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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF AZELNIDIPINE BY UV-VISIBLE SPECTROSCOPY

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ABSTRACT

Azelnidipine is a new dihydropyridine calcium channel antagonist with selectively for L-type calcium channel that has recently been approved in Japan for the treatment of patients with hypertension. The objective was to develop new UV- visible spectroscopy method for Azelnidipine which should be rapid, precise, accurate and sensitive and validate the develop method according to ICH guidelines by the use of API and tablet formulations of Azelnidipine by UV- visible spectroscopy. UV-Visible Spectrophotometric determination was performed with Systronics PC- based double beam spectrophotometer 2202. A simple, Rapid, precise, accurate and sensitive UV- visible Spectroscopy method has been developed for the determination of Azelnidipine by using methanol as a medium. The spectrum of the standard solution was run from 200-400nm range for the determination of λmax and the λmax of Azelnidipine was found at 257nm. The absorbance of 2-14µg/ml of standard drug solution was measured at λmax 257nm. Validation parameters such as linearity, range, LOD and LOQ, accuracy, precision and robustness were evaluated as per ICH guidelines. Linearity for the UV-visible spectroscopy was noted in the concentration range of 2-14µg/ml and gave a mean correlation coefficient of 0.982. Accuracy was found to be 1.09%-0.83%. The precision expressed as relative standard deviation of Intraday and Interday which was 1.03%-1.70% and 1.26%-1.67% with % RSD less than 2 and the limit of detection (LOD) and limit of quantitation (LOQ) for Azelnidipine was found to be 0.77 and 2.36 respectively. The proposed UV-visible spectroscopy method and its validation according to the ICH guidelines shows that develop method is sensitive, precise, accurate and simple for the determination of

Azelnidipine API and tablet formulation.

KEYWORDS: Azelnidipine, UV-Visible Spectroscopy method, Method validation by UV-Visible Spectroscopy, ʎ max at 257 nm, Absorbance Measure of Azelnidipine.

1. INTRODUCTION

Azelnidipine is a lipopholic dihydropyridine calcium channel blocker antagonist. Dihydropyridine (DHP) calcium channel blockers are derived from the molecule dihydropyridine and often used to reduce systemic vascular resistance and arterial pressure. IUPAC name of Azelnidipine03-[1-[di(phenyl)methyl]azetidin-3-yl]05-propan-2-yl,2-amino-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5 dicarboxylate (Figure 1).[1–3] Azelnidipine is a new and long acting dihydropyridine derivative with calcium antagonistic activity. Azelnidipine is inhibits Trans membrane Ca+2 influx through the voltage dependent channels of smooth muscle in vascular walls. They enter the cells through cell membrane, lower peripheral vascular resistance and arterial pressure.[2,4,5] It is used for treatment of essential hypertension and angina pectoris. Calcium channel blockers (CCBs) have been shown to retard atherogenesis in animal models and to prevent the development of early lesions in human coronary arteries.[2,4,6] The chemical and pharmacokinetic profiles are as shown in table 1.

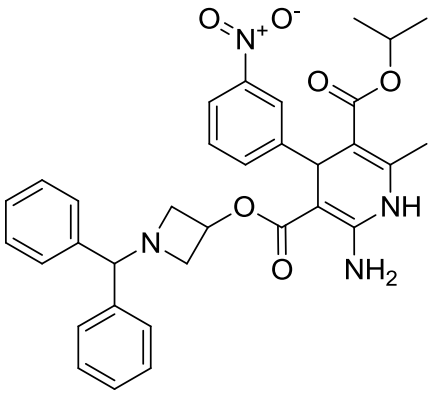

Figure 1: Chemical structure of azelnidipine.

Table 1: Drug profile of azelnidipine.

Parameters	Azelnidipine
Molecular weight	582.646 g/mol
Molecular formula	C33H34N4 O
CAS No.	123524-52-7
Absorption	Orally absorbed

Metabolism	Metabolized by cytochrome P450 (CYP) 3A4 in the liver and has no active metabolite.[4]
Bioavailability	Less than 50%
Half life	16-24 hrs
Cmax	3.0-13.1 ng/ml
Plasma protein binding	~90%

2. MATERIAL AND METHOD

2.1 Instrumentation

For the selection of analytical wavelength PC Based Double Beam UV- visible spectrophotometer with 1.0 cm matching quartz cell were used for measurement of absorbance (fig. 6). The UV spectra were recorded over the wavelength 200-400nm. All the drug and chemical were weighed on digital laboratory electronic balanced.[1,7,8]

2.2 Chemical and Reagents

Azelnidipine API sample was gifted obtain from Ajanta Pharma. Pvt. Ltd, Aurangabad India. The gifted samples of azelnidipine were slightly soluble in methanol, freely soluble in acetone, soluble in ethyl acetate and sparingly soluble in water and azelnidipine was used as standard without any further purification.[9,10] Distilled grade water methanol was used as solvent for drug. The melting point of azelnidipine was estimated by utilizing a capillary strategy on the melting point devices. The melting point of azelnidipine was seen as 119 ^{o}C while its ideal range is from 116-123^{o}C.[11,12]

2.3. Preparation of standard stock solution

A 100 mg of azelnidipine standard weighed accurately and transferred to a 100 ml volumetric flask and dissolved in diluents to give a solution containing 1000µg/ml standard stock solution of azelnidipine.[1,3,13]

2.4. Preparation of working standard stock solution

10 ml of standard stock solution was withdrawn and transferred to 100 ml volumetric flask. Volume is made up to the mark with diluents to get the working standard solution 100 µg/ml of azelnidipine.[2,6]

Characterization of drug

Melting point of Azelnidipine was checked using Melting point apparatus (Sigma Scientific) using capillary fusion method. In capillary fusion method, one side of the capillary was fused

and another side is filled with azelnidipine dry powder. Thus, filled capillary tube was inserted into Melting point apparatus and temperature at which solid changes to liquid was noted. The experiment is carried out multiple times to validate the melting point of the azelnidipine.

The FTIR technique helps in identification of various functional groups and structure of compounds using various infrared radiations. FTIR spectra of azelnidipine were characterized on Perkin Elmer 1750 FTIR spectroscopy to determine functional groups and structure combine with other spectroscopic technique or alone. The FTIR spectra were recorded in the range of 4000 and 400 cm^{-1}.[7,8,14]

2.5 Selection of wavelength (λmax)

For the selection of analytical wavelength range for method 100µg/ml azelnidipine was scanned in the spectrum mode from 200nm to 400 nm against distilled methanol as blank.[15,16] Wavelength range was selected around wavelength maxima (257nm) as shown in Fig. No.2

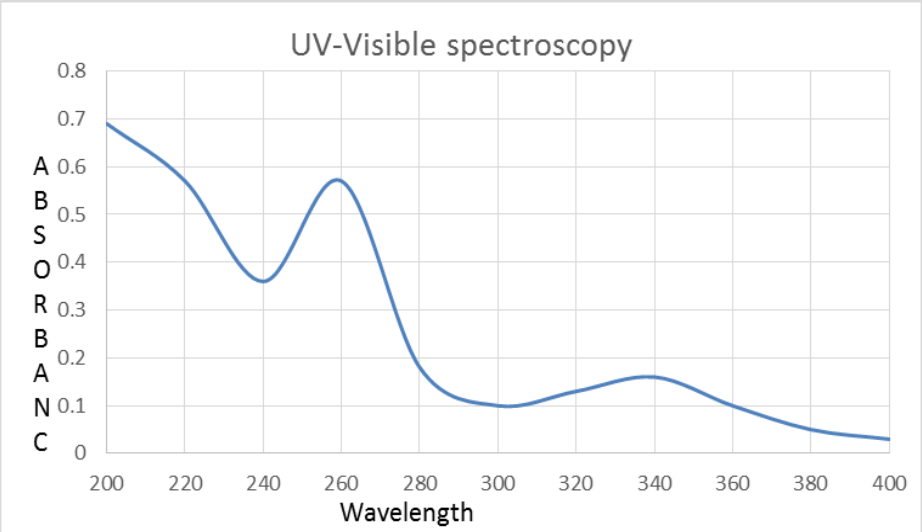

Figure 2: λ max of azelnidipine (100µg/ml).

2.6. Preparation of calibration curve of AZEL.

Appropriate volume of aliquots 2,4,6,8,10,12 and 14 µg/ml from working Azelnidipine stock solutions were transferred to different volumetric flasks of 10 ml capacity.[3] The volume was adjusted to the mark with diluents to obtain concentration of 2-14 µg/ml. The absorbance of the solutions was measured at 257nm. The calibration curve was constructed by plotting absorbance versus concentration of the drug and regression equations were computed.[17,18]

3. Analytical method validation

According to ICH Q2 (R1) guidelines, the developed method was validated to assure the reliability of results of the analysis for different parameters like linearity, Range, Specificity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), specificity and robustness.[2,19]

3.1. Linearity

The linearity was determined by analyzing absorbance of the azelnidipine standard concentration (2-14 µg/ml) at 257nm against methanol as blank. The calibration curve was plotted using concentration against absorbance. A regression equation and correlation coefficient were determined for Azelnidipine standard concentrations (2-14µg/ml).[7,8,20]

3.2. Range

The data obtained from the linearity and accuracy studies was used to assess the range of the method.[5,19]

3.3. Accuracy

Accuracy was established by preparing 3 sample of the solution 80, 100 and 120% of working standard and added known concentrations of Azelnidipine in each sample solution and dissolved in 10ml of volumetric flask with analytical grade methanol. Accuracy was assessed using a minimum of 9 determinations over a minimum of 3 concentration levels for each sample. Measure the absorbance at 257nm and finally calculate % RSD.[20–22]

3.4. Specificity

In UV-Visible spectroscopy specificity was confirmed by scanning of each azelnidipine standard solution (1-14µg/ml) in range of 200-800nm against methanol as blank.[23–25]

3.5. Repeatability

Repeatability was expresses the closeness of the results obtained with the same sample (or subsamples of the same sample), same location over a short period of time. Repeatability was expected to give the smallest possible variation in result. Select the middle concentration i.e.15µg/ml and carry out the repeatability by taking the absorbance of the solution six times. Calculate the mean of absorbance and find out standard deviation and % RSD.[1,19,26]

3.6. Precision

The Precision of the method was estimated by Interday and intraday variation studies. These two methods was determined by repeating the above methods at different time intervals (morning, afternoon and evening) on the same day (Intraday precision) and on three consecutive days (Interday precision).[1,27,28] The intraday and Interday variation for the estimation of Azelnidipine was carried out at three different concentration levels of 5, 15and 25µg/ml. calculate the mean of absorbance and % RSD.

3.7 Limit of Detection and Limit of quantification

3.8 Limit of Detection (LOD)

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. The LOD was estimated from the set of five calibration curves used to determine method linearity. LOD was calculated as follow[1,2] From the formula LOD= (3.3*SD)/slope.

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

Slope= the mean slope of the 5 calibration curves.

3.9 Limit of Quantification (LOQ)

The Quantitation limit is a parameter of quantitative assays for low levels of compounds in sample. The LOQ was estimated from the set of five calibration curves used to determine method linearity.[1,19,29]

The LOQ may be calculated as $LOQ = 10 \times (\sigma/S)$

Where, σ = Standard deviation of the Y- intercepts of the five calibration curves.

S = Mean slope of the five calibration curves.

3.10 Robustness

The robustness of an analytical procedure was a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. It was carryout by doing deliberate variation in method parameter is done (i.e. change in wavelength, analysis by person to person and changing room temperature). Absorbance of any one concentration 15 µg/ml is measured at two different wavelengths i.e. 255,256,257 nm and calculate mean and % RSD.[2,3,7,14]

Application of the proposed method for the analysis of pharmaceutical formulation

For the analysis of pharmaceutical formulation, twenty tablets were weighed accurately and finely powder. Tablet powder equivalent to 20 mg of azelnidipine was accurately weighed and transferred to a 50ml volumetric flask. A few ml diluents was added and sonicated (Leela Sonic) for 5min. volume was made up mark with methanol. An aliquot of 1ml was transferred to the 50ml volumetric flask and volume was made up to mark to obtain 8µg/ml of azelnidipine. The solution was filtered using 0.45µ Millipore PVDF Filter. This solution was prepared six times and the absorbance of each solution was determined at 257nm and the concentration of each drug in the sample solution was determined from calibration curve by using regression analysis.

3. RESULT AND DISCUSSION

Fourier Transform Infrared (FTIR) study

The FTIR absorption spectrum of sample was concordant with that of azelnidipine reference standard or reference spectrum of azelnidipine. By the interpretation of the spectra which showed the conformed presence of functional group in drug. The results of different stretching for different functional group was almost similar peak obtained by Kumari et al.[15] Peak at 3377.7 attribution to N-H stretching vibration, peak at 2850.0 was assigned to aromatic stretching and peak at 1596.5 was assigned C=C Stretching which conform the structure of azelnidipine.

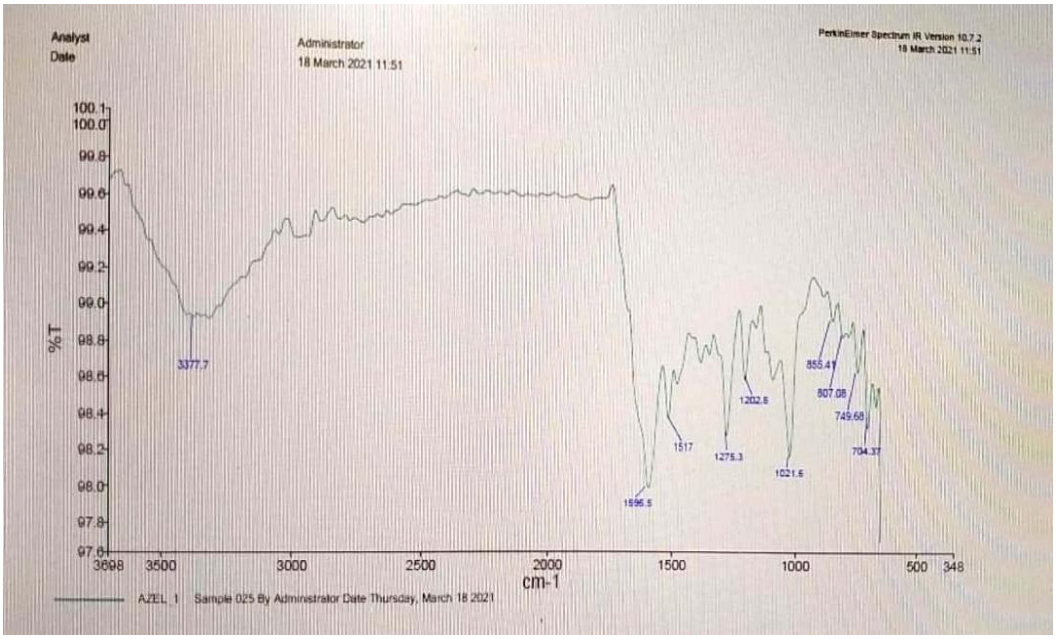

Figure 3: IR spectrum of sample AZEL.

Linearity

The linearity of the proposed method was obtained which are directly proportional to the concentration (amount) of analyte in the sample. Linearity was evaluated by visual inspection of the linear data for calibration curved which was a good linear relationship over the concentration range 2-14µg/ml for azelnidipine. The linearity of response for azelnidipine was assessed by analysis of seven independent levels of concentration in range of 2-14µg/ml and Slope, Y-intercept was found to be 0.072, 0.017 and correlation coefficient values of calibration curve was found to be 0.9826 respectively. The results of linearity are shown in table 3 and figure 4.

Table 3: Linearity calibration data.

S. N	Concentration (µg/ml)	Absorbance
1	2	0.187
2	4	0.326
3	6	0.424
4	8	0.534
5	10	0.722
6	12	0.951
7	14	1.019

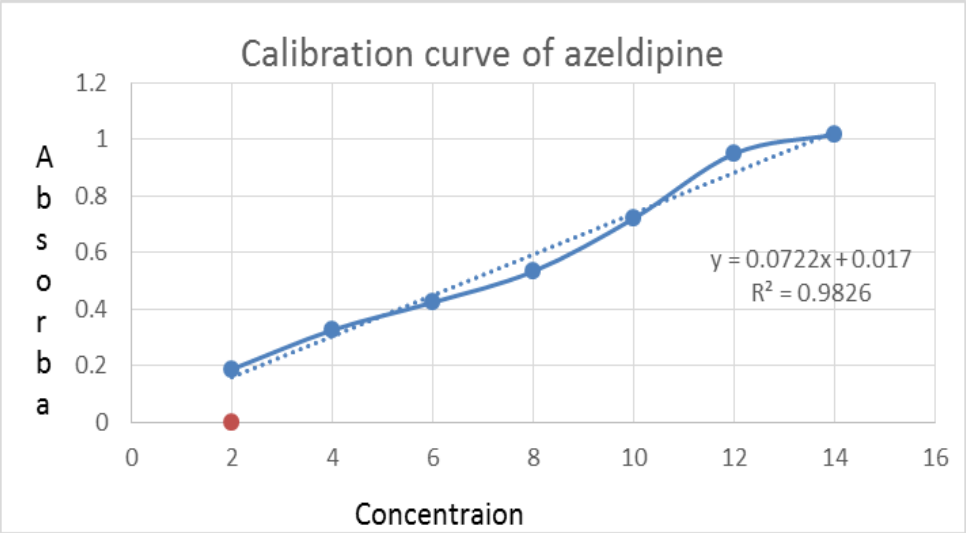

Figure 4: Calibration curve of azelnidipine at 257 nm.

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. The Accuracy of proposed method was assessed by analyzed and the RSD was found in between 1.09-0.83% which was less than 2. The results of accuracy are shown

in the table 4.

Table 4: Accuracy data.

S. N	Spike %	Total Concentration	Absorbance	Mean	Std Deviation	% RSD
1	80%	27µg/ml 27µg/ml 27µg/ml	1.001 1.010 1.023	1.011	0.011	1.09%
2	100%	30µg/ml 30µg/ml 30µg/ml	1.023 1.041 1.047	1.037	0.0124	1.20%
4	120%	33µg/ml 33µg/ml 33µg/ml	1.073 1.081 1.091	1.081	0.0090	0.83%

Repeatability

Repeatability was estimated by analyzes middle concentration 15µg/ml of azelnidipine solution for six time and the % RSD was found to be 1.13% with % RSD ≤ 2. The results of repeatability are as shown in table 5.

Table 5: Repeatability data.

S. no.	Concentration (µg/ml)	Absorbance	Mean	Std. deviation	% RSD
1	15µg/ml	0.771	0.766	0.0086	1.13%
2	15µg/ml	0.774			
3	15µg/ml	0.751			
4	15µg/ml	0.769			
5	15µg/ml	0.763			
6	15µg/ml	0.773			

Intraday and Interday Precision

Precision was determined by repeating the above methods at different time intervals (morning, afternoon and evening) on the same day (Intraday precision) and on three consecutive days (Interday precision).[1] The intraday and Interday variation for the estimation of Azelnidipine was carried out at three different concentration levels of 5, 15and 25µg/ml. The percentage relative standard (% RSD) was found to be 1.03-1.70% for Intraday and 1.26-1.67% for Interday which are under normal range i.e. % RSD ≤ 2. The Results of Intraday and Interday are as shown in table 6 and table 7.

Table 6: Intraday precision data.

S. N	Concentration µg/ml	Absorbance			Mean	Std. deviation	% RSD
		1	2	3			
1	5 µg/ml	0.436	0.441	0.432	0.436	0.0045	1.03%
2	15 µg/ml	1.267	1.311	1.289	1.289	0.022	1.71%
3	25 µg/ml	1.344	1.389	1.357	1.363	0.023	1.70%

Table 7: Interday precision data.

S.N	Concentration µg/ml	Absorbance			Mean	Std. deviation	% RSD
		1	2	3			
1	5 µg/ml	0.446	0.452	0.439	0.444	0.0056	1.26%
2	15 µg/ml	1.267	1.239	1.261	1.261	0.016	1.33%
3	25 µg/ml	1.344	1.389	1.361	1.364	0.022	1.67%

Limit of detection (LOD) and Limit of quantification (LOQ)

LOD and LOQ were estimated from linear equation according to ICH Guideline. From the linear equation, LOD Was found to be 0.77 and LOQ was found to be 2.36 for azelnidipine respectively. The results of LOD and LOQ are as shown in Table 10.

Robustness

Robustness was carryout by doing deliberate variation in method parameter was done (i.e. change in wavelength). Robustness of azelnidipine was determined by variance of wavelength, analysis by person to person and changing room temperature i.e. 255,256 &257 nm and Absorbance of any one concentration 15 µg/ml is measured. Robustness of azelnidipine was found to be 1.37%-1.92% that was less than 2 % RSD indicating the proposed method was Robust. The results are shown in table 8.

Table 8: Robustness data

S.N	Parameter	Parameter Sequence	Absorbance	Mean	Std. Deviation	% RSD
1	Wavelength	256nm	0.494	0.4963	0.0068	1.37%
		257nm	0.491			
		258nm	0.504			
2	Analysis by person to person	1st	0.476	0.484	0.0065	1.34%
		2nd	0.481			
		3rd	0.491			
3	By changing temperature	38’C	0.436	0.442	0.0052	1.92%
		43’C	0.452			
		40’C	0.439			

Assay of azelnidipine tablet formulation

The actual weight of azelnidipine was 8.00 mg and equivalent weight of each tablet (active drug) was found to contain 7.812 mg of the active drug. So that calculates the % assay of the sample of the drug. % assay = actual amount of desired material x 100 / total amount of material. The results are shown in table 9.

Table 9: Assay of azelnidipine tablet formulation.

Dosage form	Actual weight (mg)	Active weight (mg)	% Assay
Tablet	8.00mg	7.812±0.02mg	97.68%

Table 10: Summary of validation parameter.

S. N	Parameter		Normal range	Result
1	Linearity (r^2)		0.999	0.9826
	Slope (m)		-	0.072
	Y-intercept		-	0.017
2	Accuracy		% rsd ≤ 2%	1.09-0.83 %
3	Precision	Repeatability	% rsd ≤ 2%	1.13%
		Intraday		1.03-1.70 %
		Interday		1.26-1.67 %
4	Limit of detection (lod)		≥ 2 time base line	0.77
5	Limit of quantification (loq)		Signal to noise ratio 10:1	2.36
6	Robustness		% rsd≤ 2%	1.78%
7	% assay			97.67%

4. CONCLUSION

The proposed method development of UV-Visible Spectroscopy is quite, rapid, precise, and accurate and sensitive for azelnidipine tablet formulation. In validation of UV-Visible Spectroscopy, it can be concluded that spectroscopic method has been validated. All the validation parameter likes linearity, accuracy, precision and Robustness was found to be less than 2% RSD according ICH Guideline that indicate proposed method is sensitive. Correlation coefficient of Linearity is 0.9826. The value of % RSD for intra-day and Interday were within normal range and the accuracy was found to be normal range. The limit of detection and limit of quantification of the projected methodology was found to be 0.77 and 2.36 that is Simple and rapid for quantification of azelnidipine table formulation. The value of % assay was found to be less than 98% for this method that indicate Method is accurate and free from the interference used in formulation. It was concluded that developed method is simple, almost accurate, precise and reliable. In compliance with ICH guideline the method is valid and appropriate for estimation azelnidipine with excellent linearity, accuracy, precision,

and robustness. This method can be used for routing analysis of azelnidipine tablet formulation.

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CONFLICT OF INTEREST

Authors declared no conflict of interest.

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