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 C_{27}H_{28}N_{2}O_{7} and molecular weight 492.52g/mol [2-3].

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 ($\lambda_{\text{max}}$).

**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 µg/ml, 12 µg/ml and 15 µg/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 µg/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**

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Standard number</th>
<th>Absorbance @242.0 nm</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Standard Preparation -1</td>
<td>0.8652</td>
<td>0.28%</td>
</tr>
<tr>
<td>2</td>
<td>Standard Preparation -2</td>
<td>0.8673</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Standard Preparation -3</td>
<td>0.8706</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Standard Preparation -4</td>
<td>0.8662</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Standard Preparation -5</td>
<td>0.8700</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Standard Preparation -6</td>
<td>0.8708</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>Absorbance</td>
<td>0.8684</td>
<td></td>
</tr>
</tbody>
</table>

**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 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml and 25µg/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

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

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

LOD = 3.3 × (SD/Slope) & LOQ = 10 × (SD/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

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Sample number</th>
<th>Cilnidipine Tablet 5 mg</th>
<th>% RSD of Six Assay content</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sample Preparation -1</td>
<td>98.5</td>
<td>4.924</td>
</tr>
<tr>
<td>2</td>
<td>Sample Preparation -2</td>
<td>99.6</td>
<td>4.982</td>
</tr>
<tr>
<td>3</td>
<td>Sample Preparation -3</td>
<td>97.8</td>
<td>4.889</td>
</tr>
<tr>
<td>4</td>
<td>Sample Preparation -4</td>
<td>101.8</td>
<td>5.088</td>
</tr>
<tr>
<td>5</td>
<td>Sample Preparation -5</td>
<td>97.1</td>
<td>4.855</td>
</tr>
<tr>
<td>6</td>
<td>Sample Preparation -6</td>
<td>100.0</td>
<td>4.999</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>99.1</td>
<td>4.956</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Sample number</th>
<th>Cilnidipine Tablet 5 mg</th>
<th>% RSD of Overall 12 Assay content</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sample Preparation -1</td>
<td>97.7</td>
<td>4.883</td>
</tr>
<tr>
<td>2</td>
<td>Sample Preparation -2</td>
<td>99.2</td>
<td>4.962</td>
</tr>
<tr>
<td>3</td>
<td>Sample Preparation -3</td>
<td>100.2</td>
<td>5.009</td>
</tr>
<tr>
<td>4</td>
<td>Sample Preparation -4</td>
<td>98.0</td>
<td>4.900</td>
</tr>
<tr>
<td>5</td>
<td>Sample Preparation -5</td>
<td>97.9</td>
<td>4.897</td>
</tr>
<tr>
<td>6</td>
<td>Sample Preparation -6</td>
<td>97.9</td>
<td>4.893</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>98.5</td>
<td>4.924</td>
</tr>
</tbody>
</table>

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 illustrous. 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

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Precision</th>
<th>% RSD of assay of Six Preparation</th>
<th>Limit for ruggedness</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Intra Precision</td>
<td>1.701</td>
<td>NMT 2%</td>
</tr>
<tr>
<td>2</td>
<td>Inter Precision</td>
<td>1.016</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Accuracy @ 80% level</th>
<th>Recovery of Cilnidipine Tablet 5 mg % Assay content</th>
<th>% Recovery 90.0% to 110%</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sample Preparation -1</td>
<td>83.2</td>
<td>101.0</td>
<td>2.481</td>
</tr>
<tr>
<td>2</td>
<td>Sample Preparation -2</td>
<td>79.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Sample Preparation -3</td>
<td>79.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average % Assay</td>
<td></td>
<td>80.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Accuracy @ 100% level</th>
<th>Recovery of Cilnidipine Tablet 5 mg % Assay content</th>
<th>% Recovery 90.0% to 110%</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sample Preparation -1</td>
<td>102.6</td>
<td>101.9</td>
<td>1.554%</td>
</tr>
<tr>
<td>2</td>
<td>Sample Preparation -2</td>
<td>103.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Sample Preparation -3</td>
<td>100.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average % Assay</td>
<td></td>
<td>101.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Accuracy @ 120% level</th>
<th>Recovery of Cilnidipine Tablet 5 mg % Assay content</th>
<th>% Recovery 90.0% to 110%</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sample Preparation -1</td>
<td>120.9</td>
<td>100.8</td>
<td>0.145%</td>
</tr>
<tr>
<td>2</td>
<td>Sample Preparation -2</td>
<td>121.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Sample Preparation -3</td>
<td>120.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average % Assay</td>
<td></td>
<td>121.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### 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. \( \lambda \) 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 µg/ml -25 µg/ml.

Method precision for the Cilnidipine Tablet 5 mg at concentrations level 10µg/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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