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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE DETERMINATION OF CILNIDIPINE TABLET DOSAGE FORM BY RP-HPLC TECHNIQUES

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ABSTRACT

A simple, rapid, and precise reverse phase high performance liquid chromatographic (RP-HPLC) method for analysis of Cilnidipine (CLN) in a tablet dosage form has been developed and validated. This method was performed with a Symmetry C_{18} (4.6 × 150mm i.d., 5µm particle column with 35:65 (v/v) 20mM potassium dihydrogen orthophosphate buffer : methanol as mobile phase at a flow rate of 1.0 ml/min. UV detection at 225 nm CLN were eluted with retention times of 4.870min. The method was continued and validated accordance with ICH guidelines. Validation revealed the method is rapid, specific, accurate, precise, reliable, and reproducible. Calibration curve plots were linear over the concentration ranges 25-125µg/mL. Limit of detection (LOD) was 0.0375µg/ml and limit of quantification (LOQ) was 0.125µg/mL. Statistical analysis was proves the method is suitable for the analysis of CLN as a bulk, in tablet dosage form without any interference from the excipients. It was also proved study for degradation kinetics. It may be extended for its estimation in plasma and other biological fluids.

Keywords: *Metoprolol Succinate (CLN), RP-HPLC, Validation.*

INTRODUCTION

Cilnidipine (CLN) chemically 3-(2-methoxyethyl)-5-[(E)-3-phenylprop-2-enyl]-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate (Figure 1). It is a calcium channel blocker. Cilnidipine is a novel calcium antagonist accompanied with L-type and N-type calcium channel blocking function [1].

In the scientific literature, analysis of CLN has been reported as individual ingredients and in combination with other compounds. Analytical methods have included estimation of CLN [2, 3] individually. And CLN with other drugs individually have also been reported [4-13].

No other chromatographic methods are found for analysis of CLN in a tablet dosage form. The method described is rapid, economical, precise, and accurate and can be used for routine analysis of tablets. It was validated as per ICH guidelines [14-16].

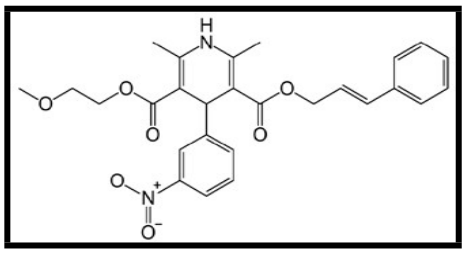

Fig. 1: Chemical structures of CLN

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MATERIALS AND METHODS

Experimental:

1. Materials and Methods:

Pharmaceutical grade working standard Cilnidipine (CLN) were obtained from Hetero Labs, Jedcharla, India. All chemicals and reagents were HPLC grade and were purchased from Merck Chemicals, Mumbai, India.

2. Instrumentation:

The analysis was performed using Waters-2695 (Modal Alliance) High Performance liquid chromatography, analytical balance (Mettler Toledo), PDA Detector (Standard cell) and data handling system (Empower 2), pH meter (lab India), Sonicator. The column used is Symmetry C_{18} (150×4.6mm, packed with 5µm) with the flow rate 1.0ml/min (isocratic).

3. Preparation of stock solution:

Accurately weighed 10 mg CLN working standard transferred into a 10ml clean dry volumetric flasks, add about 7ml of diluent to each volumetric flask and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Calibration standards at five levels were prepared by appropriately mixed and further diluted stock standard solutions in the concentration range from 10-50µg/ml. Samples in triple injections were made for each prepared concentration. Peak areas were plotted against the corresponding concentration to obtain the Linearity graphs.

4. Preparation of Standard stock solution:

The above standard stock solution was containing 1000µg/mL of CLN in separate volumetric flasks. Then transferred the 0.3ml of CLN of prepared standard stock solution into a clean 10ml volumetric flask and made upto the mark with diluent. And finally the standard solution concentration was 30µg/mL.

5. Preparation of Test solution:

For the analysis of a tablet dosage form, 20 tablets were weighed and their average mass was determined. Then, the tablets were crushed to a fine powder. The powder equivalent to 10mg of CLN was transferred to a 10mL volumetric flask and dissolved in diluent, sonication was done for 15 min with swirling. After sonication, the solution was filtered through a membrane filter

paper (#0.45µ). From the above stock solution 0.3mL was transferred in to 10mL volumetric flask and made volume upto the mark with diluent, the final concentration was 30µg/mL, then injected into the chromatographic system, and analyzed quantitatively. The analysis was repeated six times and the possibility of excipient interference with the analysis was examined.

6. Optimization of HPLC Method:

The HPLC method was optimized and developed with a method for CLN. The mixed standard solution (30mg of CLN) injected in HPLC by the followed chromatographic conditions. The chromatographic separation was achieved on a Symmetry C18 (4.6 x 150mm, 5µm). The isocratic mobile phase consisting of 20mM potassium dihydrogen orthophosphate and Methanol in the ratio of (35:65v/v) was used throughout the analysis and the pH 3.0 adjusted with orthophosphoric acid. The flow rate of the mobile phase was 1.0ml/min. Detection was monitored at wavelength of 225nm. The column temperature was kept at ambient and injection volume was 10µl.

7. Method validation:

The method validation was done according to the ICH guidelines. The following validation characteristic parameters are accuracy, precision, linearity, and specificity, LOD, LOQ and robustness.

7.1. Linearity and range:

Linearity of the method was studied by the injecting the mixed standard solutions with the concentration range from 10-50µg/ml for CLN levels of target concentrations were prepared and injected six times into the HPLC system keeping the constant injection volume. The peak areas were plotted against the concentrations to obtain the linearity graphs.

7.2. Precision:

The precision of the optimized method was evaluated by carrying out six independent assays of test sample. %RSD of six assay values was calculated. Intermediate precision was carried out the samples by using another instrument and with different analyst.

7.3. Limit of Detection and Quantification:

The LOD and LOQ procedures were performed on samples contain very lower concentrations of analytes under the ICH guidelines. By applying the visual evaluation method, LOD was expressed by establishing the lowest concentration at which the analyte can be detected. LOQ was considered as the lowest concentration of analytes that can be detected and quantified, with acceptable accuracy and precision.

7.4. Robustness:

Robustness was studied by evaluating the effect of small variations in the chromatographic conditions. The conditions studied were flow rate altered by ±0.1ml/min, mobile phase composition with methanol ±5ml.

7.5. System suitability:

The system suitability parameters with respect of tailing factor, theoretical plates, and repeatability were defined.

7.6. Specificity:

The specificity of the analytical method is the ability of the method to estimate the analyte response in the presence of additional components such as impurities, degradation products and matrix [17].

The specificity method was also evaluated to ensure that there were no interference products resulting from forced degradation studies.

8.7. Accuracy:

Accuracy was carried out by applying the method to drug sample to which known amounts of CLN standard powder corresponding to 50, 100 and 150% of label claim was added, mixed and the powder was extracted and determined by the system in optimized mobile phase. The experiment was performed in triplicate and percentage recovery, % RSD was calculated.

8.8. Analysis of marketed formulation:

The marketed formulation was assayed by above description. The peak areas were monitored at 225nm and determination of sample concentrations were using by multilevel calibration developed on the same HPLC system under the same conditions using linear regression analyzed for CLN in the same way as described above.

RESULTS AND DISCUSSION

The estimation of CLN was done by RP-HPLC and in the optimized method the mobile phase consists of buffer (350 volumes of phosphate buffer and 650 volumes of Methanol and the pH was adjusted to be 3.0. Then finally filtered using 0.45µ membrane filter paper and degassed in sonicator for 15 minutes. The detection is carried out using PDA detector at 225nm. The solutions are following at the constant flow rate of 1.0 ml/min.

The retention time for CLN was 3.516minutes. Linearity ranges for CLN was 10-50µg/mL and the result was found for in the acceptable as (R^2) = 0.9994. LOD was 0.015µg/ml and LOQ was 0.05µg/mL. The all parameters value of RSD is less than 2.0% indicating the accuracy and precision of the method. The percentage recoveries were found 100.15-100.46%.

1. Method Development and Optimization:

The HPLC procedure was optimized with a view to develop a suitable LC method for the analysis of CLN in fixed dose for bulk and combined dosage form. It was found that 35:65 v/v (20mM) potassium dihydrogen orthophosphate buffer: methanol gave acceptable retention time (3.516min for CLN), plates, and good resolution for CLN at the flow rate of 1.0ml/min (**Table. 1**; **Fig. 2 & 3**).

Table No. 1: Optimized Chromatographic Conditions

Parameters	Method
Stationary phase (column)	Symmetry C_{18} (4.6 × 150mm, packed with 3.7µm)
Mobile Phase	35:65v/v, (0.02M Phosphate Buffer : Methanol)
pH	3.0 ± 0.02
Flow rate (ml/min)	1.0
Run time (minutes)	8.0
Column temperature (°C)	Ambient
Volume of injection loop (µl)	10
Detection wavelength (nm)	225
Drugs RT (min)	3.516

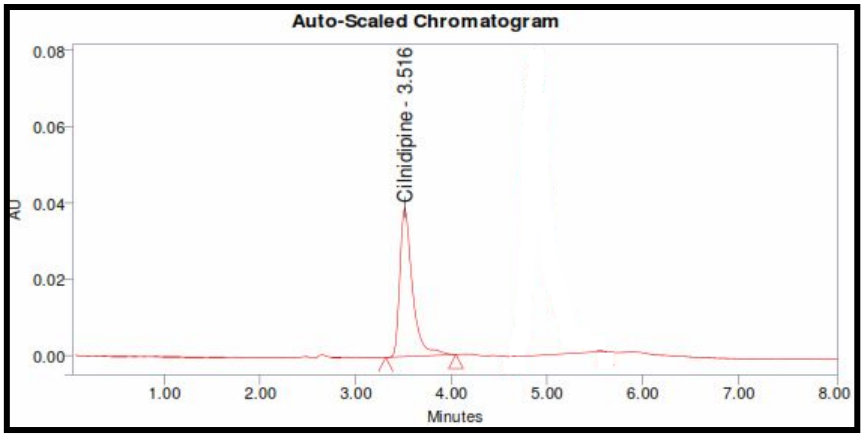

Fig. 2: Chromatogram of CLN at 225nm from bulk drug

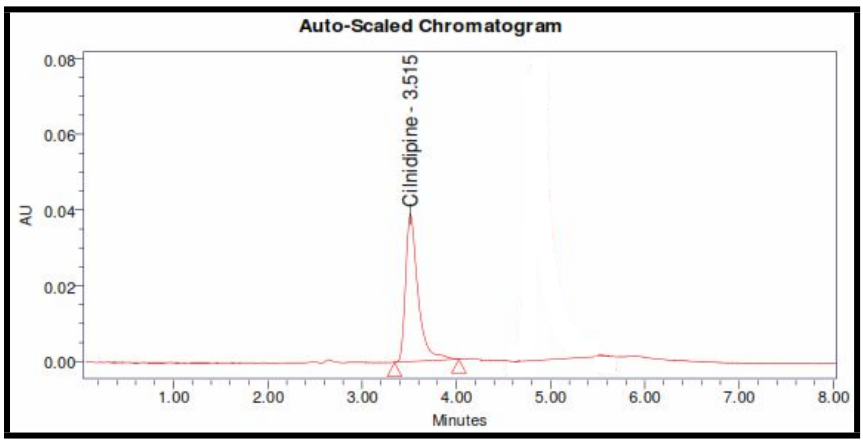

Fig. 3: Chromatogram of CLN at 225nm from pharmaceutical formulation

2. Validation of Developed method:

2.1. Linearity:

Linearity was evaluated by analysis of working standard solutions of CLN of five different concentrations. The linearity range of CLN from 10-50µg/ml (**Table. 2**). The result of correlation coefficient of CLN (R^2) = 0.9994. There was an excellent correlation between peak areas and concentrations of each drug.

Table 2: Data for linearity

Analyte	Conc. range (µg/mL)	Correlation Coefficient (R^2)	Slope	Intercept
CLN	10-50	0.9994	12079x	3689

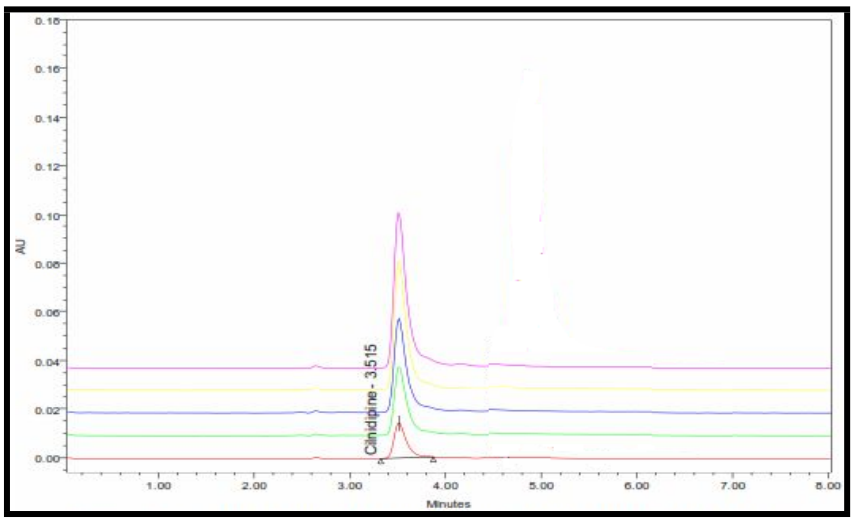

Fig. 4: Overlay linearity Chromatogram for CLN

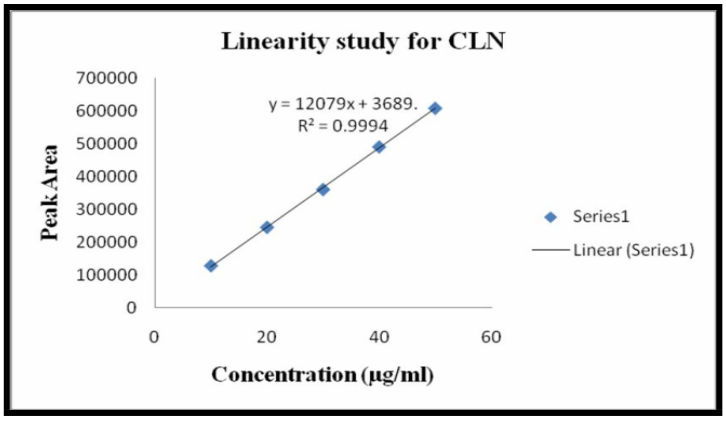

Fig. 5: Linearity Curve of Standard CLN

2.2. Precision:

The results of precision method were evaluated by carrying out six independent test samples of CLN. The percentage of RSD of six sample peak area values was calculated. Different analyst from the same laboratory conditions analyzed the intermediate precision for the optimized method. The RSD values of intra-day and inter-day studies for CLN confirming good precision of the optimized method (**Table. 3**).

Table No. 3: Intra-day and inter-day Precision results of CLN from tablets

No. of Preparation	CLN	
	Intra-day precision	Inter-day precision
Pre-1	358728	359278
Pre-2	357258	358925
Pre-3	359268	359692
Pre-4	356825	358726
Pre-5	358926	359562
Mean	358201	359236.6
St. dev.	1086.792068	410.1204701
% RSD	0.303402857	0.114164445

2.3. LOD and LOQ:

The LOD and LOQ values were found to be 0.015µg/mL and 0.05µg/mL for CLN (**Table. 5**).

2.4. Specificity:

Injected the extracted solutions commonly used excipients were performed to demonstrate for the absence of interaction with the drugs. These results are expressed that there was no interference from the other excipients in the tablet formulation; therefore, confirm the method was specific.

2.5. System suitability:

System suitability parameters such as the theoretical plates count, resolution, % RSD and peak tailing factors are determined (**Table. 5**).

Table No. 5: System suitability parameters for CLN

System suitability parameters	TLM
Retention time (min)	3.516
Repeatability of retention time; %R.S.D (n=5)	0.034
Repeatability of peak area; %R.S.D= (S.D./Mean)×100	0.319
Tailing factor (asymmetric factor)	1.48
USP plate count	3834
LOD (µg/mL)	0.015
LOQ (µg/mL)	0.05

2.6. Robustness:

To ensure the insensitivity of the optimized RP-HPLC method to small alteration in the experimental conditions. The conditions studied were flow rate altered by ±0.1ml/min, mobile phase composition with methanol ±5ml. (**Table. 6**).

Table No. 6: Robustness study for analytical method validation of CLN tablets

Parameters		Adjusted to	Mean Area [a]	Mean RT	SD	% RSD
CLN	**Flow Rate ±0.1ml/min**	**0.9 ml/min**	397724.33	3.92	489.69	0.12
		1.1ml/min	335048.50	3.22	1748.28	0.52
	Mobile Phase (35:65) (±5ml)	**30:70**	327269.83	3.86	824.87	0.25
		40:60	342643.50	3.05	1657.58	0.48

[a] = 5 *Replicates*

2.7. Solution stability studies:

The concentration of CLN (30µg/mL) was prepared from the sample solution and stored at room temperature for 24 hrs. Then injected into the HPLC system and the additional peaks were not found in the chromatograms so, it was indicating the stability of CLN tablet in the solution (**Table. 7**).

2.8. Recovery studies:

A good recovery of CLN was obtained at different added concentrations for the tablets (**Table. 8**).

Table No. 7: Solution stability study for analytical method validation of CLN tablets

Name	*Replicate (n = 5)*	*Initial*	*After 3 hrs*	*After 6 hrs*	*After 12 hrs*	*After 24 hrs*
CLN	**Mean**	358698.4	358127.4	358341.2	358155.4	356796.6
	SD	1023.571	784.3467	1188.667	739.3655	1236.244
	% RSD	0.285357	0.219013	0.331714	0.206437	0.346484

Tablet No. 8: Accuracy Results of CLN from tablets

Analyte	Recovery levels	Actual Conc. (µg/mL)	Added Conc. (µg/mL)	Theoretical Conc. (µg/mL)	Found Conc. (µg/mL)	% Recovery	% RSD	% Error [a]
CLN	**50 %**	10	5	15	15.07	100.46	0.029	0.46
	100 %	10	10	20	20.03	100.15	0.085	0.15
	150 %	10	15	25	25.08	100.32	0.129	0.32

[a][found conc. – theoretical conc./theoretical conc.] x 100.

2.9. Analysis of a commercial formulation:

Experimentally the results for the amount of CLN in tablets, expressed as a percentage of label claims were in good agreement with the label claims thereby suggesting that there is no interaction from the excipients which are commonly present in formulation of tablets.

CONCLUSION

A new RP-HPLC method described in this manuscript provides a simple, convenient and reproducible approach for the estimation and quantification of Cilnidipine in routine quality control analysis.

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