

## SIMPLE VALIDATION OF AZELNIDIPINE BY RP-HPLC METHOD

Selvadurai Muralidharan\*, Subramaniya Parasuraman and Vijayan Venugopal

Faculty of Pharmacy, AIMST University, Malaysia.

### ABSTRACT

A simple, selective, rapid, precise and economical reverse phase high pressure liquid chromatographic method has been developed for the estimation of Azelnidipine from pharmaceutical Tablet dosage form. The method was carried out on a C<sub>18</sub> (250 mm x 4.6 mm i.d., 5 μ) column with a mobile phase consisting of acetonitrile: 0.5% triethyl amine (adjusted to pH 3.5 using orthophosphoric acid) (70:30 v/v) at a flow rate of 1.0 ml/min. Detection was carried out at 254 nm. The retention time of Azelnidipine was 4.9 min. The developed method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantitation and solution stability. The proposed method can be used for the routine analysis.

**Keywords:** Azelnidipine, Method development

### INTRODUCTION

Azelnidipine, (±)-3-[1-(diphenylmethyl) azetidin-3-yl] 5-propan-2-yl 2-amino-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate, is a new dihydropyridine derivative with calcium antagonistic activity [1]. Only limited analytical methods have been reported for the determination of Azelnidipine, which includes HPLC, LC-MS and LC-ESI-MS [2-8]. The present study was validated for the routine analysis and following the ICH guidelines [9].

### EXPERIMENTAL

#### Reagents and chemicals:

Acetonitrile HPLC grade was procured from Merck KGaA, Germany. Triethyl amine AR grade were procured from SYSTEMRM, Selangor, Malaysia. Water HPLC grade was obtained from a Milli-QRO water purification system. A reference standard of azelnidipine was received from swapnroop drugs & pharmaceuticals Ltd, India.

#### Apparatus:

HPLC chromatographic separation was performed on a Shimadzu liquid chromatographic system equipped with a LC-20AD solvent delivery system (pump), SPD-20A photo diode array detector, and SIL-20A injector with 50μL loop volume. LC solution version 1.25 was applied for data collecting and processing

#### Preparation of standard solutions:

Standard stock solutions of 1.0 mg/ml Azelnidipine was prepared separately using a mixture of water and acetonitrile (1:1 v/v).

### RESULTS AND DISCUSSION

#### Method validation:

##### Accuracy and precision:

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out six times and the percentage recovery and standard deviation of the mean percentage recovery was 81.52. From the data obtained, added recoveries of standard drugs were found to be accurate.

The precision of the method was demonstrated by inter day and intra day variation studies. In the intra day studies, six repeated injections of standard and sample solutions were made and the response factor of drug peaks and % CV were calculated and presented in Table 1. In the inter day variation studies, six repeated injections of standard and sample solutions were made for three consecutive days and response factor of drug peaks and percentage % CV were calculated and presented in Table 1. From the data obtained, the developed RP-HPLC method was found to be precise.

##### Linearity and range:

The linearity of the method was determined at five concentration levels ranging from 5.0 to 30.0 mcg/ml for Azelnidipine. The calibration curve was constructed by plotting response factor against concentration of drugs. The slope and intercept value for calibration curve was ( $r^2=0.99$ ) for azelnidipine. The results show that an excellent correlation exists between response

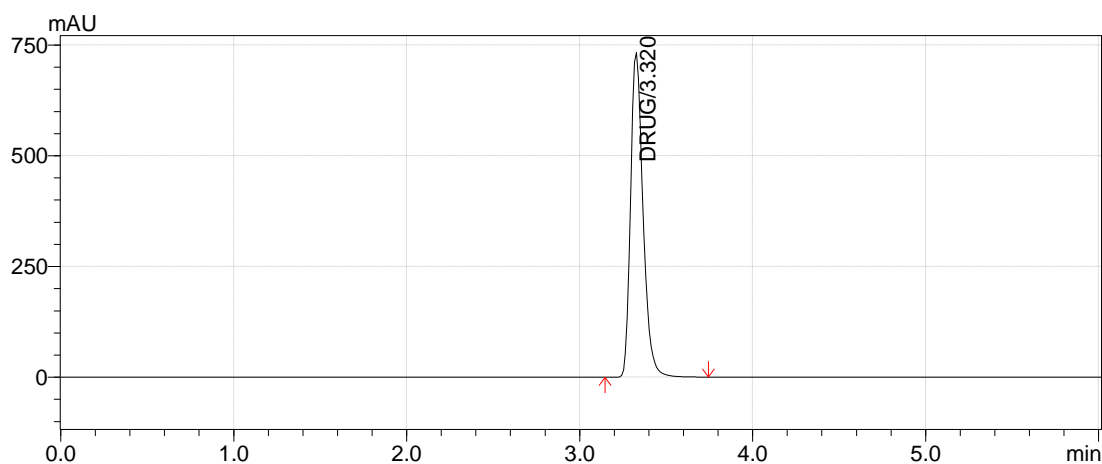
#### Address for correspondence:

Dr. Selvadurai Muralidharan,  
Faculty of Pharmacy,  
AIMST University, Semeling, Bedong,  
Malaysia.  
Email: jayaraj2775@gmail.com

factor and concentration of drugs within the concentration range indicated above.

**Table-1: Intraday and interday precision studies of Azelnidipine**

|           | Intraday studies |                |            | Interday studies |                |       |
|-----------|------------------|----------------|------------|------------------|----------------|-------|
|           | 5                | 15<br>(mcg/ml) | 30         | 5                | 15<br>(mcg/ml) | 30    |
| Mean      | 4.6834           | 14.664329      | 42.8746094 | 14.622229        | 5.055          |       |
| SD        | 0.27             | 0.13           | 0.45       | 0.41             | 0.35           | 0.39  |
| %CV       | 5.67             | 0.85           | 1.52       | 8.85             | 2.36           | 1.31  |
| %Accuracy | 93.6             | 97.7           | 98.1       | 92.19            | 97.48          | 98.35 |



**Figure- 1: Typical Chromatogram of Standard**

*Limit of Detection and Limit of Quantification:*

The Limit of Detection (LOD) and Limit of Quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3). The LOD for azelnidipine was found to be 5 mcg/ml respectively. The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified. The LOQ was 11 mcg/ml azelnidipine, respectively.

*Solution stability:*

In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 5 h at room temperature. The results show that for both solutions, the retention time and peak area of azelnidipine

remained almost unchanged and no significant degradation within the indicated period, thus indicated that both solutions were stable for at least 5 h, which was sufficient to complete the whole analytical process.

*System suitability studies:*

The column efficiency, resolution and peak asymmetry were calculated for the standard solutions. The values obtained demonstrated the suitability of the system for the analysis of this drug and the system suitability parameters fall within  $\pm 3$  % standard deviation range during routine performance of the method.

**CONCLUSION**

In conclusion, the developed method for the estimation of azelnidipine is accurate, precise, selective and linear and it can be applicable for the further research. These advantages encourage the application of this method in routine analysis Azelnidipine in pharmaceutical formulations.

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