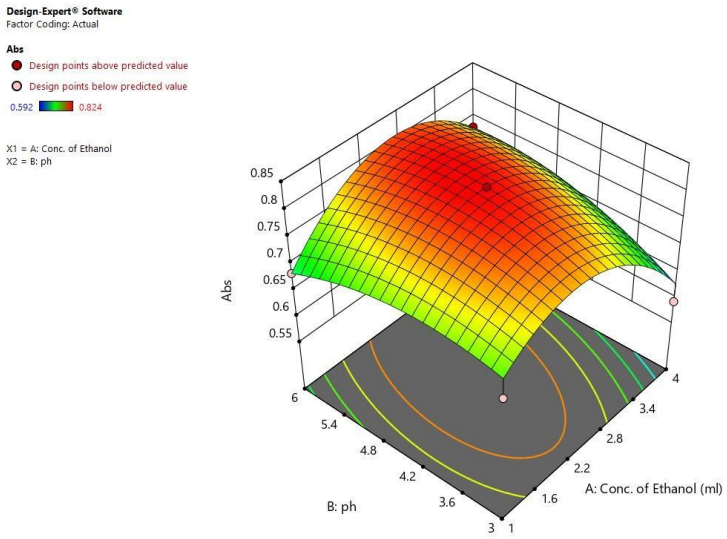


Fig 4 3-D Response surface plot for absorbance against Conc. of EtOH Vs. pH

Furthermore, the results of the ANOVA's Analysis of Variance revealed P-value, less than 0.0005, suggesting the model is appropriate to handle the variable observed data. Additionally, this result implies that the null hypothesis needs to be disproved. In addition, the lowest value seen in anticipated PRESS supported the appropriateness of the concept.

In order to estimate the risk of variability from a variety of variables, it is essential to analyse parametric calculations. When p-value < 0.05, non-zero slope is present.

Sampling interval × pH (B2) and conc. of EtOH (A) are the greatest impacting key variables.

$$Absorbance\ (Y) = 0.8240 - 0.0136\ A + 0.0149\ B + 0.0365\ AB - 0.0993\ A^2 - 0.0326 B^2$$

where, A=Conc. of EtOH, B=pH.

The optical characteristics connected to the spectrophotometric method are shown in Table 2. It was revealed that, the frequently used formulation preservatives in the tablet dose formulation is not comply with respect to the projected methodology, the developed approach displayed both specificity and selectivity. The drug showed linearity in the range of approximately 2–12 µg/ml. Regression analysis was performed on the linearity data, and the results showed a strong overall goodness of fit. The R_2, adjusted R_2, also anticipated R_2 statistical measures produced results of 0.9399, 0.8970, & 0.5729, in that order. Statistically significant p-value < 0.05, in ANOVA suggested that the method for evaluating the linearity of the data was considered sufficient.

Table 2. Optical Characteristics and Summary of validation parameters

Parameters	Obtained Values
Linearity Range (µg/ml)	2-12
Wavelength (nm)	252
Regression equation(Y=ax+b)*	0.0681x + 0.0037
Correlation coefficient(R^2)	0.9999
Molar extinction coefficient (ltr/ mol.cm)	$3.961 * 10^4$
Precision (% R.S.D., n=6)	0.0842847
Accuracy (% RSD ± S.D.)	
120%	0.162865 ± 0.002449
100%	0.691547 ± 0.009428
80%	0.199362 ± 0.002449
% Range of error	
99% confidence limit	± 0.4902
95% confidence limit	± 0.0037

RSD: Relative Standard Deviation; SD: Standard Deviation; AU: Absorbance Unit

Y= ax+b, where Y = absorbance, a = slope, b = intercept and x = concentration, (average of three determinations at each level)

DISCUSSION

The accuracy investigation's recovery rates showed an average limit of 99.6–100.1%. With regard to intra-day evaluations, %RSD showed a value significantly less than 2%, suggesting a high degree of accuracy in the recommended methodology. The results of the used

methodology are achieved within the predefined range, suggesting the availability of additives has no effect on the approach.

CONCLUSION

The QbD methodology makes it possible to measure AZDN precisely using a UV spectrophotometric method. A high calibre of analysis was ensured by the QbD process. In particular, the conc. of EtOH and pH had to be considered by the researcher when designing future trials to maintain improving the method's performance and developing control approaches. The approach appears to be novel, simple, accurate, and exact based on the data. Statistics on the method validation findings show that the developed techniques can be used in quality control labs. This method can be used to monitor the dosage form of AZDN tablets, which are commonly used fillers without causing any issues. This means that this approach in particular can be used for typical analytical objectives.

ACKNOWLEDGMENT

The study group is grateful to Laksh Finechem Pvt. Ltd. for graciously supplying the provision sample of AZDN, a standard drug. The researchers also express their gratitude to ARKA JAIN University's School of Pharmacy in Jamshedpur, Jharkhand, for granting them access to the necessary lab space.

CONFLICT OF INTEREST

The researchers do not have any conflicts of interest.

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