

## Research Article



## Development and Validation of Stability Indicating RP-HPLC Method for the Estimation of Cilnidipine in Bulk and Pharmaceutical Dosage Form.

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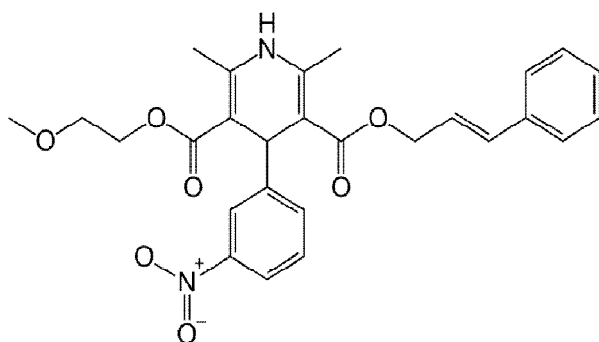
### ABSTRACT

A simple, rapid, specific and highly sensitive stability indicating RP-HPLC method with PDA detection was developed for the estimation of Cilnidipine from bulk and its tablet dosage form. Efficient chromatographic separation was achieved on Kromasil C<sub>18</sub> column (250mm X 4.6mm, 5µm) using isocratic elution and 240nm as a detection wavelength. The optimized mobile phase consists of methanol, sodium di-hydrogen ortho phosphate buffer (PH=3) and acetonitrile [75:18:7] with a flow rate of 1 mL/min. The reliability and analytical performance of the proposed HPLC method were validated for limit of detection, limit of quantification, linearity, range, precision, accuracy, specificity and system suitability. The developed method is linear in the range of 5-15 µg/mL with correlation coefficient being 0.9996. The relative standard deviation for intra and inter day precision were below 1.2%. Forced degradation of the drug was carried out under acidic, basic, oxidative, photolytic and thermal conditions and with the help of developed method the drug was successfully resolved from all degradation products.

**Keywords:** stability indicating, Cilnidipine, RP-HPLC.

### INTRODUCTION

Cilnidipine (CILNI) chemically, 1,4-Dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinecarboxylic acid 2-methoxy ethyl (2E)-3-phenyl-propenyl ester is a dual blocker of L-type voltage-gated calcium channels in vascular smooth muscle and N-type calcium channels in sympathetic nerve terminals that supply blood vessels. It dilates both arteriole and venuoles as a result the pressure in the capillary bed is reduced and the accumulated fluid in the tissues flows back to veins, thus Cilnidipine minimizes the incidence of pedal edema.<sup>1-3</sup>



**Figure 1:** Chemical Structure of Cilnidipine

The presence of ester, methoxy, nitro and pyridine groups make the drug prone to the degradation during various stages of formulation development, which can lead to the appearance of degradation related impurities in the final product<sup>4-5</sup>.

Literature survey reveals that few analytical methods are available for the estimation of CILNI viz. UV spectrophotometry<sup>6-8</sup>, HPTLC<sup>9</sup>, and RP-HPLC<sup>10</sup>. The available methods have certain drawbacks such as less

sensitivity, less accuracy and precision. Also there are no methods reported for stability indicating assay of CILNI. Hence an attempt has been made to develop and validate a stability indicating High Performance Liquid Chromatographic method for the estimation of CILNI which is more sensitive, accurate and precise.

### MATERIALS AND METHODS

#### Instrumentation

The analysis of the drug was carried out on a Jasco LC system equipped with 2089 pump and photo-diode array detector (PDA). The output of signal was monitored and integrated using ChromNAV software. Weighing was performed with microbalance Mettler-Toledo (MT-5) and analytical balance Mettler-Toledo (AB 104-S).

#### Chemicals, Reagents, and Samples

CILNI was obtained from Macleods Pharmaceuticals Pvt. Ltd., Its marketed formulation containing 10mg of CILNI obtained from JB chemicals, HPLC Grade Methanol (MeOH) and Acetonitrile (ACN), Ortho-phosphoric acid (GR Grade), Sodium dihydrogen ortho phosphate (GR Grade) were procured from Merck, Mumbai. HPLC grade water was obtained from Milli-Q purification system.

#### Preparation of Solutions

##### Buffer

1.56 grams of Sodium di-hydrogen ortho phosphate was accurately weighed and transferred in 1000 ml of purified water and mixed. PH was adjusted to 3 (±0.05) with dilute ortho-phosphoric acid solution. This solution was filtered through 0.45µm membrane filter.



### Mobile phase

Mixture of Methanol, Buffer and Acetonitrile in the ratio of 75:18:7v/v/v was prepared and degassed by sonication for 30min.

### Standard solution

10mg of working standard of CILNI was accurately weighed and transferred to 10ml of volumetric flask, about 4 ml of Methanol was added, sonicated to dissolve and volume was made up with methanol to give stock solution of 1000µg/ml. Further dilutions were done with methanol.

### Test solution

Twenty tablets of CILNI (label claim-10 mg) were weighed and triturated. Powder equivalent to 10 mg of Cilnidipine was weighed and dissolved in Methanol in 10ml volumetric flask (1000 µg/ml). Further dilutions were made with methanol.

### Chromatographic conditions

HPLC measurements were carried out using Kromasil C<sub>18</sub> column (250mm X 4.6mm, 5µm) operated at ambient column temperature.

The isocratic mobile phase was pumped through the column at a flow rate of 1.0 ml/min with the sample injection volume was 20µl.

The photodiode array detector was set to a wavelength of 240nm for the detection and Chromatographic runtime was 10 minutes.

### Forced degradation conditions

Forced degradation of the CILNI drug substance was performed under neutral, acid, alkaline, oxidative, thermal, and photolytic stress conditions.

In all stress conditions, the drug concentration used was 50µg/mL. Blank solutions for each degradation conditions were prepared at the same time with the preparation of stock solutions (1000µg/mL).

### Hydrolysis

CILNI stock solutions were prepared in 0.1N HCl (acidic), 0.1N NaOH (basic) and water (neutral) at room temperature. This mixture was refluxed for 2hrs at 60°C. Samples (500µL) were withdrawn at different intervals and diluted to 10 mL with methanol (50µg/mL).

Samples from acidic hydrolysis were neutralized with 0.1 N NaOH and samples from base hydrolysis were neutralized with 0.1 N HCl.

### Oxidation

CILNI stock solution was prepared in 10% of hydrogen peroxide. This mixture was refluxed for 1 hr at 60°C. Samples were withdrawn, diluted with methanol to 50µg/mL and analyzed.

### Thermal degradation

Drug stock solution was refluxed for 4hrs at 60°C. Further dilutions were made with methanol to obtain a sample (50µg/mL) for analysis.

### Photolytic degradation

Photo degradation studies were conducted by exposing a stock solution of the CILNI to UV radiation for 24hrs. After degradation, the sample was diluted with methanol to 50µg/mL and analyzed under the optimized conditions.

In all of the degradation studies, the percentage degradation of CILNI was calculated from the peak areas of drug and degradation products.

## RESULTS AND DISCUSSION

### Method Development and Optimization of the Chromatographic Conditions

In preliminary experiments, the drug was subjected to the reversed-phase separation using a C18 column (Kromasil, 250 x 4.6 mm, 5µ) and mobile phases consisting of water and methanol by varying the % aqueous phase from 10% to 30%. The drug was retained on the column, but the peak shape was not good and run to run variation was seen. To overcome run to run variation and to obtain a good peak shape sodium dihydrogen ortho phosphate buffer is used to maintain mobile phase at pH=3. It was noted that with methanol and buffer the drug was taking more time for elution while with addition of acetonitrile to the above combination decreases the elution time. Depending upon the retention time, resolution and good peak symmetry optimized mobile phase was Methanol, sodium di-hydrogen ortho phosphate buffer and Acetonitrile (75:18:7 %v/v/v) adjusted to pH 3.0 with ortho phosphoric acid. The flow rate was 1.0 mL/min. The injection volume was 20µL and the PDA detection wavelength was at 240 nm. The chromatogram obtained in the optimized condition is shown in Fig. 2.

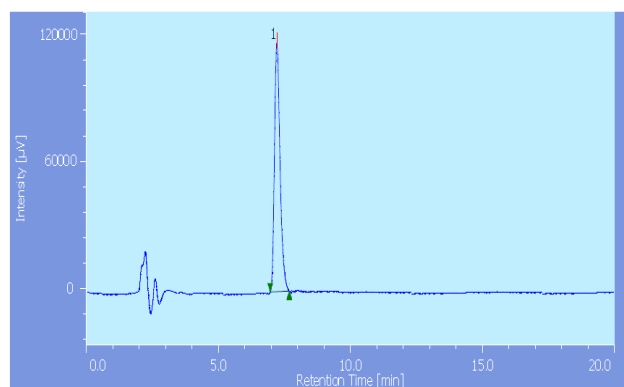


Figure 2: A typical HPLC Chromatogram showing the Peak of CILNI

### System suitability studies

System performance parameters of the developed HPLC method were determined by analyzing working standard solution (six replicates). Chromatographic parameters,

such as retention time, number of theoretical plates and peak asymmetry were calculated. The results are shown in Table 1.

**Table 1:** System suitability parameters for Cilnidipine by proposed method

Name Of Compound	Number Of Theoretical Plates	Retention Time	Symmetry Factor
Cilnidipine	7667	7.04	1.25

### Method validation

The method was validated as per ICH Q2 (R1) guidelines with respect to linearity, accuracy, precision, specificity, robustness, limit of detection, and limit of quantification.<sup>11</sup>

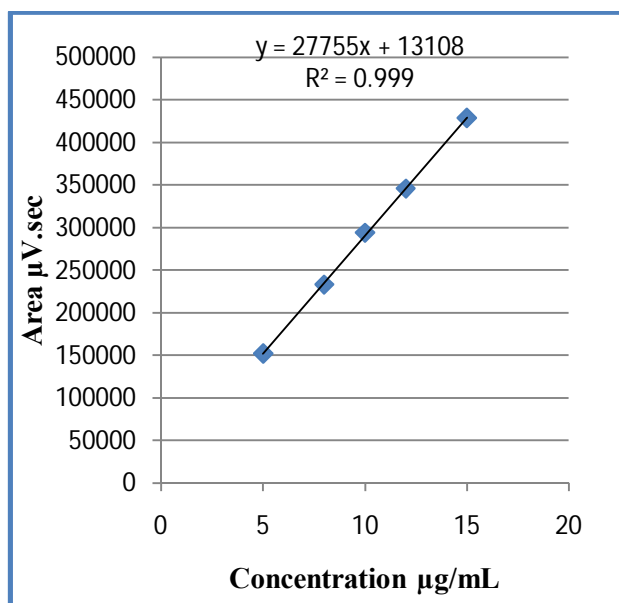
### Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ were determined on the basis of signal-to-noise ratio (S/N). The LOD was taken as the amount for which S/N was 3:1, whereas for the LOQ the S/N was 10:1. The LOD and LOQ were 0.2 and 0.6 µg/mL respectively.

### Linearity and Range

The linearity of the detector response to different concentrations of CILNI was studied in the range of 5–15 µg/mL. Samples were analyzed in six replicates at five different concentrations, such as 5, 8, 10, 12 and 15 µg/mL.

The correlation coefficient ( $r^2$  value) obtained was 0.9996, indicating a linear response of CILNI.



**Figure 3:** Calibration Curve for Cilnidipine

### Accuracy

Accuracy was determined by recovery studies using drug concentration in the range of 80, 100, and 120% of the sample concentration 10 µg/mL. Each concentration was

analyzed in 6 replicates. Results of the recovery studies were between 98.97 to 101.15% and results are shown in Table 2.

### Precision

Results from the study of intraday and interday precision were obtained by analysis of multiples of the concentrations 8, 10 and 12 µg/mL.

For intraday precision 6 replicates were studied with % CV 0.35 to 0.95. For interday precision 12 replicates were studied with % CV 0.39 to 0.98.

This indicates that the method was sufficiently precise. Results are shown in Table 3.

**Table 2:** Accuracy data of 6 replicates

Conc. (µg/mL)	Mean Recovered Conc. (µg/mL)	% Recovery	% C V
8.00	7.91	98.97	0.67
10.00	10.11	101.15	0.35
12.00	11.98	99.87	0.95

**Table 3:** Intraday and Interday precision data

Conc. (µg/mL)	Intraday 6 Replicate			Interday 12 Replicate		
	Mean Recovered Conc. (µg/mL)	±SD	%CV	Mean Recovered Conc. (µg/mL)	±SD	%CV
8.00	7.918	0.054	0.677	7.911	0.071	0.893
10.00	10.116	0.036	0.356	10.112	0.040	0.394
12.00	11.985	0.114	0.954	11.980	0.118	0.987

### Specificity

Forced degradation studies were performed on CILNI to support the specificity of the stability-indicating method. The study was employed on the degradation of CILNI by acidic hydrolysis, basic hydrolysis, oxidation, thermal degradation and photo degradation.

The method was proven to be specific by separating the drug from degradation products formed under the stress conditions.

### Robustness

The robustness of the method was determined by the analysis of samples under a variety of changed method conditions, such as flow rate ( $\pm 0.1$  mL), organic solvent in the mobile phase ( $\pm 2\%$ ), and pH ( $\pm 0.2$ ). The method was robust for all of the conditions for a robust run. The RSD value for the assay was less than 2% for all of the robust tests.

### Assay of marketed formulation

The validated method was successfully applied to estimate CILNI from marketed formulation. The developed method has specifically separated the drug from other excipients. The % content was found to be 100.7%.

**Forced degradation behavior**

**Acid degradation**

CILNI on exposure with 0.1N HCl for 2 hrs at 60°C resulted in to three degradation products. Drug was degraded by 12.46% in acidic conditions after 2 Hrs.

**Base degradation**

CILNI was 24.70% degraded on treatment with 0.1N NaOH for 2 hrs at 60°C, results in formation of six degradation products.

**Oxidative degradation**

On treatment with 10% hydrogen peroxide at 60°C for 1 hr. 11.23% of drug got degraded with formation of 2 degradants.

**Thermal degradation**

CILNI on heating at 60°C resulted in formation of three degradants after 4 hrs. On thermal degradation 14.06% of drug got degraded.

**Photolytic degradation**

24 hrs UV exposures resulted in the formation of three degradants. CILNI was degraded by 10.45%

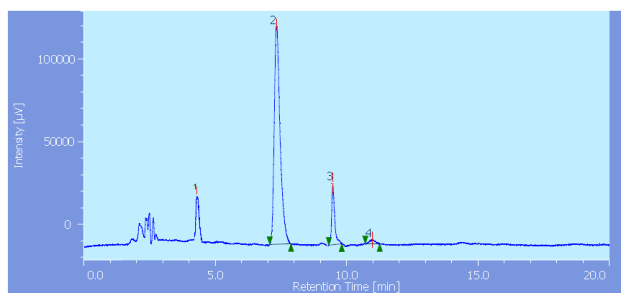


Figure 4 (d)

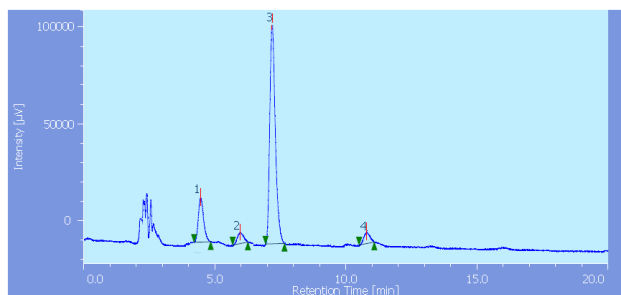


Figure 4(e)

Figure 4: a) Acid Degradation, b) Base Degradation, c) Peroxide Degradation, d) Thermal Degradation, e) Photolytic Degradation

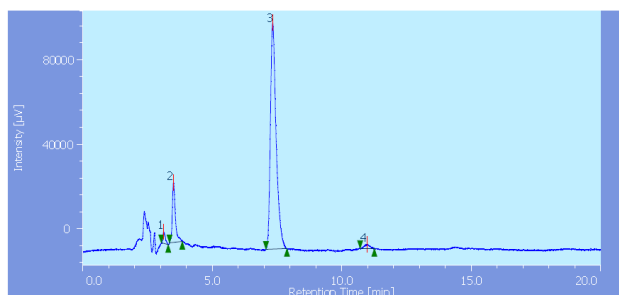


Figure 4 (a)

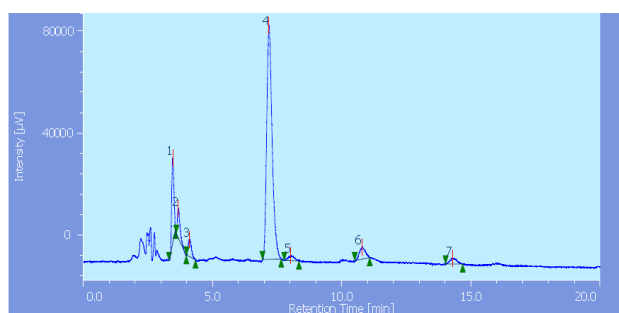


Figure 4 (b)

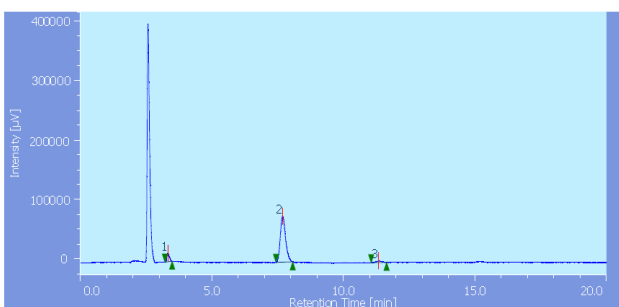


Figure 4 (c)

Table 4: Forced degradation data

	Peak No.	Peak Name	Rt	NTP	Resolution	% Drug Degraded
Acid	1	Degradant 1	3.12	3393	2.12	12.46%
	2	Degradant 2	3.51	5418	13.38	
	3	Cilnidipine	7.33	6836	9.16	
	4	Degradant 3	10.98	3975	N/A	
Base	1	Degradant 1	3.45	6918	2.01	24.70%
	2	Degradant 2	3.67	6385	2.06	
	3	Degradant 3	4.1	4844	10.15	
	4	Cilnidipine	7.1	5989	2.03	
	5	Degradant 4	8.03	4167	5.87	
	6	Degradant 5	10.70	9563	6.96	
	7	Degradant 6	14.29	6095	N/A	
Peroxide	1	Degradant 1	3.32	5569	16.27	11.23%
	2	Cilnidipine	7.69	7304	9.10	
	3	Degradant 2	11.33	5415	N/A	
Thermal	1	Degradant 1	4.3	4276	11.41	14.06%
	2	Cilnidipine	7.52	9219	5.09	
	3	Degradant 2	9.6	5182	3.89	
	4	Degradant 3	11.03	4619	N/A	
photolytic	1	Degradant 1	4.5	5521	3.34	10.45%
	2	Degradant 2	6.0	4673	2.94	
	3	Cilnidipine	7.3	7981	4.87	
	4	Degradant 3	10.89	4662	N/A	

**CONCLUSION**

An isocratic, specific, sensitive, accurate and precise stability-indicating RP-HPLC method has been developed for the estimation of Cilnidipine in bulk and pharmaceutical dosage form and it is validated as per the ICH guidelines. The method is specific and remains unaffected by the presence of degradants from various



stress conditions, indicating the suitability of the method for the stability assay of Cilnidipine.

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