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Original Article

# Azilsartan Medoxomil and Cilnidipine Spectrophotometric Determination in Bulk and Formulation: A Validated Dual Wavelength Method

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## ABSTRACT

**Introduction:** If there is a rise in blood pressure then that condition is called hypertension. Azilsartan medoxomil and Cilnidipine is a new combined dosage form used in the treatment of Stage II Hypertension. **Objectives:** The main intention of the research is to reinforce a validated dual wavelength spectrophotometric approach for the combined evaluation of AZL and CIL in bulk and formulation. **Materials and Methods:** In the dual wavelength approach, for each drug two wavelengths have been chosen such that they exhibit the same absorbance or the difference in absorbance for each drug is 0. Two wavelengths 229.5 nm and 256.5 nm opted for the detection of AZL while CIL exhibits the same absorbance. Similarly, 238 nm and 258 nm opted to determine CIL while AZL exhibits the same absorbance at both of these wavelengths. The solvent employed was ethanol. **Results:** Linearity lay between the quantitation range of 1-5 µg/mL for AZL and 4-20 µg/mL for CIL. LOD and LOQ for AZL and CIL were revealed to be 0.6278 µg/mL and 0.0931 µg/mL and 1.9026 µg/mL and 0.2821 µg/mL respectively. The calculated % recovery was found to be 99.97±0.45 for AZL whereas 100.15±0.035 for CIL. **Conclusion:** The validated method was found to be simple, sensitive, easy and affordable for a quantitative estimate of AZL and CIL. Through the use of ICH criteria, the method was validated.

**Keywords:** Azilsartan medoxomil, Cilnidipine, Dual Wavelength Method, Hypertension.

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## INTRODUCTION

The term "silent killer" is used to characterize hypertension. Hypertension is a condition that occurs when blood pressure rises and is characterized by increased pressure on the blood vessels. When the pressure is higher, the heart has to work harder to pump blood. Abnormally elevated systolic and/or diastolic blood pressure readings that are at or beyond the permissible range (140 mm Hg for systolic and 90 mm Hg for diastolic). Over the past three decades, the reported rate of increase in patients suffering from hypertension ranges from 650 M to 1.28 B.[1,2] The condition affects more than a billion people worldwide-roughly 1 in 4 men and 1 in 5 women-and is a substantial cause of premature death.[3] Antihypertensive drugs are therefore used to treat this hypertension.

Chemically, *Azilsartan medoxomil* is a 5-methyl-2-oxo-1, 3-dioxol-4-yl, 5-ethoxy-1-([2'-(5-oxo-4,5-dihydro-1, 2, 4-oxadiazol-3-yl) biphenyl-4-yl) methyl)-1H-benzimidazol e-7-carboxylate (Figure 1). AZL molecular formula is $C_{30}H_{24}N_4O_8$. It is white, amorphous solid, easily soluble in acetic acid, methanol, ethanol, DMSO, and DMFA but essentially insoluble in water. It is an antagonist of the Angiotensin II type-I receptor.[4]

*Cilnidipine* is 1,4-Dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridine dicarboxylic acid. 3-phenyl-2-propenyl-2-methoxyethyl ester, 2-methoxyethyl (Figure 2). $C_{27}H_{28}N_2O_7$ is the chemical formula for CIL. It is an amorphous solid with a light yellow to white colour that is easily soluble in acetone, methanol, and ethanol but essentially insoluble in water. It is a $Ca^{++}$ channel blocker of the L- and N-type.[5]

In the dual wavelength approach, a mixture that contains both the chemical substance of interest and an undesirable intervening chemical component is utilized to estimate the unknown amount of the desired chemical component present in the mixture. This is independent of the chemical substance in between and directly proportional to the concentration of the relevant chemicals. The absorbance difference between two spots on the mixture spectra

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is exactly proportional to the concentration of the component of interest, according to the guiding principle of dual wavelength method. The methods are validated according to ICH guidelines.[6-9]

Literature survey revealed that, in April 2020 CDSCO approved AZL and CIL as a mixed dosage form. UV spectroscopy, RP-HPLC, HPTLC and stability-indicating methods have been reported for AZL and CIL drugs individually and along with other drugs.[10-15] The review of published data also showed that only one simultaneous equation approach for UV spectroscopy and one RP-HPLC method for estimating AZL and CIL in synthetic mixtures were disclosed.[16-19] Hence, an effort has been made to develop a straightforward, sensitive and affordable method for the combined estimation of AZL and CIL in tablet dosage forms.

## MATERIALS AND METHODS

### Chemicals and reagents

Ethanol of A.R. grade was collected from S.D. Fine Chem Ltd., Mumbai, India. Whatmann filter paper no 41 (Millipore, USA) was used as a filtering aid. Pure Azilsartan medoxomil was obtained as a gift sample from APL Research Centre-II, Hyderabad, Telangana, India. Pure Cilnidipine procured from J B Chemicals Pvt. Ltd., Mumbai, India. MYOTAN ® CN 40/10 tablets containing AZL 40 mg and CIL 10 mg manufactured by Synokem pharmaceuticals, Haridwar. These are procured from TATA 1mg Healthcare Solutions through online.

### Instrument

Shimadzu model 1800 double beam with a spectral bandwidth of 1±0.2 nm, wavelength precision of ±0.3 nm UV/VISIBLE Spectrophotometer attached to UV-probe 2.0 system softwares was used as an instrument for spectral measurement. A pair of quartz cuvettes having a 1 cm path length, electronic analytical balance (Type Ax200, Shimadzu Corporation), sonicator and calibrated glassware's was also used.

### Experimental

#### *Selection of Solvent*

Separately, a little amount of the pure pharmaceuticals AZL and CIL were dissolved in distilled water, methanol, ethanol and methanol: distilled $H_2O$ (50:50). It was discovered that ethanol has the highest solubility of AZL and CIL. As a result, ethanol was chosen as a typical solvent for making standard and working stock solutions at 28°±1°C.

### Standard Stock Solution Preparation of AZL and CIL pure drugs

Weigh 100 mg of AZL and 100 mg of CIL, transfer them into two separate 100 mL volumetric flasks, dissolve in 50 mL of ethanol, subject them for sonication and fill the remaining volume with ethanol to get an evaluation range of 1000 µg/mL. Each stock "A" and "A1" solution has a 1 mg/mL concentration.

From the above prepared stock 'A' and stock 'A[1]' solutions, 10 mL of aliquots were pipetted out and transferred into two different 100 mL VF and diluted up to the mark with ethanol. Each Stock 'B' and Stock 'B[1]' solution contains 100 µg/mL.

### Analytical Wavelength Selection for Dual Wavelength method

After making choice of suitable solvent and preparation of standard and working stock solutions, it is important to fix the $\lambda_{max}$ for the proposed method. From stock B and B[1], serial dilutions ranging from 4-20 µg/mL for AZL, 1-5 µg/mL for CIL were prepared, scanned in the wavelength region 400-200 nm. At 249.0 nm AZL showed maximum absorbance and at 241.0 nm CIL shows maximum absorbance with more linearity and repeatability. The overlaid drug spectra suggested that the best method for simultaneously determining AZL and CIL in bulk drug and pharmaceutical formulation was a dual wavelength spectrophotometric method.

From the overlaid drug spectra, the set of dual wavelengths 229.5 nm and 256.5 nm were chosen as $\lambda_1$ and $\lambda_2$ for the evaluation of AZL as CIL shows equal absorbance at the wavelengths. In a similar way, set of 2 wavelengths 238.0 nm and 258.0 nm selected as $\lambda_3$ and $\lambda_4$ for the evaluation of CIL as AZL shows equal absorbance at these wavelengths (Figure 3).

### Selection and construction of concentration range and calibration curve for AZL and CIL in pure drug

From stock B and B[1] solutions, appropriate aliquots were pipetted out into a succession of 10 mL VF. Ethanol was used to raise the volume to the necessary amount so that a set of solutions could be created having the fixation range of 4, 8, 12, 16 and 20 µg/mL of AZL and 1, 2, 3, 4 and 5 µg/mL of CIL. The above prepared solutions were individually scanned in "Spectrum Mode" from 400 nm to 200 nm. With the help of an overlaid drug spectrum, the absorbance of the above solutions was recorded at 229.5 nm and 256.5 nm as $\lambda_1$ and $\lambda_2$ for the evaluation of AZL as CIL shows equal absorbance at these two wavelengths. Similarly, absorbances were recorded at 238.0 nm and 258.0 nm as $\lambda_3$ and $\lambda_4$ for the evaluation of CIL as AZL shows equal absorbance. AZL and CIL drugs followed Beer's law in the fixation range of 4-20 µg/mL and 1-5 µg/mL for AZL and CIL respectively. By plotting absorbance

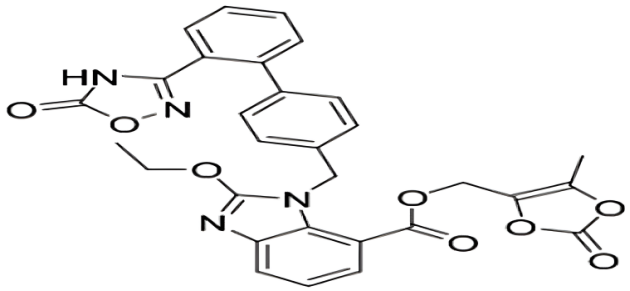


**Figure 1:** Structure of Azilsartan Medoxomil (AZL).

against concentration, the calibration curve was constructed (Tables 1 and 2, Figures 4 and 5). Statistical validation data were determined.

## Absorptivity calculation of both the drugs at chosen wavelengths

The absorptivity at the selected wavelength was calculated by preparing five working standard drug solutions.

## Sample stock solution preparation using tablet formulation

An average weight of MYOTAN ® CN 40/10 twenty tablets was determined and crushed into fine powder. In 50 mL of ethanol, a powder weighing 40 mg of AZL and 10 mg of CIL was dissolved after being weighed and the mixture was then subjected to 15 min sonication. It is diluted with 100 mL ethanol to get 400 µg/mL AZL and 100 µg/mL for CIL (sample stock 'A[11]' solution). Filter the solution using Whatmann filter paper No. 41. The above prepared stock 'B[11]' solution containing AZL (40 µg/mL), CIL (10 µg/mL) were prepared.

## Pure Drug Mixture Analysis

Standard stock B and B[1] solutions were combined to get 8 µg/mL of AZL and 2 µg/mL of CIL. Six replicates were prepared and analysed.

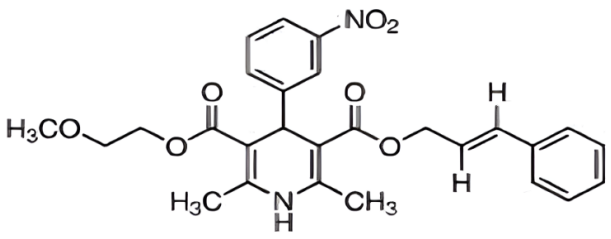


**Figure 2:** Structure of Cilnidipine (CIL).

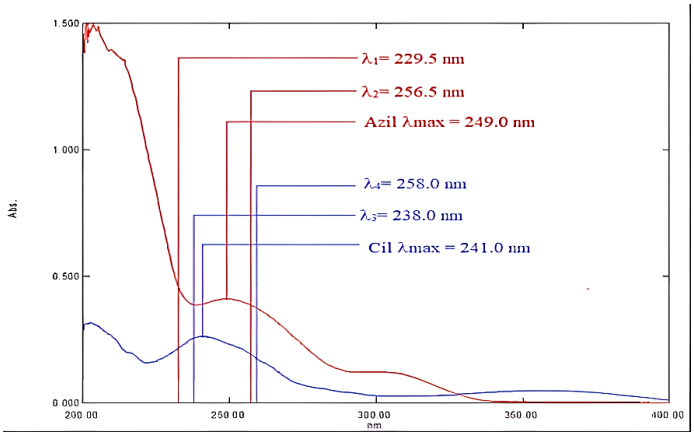


**Figure 3:** Overlaid Spectra of AZL at $_1$ 229.5 nm and $_2$ 256.5 nm and CIL at $_3$ 238.0 nm and $_4$ 258.0 nm.

## Tablet Formulation Analysis

From sample stock "B[11]" solutions six replicates containing 12 µg/mL of AZL and 3 µg/mL of CIL were prepared and analysed. Assay results of tablet formulation were computed and Statistical validation data were determined (Table 3).

# RESULTS

## Method Validation

### *Linearity and Range*

As the dual wavelength method adhered to Beer's law in the opted fixation range, linearity was attained in the fixed range 4-20 µg/mL for AZL and 1-5 µg/mL for CIL respectively.[9]

## Accuracy

By adding 80%, 100%, and 120% of the standard drug solutions of AZL and CIL to the sample stock solutions, recovery studies were conducted. The results were contrasted and statistically verified. The recovery rates for AZL and CIL using this approach were 99.78% and 100.15%, respectively. There were three determinations made for each recovery study level. The accuracy was determined to be well within the 95-105% acceptability range.

**Table 1: Result of calibration curve of absorbance difference of AZL at 229.5 nm and 256.5 nm where CIL showed the same absorbance.**

| Concentration (µg/mL) | Absorbance difference of AZL at 229.5 nm and 256.5 nm Mean±Std. Deviation (*n*=6) | %CV |
|---|---|---|
| 4 | 0.063±0.00063 | 1.0038 |
| 8 | 0.124±0.00116 | 0.9364 |
| 12 | 0.189±0.00187 | 0.9872 |
| 16 | 0.248±0.00231 | 0.9309 |
| 20 | 0.310±0.0025 | 0.8066 |

*n**=6.

**Table 2: Result of calibration curve of absorbance difference of CIL at 238.0 nm and 258.0 nm where AZL showed the same absorbance.**

| Concentration (µg/mL) | Absorbance difference of CIL at 238.0 nm and 258.0 Mean±Std. Deviation (*n*=6) | %CV |
|---|---|---|
| 1 | 0.025±0.0004 | 1.6221 |
| 2 | 0.049±0.0008 | 1.6902 |
| 3 | 0.076±0.0010 | 1.3530 |
| 4 | 0.099±0.0018 | 1.8502 |
| 5 | 0.124±0.0021 | 1.7210 |

*n**=6.

**Table 3:** Statistical Validation Data for Tablet Formulation.

| Components | Mean | Std. Deviation | Co-efficient of variation | Standard error |
|---|---|---|---|---|
| AZIL | 100.75 | 0.8821 | 0.8755 | 0.3601 |
| CIL | 99.72 | 1.2030 | 1.2063 | 0.4911 |

$n^*$=6.

**Table 4:** Summary of the proposed method.

| Parameter | Azilsartan medoxomil at 229.5 nm and 256.5 nm | Cilnidipine at 238.0 nm and 258.0 nm |
|---|---|---|
| Wavelength Range | 229.5-256.9 | 238-258 |
| Linear Range (µg/mL) | 4-20 | 1-5 |
| Regression equation (y=mx + c) | Y=0.0154x-0.0011 | Y=0.0247x-0.0001 |
| Slope (m) | 0.0154 | 0.0247 |
| Intercept (c) | 0.0011 | 0.0001 |
| Correlation Coefficient ($r^2$) | 0.9995 | 0.9998 |
| Accuracy ($n$=3) %RSD | 0.5625 | 1.3684 |
| Precision ($n$=3) (% RSD) | 0.6919 | 1.2052 |
| Limit of Detection (µg/mL) | 0.6278 | 0.0931 |
| Limit of Quantification (µg/mL) | 1.9026 | 0.2821 |
| % Assay±S.D ($n$=6) | 99.97±0.45 | 100.15±0.035 |

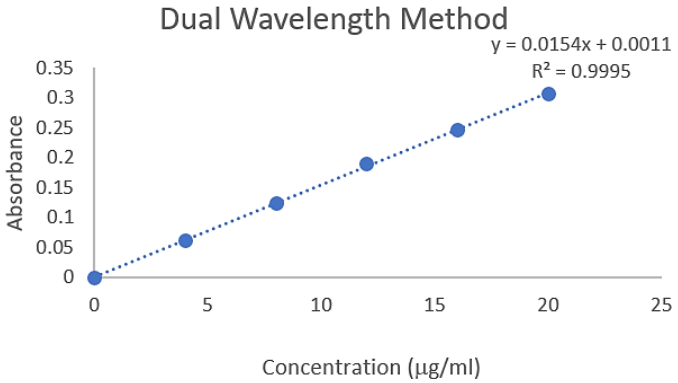


**Figure 4:** Calibration curve for absorbance difference of AZL at 229.5 nm and 256.5 nm where CIL showed the same absorbance.

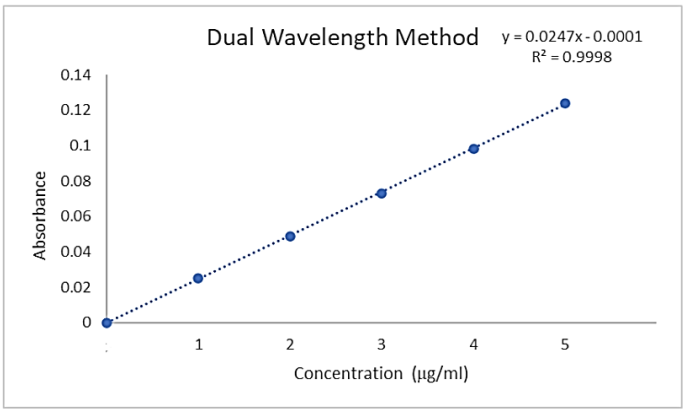


**Figure 5:** Calibration curve for absorbance difference of CIL at 238.0 nm and 258.0 nm where AZL showed the same absorbance.

## Precision

For the sample mixture containing 16 µg/mL of AZL and 4 µg/mL of CIL, intraday and interday precision studies were carried out. The sample mixture was analyzed six times on the same day at different time intervals and on 3 different days at their chosen analytical wavelengths, and absorbances were measured at 229.5 and 256.5 nm as $\lambda_1$ and $\lambda_2$ for the estimation of AZL as CIL shows the same Likewise, absorbances at 238.0 and 258.0 nm were measured as $\lambda_3$ and $\lambda_4$ for the estimate of CIL since AZL exhibits the same absorbance at these wavelengths. The fluctuation of the results on three distinct days as well as on the same day was examined and statistically validated. For AZL and CIL, the assay's % RSD was determined to be 0.6919% and 1.2052%, respectively. Results for intra-day and inter-day precision were found to be good, with %RSD for both AZL and CIL being less than 2.0%.

## Limit of detection

The LOD was estimated using the response's standard deviation and slope, and it was discovered to be 0.6278 µg/mL for AZL and 0.0931 µg/mL for CIL, respectively.

$$LOD = \frac{3.3\,\sigma}{S}$$

Where,

σ=Response of the standard deviation,

S=Slope of the calibration curve.

## Limit of quantitation

Response standard deviation and slope were used to compute the LOQ, which was recorded to be 1.9026 µg/mL for AZL and 0.2821 µg/mL for CIL, respectively.

$$LOQ = \frac{10\,\sigma}{S}$$

Where,

σ=Response of the standard deviation,

S=Slope of the calibration curve.

## DISCUSSION

The main aim and objective of the intended work is to develop a simple, sensitive, accurate and validated Dual wavelength UV-visible spectrophotometric method for the simultaneous estimation of AZL and CIL in bulk and in combined dosage form. From overlapped spectra, two wavelengths ($\lambda_1$ and $\lambda_2$) at 229.5 and 256.5 nm were chosen for AZL because they displayed different absorbances compared to CIL, which had the same absorbance. Similarly, two wavelengths at 238 and 258 nm ($\lambda_3$ and $\lambda_4$) were selected on overlay spectra at which CIL displayed a change in absorbance whereas AZL had the same absorbance. Linearity for dual-wavelength method was achieved at 4-20 µg/mL for AZL and 1-5 µg/mL for CIL. In the present method, AZL showed a percentage recovery of 99.97% and CIL showed 100.15% in which accuracy was found to be well within the acceptance limit of 95-105%. The intra-day precision and inter-day precision results were found to be 0.6919 for AZL and 1.2052 for CIL which was less than 2% according to the percentage relative standard deviation limit. The LOD and LOQ were determined by standard deviation of the response and the slope and shown in Table 4.

## CONCLUSION

The present method shows a high % recovery greater than 98% indicating that the method is free from the interference of excipients used in the formulation. The value of standard deviation and % R.S.D. were found to be <2%, indicating the high precision of the method. High % recovery and low % RSD suggest that the above methods can be applicable for the routine analysis of AZL and CIL in formulations. The developed methods were validated for various parameters as per ICH guidelines like Linearity, accuracy, precision, LOD and LOQ. The results obtained were within the acceptance criteria for the parameter. The proposed method was applied for simultaneous estimation of AZL and CIL in marketed formulations. The assay results conformed to the label claim of the formulation. Hence the proposed method is found to be satisfactory and could be used for the routine analysis of AZL and CIL in bulk drug and pharmaceutical formulations.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## ABBREVIATIONS

**AZL:** Azilsartan Medoxomil; **CIL:** Cilnidipine; **ML:** Milli Liter; **µg:** Microgram.

## SUMMARY

Dual Wavelength UV-visible Spectrophotometric method for the simultaneous estimation of Azilsartan medoxomil and Cilnidipine has been developed and validated using Shimadzu model 1800 double beam UV Spectrophotometer with spectral band width of 1±0.2 nm, wavelength accuracy of±0.3 nm and a pair of quartz cuvettes having 1cm path length and ethanol was used as solvent. The proposed method is validated through laboratory studies for various parameters as per ICH guidelines, viz, linearity, accuracy, precision, LOD and LOQ. The results indicated that all the values were well within the acceptance limit (Table 4). Hence the methods are found to be linear, accurate and precise.

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