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Spectrophotometric Estimation of Azelnidipine in Bulk drug and Pharmaceutical Dosage form by First Order Derivative and Area Under Curve Methods

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ABSTRACT

Simple and precise UV- spectrophotometric methods, first order derivative and area under curve [AUC], have been developed and validated for the estimation of azelnidipine in bulk drug and its tablet formulation. The standard and sample solutions of azelnidipine were prepared in methanol. Azelnidipine was estimated at 242.6 nm for the first order derivative UV-spectrophotometric method (A) while in area under curve (AUC) method (B) the zero order spectrum of azelnidipine was measured in between 250.5 nm to 258.8 nm. Beer's law was obeyed in the concentration range of 1 to 20 µg / ml with coefficient of correlation value 0.9993 for first order derivative method. Similarly in AUC method, Beer's law was obeyed in the concentration range of 1 to 20 µg / ml with coefficient of correlation value 0.9991. These methods were tested and validated for various parameters according to ICH guidelines. The precision expressed as relative standard deviation, which was within the range of 0.1157 to 0.9995 % for the above two methods. The proposed methods were successfully applied for the determination of azelnidipine in pharmaceutical formulation. Results of the analysis were validated statistically and were found to be satisfactory. The proposed methods are simple, easy to apply, low-cost and require relatively inexpensive instruments.

Keywords: Azelnidipine, Derivative spectroscopy, Area under curve method.

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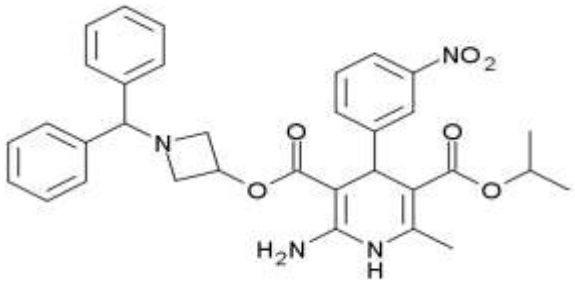
INTRODUCTION

Azelnidipine is a lipophilic calcium channel antagonists. Its chemical name is O-3-[1-[di (phenyl) methyl] azetidin-3-yl] O-5-propan-2-yl 2-amino-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate. Azelnidipine can restrain Ca^{+} ions outside the cardiac muscle and vascular smooth muscle. They enter the cells through cell membrane; it expands blood vessel, lower peripheral vascular resistance and arterial pressure. In clinic, it is used for treatment of essential hypertension and angina pectoris[1].

This drug is not official in any pharmacopoeia. In literature survey only HPLC[2,3], spectrophotometric[4] and titrimetric[5] methods have been reported for its validation of drugs. Simple, sensitive and reproducible UV spectrophotometric methods have been developed here for the estimation of azelnidipine from bulk drug and pharmaceutical formulation. The developed methods will useful for routine analysis in pharmaceutical industries and research organizations. The structure of azelnidipine is as shown.



Chemical structure of Azelnidipine

MATERIALS AND METHOD

Instrument and reagents

Spectral scan was made on a Shimadzu UV-spectrophotometer, model 1800 (Shimadzu, Japan) with spectral band width of 0.5 nm with automatic wavelength corrections by using a pair of 10 mm quartz cells. All spectral measurements were done by using UV-Probe 2.42 software. Reference standard of azelnidipine was obtained from reputed firm with certificate analysis.

Preparation of standard drug solution

100 mg standard azelnidipine was weighed accurately and transferred to a 100 ml volumetric flask and sonicated with 30 ml of methanol for 15 minutes. The volume was made up to the mark with methanol to give a stock solution of concentration 1000 µg /ml. From this solution, 10 ml of solution was pipetted out and transferred into 100 ml volumetric flask. The volume was made up to mark with methanol to give a working standard solution of concentration 100 µg / ml.

Estimation from tablets

Twenty tablets of labeled claim 8 mg of azelnidipine were weighed accurately and average weight of each tablet was determined. Powder equivalent to 10 mg of azelnidipine was weighed and transferred in 100 ml of volumetric flask. A 30 ml of methanol was added and sonicated for 15 minutes and filtered. The filtrate and washing were diluted up to the mark with methanol to give concentration as 100 µg /ml. Such solution was used for analysis.

EXPERIMENTAL

Method A: First order derivative method

For the selection of analytical wavelength, 20 µg/ml solution of azelnidipine was scanned in the spectrum mode from 320 nm to 200 nm by using methanol as blank. The first order derivative spectrum was obtained by using derivative mode by UV probe 2.42 software. (Figure 1) From the spectrum, the amplitude of the derivative spectrum was measured at 242.6 nm.

Into series of 10 ml graduated flask, varying amount of sample solutions of azelnidipine were pipette out and volume was adjusted with methanol. Solutions were scanned between 320 nm to 200 nm in spectrum mode. The first order derivative spectra were obtained by using derivative mode. Amplitudes of the resulting solutions were measured at 242.6 nm by using methanol as blank. The overlay spectrum was given in figure 2. The calibration curve for azelnidipine was plotted in the concentration range of 1 to 20 µg/ml was given in figure 3. Results of the analysis are given in table 2.

Method B: Area under curve (AUC) method

Area under curve method involves the calculation of integrated value of absorbance with respect to the wavelength between two selected wavelengths such as λ_1 and λ_2. The area under curve between λ_1 and λ_2 was calculated by UV probe 2.42 software. In this method, 20 µg/ml solution of azelnidipine was scanned in the spectrum mode from 320 nm to 200 nm. From zero order spectrum the AUC calculation was done. The AUC spectra were measured between 250.5 nm to 258.8 nm. It is given in figures 4a, b, c.

Into series of 10 ml graduated flask, varying amount of sample solutions of azelnidipine were pipette out and volume was adjusted with methanol. Solutions were scanned between 320 nm to 200 nm in spectrum mode. The AUC calculations were done and the calibration curve for azelnidipine was plotted in the concentration range of 1 to 20 µg/ml. It is given figure. 5.

Results of the analysis are given in table 2.

VALIDATION

Accuracy

Accuracy of the proposed methods was carried as on the basis of recovery studies. It is performed by the standard addition method. Recovery studies were performed by adding standard drug at different levels to the pre-analyzed tablets powder solution and the proposed method was followed. From the amount of the drug estimated, the percentage recovery was calculated. The results of the analysis are shown in table 3 and 4.

The methods precision were established by carrying out the analysis of homogenous powder blend of tablets. The assay was carried out of drug by using proposed analytical methods in seven replicates. The values of relative standard deviation lie well within the limits indicated the sample repeatability of the methods. The results obtained are tabulated in table 5.

Inter-day and intra-day precision

An accurately weighed quantity of tablets powder equivalent to 10 mg of azelnidipine was transferred to 100 ml of volumetric flask. A 30 ml of methanol was added and sonicated for 15 minutes and filtered. The filtrate and washing were diluted up to the mark with methanol to give concentration as 100 µg /ml. Such solution was used for analysis.

For first order derivative method

Solution was scanned between 320 nm to 200 nm in spectrum mode. The first order derivative spectrum was obtained by using derivative mode. Amplitude of the resulting solution was measured at 242.6 nm by using methanol as blank. The amplitude of final solution was read after 0 hr., 3 hrs. and 6 hrs. in 10 mm cell at 242.6 nm for first order derivative (method A). Similarly the amplitude of the same solution was read on 1^{st}, 2^{nd} and 5^{th} day. The amount of azelnidipine was estimated by comparison with standard at 242.6 nm for first order derivative. The results obtained are tabulated in table 6.

For area under curve method

Solution was scanned between 320 nm to 200 nm in spectrum mode. The area under curve of resulting solutions was measured at between 250.5 nm to 258.8 nm. by using methanol as blank. The area under curve of final solutions was read after 0 hr., 3 hrs. and 6 hrs. in 10 mm cell at 250.5 nm to 258.8 nm. (Method B). Similarly area under curve of the same solution was read on 1^{st}, 2^{nd} and 5^{th} day. The amount of azelnidipine was estimated by comparison with standard at 250.5 nm to 258.8 nm. The results obtained are tabulated in table 6.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The limit of detection (LOD) is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from back-ground levels. In this study, LOD and

LOQ were based on the standard deviation of the response and the slope of the corresponding curve using the following equations-

$$LOD = 3.3\ \sigma/S \quad \text{and} \quad LOQ = 10\ \sigma/S$$

Where σ is the standard deviation of the signal to noise ratio of the sample and S is the slope of the related calibration graphs.

The limit of quantification (LOQ) is defined as the lowest concentration of the standard curve that can be measured with an acceptable accuracy, precision and variability .The values of LOD and LOQ are given in table 7.

Ruggedness

The ruggedness of the method is defined as degree of reproducibility of results obtained by analysis of azelnidipine sample under variety of normal test conditions such as different laboratories, different analysts and different lots of reagents. Quantitative determination of azelnidipine was conducted spectrophotometrically on one laboratory. It was again tested in another laboratory using different instrument by different analyst. The assays obtained in two different laboratories were well in agreement. It proved ruggedness of the proposed methods.

RESULTS AND DISCUSSION

The first order derivative and area under curve UV-spectroscopic methods are useful for routine analysis of azelnidipine in bulk drug and formulation. The derivative spectroscopy method applied has the advantage that it locates hidden peak in the normal spectrum. It eliminates the interference caused by the excipients and the degradation products present, if any, in the formulation.

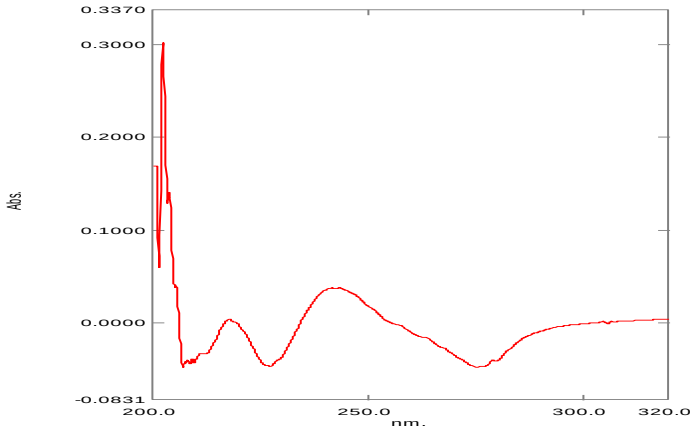


Figure. 1. First order derivative spectrum of azelnidipine (20 µg/ml) showing Absorbance at 242.6 nm

The method was validated according to International Conference on Harmonization guidelines for validation of analytical procedure [6]. Azelnidipine has the absorbance maxima at 242.6 nm

(method A) (figure.1) and in the AUC spectrum method areas were measured between 250.5 nm to 258.8 nm. (Method B) (Figure.4 a, b, c). The polynomial regression data for the calibration plots showed good linear relationship in the concentration range of 1 to 20 µg/ml and given in table 1 and 2. Recovery studies were carried out by adding the pure drug to the previously analyzed tablet powder sample and shown in table 3, 4. The percentage recovery value indicates non interference from excipients used in formulation. The reproducibility and accuracy of the methods were found to be good, which was evidenced by low standard deviation.

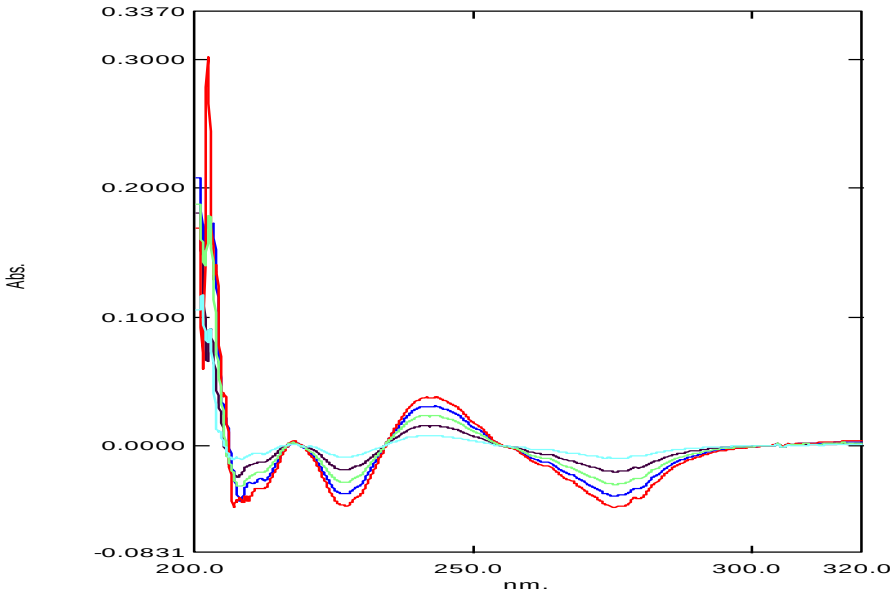


Figure.2: The overlay spectra of concentration from 4- 20 µg/ ml of standard azelnidipine.

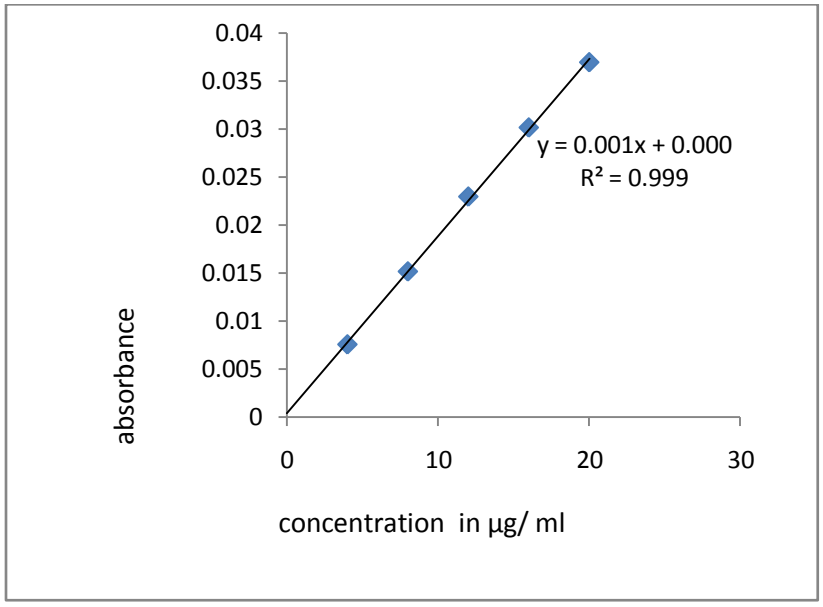


Figure.3. Calibration curve for azelnidipine at 242.6 nm by first order derivative spectroscopy

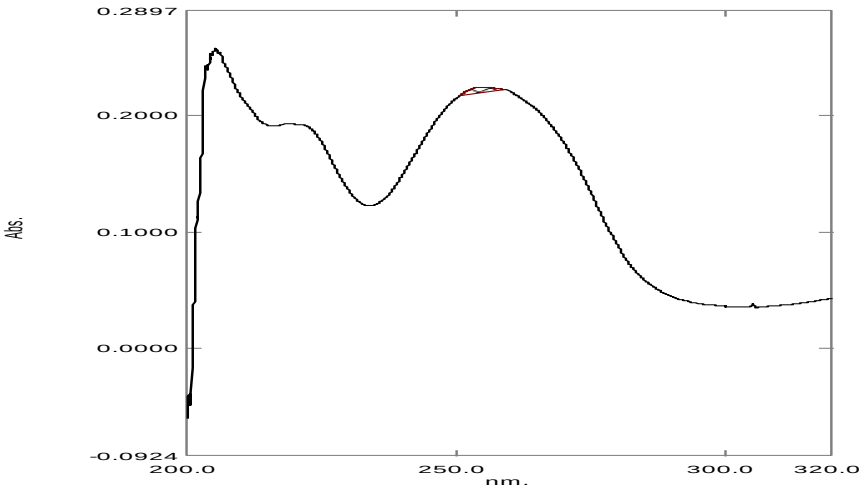


Figure.4a. Area under curve spectrum of azelnidipine (4 µg/ml) showing area from 250.5 nm to 258.8 nm.

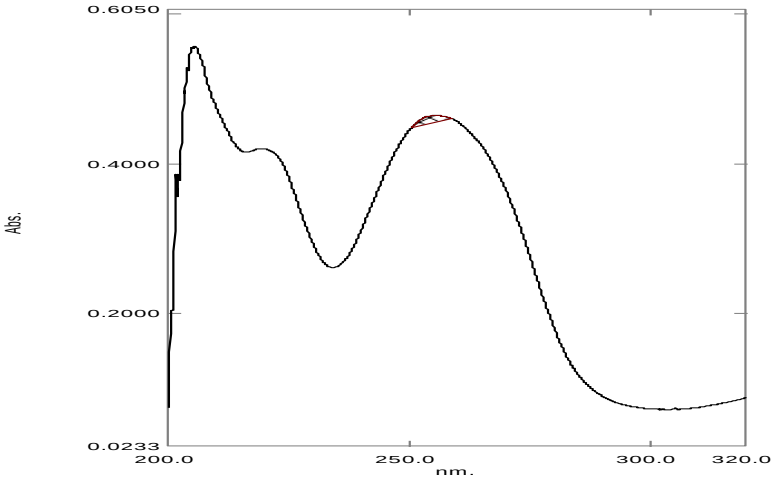


Figure.4b. Area under curve spectrum of azelnidipine (8 µg/ml) showing area from 250.5 nm to 258.8 nm.

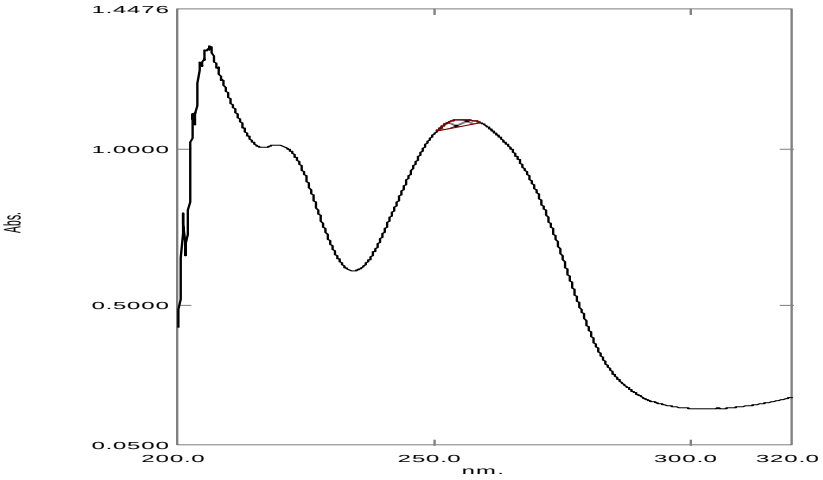


Figure.4c. Area under curve spectrum of azelnidipine (20 µg/ml) showing area from 250.5 nm to 258.8 nm.

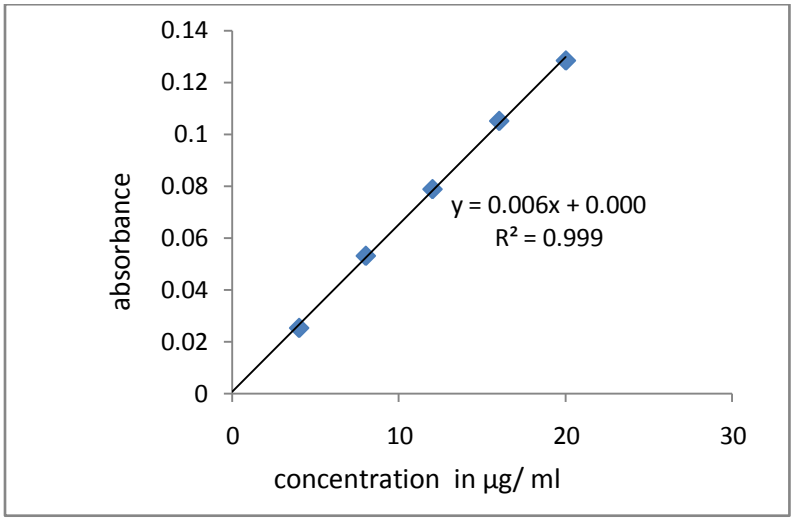


Figure.5. Calibration curve for azelnidipine by area under curve spectroscopy

Table 1: Values of linearity

Sr.No.	Concentration in µg/ml	First order Derivative method (Absorbance)	Area under curve method (Absorbance)
1	4	0.0076	0.0254
2	8	0.0152	0.0532
3	12	0.0230	0.0789
4	16	0.0301	0.1052
5	20	0.0370	0.1285

Table 2: Values of results of optical and regression of drug

Parameter	First order derivative method	Area under curve (AUC) method
Detection Wavelength (nm)	242.6	250.5-258.8
Beer Law Limits (µg/ml)	1-20	1-20
Correlation coefficient (r^2)	0.9993	0.9991
Regression equation (y=b+ac)		
Intercept (b)	0.0005	0.0008
Slope (a)	0.0018	0.0065

Table 3: Results of recovery of azelnidipine for first order derivative method

Amount of sample added µg/ ml	Amount of standard added µg/ ml	Total amount recovered	Percentage recovery	Standard deviation	Percentage standard deviation
4	0	4.0090	100.225	0.03683	0.9187
4	4	8.0302	100.377	0.5167	0.6435
4	8	12.060	100.500	0.12054	0.9995
4	12	16.0298	100.18	0.06700	0.4180
				Mean=0.1852	Mean=0.7449

Table 4: Results of recovery of azelnidipine for area under curve (AUC) method

Amount of sample added	Amount of standard added	Total amount recovered	Percentage recovery	Standard deviation	Percentage standard deviation

µg/ ml	µg/ ml				
4	0	4.0065	100.160	0.2196	0.5481
4	4	7.9952	99.940	0.2746	0.3435
4	8	12.002	100.016	0.1637	0.1363
4	12	16.002	100.012	0.1852	0.1157
				Mean=0.2107	Mean=0.2859

Table 5: Precision- method precision

Experiment no.	Weight of Azelnidipine taken in mg.	Content in mg. of azelnidipine first order derivative method	area under curve method
1	8	8.0529	8.001
2	8	8.002	7.969
3	8	8.1059	7.969
4	8	8.0529	8.030
5	8	8.0529	7.969
6	8	7.947	8.030
7	8	8.002	8.001
Standard deviation		0.3866	0.2381
%RSD		3.8641	2.3732

Table 6: Summary of validation parameter for intra-day and inter-day

Sr. no	Parameters	First order derivative method	Area under curve method
(A)	Intra-day precision (n=3) Amount found ±	99.60 %	99.45%
	%RSD	0.24847	0.03446
(B)	Inter-day precision (n=3) Amount found ±	98.484 %	98.762 %
	%RSD	0.13607	0.00768
(C)	Ruggedness Analyst to analyst (n=3)	100.12 %	99.87 %
	%RSD	0.06786	0.00812

Table 7: Values of results of LOD and LOQ of drug

Parameter	First order derivative method	Area under curve method
Limit of Detection (µg/ml)	0.17891	0.07093
Limit of Quantification (µg/ml)	0.54216	0.21496

CONCLUSION

The most striking features of two methods are its simplicity and rapidity, not requiring tedious sample solutions preparations which are needed for other instrumental methods. From the results obtained it can be concluded that the proposed methods are fully validated and found to be simple, sensitive, accurate, precise, reproducible, rugged and robust and relatively inexpensive. So, the developed methods can be easily applied for the routine quality control analysis of azelnidipine in pharmaceutical formulation.

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