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DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPIC METHOD FOR THE ESTIMATION OF AZELNIDIPINE IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

Simple, precise and accurate area under curve spectroscopic method has been developed and validated for the estimation of Azelnidipine in bulk and pharmaceutical dosage form. The drug shows maximum absorption (λ_{max}) at 254nm in acetonitrile solution and Area under Curve [AUC] in absorption spectra were measured between the wavelength range 249 to 259nm which obeys Beer's law in the concentration range of 2-12µg/ml. The linearity study carried and regression coefficient was found to be 0.9993 and it has showed good linearity, precision during this concentration range. The % recovery was found to be 98.6-101.9. The LOD and LOQ were found to be 0.04 and 0.13µg/ml. The % relative standard deviation were found less than 2. According to ICH guidelines the method has been validated for linearity, precision, accuracy, robustness, ruggedness, LOD and LOQ. The developed and validated method can be successfully applied for reliable quantification of Azelnidipine in bulk form and pharmaceutical dosage form.

KEYWORDS: Azelnidipine, Area under curve spectroscopy, Validation, Pharmaceutical formulations.

INTRODUCTION

Azelnidipine is a new lipophilic dihydropyridine calcium channel blocker. Selectively for L – type calcium channel that has been recently used for the treatment of patients with hypertension.[1] Azelnidipine is often used to reduce systemic vascular resistance and arterial pressure. Azelnidipine is inhibits trans membrane Ca^{+2} influx through the voltage dependent channels of smooth muscles in vascular walls. Ca 2+ channels are classified in to various categories, including L-type, T-type, N-type, P/Q-type and R-type Calcium channels. The L-type Ca 2+ channels. Normally, calcium induces smooth muscles contraction, contributing to hypertension. When calcium channels are blocked, the vascular smooth muscles do not contract, resulting in relaxation of vascular smooth muscles walls and decreased blood pressure. It is used for the treatment of essential hypertension and angina pectoris.[2] It is classified as a class II drug according to the biopharmaceutical classification systems (BCS).

Chemically Azelnidipine is 3-(1-Benzhydrylazetidine-3-yl) 5-isopropyl 2-amino-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3, 5-dicarboxylate.[4] Azelnidipine is a pale-yellow powder available in amorphous solid form and it is soluble in organic solvents such as methanol, DMSO, ethanol, acetonitrile, acetone and slightly soluble in water. Its molecular formula is $C_{33}H_{34}N_4O_6$ and its molecular weight is 582.6g/mol and having melting point in the range of 122 - 123˚C.

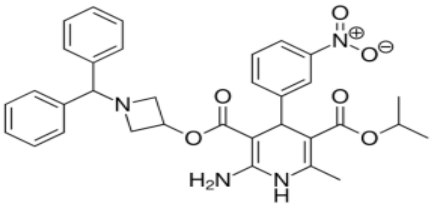

Figure 1: Structure of azelnidipine.[3]

Literature survey revealed that there were few analytical methods have been reported for the determination of Azelnidipine in pure drug and pharmaceutical dosage forms by using UV[5-9], HPLC[10-17], and HPTLC[18] so far. The aim of present work is to develop and validate a novel, rapid, simple, precise and specific Area under curve UV Spectrophotometric method for estimation of Azelnidipine in bulk and tablet dosage form.

MATERIALS AND METHODS

Instrument: UV-Visible double beam spectrophotometer, SHIMADZU (model UV-1800) with UV probe software. All weights were taken in analytical balance.

Chemicals: Azelnidipine pure drug was obtained as a gift sample from 4Care Lifescience Pvt Ltd., Bagdol and its pharmaceutical dosage Azelnidipine 20 tablets (Azovas-16) labelled claim 16mg from local pharmacy manufactured by Synokem Pharmaceuticals Ltd.

Solvent: Acetonitrile is used as a solvent.

Selection of analytical wavelength: Appropriate dilutions of Azelnidipine were prepared from standard stock solution and using spectrophotometer solution was scanned in the wavelength range 200-400nm. Area under Curve [AUC] in absorption spectra were measured between the wavelength range of 249 to 259nm as the wavelength for detection (Fig-2).

Preparation of standard stock solution: 100mg of Azelnidipine was weighed accurately and transferred into 100ml volumetric flask and diluted in acetonitrile up to mark. From this, the solution was further diluted into 100µg/ml and pipetted 0.2, 0.4, 0.6, 0.8, 1.0, and 1.2ml into 10ml individual volumetric flask and diluted in acetonitrile up to mark, this gives 2, 4, 6, 8, 10, and 12µg/ml concentration.

Preparation of sample solution: 20 tablets of Azelnidipine marketed formulations was weighed and powdered. A quantity of tablet powder equivalent to 100mg of Azelnidipine was transferred into a 100ml of volumetric flask then it was diluted with acetonitrile and made up to the mark.

METHOD AND VALIDATION

The method was validated according to ICH guidelines.[19-21]

RESULTS AND DISCUSSION

Method: Area under curve spectroscopy

Linearity: The linearity of an analytical method is its capacity to show the test results that are directly proportional to the concentration of the analyte in the sample within the range. The linearity was established in the range of 2-12µg/ml and Area under Curve [AUC] in absorption spectra were measured between the wavelength of 249 to 259nm as absorbance values are shown in table-1 (Fig-3). The calibration curve was prepared by plotting graph against the concentration and absorbance and therefore the graph shown in (Fig-4). Statistical parameter like slope, intercept, regression equation, correlation coefficient and Sandell's sensitivity were determined. (table-2).

Precision: The precision of an analytical method expresses the closeness of a series of individual analyte measurements obtained from multiple sampling of the equivalent sample. Precision was determined by intra-day and inter-day study. Intra-day precision was determined by analysing the same concentration for six times in a same day. Inter-day precision was determined by analysing the same concentration daily for six days. (table-3).

Accuracy: The accuracy of an analytical method says that closeness of test results obtained by that method to the true value. To assess the accuracy of the developed method, recovery studies were carried out at three different levels as 50%, 100% and 150%. In which the formulation concentration kept constant and varied pure drug concentration. (table-4).

Ruggedness: The ruggedness is defined as the reproducibility of results when the method is performed under the variation in conditions. This includes different analyst, laboratories, instruments, temperature etc. Ruggedness was determined between different analyst, the value of %RSD was found to be less than 2.(table-5)

LOD and LOQ: The limit of detection is an individual analytical method is the smallest amount of analyte in a sample which can be reliably detected by the analytical method. The limit of quantitation is an individual analytical procedure is the smallest amount of analyte in a sample which can be quantitatively determined. LOD and LOQ were calculated using formula.

LOD = 3.3(SD)/S and LOQ = 10(SD)/S

LOD and LOQ value of Azelnidipine were found be 0.04 and 0.13µg/ml.

Tables

Table 1: Results of calibration curve at 249-259nm by Area under curve method

Sl. No.	Concentration in µg/ml	Absorbance ± Standard deviation*
1	0	0
2	2	0.113±0.0008
3	4	0.226±0.0010
4	6	0.313±0.0012
5	8	0.422±0.0022
6	10	0.533±0.0008
7	12	0.635±0.0014

**Average of six determinations.*

Table 2: Regression parameter for Azelnidipine at 249-259nm by Area under curve method.

Regression parameter	Results
Range(µg/ml)	2-12
λ_{max}(nm)	249-259
Regression Equation	0.0525x±0.0052
Slope(b)	0.0525
Intercept(a)	0.0052
Correlation coefficient(r^2)	0.9993
Sandell's equation	0.019
Limit of detection(µg/ml)	0.04
Limit of quantitation(µg/ml)	0.13

Table 3: Determination of precision results for Azelnidipine at 249-259nm by Area under curve method.

Concentration (µg/ml)	Intra-day Absorbance ±Standard deviation*	%RSD**	Inter-day Absorbance ±Standard deviation*	%RSD**
2	0.113±0.0001	0.72	0.107±0.0008	0.76
4	0.226±0.0016	0.44	0.223±0.0014	0.65
6	0.313±0.0012	0.38	0.309±0.0031	1.02
8	0.422±0.0022	0.52	0.480±0.0018	0.43
10	0.533±0.0008	0.16	0.528±0.0018	0.35
12	0.635±0.0014	0.22	0.628± 0.0015	0.24

**Average of six determinations, **percentage relative standard deviation.*

Table 4: Determination of Accuracy results for Azelnidipine at 249-259nm by Area under curve method.

Spiked Levels	Amount of Sample (µg/ml)	Amount of Standard (µg/ml)	Amount Recovered	% Recovery ±Standard deviation*	%RSD**
50	6	3	9.08	100.9±0.218	0.217
100	6	6	11.81	98.6±0.226	0.229
150	6	9	18.13	100.6±0.225	0.223

**Average of six determinations, **percentage relative standard deviation.*

Table 5: Determination of Ruggedness results for Azelnidipine at 249-259nm by Area under curve method.

Analysts	Analyst 1	Analyst 2
Mean absorbance	0.309	0.309
±Standard deviation*	0.003	0.003
%RSD	0.38	0.38

**Average of six determinations, **percentage relative standard deviation.*

Figures

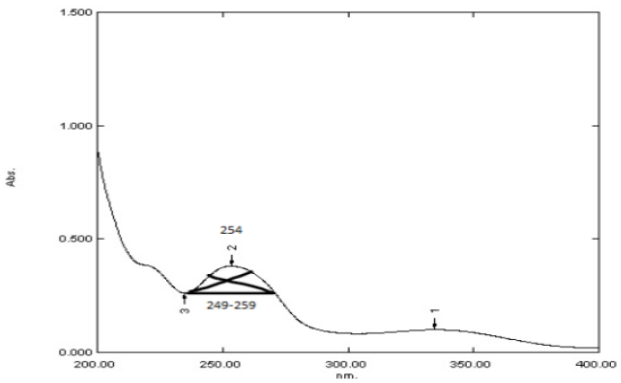

Fig. 2: Area under curve spectrum of Azelnidipine at 249-259nm.

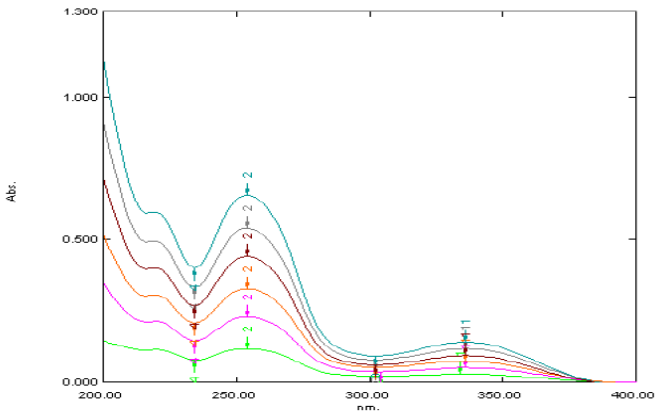

Fig. 3: Area under curve overlain spectra of Azelnidipine showing absorbance at 249-259nm

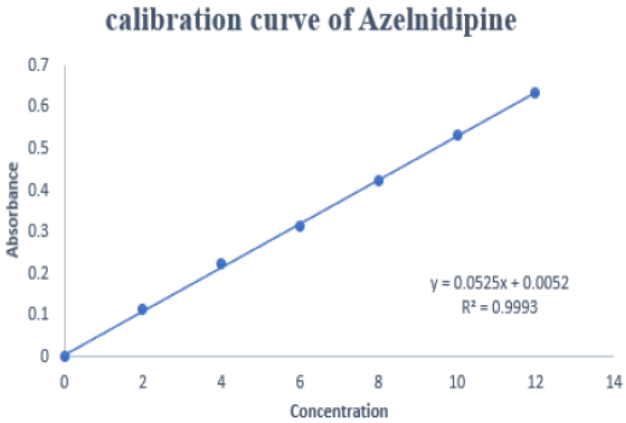

Fig. 4: Calibration curve of Azelnidipine at 249-259nm by Area under curve method.

CONCLUSION

As per ICH guidelines, the present analytical was carried and met the acceptance criteria. It was concluded that the developed analytical method was simple, specific, accurate, economical and sensitive and can be used for routine analysis of Azelnidipine in bulk drug and in pharmaceutical dosage forms.

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