



A SIMPLE, SIGNIFICANT UV-SPECTROSCOPIC ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF FORMULATION DRUG PRODUCT- CILNIDIPINE TABLET (ORAL DOSAGE FORM)

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ABSTRACT

An easy, simple, specific, fast, precise and accurate UV Spectrophotometric method have been developed and validated for estimation of Cilnidipine. Drug Cilnidipine confirmed the absorption maxima in at 242 nm and found was linear for a range of 5 µg/ml –25 µg/ml with correlation coefficient of 0.9996. The limit of detection (LOD) of Cilnidipine was found to be 2.3µg/ml and the limit of quantification (LOQ) of Cilnidipine was found to be 7.6µg/ml. The analytical method validation of the above proposed method was performed by carrying out precision and accuracy studies. The Accuracy percentage recovery on three different levels i.e. 80%, 100% and 120% was found to be 80.8%, 101.9% and 121.0% respectively. The projected analytical method established good Intra precision (Repeatability) with relative standard deviation 1.701% and Inter precision with relative standard deviation is 1.016% which is less than 2. The projected analytical method was validated for the test parameter Specificity, Precision, Linearity and range, Ruggedness, Accuracy and recovery. Hence anticipated analytical method for estimation of Cilnidipine formulation drug in tablet dosage forms by UV spectrophotometer in pharmaceutical found simple, easy, accurate, precise and reproducible, economical and can be applied for the everyday quality control analysis.

KEY WORDS

Cilnidipine Tablet, Method development, Validation, UV Spectrophotometer.

INTRODUCTION

Cilnidipine is an Anti-hypertensive drug belongs to calcium channel blocker ^[1] used in effective hypertension, decreases the blood pressure. Due to its blocking action at N-type and L-type calcium channel, Cilnidipine delates both arterioles and venules, reducing the pressure in the capillary bed. The IUPAC name is 3-(E)-3-Phenyl-2-propenyl 5-(2-methoxyethyl) 2, 6-dimethyl-4-(m-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate. Cilnidipine having molecular formula C₂₇H₂₈N₂O₇ and molecular weight 492.52g/mol ^[2-3]. Literature assessment divulges that a small number of analytical methods are existing counting HPTLC ^[4], HPLC ^[5-9] and UV Spectrophotometry ^[10-21].

In the current assessment, an easy, simple, accurate and sensitive method for estimation of Cilnidipine in drug substance and drug product (Tablet Dosage Form) was introduced. No simple and rapid work has been reported for estimation of Cilnidipine. All these reported methods either took a long time for analysis or employ mobile phases preparation with pH adjustment of Buffer solutions, carefully weighing of crushed tablet powder avoiding lumps/granules of fine material all through sample preparation, Fine crushing of tablet, which is tedious and anomalous ^[4-21], especially for routine testing of quality control samples of assay estimation study. Hence it was felt necessary to build up an easy, simple, rapid, economical and precise

Spectrophotometric method for the direct determination of Cilnidipine Tablet.

The existing research work for development of UV Spectrophotometric method and its validation as per validation reference guideline [22-24]. The developed

method was found to be easy, simple, specific, stable, rapid, accurate, precise, reliable, less expensive and time saving by UV Spectrophotometric method [10-21] for the determination of Cilnidipine content in drug substance and drug product (Tablet Oral Dosage Form).

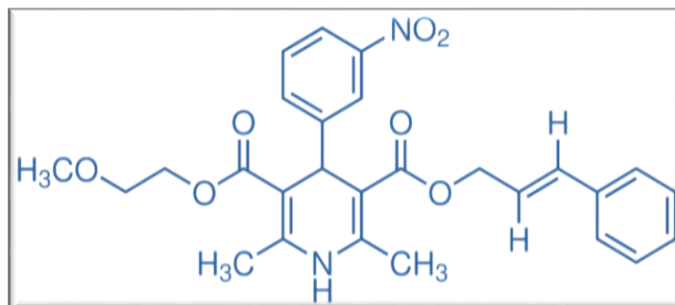


Figure 1: Chemical structure of Cilnidipine

MATERIALS AND METHODS

Instrumentation and Materials:

U.V. visible double beam spectrophotometers SL 210 Elico with Spectra treat software having path length 1cm U.V. matched quartz cells were used. CILNY 5 (Cilnidipine Tablets 5 mg, B.No. NX0056, Manufacturer - Intas Pharmaceuticals Sikkim) sample obtained from market and Cilnidipine Standard from Omicron Pharmaceuticals Surat, Gujarat. All chemicals, solvents and reagents i.e. Acetic acid, Ethanol, Water and Methanol used, were analytical grade and purchased from qualigens, Merck Ltd, India S.D. Fine Chem Ltd.

Method Development: Preparation of Diluent Solution

Transferred about 400 ml of water to the 1000 ml volumetric flask, then gradually added about 100 ml of Ethanol with stirring and add 2 ml of Acetic Acid, stir and

mixed well to dissolve completely, then with even stirring gradually added Methanol up to mark to make volume 1000 ml. used this solution as diluent.

Preparation of Standard Solution

Weighed accurately about 100 mg of Cilnidipine and transferred to 200 ml amber volumetric flask. Dissolved in diluent about 150 ml with slight warm heating (for about 1-2 minute with shaking to obtain clear solution) cool the solution to room temperature and made up the volume to 200 ml, further transferred 2 ml of solution to 100 ml amber volumetric flask. Made volume upto the mark to get a concentration 10µg/ml.

Selection of wavelength for analysis of Cilnidipine

The standard solution having concentration 10µg/ml was scanned at 200 nm to 400 nm with diluent as the blank to detect maximum wavelength (Figure-2).

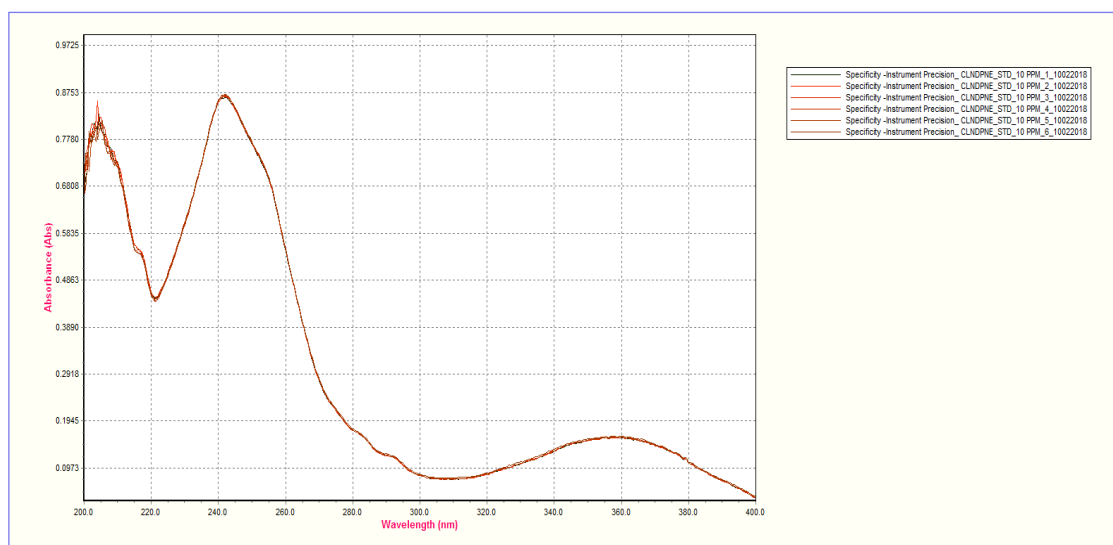


Figure 2: Estimation of Maxima of Cilnidipine

From the above (Figure-2) spectra of Cilnidipine wavelength maxima identified for quantification were 242 nm (λ_{max}).

Validation of proposed Analytical Method

Analytical method validation is a planned and documented course of action to institute its concert uniqueness. Parameters that illustrate each analytical method comprise of specificity, linearity and range, detection limit (LOD), Quantitation limit (LOQ), precision, ruggedness, solution stability and accuracy (recovery).

The analytical procedures presented in this evaluation have been validated in terms of vital stricture i.e. specificity, linearity and range, detection limit (LOD), Quantitation limit (LOQ), precision, ruggedness, solution stability and accuracy (recovery). The statistics acquired makes it achievable to desire the appropriate analytical procedure, adapted to the variety of sample (bulk, pharmaceutical preparations), method of the determination or detection.

The proposed method was validated according to International Conference on Harmonization (ICH) guidelines for validation of analytical procedures [22-24]. Analysis of variance was used to ensure the validity and performance effectiveness of the proposed analytical methods.

Specificity

Specificity is the competence to assess apparently the analyte in the existence of components which could be predictable to be present. In general, these might consist of impurities, degradants, matrix, etc. Specificity was performed by scanning of Diluent solution and Cilnidipine Standard solution of concentrations 10 $\mu\text{g/ml}$, 12 $\mu\text{g/ml}$ and 15 $\mu\text{g/ml}$ in Spectrophotometric range from 200 nm to 400 nm to substantiate specific absorption maxima at predefined wavelength i.e. 242 nm and solution stability study executed to assess the solution stability at different time interval up to 24 hrs.

Instrument Precision

Instrument precision was performed to evaluate the appropriateness of the developed analytical method with respect to competence of instrument reliability to furnish the precise wavelength maxima when scanned the Cilnidipine Standard solution of having concentrations 10 $\mu\text{g/ml}$ in the UV range from 200 nm to 400 nm. To craft sure specific absorption maxima at predefined wavelength 242.0 nm with reproducible absorption detection. Six separate standard preparations were scanned / analyzed as per the proposed analytical method of analysis.

The % RSD due to Cilnidipine concentration for the six standards was found 0.280%. The % RSD due to Cilnidipine concentration for the instrument precision meets the requirements. Results are tabulated in the Table 1.

Table 1- Instrument Precision

Sr.No.	Standard number	Absorbance @242.0 nm	% RSD
1	Standard Preparation -1	0.8652	0.28% Limit < 2%
2	Standard Preparation -2	0.8673	
3	Standard Preparation -3	0.8706	
4	Standard Preparation -4	0.8662	
5	Standard Preparation -5	0.8700	
6	Standard Preparation -6	0.8708	
Average Absorbance →		0.8684	

Linearity and Range

The linearity of an analytical method is the capability to elicit test results, which are directly comparative to the concentrations of drug in a prearranged range. Linearity justifies the use of single-point calibrations. The correlation coefficient of the regression line for was found that 0.9996.

Five levels of five different concentrations of Cilnidipine Standard solution with concentrations range from

5 $\mu\text{g/ml}$, 10 $\mu\text{g/ml}$, 15 $\mu\text{g/ml}$, 20 $\mu\text{g/ml}$ and 25 $\mu\text{g/ml}$, in the range relative to the working concentrations, were prepared and recorded the absorbance as per proposed method of analysis. A linear regression curve was drawn, the correlation coefficient (R²) and assessment value calculated. The correlation coefficient (R²) for Cilnidipine obtained is 0.9996. The plot is a straight line and the results are tabulated in the Table 2 and Curve is shown in the Figure 3.

Table 2- Linearity and Range

Sr.No.	Standard Concentration (µg/ml)	Absorbance @ 242.0 nm	Correlation coefficient
1	5	0.4039	0.9996 Limit ≥0.999
2	10	0.8621	
3	15	1.2470	
4	20	1.7185	
5	25	2.1162	


Figure 3: Linearity and Range of Cilnidipine

Limit of Detection and Limit of Quantification

Limit of detection (LOD) is the minimum concentration of the analyte which can be detected by the instrument. Limit of quantification (LOQ) is the minimum concentration of the analyte that can be reliably quantified. The Limit of detection (LOD) and Limit of quantification (LOQ) were measured using formula as below

$$\text{LOD} = 3.3 \times (\text{SD}/\text{Slope}) \quad \& \quad \text{LOQ} = 10 \times (\text{SD}/\text{Slope})$$

Where, SD = Standard deviation of the 5 calibration curves.
Slope = Mean slope of the 5 calibration curves.

To assess Limit of Detection and Limit of Quantification Standard solution of Cilnidipine were used of concentration's range from 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml and 25µg/ml, in view of the virtual series of the working concentrations using the slope ratio, Limit of detection of Cilnidipine were found to be 2.3µg/ml and Limit of Quantitation of Cilnidipine were found to be 7.6µg/ml.

Analytical Method Precision

The precision of an analytical method articulates the degree of conformity enclosed by individual test results

when the method is applied to numerous sampling of a homogenous illustration.

Method of analysis for Tablet Formulation:

Determined the weight of 10 tablets and transferred to 200 ml amber volumetric flask. Dissolved in about 150 ml diluent with sonication for about 20 minutes with intermittent shaking then with slight warm heating (for about 1-2 minute with shaking) cool the solution at room temperature and made up the volume to 200 ml with diluent. The solution was filtered through Whatmann filter paper and removed first few ml of filtrate, and further transferred 2 ml of solution to 50 ml amber volumetric flask.

Intra Precision (Repeatability)

This parameter concludes the repeatability of Cilnidipine Tablet 5 mg assay results under the similar operating circumstances greater than a short period of time. The % RSD due to Cilnidipine Tablet 5 mg concentration for the six samples was instituted to be 1.701%. Six separated sample measures were analyzed as per the proposed method of analysis. The % RSD due to Cilnidipine Tablet 5 mg concentration for the assay meets the requirements. Results are tabulated in the Table 3.

Table 3 -Intra Precision (Repeatability) Results

Sr.No.	Sample number	Cilnidipine Tablet 5 mg		% RSD of Six Assay content
		% Assay content	Assay content in mg	
1	Sample Preparation -1	98.5	4.924	1.701% Limit < 2%
2	Sample Preparation -2	99.6	4.982	
3	Sample Preparation -3	97.8	4.889	
4	Sample Preparation -4	101.8	5.088	
5	Sample Preparation -5	97.1	4.855	
6	Sample Preparation -6	100.0	4.999	
Average % Assay		→ 99.1	4.956	

Inter Precision (Repeatability)

This parameter concludes the Intermediate repeatability of Cilnidipine Tablet 5 mg, assay results under the similar operating circumstances test performed on a different day, using different makes of reagents and solvents. The % RSD due to Cilnidipine

Tablet 5 mg concentration for the six samples was instituted to be 1.016%. Six separated sample preparations were analyzed as per the proposed method of analysis. The % RSD due to Cilnidipine Tablet 5 mg concentration for the assay meets the requirements. Results are tabulated in the Table 4.

Table 4- Inter Precision (Repeatability) Results

Sr. No.	Sample number	Cilnidipine Tablet 5 mg		% RSD of Six Assay content
		% Assay content	Assay content in mg	
1	Sample Preparation -1	97.7	4.883	1.016% Limit < 2%
2	Sample Preparation -2	99.2	4.962	
3	Sample Preparation -3	100.2	5.009	
4	Sample Preparation -4	98.0	4.900	
5	Sample Preparation -5	97.9	4.897	
6	Sample Preparation -6	97.9	4.893	
Average % Assay		→ 98.5	4.924	

Ruggedness

Ruggedness of the method was determined by carrying out the analysis on different days, different makes of reagents and solvents. The respective test assay results of Cilnidipine Tablet 5 mg having concentration as

10µg/ml was illustrious. The result is expressed as shown in table 4. The developed method for estimation of Cilnidipine Tablet 5 mg was found to be rugged as Shown in Table 5.

Table 5- Ruggedness

Sr. No.	Precision	% RSD of assay of Six Preparation	Limit for Ruggedness
1	Intra Precision	1.701	NMT 2%
2	Inter Precision	1.016	
% RSD of Overall 12 Assay content		1.387	

Accuracy

This parameter concludes the accuracy of the assay results under the similar operating circumstances test. A Cilnidipine Tablet 5 mg sample was analyzed for the accuracy with known quantity of standard samples of Cilnidipine at 80%, 100%, 120% concentration levels and assayed per the method stated under proposed

analytical Methods correspondingly. Three determinations were performed under each concentration levels respectively. Results are shown in Tables 6, 7, 8. The % RSD due to recovery of Cilnidipine at 80%, 100%, 120% concentration levels was found to be 80.8%, 101.9% and 121.0% respectively. Nine sample preparations were analyzed according to the proposed

method of analysis. The %RSD due to Cilnidipine Tablet 5 mg concentration for the assay meets the requirement and accuracy of recovery is within 90.0% to 110%. Results are tabulated in the Table 6, 7, 8.

Table 6- Accuracy and Recovery Results @ 80 % Concentration level

Sr. No.	Accuracy @ 80% level	Recovery of Cilnidipine Tablet 5 mg % Assay content	% Recovery 90.0% to 110%	% RSD
1	Sample Preparation -1	83.2		
2	Sample Preparation -2	79.7		
3	Sample Preparation -3	79.7	101.0	2.481
Average % Assay →		80.8		

Table 7- Accuracy and Recovery Results @ 100 % Concentration level

Sr. No.	Accuracy @ 100% level	Recovery of Cilnidipine Tablet 5 mg % Assay content	% Recovery 90.0% to 110%	% RSD
1	Sample Preparation -1	102.6		
2	Sample Preparation -2	103.0	101.9	1.554%
3	Sample Preparation -3	100.1		
Average % Assay →		101.9		

Table 8- Accuracy and Recovery Results @ 120 % Concentration level

Sr. No.	Accuracy @ 120% level	Recovery of Cilnidipine Tablet 5 mg % Assay content	% Recovery 90.0% to 110%	% RSD
1	Sample Preparation -1	120.9		
2	Sample Preparation -2	121.2		
3	Sample Preparation -3	120.9	100.8	0.145%
Average % Assay →		121.0		

Solution Stability

Solution stability of the Cilnidipine Tablet 20 mg sample solution was performed up to 24hrs with different time interval and found the solution is constant viewing cumulative % RSD of different time interval is 0.419 which is less than the 2. Hence the Cilnidipine Tablet 5 mg sample solution is found stable up to 24 hrs at room temperature and recommended for 24 hrs solution stability (%RSD 0.419).

RESULTS AND DISCUSSION

The method conversed in the current work endows with a simple, constant, easy, reliable, precise, accurate, less expensive (Economical), rapid, time *saving* and suitable method for the analysis of Cilnidipine Tablet 5 mg using U.V. Spectrophotometry. λ max selected for quantitation was 242.0 nm. In the developed analytical method, the linearity was observed 0.9996 in the concentration range of 5 μ g/ml -25 μ g/ml.

Method precision for the Cilnidipine Tablet 5 mg at concentrations level 10 μ g/ml was found in the range of 97.1%-101.8%. Accuracy of the projected method was established by recovery studies and the results were articulated as percent recovery and were found in the Range of 100.8%-101.9%. Values of standard deviation and coefficient of inconsistency was satisfactorily indicating the accuracy of the method. Intra-day and Inter-day precision studies were carried out by analyzing the sample of Cilnidipine Tablet 5 mg at different time interval on the similar day and on different days respectively. Standard deviation and coefficient of distinction for Intra-day and Inter-day precision studies was found to be less than 2 demonstrating precision of the projected method.

Based on the conclusion of analytical method development and analytical substantiation study test results, it was found that, the projected analytical method for determination of Cilnidipine and Cilnidipine Tablet 5mg by UV Spectrophotometry is accurate,

precise, Reproducible, Stable, Easy, Simple, Rapid Time saving and less expensive (Economical). The analytical method can be employed for routine analysis in quality control of Cilnidipine and Cilnidipine Tablet in pharmaceutical.

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