*ejpmr, 2025, 12(6), 294-300*

**SJIF Impact Factor 7.065**

**EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH**

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

# RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR ACCURATE ESTIMATION OF ANTIHYPERTENSIVE IN PHARMACEUTICAL DOSAGE FORM

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**Article Received on 06/04/2025** **Article Revised on 27/04/2025** **Article Published on 18/05/2025**

**ABSTRACT**

Azelnidipine is a new dihydropyridine derivative with calcium antagonistic activity. It is a first-pass hepatic metabolism drug, primarily metabolized by hepatic cytochrome P450 (CYP) 3A4, and has no active metabolite product. Azelnidipine is lipophilic and has a potent affinity for vascular smooth muscle cell membranes. It is a drug that has undergone preclinical and clinical studies and was launched as CALBLOCK® in Japan in 2003. The study aims to develop and validate the RP-HPLC method for estimating Azelnidipine in Pharmaceutical Dosage Form, focusing on the application of suitable analytical techniques, optimization, and validation in accordance with ICH guidelines. The study will also focus on selecting the appropriate drug and developing an analytical methodology. In this study, we found that the pharmaceutical dose tablet formulations containing Azelnidipine (AZP) may be accurately measured using the RP-HPLC method. The RP-HPLC technique is sensitive, accurate, precise, and repeatable; it also demonstrates high repeatability. Azelnidipine (AZP) tablet dosage formulation analysis may also be conducted with success. These techniques do not experience any influence from additives, matrices, etc. To further understand these trials, additional research on other medication formulations is needed.

**KEYWORDS:** Azelnidipine; RP-HPLC; CALBLOCK; Pharmaceutical Dosage Form.

## 1. INTRODUCTION

Azelnidipine, (±)-(3)-(1- diphenyl methyl azetidin- 3-yl)-5-isopropyl-2-amino-1, 4-dihydro-6- methyl-4-(3-nitrophenyl)-3, 5-pyridine dicarboxylate (Figure 1), is a new dihydropyridine derivative with calcium antagonistic activity. Although all the existing dihydropyridine calcium blockers have two methyl groups located at the 2- and 6- positions of the dihydropyridine ring, one methyl group at the 2-position is substituted by an amino group in the azelnidipine molecule. Consequently a conduct of a series of preclinical and clinical studies, this drug was launched into the market as CALBLOCK® in Japan in 2003. Azelnidipine occurs as two enantiomers due to an asymmetric carbon at the 4-position of the 1,4 dihydropyridine ring.[1-4]

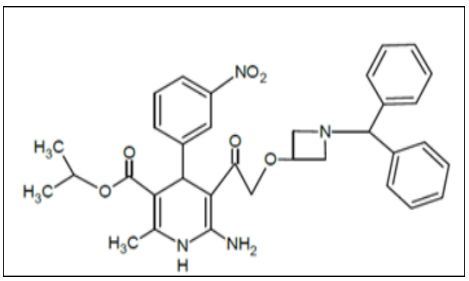


**Figure 1: Azelnidipine.**

Like most members of its class, azelnidipine primarily undergoes first-pass hepatic metabolism. Azelnidipine is metabolized by hepatic cytochrome P450 (CYP) 3A4 and has no active metabolite product. It may interact with other drugs or compounds that are substrates for this enzyme. Azelnidipine is lipophilic and has a potent affinity for membranes of vascular smooth muscle cells.[5-10]

The aim of the present study is to develop and validate the RP-HPLC method for estimating Azelnidipine in Pharmaceutical Dosage Form, focusing on the application of suitable analytical techniques, optimization, and validation in accordance with ICH guidelines, while selecting the appropriate drug and developing an analytical methodology.

## 2. MATERIALS AND METHODS

### 2.1 Procurement of the Drug

Azelnidipine, a medication from Arch Pharma labs Ltd Thane, is available in a 10g package with a purity of 99.8 to be used as Reference drug while Muvera 15, Sun Pharma Lab. Ltd India which contains 15 mg dosage of Azelnidipine to be used as test drug.

### 2.2 Method and Procedure

#### 2.2.1 Identification and characterization of drug

Azelnidipine (AZP) were selected as model drug candidate for method development and validation. The drugs were kindly gifted from Pharmaceutical industry India. The procured drug was analyzed for different physical properties viz. color, odor, melting point, etc. The IR absorbance spectrum of MLC was recorded using FTIR 8400S spectrometer (Shimadzu) over range of 4000 to 400 $cm^{-1}$.[11-15]

#### 2.2.2 Selection of Mobile Phase

The mobile phases tested include methanol: water (90:10), methanol: water (80:20), acetonitrile: water (90:10), acetonitrile: phosphate buffer 10mm (90:10), acetonitrile: phosphate buffer 10mm (80:20), and acetonitrile: phosphate buffer (75:25) with pH 4.5.

#### 2.2.3 Chromatographic Conditions

The chromatographic conditions were established through trial and error, maintaining constant consistency throughout the method. The column was Inertsil 4.6 x 250 mm, with a particle size of 5 µm, stationary phases of C18 Inertsil, mobile phase of Acetonitrile: Phosphate Buffer (75:25), pH 4.5, and a sample size of 20 µL.

#### 2.2.4 Validation of the Method

Adjusting several UFLC settings (FDA, 1995, 1997, 2000, 1994, 1987; USP, 2000) confirmed the reliability of the UFLC approach.[16] Calibration plot least-squares linear regression analysis verified the UFLC method's linearity[17], the limits of detection and quantification for the medicines mentioned were determined to be three and five epochs, respectively, above and below the baseline noise, The process adhered to the guidelines established by the United States Pharmacopoeia (USP, 2000), specificity[17], precision[18] accuracy[19], robustness[20] and ruggedness[21] were determined.

## 3. RESULTS AND DISCUSSION

### 3.1 Characterization of the Drug

Azelnidipine is typically a light orange to yellow to green powder or crystal. It is soluble in acetone and acetic acid, and also soluble in ethanol and ethyl acetate. It has limited solubility in water and methanol. Azelnidipine has an apparent partition coefficient (log P)app = 0.51 in n-octanol/buffer pH 7.4. Azelnidipine has pKa values of 1.9 and 4.9. Melting point of the Azelnidipine is found to be 122.75 ± 0.188 ºC.

### 3.2 Identification of the Drug

#### 3.2.1 UV Spectra Analysis

The ultraviolet absorption spectrum of MLC was obtained using Shimadzu1800- UV visible spectrophotometer and 1cm quartz cells, over a wavelength range of 400 to 200 nm, which was found to be 255 nm (Figure 2)

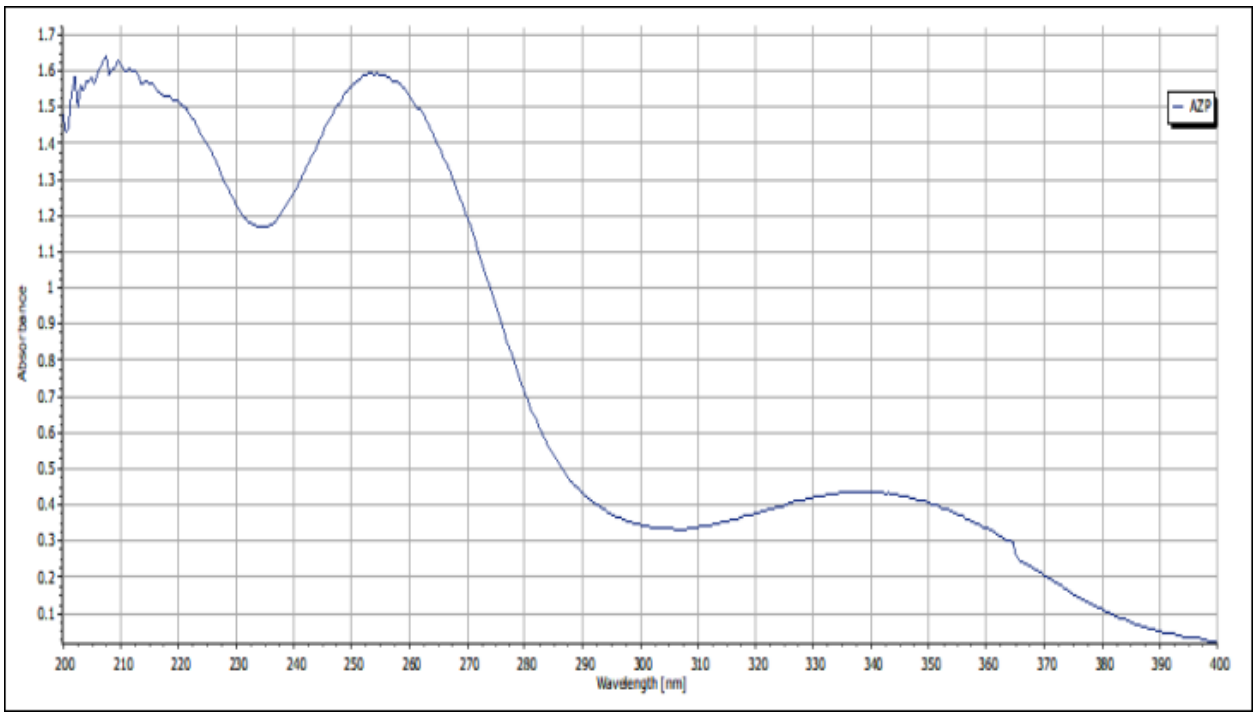


**Figure 2: UV Spectra Analysis.**

#### 3.2.2 FT-IR of Azelnidipine

The IR absorbance spectrum of Azelnidipine (AZP) was recorded using FTIR 8400S spectrometer (Shimadzu) over range of 4000 to 400 cm-1 (Figure 3).

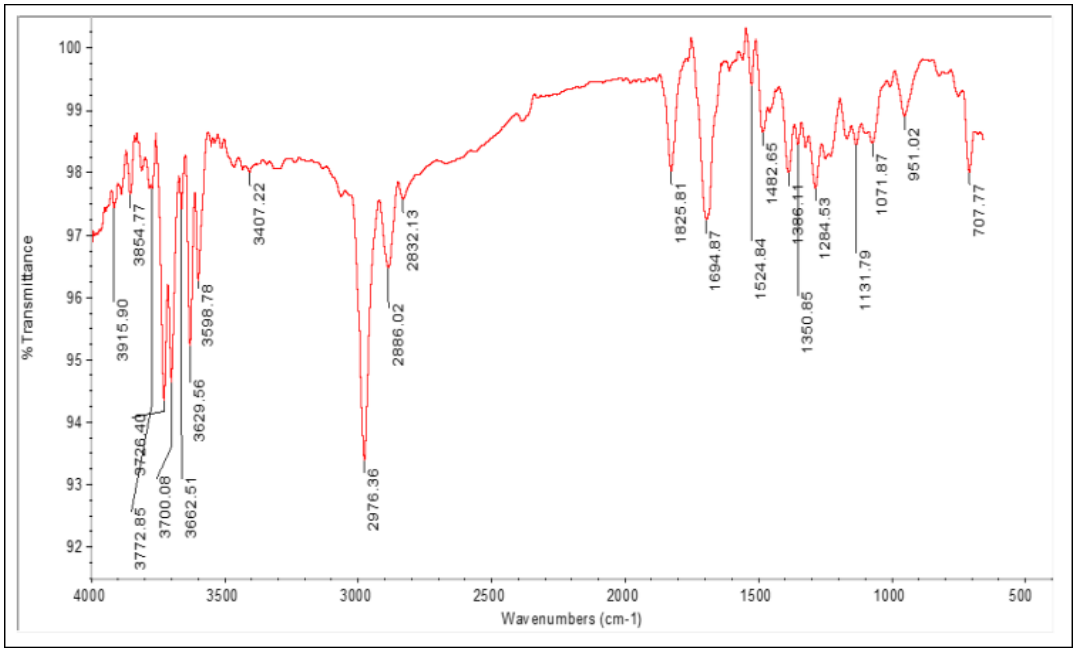


**Figure 3: FT-IR study of Azelnidipine.**

### 3.3 Selection of the Mobile Phase

From various mobile phases tried, mobile phase containing Acetonitrile: Phosphate Buffer (65:35) pH 4.5 was selected, since it gives sharp reproducible retention time for MLC (Figure 4-7).

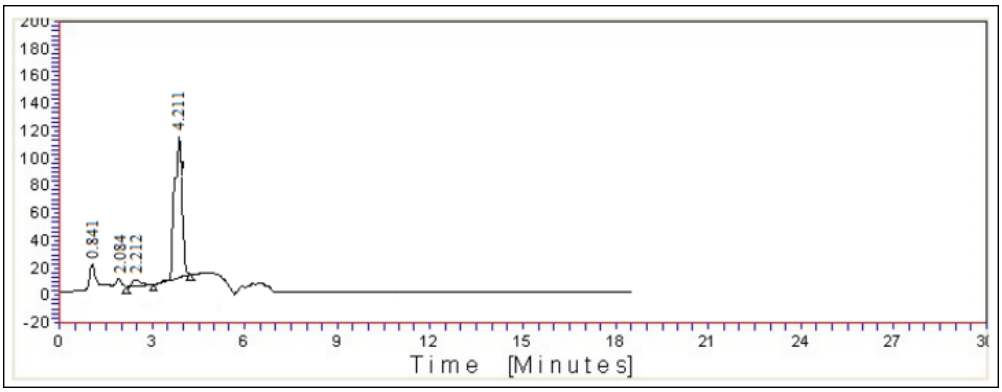


**Figure 4: Trial Chromatogram obtained by using Acetonitrile: water (80:20) as mobile phase.**

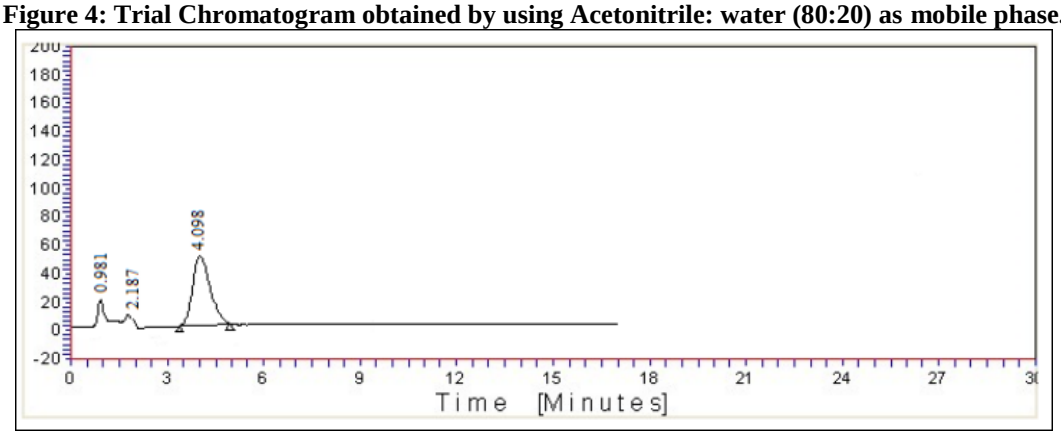


**Figure 5: Trial Chromatogram obtained by using Acetonitrile: Phosphate Buffer 10mm (90:10) pH 5.5 as mobile phase.**

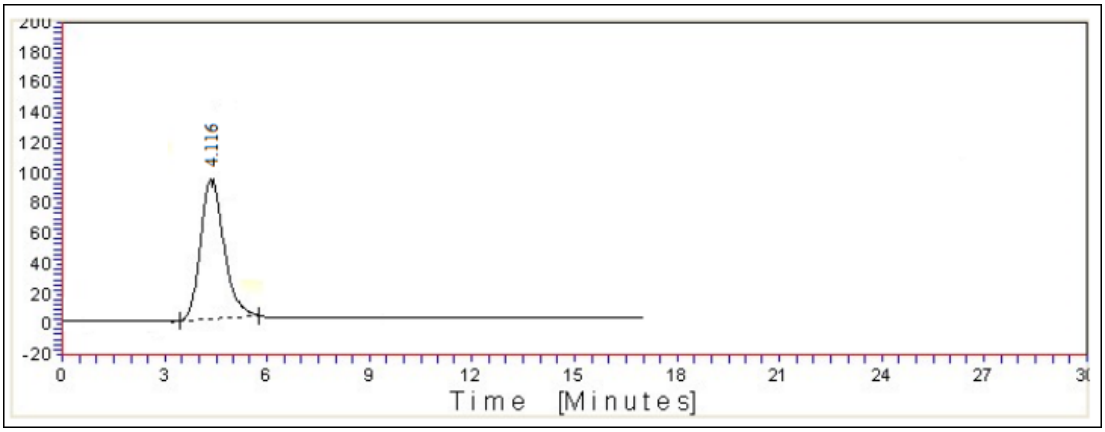


**Figure 6: Trial Chromatogram obtained by using Acetonitrile: Phosphate Buffer 10mm (65:35) pH4.5 as mobile phase of MLC.**

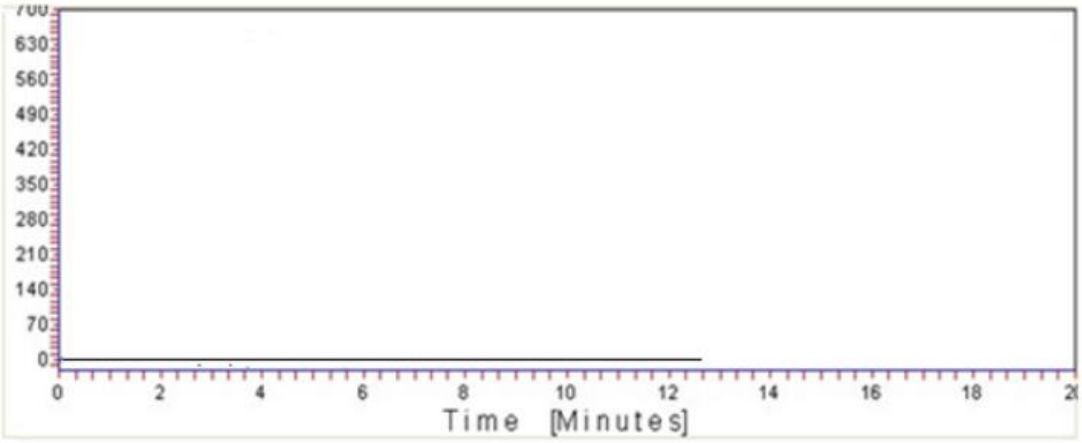


**Figure 7: Blank Chromatogram obtained by using Acetonitrile: Phosphate Buffer 10mm (65:35) pH4.5 as mobile phase.**

**3.5 Validation of the Method**

Accuracy was ascertained on the basis of recovery studies performed by standard addition method (Table 2). Precision of an analytical method is expressed as S.D or R.S.D of series of measurements. It was ascertained by replicate estimation of the drugs by proposed method (Table 3). Specificity was measured as ability of the proposed method to obtain well separated peak for MLC without any interference from component of matrix. Mean retention time for – MLC – 3.981 The values obtained were very close to that in standard laboratory mixture indicates no interference from the component of matrix. Linearity and range: According to USP tablet powder equivalent to 80, 90, 100, 110, 120 % of label claim was taken and dissolved & diluted appropriately with mobile phase to obtain a concentration in the range of 80%-120% of the test concentration. The chromatograms of the resulting solutions was recorded. MLC marketed formulation was found to be linear in the range ± 20% of the test concentration of the respective drug (Table 4). The robustness study indicated that the factors selected remained unaffected by small variation of organic composition of mobile phase, wavelength and the flow rate. The system suitability results should lie within the limit. Hence the method was robust (Table 5). Limit of detection is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. Limit of quantitation is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision accuracy (Table 6). After establishing the chromatographic conditions, standard laboratory mixture was prepared and analysed by following procedure described under experimental and results. It gave accurate, reliable results and was extended for estimation of drugs in marketed tablet formulation.

**Table 2: Results and Statistical Data for Recovery study of MLC.**

| Sr. No | wt. of formulation | Amount of Drug Added in (µg/ml) | Peak Area of stand. | Peak Area of sample | % Recovery |
|---|---|---|---|---|---|
| | AZP | AZP | AZP | AZP | AZP |
| 1 | 110 | 1 | 62467.2 | 62092.4 | 99.4 |
| 2 | | 1 | | 62342.3 | 99.8 |
| 3 | | 1 | | 62279.8 | 99.7 |
| 4 | | 2 | | 62217.3 | 99.6 |
| 5 | | 2 | | 62966.9 | 100.8 |
| 6 | | 2 | | 63029.4 | 100.9 |
| 7 | | 3 | | 63154.3 | 101.1 |
| 8 | | 3 | | 63404.2 | 101.5 |
| 9 | | 3 | | 62592.1 | 100.2 |

**Table 3: Results and Statistical Data of Precision Study.**

| Sr. No. | Weight of Standard (mg) | Weight of Sample (mg) | Peak Area of Stand. | Peak Area of Sample | % Label claim |
|---|---|---|---|---|---|
| | AZP | AZP | AZP | AZP | AZP |
| 1 | 10 | 110 | 62467.2 | 62404.7 | 99.9 |
| 2 | | 110 | | 62529.7 | 100.1 |
| 3 | | 109.9 | | 62217.3 | 99.6 |

**Table 4: Observations of Linearity and range study for MLC.**

| Sr. No. | % Label claim | Peak area AZP |
|---|---|---|
| 1 | 80 | 49973.76 |
| 2 | 90 | 56220.48 |
| 3 | 100 | 62467.2 |
| 4 | 110 | 69713.92 |
| 5 | 120 | 74960.64 |

**Table 5: Result of Robustness study of MLC.**

| Sr. No. | Condition | Parameter | Peak Area | RT |
|---|---|---|---|---|
| 01 | Change of wavelength | 253 nm | 62207.1 | 4.117 |
| 02 | | 255 nm | 62467.2 | 4.116 |
| 03 | | 257 nm | 62645.7 | 4.118 |
| 04 | Change in Temperature | 30 ºC | 62101.2 | 4.115 |
| 05 | | 25 ºC | 62467.2 | 4.116 |
| 06 | | 20 ºC | 62187.2 | 4.116 |
| 07 | Change in Flow rate | 0.8 ml/min | 62467.2 | 4.121 |
| 08 | | 1ml/min | 62489.1 | 4.116 |
| 09 | | 1.2 ml/min | 62441.9 | 4.111 |
| 10 | Change in Mobile Phase | 75:25 | 62407.4 | 4.121 |
| 11 | | 65:35 | 62467.2 | 4.116 |
| 12 | | 55:45 | 62454.56 | 4.112 |

**Table 6: Limit of detection (LOD) and Limit of quantitation (LOQ).**

| Sr. No. | Drug Name | LOD µg/ml | LOQ µg/ml |
|---|---|---|---|
| 1 | AZP | 0.028 | 1.104 |

## 4. CONCLUSIONS

In this study, we found that the pharmaceutical dose tablet formulations containing Azelnidipine (AZP) may be accurately measured using the RP-HPLC method. The RP-HPLC technique is sensitive, accurate, precise, and repeatable; it also demonstrates high repeatability. Azelnidipine (AZP) tablet dosage formulation analysis may also be conducted with success. These techniques do not experience any influence from additives, matrices, etc. To further understand these trials, additional research on other medication formulations is needed.

## 5. Conflict of Interest

None.

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