<u>**Research Article**</u>

UV-SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION FOR DETERMINATION OF AZELNIDIPINE IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple and reproducible method was developed for the assay of Azelnidipine from tablet formulation. The solvent system and wavelength of detection were optimized in order to maximize the sensitivity of the proposed method. Azelnidipine shows the maximum absorbance at 255 nm and the linearity was observed in the concentration range of 2-14 μg/ml and gave a mean correlation coefficient 0.999. The excipients in the commercial tablet preparation did not interfere with the assay. The developed UV Spectrophotometric method was found to be accurate, sensitive, precise, and was successfully applied to a pharmaceutical tablet formulation for quantitative estimation of Azelnidipine.

Keywords: Azelnidipine, UV Spectrophotometry

INTRODUCTION

Azelnidipine (AZEL) is chemically (±)-3-(1-diphenylmethylazetidin-3-yl) 5-isopropyl12-amino-1, 4-dihydro-6-methyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate. It is a dihydropyridine (DHP) type of calcium channel blocker (CCB) used for the treatment of hypertension. AZEL has two enantiomers due to an asymmetric carbon at the 4-position of the DHP ring. The pharmacological action of AZEL resides in the (R)-enantiomer. This is in marked contrast to other CCBs in which the (S)-enantiomer is responsible for the biological activity [1]. The peculiar three-dimensional structure of the active enantiomer of AZEL may be related to its unique pharmacological features that are not shared by other DHPs such as long lasting reduction in blood pressure, decreased heart rate and antiatherosclerotic effect. AZEL also shows diuretic effect by increasing urine volume and thus reduction in retention of ions [2].

A literature survey revealed that analytical methods like LC with tandem mass spectroscopy [3, 4], LC-ESI-MS [5] in biological samples and one HPLC method [6] for the determination of AZEL in tablets have been reported. These methods offered the required sensitivity and selectivity for the analysis of AZEL; however, their sophisticated instrumentation and high analysis cost limited their routine use in quality control laboratories for analysis of in its pharmaceutical dosage form such as tablets. A UV-Spectrophotometric method for the analysis of AZEL has not been reported yet. Therefore this paper describes a specific and sensitive UV-Spectrophotometric method developed and successfully applied for the estimation of AZEL in the tablet formulation.

MATERIAL AND METHOD

Instrumentation

A SHIMADZU 1800 UV-VISIBLE spectrophotometer with 1.0 cm matching quartz cells were used for absorbance measurements. The UV spectra were recorded over the wavelength 200 400 nm. All the drugs and chemicals were weighed on Metter torledo model (AX 205 series) weighing balance.

Chemicals and reagents

Gift sample of analytically pure AZEL and tablet formulation containing 8mg AZEL was obtained from Cadila Healthcare Ltd. Ahmedabad, India. Methanol of AR grade reagent was obtained from Merck chemicals, India.

Preparation of standard stock solution

20 mg of AZEL was accurately weighed and transferred to 50 ml volumetric flask containing a few ml of diluent methanol. The mixture was sonicated and volume was made up to the mark with the diluent to yield a solution of concentration 400 μg/ml. Standard stock solution was prepared by taking an aliquot of 5ml and diluting to 50ml to obtain solution of 40 μg/ml AZEL. From the stock solution 2ml was transferred to 10 ml volumetric flask and diluted up to mark with methanol to obtain final concentration of 8μg/ml. Absorbance was determined at 255 nm.

Calibration Curve for AZEL

Appropriate aliquots of AZEL working standard solution were taken in different 10 ml volumetric flasks. The volume in each flask was made up to the mark with the diluent to obtain final concentration of 2, 4, 6, 8, 10, 12, and 14μg/ml of AZEL. The calibration curve was constructed by plotting absorbance versus concentration of the drug and regression equations were computed.

Analysis of marketed formulations

Twenty tablets were weighed accurately and finely powdered. Tablet powder equivalent to 20 mg of AZEL was accurately weighed and transferred to a 50 ml volumetric flask. A few ml of diluent was added and sonicated for 5 min. Volume was made upto the mark with methanol. An aliquot of 1ml was transferred to a 50ml volumetric flask and the volume was made up to the mark to obtain 8μg/ml of AZEL. 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 255 nm and the concentration of drug in sample solution was determined from calibration curve.

Method Validation

The method was validated for linearity, accuracy, precision and specificity, limit of detection, and limit and quantitation.

Accuracy

The accuracy of the method was assessed by determination of the recovery of the method at 3 different concentrations (50%, 100% and 150% concentration) by addition of known amount of standard to the placebo. For each concentration three sets were prepared.

Precision

The instrument precision was evaluated by determining the absorbance of the standard solution of AZEL six times repeatedly. The results are reported in terms of relative standard deviation. The intra- and inter-day variation for the determination of AZEL was carried out in triplicate for the standard solution.

Specificity

The specificity was estimated by spiking the placebo with a pre-weighed quantity of drug. The spectrum was taken by appropriate

dilutions and the quantity of drug was determined.

Limit of detection and quantification

LOD and LOQ were calculated using following equation as per ICH guidelines. LOD = 3.3 σ /S and LOQ = 10σ /S, where σ is the standard deviation of response and S is the slope of the calibration curve.

Solution Stability

Stability of the standard solution was evaluated after storing for 12, 24, 36, 48 hrs. The absorbance of the aged solution was evaluated by comparing with freshly prepared solution.

RESULTS AND DISCUSSION

In this work an analytical UV-Spectrophotometric method for assay of AZEL in tablet formulation was developed and validated. Methanol as diluent was selected which gave sharp spectra of AZEL (Figure 1).
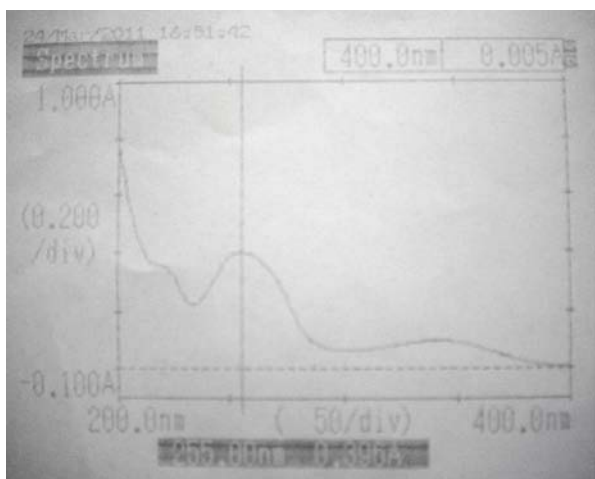

Fig. 1: Spectra of Azelnidipine

The λmax was found to be 255nm which was selected as the detection wavelength. The calibration curve was found to be linear over the range of 2-14 µg/ml for AZEL (Figure 2).
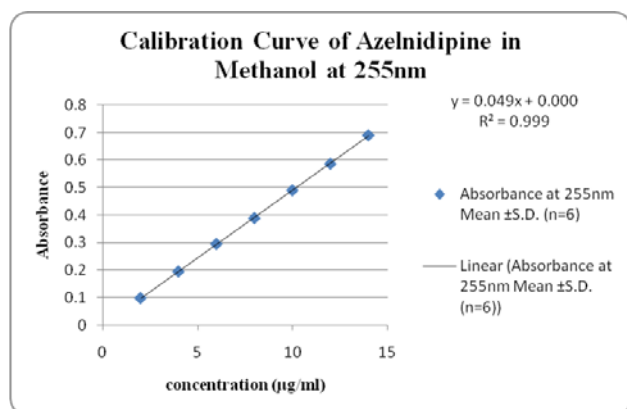

Fig. 2: Calibration curve of Azelnidipine at 255 nm

The data of regression analysis of the calibration curve are shown in table 1.

Table 1: Regression analysis data of Calibration curve

Parameters	AZEL
Wavelength (nm)	255
Linearity range (µg/ml)	2 - 14
Slope	0.049
Intercept	0.00
Correlation Coefficient	0.999

The accuracy of the method was determined by calculating recoveries which were found to be 98.33 – 99.16%. The high values indicate that the method is accurate. Instrument precision was determined by performing injection repeatability test and the RSD value for AZEL were found to be 1.03%. The low RSD values indicate that the method is precise. The detection limit for AZEL was 0.37µg/ml while quantitation limit was 1.12 µg/ml. The validation parameters are summarized in table 2.

Table 2: Summary of validation parameters

Parameters	AZEL
Accuracy (%)	98.33 - 99.16
Instrument Precision(% RSD)	1.03
Inter-day Precision (% RSD)	0.461
Intra-day Precision (% RSD	0.211
Specificity (8µg/ml at 255nm)	No interference
Limit of Detection (µg/ml)*	0.37
Limit of Quantification (µg/ml)*	1.12

*Based on S.D. of response and slope of regression curve

The solution stability study revealed that AZEL solution was stable for 48hrs without detectable degradation. The proposed method was applied to the determination of AZEL in tablet dosage form. The result for AZEL was comparable with the corresponding labelled amount (table 3).

Table 3: Assay Results of Tablet Dosage Form

Formula tion	Actual Amount (mg)	Amount Found ± SD (mg)	% of Drug Found ± SD
Tablet	8	7.97± 0.01	99.62 ± 00.12

CONCLUSION

This UV-Spectrophotometric method for assay of AZEL in tablet dosage form was successfully developed and validated for its intended purpose. The method was shown to be specific, linear, precise, and accurate. The sample recovery in tablet formulation was in good agreement with the label claim and thus suggested the validity of the method and non-interference of formulation excipients present. So this method can be applied for routine analysis of AZEL in pure form and in its formulations without interference.

ACKNOWLEDGEMENT

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