Development and Validation of Ultraviolet and Reverse Phase-high Performance Liquid Chromatographic Method for Estimation of Cilnidipine

Biswajit Samantaray a, Jagannath Panda b, Satyapriya Mahapatra c, Kajal Ray c and Satish Kanhar a*

a School of Pharmacy and Life Sciences, Centurion University of Technology and Management, Odisha, India.
b School of Pharmaceutical Sciences, Siksha ‘O’ Anusandhan University, Odisha, India.
c Royal College of Pharmacy and Health Sciences, Odisha, India.

Authors’ contributions
This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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 Cilnidipine. Action separation of Cilnidipine was achieved by employing a C18 column. Mobile phase 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.

Results: The standardization curve was linear within the varying concentration of 2-10 mg/ml for Cilnidipine with parametric statistic ($r^2$) equal to 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 [3-4]. 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 [5-6]. 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. MATERIALS AND METHODS

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 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 following modified dissolution method using different solvents such as dist. water, methanol, ethanol, acetonitrile, HCl (0.1 N) and NaOH (0.1 N) in dissolution test apparatus (Electrolab, India). The amount of drug released was determined at fixed time interval of 5, 10, 20, 40, 80 and 120 min at 241 nm in UV-Vis spectrophotometer (UV-1800 Shimadzu). Based on the results of solubility, CL was dissolved in suitable solvent [7].

2.2.2 Determination of working wavelengths

Different solutions of CL (2-10 mg/ml) were prepared in methanol-water (90:10) and were scanned using double beam UV-spectrophotometer (SHIMADZU 1800) at 200-400 nm. Methanol-water (90:10) was used as blank solution for the determination of maximum absorbance wavelength (λ_max) [8-9].

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 (1000 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 and 10 ppm) of working solution [8-9].

2.2.4 Calibration curve

The maximum absorbance (λ_max) of the working solutions (2-10 ppm) was recorded and calibration curve was plotted [8-9].

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 [10].

2.3.2 Chromatographic condition

An isocratic high pressure liquid chromatography system (Simadzu, 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 µm). 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 [10].

2.3.3 Preparation of mobile phase

Based on peak separation and tailing factor, the mixture of methanol-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 and 10 ppm) of working solution [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 and 10 ppm). Linearity was determined as regression constant (r²), slope, and intercept of calibration curves [11].

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 [11].

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 [12].

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. After that, the data was statistically analyzed, and the results were represented in terms of mean, standard deviation, and % RSD [12].

2.4.5 Sensitivity

Limit of detection (LOD) and limit of quantification (LOQ) was used to determine the sensitivity of the method [13]. LOD and LOQ were calculated as

$$\text{LOD} = 3.3 \sigma / S$$
$$\text{LOQ} = 10 \sigma / S$$

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 (methanol-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). The data was statistically analyzed and the results were represented in terms of mean, standard deviation, and % RSD [13].
3. RESULTS 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 by using dissolution method. After 120 min of dissolution study, maximum drug release was found to be 96.73% in methanol-water (90:10) than other solvents or combinations of solvents. So, the optimum solubility of CL was reported in methanol-water as represented in Fig. 1.

3.2 Spectroscopic Method

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

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, 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.

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. Linear regression analysis study showed linear relationship between different concentrations and peak area of CL under optimized experimental condition.

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). The results confirmed that % recovery of CL was acceptable and the method was established to be accurate for CL.

3.3.4 Precision

Precision of a method to determine the degree of agreement among individual test results under same experimental conditions. 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 for CL (Table 2).

![Fig. 1. Solubility study of Cilnidipine (CL)](image-url)
Fig. 2. UV overlay spectrum of Cilnidipine

Fig. 3. RP-HPLC overlay spectrum of Cilnidipine
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)      | $76.382 \times 10^3$    |
| Slope                                           | 0.0956                  |
| Intercept                                       | 0.0062                  |
| Correlation coefficient                         | 0.9993                  |

Table 2. Determination of accuracy and precision of CL in RP-HPLC

| Sl. No. | Concentration (PPM) | Peak area (PPM) | Calculated concentration (PPM) | Statistical parameter |
|---------|---------------------|-----------------|-------------------------------|-----------------------|
| 1       | 6                   | 963.71          | 5.95                          |                       |
| 2       | 6                   | 961.20          | 6.02                          |                       |
| 3       | 6                   | 960.18          | 6.09                          |                       |
| 4       | 6                   | 964.22          | 6.12                          | % RSD = 1.47          |
| 5       | 6                   | 965.37          | 6.07                          |                       |
| 6       | 6                   | 962.46          | 6.18                          |                       |

Intraday precision

| Sl. No. | Concentration (PPM) | Peak area (PPM) | Calculated concentration (PPM) | Statistical parameter |
|---------|---------------------|-----------------|-------------------------------|-----------------------|
| 1       | 6                   | 964.09          | 6.21                          |                       |
| 2       | 6                   | 963.83          | 5.95                          |                       |
| 3       | 6                   | 962.66          | 6.06                          | % RSD = 1.65          |
| 4       | 6                   | 960.12          | 5.97                          |                       |
| 5       | 6                   | 961.57          | 6.06                          |                       |
| 6       | 6                   | 962.78          | 6.11                          |                       |

Interday precision

| Sl. No. | Concentration (PPM) | Peak area (PPM) | Calculated concentration (PPM) | Statistical parameter |
|---------|---------------------|-----------------|-------------------------------|-----------------------|
| 1       | 6                   | 967.20          | 6.18                          |                       |
| 2       | 6                   | 966.38          | 6.13                          |                       |
| 3       | 6                   | 965.81          | 5.97                          | % RSD = 1.38          |
| 4       | 6                   | 966.25          | 6.02                          |                       |
| 5       | 6                   | 967.62          | 6.07                          |                       |
| 6       | 6                   | 968.49          | 6.06                          |                       |

3.3.5 Ruggedness

Ruggedness of the method was used to determine repeatability of the results under different experimental conditions of same sample. In the present study, the flow rate was varied (1 ml/min to 0.9 ml/min) and % RSD was found to be 1.31 (Table 3). The results proved the ruggedness of the experimental method for validation of CL.

Table 3. Determination of ruggedness of CL in RP-HPLC

| Sl. No. | Concentration (PPM) | Peak area (PPM) | Calculated concentration (PPM) | Statistical parameter |
|---------|---------------------|-----------------|-------------------------------|-----------------------|
| 1       | 6                   | 971.82          | 5.95                          |                       |
| 2       | 6                   | 970.33          | 6.02                          |                       |
| 3       | 6                   | 972.41          | 6.09                          | % RSD = 1.31          |
| 4       | 6                   | 973.64          | 6.12                          |                       |
| 5       | 6                   | 972.11          | 6.07                          |                       |
| 6       | 6                   | 974.38          | 6.18                          |                       |
Table 4. Determination of robustness of CL in RP-HPLC

| Parameters                  | Variations      | Mean ± SD       | % RSD |
|-----------------------------|-----------------|-----------------|-------|
| Mobile phase composition    | 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  |

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 µg/ml and 3.16 µg/ml respectively and the method was found to be sensitive. Thus, the method was proved to be sensitive for precise determination of CL.

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 less than 1 and calculated to be 0.66, 0.58 and 0.81 respectively. Thus suggested the robustness of the method (Table 4).

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.

DISCLAIMER

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.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Chandra KC, Ramesh G. The fourth-generation calcium channel blocker: Cilnidipine. Indian Heart J. 2013;65(6):691-95.
2. Aoki S, Hosomi N, Nezu T, Teshima, T, Sugii H, Nagahama S, Kurose Y, Maruyama H, Matsumo M. Blood pressure control with Cilnidipine treatment in Japanese post-stroke hypertensive patients: The CA-ATTEND study. Clin. Exp. Hypertens. 2017;39(3):225-34.
3. Balakrishna T, Mrunal SK, Amol K. Development and validation of stability indicating RP-HPLC method for estimation of Cilnidipine. J. Drug Deliv. Ther. 2020;10(1):97-100.
4. Kokilambigai KS, Lakshmi KS. Analytical methodologies for determination of Cilnidipine: An overview. Int. J. Pharm. Pharm. 2014;6(6):37-38.
5. Pawar VT, Pawar SV, More HN, Kulkarni AS, Gaikwad DT. RP-HPLC Method for Simultaneous Estimation of Cilnidipine and chlorthalidone. Res J Pharm Technol. 2017;10(11):3990-96.
6. Kumar A, Singh SK, Gulati M, Kumar B, Prasher R, Gupta, J, Mankotia, D, Pandey
NK, Singh S. Method development and validation for the estimation of Cilnidipine by using Reverse phase high-performance liquid chromatography. Res J Pharm Technol. 2021;14(1):185-88.

7. Behera A, Samal HB, Sharma DK, Kanhar S, Kadam A, Khamkar P, Behera S. Anti-inflammatory activity of herbal tablet of *Phyllanthus emblica* on Carrageenan induced paw edema in Wistar rats. J. Pharm. Res. Int. 2021;33(54B):155-167.

8. Pattanaik S, Adhikari L, Ghosh G, Si SC, Nayak SS. Antioxidant activity and estimation of quercetin from methanolic extract of *Euphorbia nerifolia* leaves by RP-HPLC. Asian J. Chem. 2015;27(2):482-86.

9. Alam MI, Siddiqui AR. Response surface method aided development and validation of stability indicating RP-HPLC-UV method for impurities of dextromethorphan hydrobromide in API and three marketed formulations. Asian J. Chem. 2021;33(11):2737-45.

10. Ali SNS, Mobina L, Mehfuza M, Patel S, Ahmed A, Khan GJ. Analytical method development and validation and forced degradation stability-indicating studies of Favipiravir by RP-HPLC and UV in bulk and pharmaceutical dosage form. J. Pharm. Res. Int. 2021;33:254-71.

11. Patel SA, Sayyed NS, Lajporiya MI, Manjra MU, Ahmed A, Khan GJ. An eco-friendly RP-HPLC and UV-method development and validation for an estimation of Tolvaptan in bulk and tablet dosage form followed by forced degradation studies. J. Pharm. Res. Int. 2021;33:271-86.

12. Chaudhary AA, Shelke AV, Jadhav AG. Development and validation of RP-HPLC method of Cabozantinib in active pharmaceutical ingredient and pharmaceutical dosage form. J. Pharm. Res. Int. 2021;33(11):81-90.

13. Sangeetha G, Manickam MS, Kumar PS. RP-HPLC method development and validation of Tapentadol hydrochloride in bulk and pharmaceutical formulations. J. Pharm. Res. Int. 2021;33(11):7-16.

© 2021 Samantaray et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

**Peer-review history:**
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/78814