# *Original Research Article*

**Development and validation of ultraviolet and reverse phase-high performance liquid chromatography method for estimation of Cilnidipine**


## ABSTRACT

**Aim:** The current experiment was to develop and validate a straight forward RP-HPLC methodology for the determination of Cilnidipine.

**Methodology:** UV spectroscopy was used to estimate clini. Action separation of Cilnidipine was achieved by employing a C18 column. Mobile phase containing a combination of methanol: water (90:10 *v/v*) was tense at the flow of 1 mL min. Detection was performed at 241 nm. Validation parameters were evaluated in line with the International conference on harmonization (ICH) Q2R1 guidelines.

**Result:** The standardization curve was linear within the varying concentration of 2-10 mg $mL^{-1}$ for Cilnidipine with parametric statistic $R^2 = 0.999$. The tactic was found to be accurate (101.66% recovery), precise (intraday, 1.65 and inter day, 1.38) and robust (% RSD was calculated to be 0.66, 0.58 and 0.81 for variation in mobile phase composition, wave length and flow velocity respectively) for the analysis of Cilnidipine.

**Conclusion:** The developed method has passed all the validation tests and can be successfully applied to estimate the presence of Cilnidipine in bulk as well as in pharmaceutical formulations.



*Keywords: UV spectroscopy, RP-HPLC, Cilnidipine, hypertension, linearity, precision*


## 1. INTRODUCTION

In the current *scenario*, hypertension or high blood pressure is one of the most fatal cardiovascular diseases. Also, it is called silent killer because its symptoms are unrevealed till any major damage occurs in the body. It is a multifaceted disease associated with kidney As a result, it is one of the most under-diagnosed and under-treated medical disorders over the world. High blood pressure can affect anyone at any age, but it is more common in persons who have a family history of the condition, who are overweight or obese and have diabetes. The prevalence of hypertensive cases may be minimalized by use of several antihypertensive drugs e.g. calcium channel blockers, β-blockers, α-blockers, angiotensin converting enzyme inhibitors, diuretics, and angiotensin II type 1 receptor blockers [1]. Cilnidipine (CL) has been extensively studied and demonstrated as calcium channel blocker in preclinical and clinical development phases. It blocks the N-type and L-type calcium channels and dilates both arterioles and venules resulting in lowering the pressure in the capillary bed [2].

According to review of literature, various spectroscopic approaches for CL estimation by RP-HPLC have already been documented. Despite the fact that various methods have been discovered, the most of them are multicomponent estimation methods, there is still a preliminary need for a good RP-HPLC method for CL analysis [3]. As a result, efforts were made to establish a new method for estimating CL that is fast, precise, and accurate. Validation is an important step in determining and documenting the capabilities of a new method so that the developed approach can be used to determine the drug content in commercially available formulations.

## 2. MATERIAL AND METHOD

### 2.1. Chemical and reagents

All the chemicals and reagents were purchased from Spectrochem Ltd., Mumbai, Lobie Chem Pvt.Ltd., India and Merck Pvt. Ltd., India, and were of analytical grade. Methanol and acetonitrile were procured from Thomas Baker Ltd, Mumbai. Cilnidipine (CL) was received

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as gift sample from Signova Pharma Pvt. Ltd., India.

**2.2. UV-*Vis* spectrophotometric method for the estimation of CL**

**2.2.1. Solubility**

Prior to estimation of CL, its solubility was tested by using different solvents such as dist. water, methanol, ethanol, acetonitrile, HCl conc. (0.1 N) and NaOH conc. (0.1 N). Based on the results of solubility, CL was dissolved in suitable solvent.

**2.2.2. Determination of working wavelengths**

Different solutions of CL (2-10 mg $mL^{-1}$) were prepared in methanol-water (90:10 *v/v*) and were scanned using double beam UV-spectrophotometer (SHIMADZU 1800) at 200-400 nm. Methanol-water (90:10 *v/v*) was used as blank solution for the determination of maximum absorption wavelength ($\lambda_{max}$)[4-10].

**2.2.3. Preparation of stock solutions**

CL (10 mg) was dissolved in methanol-water (90:10) in 10 ml of volumetric flask and sonicated in a sonicator (PCi, PKS250F) for 5 min to obtain the stock solution (1.000 ppm). The stock solution (1 mL) was diluted up to 10 mL in a volumetric flask to get required solution (100 ppm). Then, the aforesaid solution was diluted in volumetric flask to get different concentrations (2, 4, 6, 8 and10 ppm) of working solution [4-10].

**2.2.4. Calibration curve**

The maximum absorbance ($\lambda_{max}$) of the working solutions (2-10 ppm) was recorded and calibration curve was plotted [4-10].

**2.3. RP-HPLC method for the estimation of CL**

**2.3.1. Selection of mobile phase**

Cilnidipine was injected with several types of mobile phases in various combination ratios at varied flow rates until crisp peaks with no interference peaks containing spectra were achieved. The varied mobile phases contained mixture of methanol and water in varying ratios [4-10].

**2.3.2. Chromatographic condition**

An isocratic high pressure liquid chromatography system (Shimadzu, LC-20AT) was used to perform reverse phase-high pressure liquid chromatography (RP-HPLC). Under reverse phase chromatographic conditions, separation was done using a Thermo Scientific C18 (ODS-octa decyl silane) column (diameter, 250 mm and particle size, 5 m). The mobile was passed through a 0.45 µm membrane filter and purged to remove any particulate debris and air bubbles. At a flow rate of 1 ml/min, the mobile phase was passed through the column. The injection volume was 20 µL, temperature of the column was fixed to 30˚ C and the eluent was measured at 241 nm [4-10].

**2.3.3. Preparation of mobile phase**

Based on peak separation and tailing factor, the mixture of metanol-water (90:10) was selected as the mobile phase for RP-HPLC. Prior to use, it was mixed vigorously and sonicated for 30 min.

**2.3.4. Preparation of the standard solution**

CL (10 mg) was dissolved in acetonitrile-water (90:10) and sonicated on a sonicator (PCi, PKS250F) for 5 min and final volume was adjusted in a 10 ml of volumetric flask to obtain stock solution (1000 ppm). Then, stock solution (1 mL) was transferred into 10 mL of volumetric flask and diluted with same solvent to form standard solution (100 ppm). Then, the standard solution was diluted in volumetric flask to get different concentrations (2, 4, 6, 8 and10 ppm) of working solution [4-10].

**2.4. Validation of RP-HPLC**

**2.4.1. Linearity**

Linearity of the method was analyzed by plotting calibration curve with peak areas of different working solutions (2, 4, 6, 8 and10 ppm). Linearity was determined as regression constant ($R^2$), slope, and intercept of calibration curves [4-10].

**2.4.2. Accuracy**

The accuracy of the suggested approach was determined by assessing percent recovery of CL using the standard addition recovery method in six sample solutions of the same concentration (50%). The experiment was performed by mixing a known amount of the sample solution with the standard stock solution to determine mean, standard deviation, and % RSD [4-10].

**2.4.3. Precision**

Intra-day and inter-day fluctuation studies utilizing only one concentration of CL (6 ppm) for numerous times were used to assess the precision of the suggested approach. Inter-day studies were determined by evaluating single sample solution (6 ppm) for six consecutive days, whereas intra-day studies were determined by evaluating single sample solutions (6 ppm) for six times on the same day. We determined the mean, standard deviation and % RSD [4-10].

**2.4.4. Ruggedness**

It refers to the degree to which the results acquired under a variety of settings are repeatable. The method's robustness was tested by determining CL with a flow rate of 0.9 ml/min instead of 1 mL $min^{-1}$. After that, the data was statistically analyzed, and the results were represented in terms of mean, standard deviation, and % RSD [4-10].

**2.4.5. Sensitivity**

Limit of detection (LOD) and limit of quantification (LOQ) was used to determine the sensitivity of the method [4-10]. LOD and LOQ were calculated as

$LOD = 3.3\ \sigma/S$

$LOQ = 10\ \sigma/S$

Where: "S" is the slope of the calibration curve and "σ" is the standard deviation of the regression line.

**2.4.6. Robustness**

Robustness of the method was validated by three critical experimental variables such as mobile phase composition (acetonitrile-water, 90:10, 90:11 and 90:9 *v/v*), wavelength (240, 241 and 242 nm), and flow velocity (1, 1.5 and 0.5 mL $min^{-1}$). The data was statistically analyzed and the results were represented in terms of mean, standard deviation, and % RSD [4-10].

## 3. RESULT AND DISCUSSION

### 3.1. Solubility

Prior to UV and RP-HPLC analysis, it is necessary to solubilize the drug in suitable solvent. So, solubility of CL was analyzed in different solvents. The optimum solubility of it was reported in methanol: water (90:10).

### 3.2. Spectroscopic method

UV spectrophotometric methods for CL was developed in methanol: water (90:10). CL solution obeyed Beer's law over a concentration range of 2-10 mg mL (Table 1). The regression equation and regression constant ($R^2$) was recorded as y = 0.0956x-0.0062 and 0.9993 respectively (Fig. 1). Figure 2 represents the overlay spectra of CL in UV spectrophotometric method.

**Table 1.** UV spectral characteristics of Cilnidipine.

| Parameters | Values |
|---|---|
| Beer-Lambert's limits (mg/ml) | 2-10 |
| $\lambda_{max}$/Amplitude range (nm) | 241 |
| Molar extinction coefficient (Liter/mol. cm) | $76.382 \times 10^3$ |

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Example:

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https://doi.org/10.22270/jddt.v8i6-s.2205
http://dx.doi.org/10.5958/0974-360X.2020.00427.8

| | |
|---|---|
| Slope | 0.0956 |
| Intercept | 0.0062 |
| Correlation coefficient | 0.9993 |

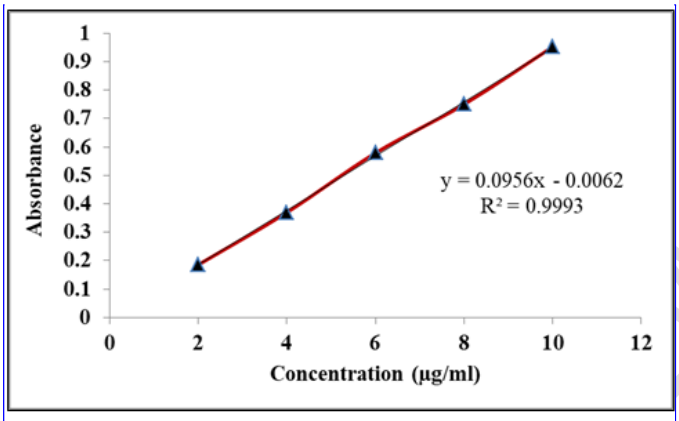


**Fig. 1.** UV calibration curve of Cilnidipine

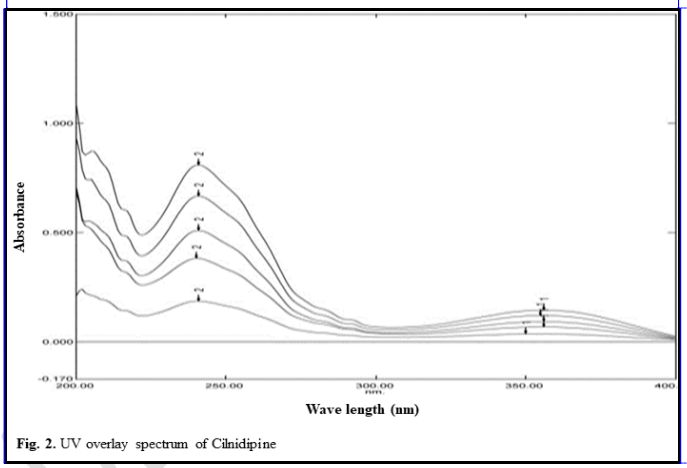


**Fig. 2.** UV overlay spectrum of Cilnidipine

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### 3.3. RP-HPLC method

#### 3.3.1. Optimization of mobile phase

Sample of CL was analysed using different experimental methods to optimize mobile phase for RP-HPLC. The experimental conditions such as wave length (241 nm), flow rate (1 ml/min), injection volume (20 µL) and column (C18) were used same for all whereas mobile phase composition and run time was varied for different conditions (Table 2). In experimental method (F), mobile phase was methanol: water (90:10) and run time was maintained at 10 min that developed well resolved sharp peak with symmetry (Fig. 3). So, the mobile phase methanol: water (90:10) was selected as best mobile phase for the study.

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**Table 2.** Optimization of mobile phase for RP-HPLC

| Conditions | A | B | C | D | E | F |
|---|---|---|---|---|---|---|
| Mobile phase | Methanol: water (50:50) | Methanol: water (60:40) | Methanol: water (70:30) | Methanol: water (80:20) | Methanol: water (90:10) | Methanol: water (90:10) |
| Wave | 241 nm | 241 nm | 241 nm | 241 nm | 241 nm | 241 nm |

| | | | | | | |
|---|---|---|---|---|---|---|
| length | | | | | | |
| Flow rate | 1.0 ml/min | 1.0 ml/min | 1.0 ml/min | 1.0 ml/min | 1.0 ml/min | 1.0 ml/min |
| Injection volume | 20 µl | 20 µl | 20 µl | 20 µl | 20 µl | 20 µl |
| Run time | 20 min | 20 min | 10 min | 10 min | 10 min | 8 min |
| Column | Symmetry C18 (250 mm×4.6 mm, 5 µm particle size) | Symmetry C18 (250 mm×4.6 mm, 5 µm particle size) | Symmetry C18 (250 mm×4.6 mm, 5 µm particle size) | Symmetry C18 (250 mm×4.6 mm, 5 µm particle size) | Symmetry C18 (250 mm×4.6 mm, 5 µm particle size) | Symmetry C18 (250 mm×4.6 mm, 5 µm particle size) |

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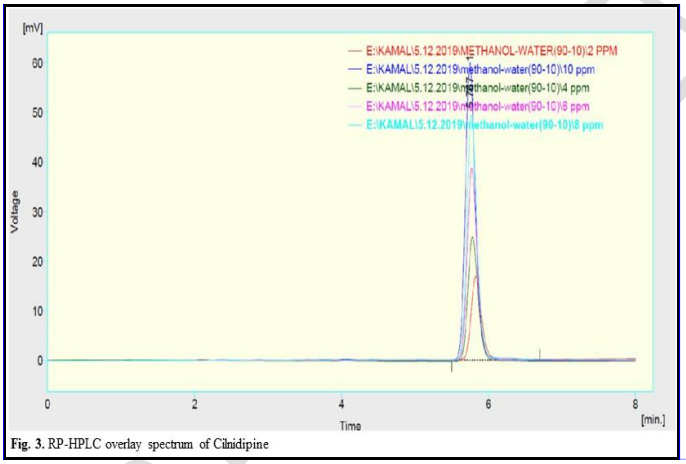


**Fig. 3.** RP-HPLC overlay spectrum of Cilnidipine

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### 3.3.2. Linearity

Linearity study was performed by using various concentrations of CL and peak areas. The regression equation was determined to be y = 47.024x – 3.034 and regression constant was found to be 0.9997 (Fig. 4).

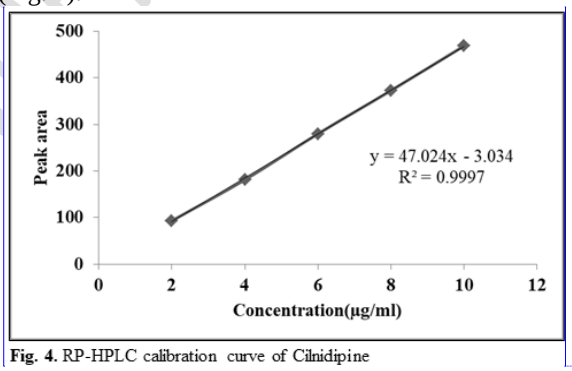


**Fig. 4.** RP-HPLC calibration curve of Cilnidipine

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### 3.3.3. Accuracy

Accuracy was established by recovery studies. The known amount of sample solution was added with standard stock solution. The average recovery was found to be 101.66% and % RSD was determined to be 1.47 (Table 3).

### 3.3.4. Precision

Precision was measured as variation in peak areas of standard solution in intraday and inter day. The % RSD for intraday and inter day was calculated to be 1.65 and 1.38 respectively and the results indicated that the proposed method was precise (Table 3).

**Table 3.** Determination of accuracy and precision of CL in RP-HPLC

| Accuracy | | | | |
|---|---|---|---|---|
| **Sl. No.** | **Concentration (PPM)** | **Absorbance** | **Calculated concentration (PPM)** | **Statistical parameter** |
| 1 | 6 | 0.572 | 5.95 | 6.10 ± 0.09<br>% RSD = 1.47 |
| 2 | 6 | 0.582 | 6.02 | |
| 3 | 6 | 0.585 | 6.09 | |
| 4 | 6 | 0.588 | 6.12 | |
| 5 | 6 | 0.584 | 6.07 | |
| 6 | 6 | 0.594 | 6.18 | |
| **Intraday precision** | | | | |
| 1 | 6 | 0.596 | 6.21 | 6.06 ± 0.10<br>% RSD = 1.65 |
| 2 | 6 | 0.572 | 5.95 | |
| 3 | 6 | 0.582 | 6.06 | |
| 4 | 6 | 0.574 | 5.97 | |
| 5 | 6 | 0.582 | 6.06 | |
| 6 | 6 | 0.587 | 6.11 | |
| **Inter day precision** | | | | |
| 1 | 6 | 0.594 | 6.18 | 6.07 ± 0.08<br>% RSD = 1.38 |
| 2 | 6 | 0.594 | 6.13 | |
| 3 | 6 | 0.574 | 5.97 | |
| 4 | 6 | 0.578 | 6.02 | |
| 5 | 6 | 0.584 | 6.07 | |
| 6 | 6 | 0.582 | 6.06 | |

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### 3.3.5. Ruggedness

Ruggedness of the method was used to determine repeatability of the results. In the present study, the flow rate was varied (1 mL $min^{-1}$ to 0.9 mL $min^{-1}$) and % RSD was found to be 1.31 (Table 4). The results proved the ruggedness of the method for validation of CL.

**Table 4.** Determination of ruggedness of CL in RP-HPLC

| Ruggedness | | | | |
|---|---|---|---|---|
| **Sl. No.** | **Concentration (PPM)** | **Absorbance** | **Calculated concentration (PPM)** | **Statistical parameter** |
| 1 | 6 | 0.572 | 5.95 | 6.07 ± 0.08<br>% RSD = 1.31 |
| 2 | 6 | 0.582 | 6.02 | |
| 3 | 6 | 0.585 | 6.09 | |
| 4 | 6 | 0.588 | 6.12 | |
| 5 | 6 | 0.584 | 6.07 | |
| 6 | 6 | 0.594 | 6.18 | |

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### 3.3.6. Sensitivity

Sensitivity of the method was calculated by using std. deviation (σ) and slope (S) of calibration curve. LOD and LOQ were calculated to be 1.04 mg $mL^{-1}$ and 3.16 mg $mL^{-1}$ respectively and the method was found to be sensitive (Fig. 4).

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### 3.3.7. Robustness

Robustness of the method was established under different analytical conditions. The variation in mobile phase composition, wave length and flow velocity were not affected and the results

were in accordance with initial experimental conditions. The % RSD was calculated to be 0.66, 0.58 and 0.81 respectively and suggested the robustness of the method (Table 5).

**Table 5.** Determination of robustness of CL in RP-HPLC

| Parameters | Variations | Mean ± SD | % RSD |
|---|---|---|---|
| Mobile phase composition (methanol-water) | 90:10, 90:11 and 90:9 | 6.03 ± 0.04 | 0.66 |
| Wave length | 240, 241 and 242 nm | 6.1 ± 0.2 | 0.58 |
| Flow velocity | 1, 1.5 and 0.5 ml/min | 6.08 ± 0.09 | 0.81 |

## 4. CONCLUSION

In this investigation, a new method for estimating CL was developed. The proposed method was precise and accurate. The validation of the suggested approach was carried out in compliance with ICH guidelines. The results demonstrated that the proposed method for drug analysis was effective. For the analysis of CL, a highly specific and sensitive stability prediction RP-HPLC approach has been devised and validated. The findings suggested that the method may be used to assay and analyze CL formulations. Furthermore, this newly developed RP-HPLC method for estimating CL has outstanding sensitivity, precision, and reproducibility.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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