DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR THE SIMULTANEOUS ESTIMATION OF CILNIDIPINE AND TELMISARTAN IN BULK DRUGS AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT
A simple, robust, precise, UV spectroscopic method has been developed for the simultaneous estimation of CIL and TEL in bulk and tablet dosage forms. In this paper the estimation of those drugs was carried out by simultaneous equation method. Literature survey revealed that cilnidipine can be estimated by spectrophotometry and by liquid chromatographic methods individually or in combination with other drugs, and telmisartan can be estimated by spectrophotometry. This method is based on measurement of absorption at 243nm and 295nm i.e, \( \lambda_{\text{max}} \) of CIL and TEL respectively. The linearity observed for Cil is in the range of 2-10 μg/ml and for Tel is in the range of 8-40 μg/ml. The accuracy of methods was assessed by recovery studies and was found to be within the range of 99.5%-100.5% for both CIL and TEL. The developed methods were validated with respect to linearity, accuracy (recovery), and precision. The method can be employed for estimation of pharmaceutical formulations with no interference from any other excipients and diluents. The results were validated as per ICH guidelines. Cilnidipine and telmisartan in their combined dosage form. Dual wavelength spectrophotometric method is considered to be a good alternative, and it should be widely explored as an important tool in routine drug analysis. In both the methods linearity for detector response was observed in the concentration range of 2-10μg/ml (for CIL) and8-40μg/ml (for TEL). In both the methods linearity for detector response was observed in the concentration range of 2-10μg/ml (for CIL) and 8-40μg/ml (for TEL). In both the methods linearity for detector response was observed in the concentration range of 2-10μg/ml (for CIL) and 8-40μg/ml (for TEL). Absorptivity coefficient were calculated for both the drugs at selected wavelengths and substituted in equations for determining concentration of CIL and TEL in its tablet dosage form. This method is very useful for quality control analysis of CIL and TEL in various pharmaceutical laboratories.

KEYWORDS: Cilnidipine, Telmisartan, ICH, Simultaneous estimation, absorbance ratio, Validation.

INTRODUCTION
Cilnidipine (CIL) is a light yellowish powder. Chemically it is 1,4-di-hydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5- pyridinedicarboxylic acid2-methoxyethyl(2E)-3-phenyl-2-prpenyl ester (Fig.1.A). It is antihypertensive agent and calcium channel blocker. Cilnidipine is a dual L-/N-type calcium channel protein inhibitor and blocker. Cilnidipine has displayed renal and vascular protective effects and improved baroreflexsensitivity in patients with hypertension.1,2,3,4,5 Telmisartan (TEL) is white crystalline powder. Chemically, it is 4 ′ -[4-Methyl-6-(1-methyl-1Hbenzimidazol-2-yl)-2-propyl-1H-benzimidazol-1- yl][methyl]biphenyl-2-carboxylic acid(6) (Fig. 1: B). It is very soluble in methanol and practically insoluble in water. Cilnidipine is a dual blocker of L-type voltage-gated Ca2+ channels in vascular smooth muscle and N-type Ca2+ channels in sympathetic nerve terminals that supply blood vessels. The inhibition of N-type Ca2+ channels may provide a new strategy for the treatment of cardiovascular diseases. L-type calcium channels are the main targets of the CCB. N-type calcium is distributed along the nerve and in the brain, cilnidipine is anticipated to exert specific action on nerve activity, such as inhibition of the sympathetic nervous system. It inhibits the Ca2+ influx in both in vessel & in the nerve. So causes the Vasodilation & inhibits the release of nor epinephrine, which causes the Vasodilation and decreases the heart rate & also decreases cardiac contraction in heart. So, used in treatment of
hypertension. It isAngiotensin-converting Enzyme Inhibitors and Angiotensin II Type 1 Receptor Blockersagents. The mechanism by which Telmisartan is an angiotensin II receptor blocker (ARB)that shows high affinity for the angiotensin II receptor type 1 (AT1), with a binding affinity3000 times greater for AT1 than AT2. It has the longest half-life of any ARB (24 hours) and the largest volume of distribution. The combination of CIL and TEL is indicated as antihypertensive agents as well as in combination they improves vascular damage in hypertensive patients.\cite{8,9} Literature survey revealed that cilnidipine can be estimated by spectrophotometry\cite{10,11} and by liquid chromatographic methods\cite{12,13,14} individually or in combination with other drugs, and telmisartan can be estimated by spectrophotometry\cite{15,16,17,18,19} and by liquid chromatographic methods individually or in combination with other drugs.\cite{20,21,22}

![Fig.1.A: Structure of Cilnidipine.](image)

![Fig. 1: B) Structure of Telmisartan.](image)

In this study we develop and validate uv spectrometry method for the simultaneously estimation of CIL and TEL. There are several UV spectroscopy methods\cite{23,24} have been reported for the estimation of cilnidipine and telmisartan in their combined dosage form. Dual wavelength spectrophotometric method is considered to be a good alternative, and it should be widely explored as an important tool in routine drug analysis. Hence it was proposed to develop economical, rapid and simple uv spectrophotometric methods for the simultaneous estimation of these drugs in dosage forms. Materials the aim of the present work was to develop an accurate, repeatable, sensitive and cost effective UV spectrophotometric method for the determination of CIL and TEL in formulation as stipulated by the ICH guidelines. The methods discussed in the present work provide a convenient and accurate way for simultaneous estimation of CIL and TEL. In simultaneous equation method, wavelengths selected for analysis were 243nm (λ max of CIL) and 295nm (λ max of TEL). The proposed method was validated according to ICH guidelines.

**MATERIALS AND METHODS**

**Instruments**
Shimadzu UV-1800 double beam spectrophotometer was used to record the spectra of sample and reference solutions using pair of quartz cells of 10 mm path length. All weighing was carried out on Sansui Vibra DJ-150S-Sweighing balance. Sonicator of Fast Clean is used for the purpose of sonication, Filter papers of Sartorius Stedim Biotech of grade 292 are used for the filtration purpose

**Chemicals And Reagent**
Cilnidipine(10 mg) and Telmisartan(40 mg) pure drugs were obtained as a gift sample fromJ. B. Chemicals and Pharmaceuticals India. The combined formulation Lupin (10 mg/40 mg) of the two drugs purchased from Chandan Medicals, Bhusawal. Dibasic Sodium phosphate, Mnobasic sodiumphosphate and water used and All other chemicals are ofanalytical grade and purchased from Avantor Chemicals Pvt. Ltd. Mumbai.

**PREPARATION OF STOCK SOLUTION AND SELECTION OF WAVELENGTH**

**Cilnidipine stock solution**
An accurately weighed quantity of CIL(10 mg) was taken in 10 mL volumetric flask and dissolved in methanol (3 mL) with the help of ultra sonication for about 10 min. Then the volume was made up to the mark using Phosphoric acid buffer pH 8.0 to get CIL standard stock solution (1 mg / mL).

**Cilnidipine working solution**
CIL standard stock solution (1 mL) was diluted to 10 mL using 30% methanolic phosphoric acid buffer pH 8.0 to get working standard solution (100 µg/mL)

**Telmisartan stock solution**
An accurately weighed quantity of TEL (10 mg) was taken in 10 mL volumetric flask and dissolved in methanol (3 mL) with the help of ultra sonication for about 10 min. Then the volume was made up to the mark using Phosphoric acid buffer pH 8.0 to get Tel standard stock solution (1 mg / mL).

**Telmisartan working solution**
TEL standard stock solution (1 mL) was diluted to 10 mL using 30% methanolic Phosphoric acid bufferpH 8.0 to get working standard solution (100 µg / mL)

**Determination of λ. Max of Individual Component**
An appropriate aliquot portion of CIL and TEL were transferred to two separate 10 mL volumetric flasks, the volume was made up to the mark using 30 % methanolic Phosphoric acid bufferpH 8.0 to obtain CIL (2 µg/mL)
and TEL (8µg/mL). Drug solutions were scanned separately between 200 nm to 400 nm. CIL shows the \( \lambda_{\text{max}} \) at 243 nm while TEL shows \( \lambda_{\text{max}} \) at 295 nm.

**Overlay spectra of Cilnidipine and Telmisartan**

The overlay spectra of both drugs were recorded and two wavelengths 243 nm (\( \lambda_{\text{max}} \) of Cilnidipine) and 295 nm (\( \lambda_{\text{max}} \) of Telmisartan) were selected for further study.

**Linearity study for Cilnidipine**

An accurately measured aliquot portion of working standard solution of CIL was transferred to seven separate 10 mL volumetric flasks. The volume was made up to the mark using 30% methanolic phosphoric acid buffer pH 8.0 to obtain concentrations of CIL (2µg/ml, 4µg/ml, 6µg/ml, 8µg/ml, 10µg/ml). Absorbance of these solutions was measured at 243 nm. Calibration curve was plotted, absorbance vs concentration.

**Linearity study for Telmisartan**

Accurately measured aliquot portions of working standard solution of TEL were transferred to seven separate 10 mL volumetric flasks. The volume was made up to the mark using 30% methanolic phosphoric acid buffer pH 8.0 to obtain concentrations (8µg/ml, 16µg/ml, 24µg/ml, 32µg/ml, 40 µg/ml) Absorbance of these solutions was measured at 295 nm. Calibration curve was plotted, absorbance vs concentration. The results are shown in the Table No.1.

**Table No. 1: Regression and Optical characteristics of Cilnidipine and Telmisartan.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Value for CIL</th>
<th>Value for TEL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beer’s law limit (µg/ml)</td>
<td>2-10 µg/ml</td>
<td>8-40 µg/ml</td>
</tr>
<tr>
<td>Regression Coefficient (R²)</td>
<td>( R^2 = 0.997 )</td>
<td>( R^2 = 0.998 )</td>
</tr>
<tr>
<td>Regression equation</td>
<td>( y = 0.037x + 0.017 )</td>
<td>( y = 0.017x + 0.022 )</td>
</tr>
<tr>
<td>Slope</td>
<td>0.037</td>
<td>0.017</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.017</td>
<td>0.022</td>
</tr>
</tbody>
</table>

The study of regression and optical characteristics of CIL and TEL are carried out in which Regression Coefficient (R²) of CIL is 0.997 and of TEL is 0.998. The slope of CIL 0.037 and slope of TEL is 0.017 with Intercept of CIL 0.017 and for TEL 0.022, therefore, concentration vs absorbance are fairly linear between both co-ordinates by statistical manner and obey guidelines.
Estimation of Laboratory mixture by proposed method

Method: Simultaneous Estimation Method\textsuperscript{25}

If a drug sample contains two absorbing drugs (X and Y) each of this absorbs at the $\lambda_{\text{max}}$ of the other. Then, it may possible to estimate both drugs by this method. The scanning spectra of 10µg/ml solution of CIL and TEL show clear peaks at 243nm and 295nm respectively. Amount of each drug was estimated using following equations,

\begin{align*}
C_x &= \frac{A_2 \times ay_1 - A_1 \times ay_2}{ax_2 ay_1 - ax_1 ay_2} \\
C_y &= \frac{A_1 \times ax_2 - A_2 \times ax_1}{ax_2 ay_1 - ax_1 ay_2}
\end{align*}

Where:

A1 and A2 are the absorbance of diluted mixture at $\lambda_1$ and $\lambda_2$

Cx and Cy are the concentration of X and Y respectively $ay_1$ and $ay_2$ are absorptivities of X at $\lambda_1$ and $\lambda_2$ respectively $ax_1$ and $ax_2$ are absorptivities of Y at $\lambda_1$ and $\lambda_2$ respectively.

The results are determined in the Table No. 2

\begin{table}[h]
\centering
\begin{tabular}{|l|c|c|}
\hline
\textbf{Analyte} & \textbf{% Concentration estimated (Mean ± S.D)} & \textbf{% R.S.D} \\
\hline
CIL & 99.7 ± 0.1277 & 0.1280 \\
TEL & 99.6 ± 0.1095 & 0.1098 \\
\hline
\end{tabular}
\caption{Results of Estimation of Cilnidipine and Telmisartan in standard laboratory mixture.}
\end{table}

The estimation of CIL and TEL in standard laboratory mixture are carried out in which % concentration of CIL and TEL were found to be 99.7 and 99.6 respectively. Those values are fairly accurate by statistical manner and are as per ICH guidelines.
Application of proposed method for Estimation of drugs in tablets

Ten ‘Telista CL’ Tablets containing Cilnidipine (10 mg) and Telmisartan (40 mg) were weighed and ground to fine powder. A quantity of sample equivalent to CIL (10 mg) and TEL (40 mg) was transferred into 100 mL volumetric flask containing methanolic phosphoric acid buffer pH 8.0 (60 mL), sonicated for 15 min and the volume was made up to the mark and filtered through Whatman filter paper (No. 45). This solution was (1 mL) transferred to 10 mL volumetric flasks, dissolved and volume was adjusted to the mark. The absorbances of the solutions were measured at 243 nm and 295 nm against blank. The concentrations of two drugs in sample were determined by using simultaneous equations. The results are shown in the Table No.3.

Table No. 3: Results of Estimation of Cilnidipine and Telmisartan in tablets dosage form.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Label claim(mg/tab)</th>
<th>% Label claim estimated (Mean±S.D)</th>
<th>% R.S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIL</td>
<td>10</td>
<td>99.84 ± 0.1140</td>
<td>0.114</td>
</tr>
<tr>
<td>TEL</td>
<td>40</td>
<td>99.76 ± 0.167</td>
<td>0.167</td>
</tr>
</tbody>
</table>

The results of Estimation of CIL and TEL in tablets dosage shows the % purity 99.84 to 99.76 with SD and RSD bellow 2 which is fairly accurate by statistical manner and are as per ICH guidelines.

Validation of proposed method

The proposed method was validated as per ICH guidelines.\(^{26,27}\)

Accuracy (Recovery study)

Accuracy of proposed method was ascertained on the basis of recovery study performed by standard addition method. A known amount of standard drug solutions were added to the tablet powder to make final concentrations in the range of 80%, 100% and 120% and re-analyzed it by the proposed method. The absorbance recorded and the % recoveries were calculated using formula.

\[
% \text{ Recovery} = \left[ \frac{A - B}{C} \right] \times 100
\]

Where,

A = Total amount of drug estimated
B = Amount of drug found on preanalyzed basis
C = Amount of Pure drug added

The results are shown in the Table No.4

Table No. 4: Recovery study.

<table>
<thead>
<tr>
<th>Drug in mixture solution (µg/ml)</th>
<th>% Recovery ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CIL</td>
</tr>
<tr>
<td>CIL</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>5.82 ± 0.0870</td>
</tr>
<tr>
<td></td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>9.86 ± 1.855</td>
</tr>
<tr>
<td></td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>9.76 ± 1.301</td>
</tr>
</tbody>
</table>

The results of Recovery study of CIL and TEL are found to be fairly accurate between 99.55 to 99.86% for CIL 99.60 to 99.96 % for TEL between various concentrations under observation by statistical way and are obey ICH guidelines.

Precision

Precision was determined as intra-day and inter-day variations. Intra-day precision was determined by analyzing CIL (4, 6, and 8 µg/mL) and TEL (16, 24, and 32 µg/mL) for three times on the same day. Inter-day precision was determined by analyzing the same concentration of solutions for three different days over a period of week. The results are shown in the Table No. 5.

Table No.5: Precision Study.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Conc. [µg/mL]</th>
<th>Intra-day Amount Found</th>
<th>Inter-day Amount Found</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ± S.D. [n = 5]</td>
<td>% R.S.D.</td>
</tr>
<tr>
<td>CIL</td>
<td>6</td>
<td>5.82 ± 0.0870</td>
<td>0.8852</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>7.82 ± 0.2108</td>
<td>1.063</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>9.93 ± 0.4624</td>
<td>1.158</td>
</tr>
<tr>
<td>TEL</td>
<td>24</td>
<td>23.8 ± 0.082</td>
<td>0.840</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>31.8 ± 0.1262</td>
<td>1.6352</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>39.8 ± 0.2590</td>
<td>0.6502</td>
</tr>
</tbody>
</table>

The Precision Study of CIL and TEL were carried out and Results are found to be fairly accurate by statistical manner and obeys ICH guidelines.

Ruggenedness

Ruggenedness of the proposed method was determined by analysis of aliquots from homogenous slot by two
different analyst using same operational and environmental conditions. The results are shown in Table No. 6.

Table No. 6: Ruggedness study.

<table>
<thead>
<tr>
<th></th>
<th>Cilnidipine 10 µg/ml</th>
<th>Telmisartan 40 µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± S.D. (n=3)</td>
<td>R.S.D.</td>
</tr>
<tr>
<td>Analyst I</td>
<td>9.96 ± 0.2066</td>
<td>0.2584</td>
</tr>
<tr>
<td>Analyst II</td>
<td>9.75 ± 0.4686</td>
<td>0.5876</td>
</tr>
<tr>
<td>Day I</td>
<td>9.97 ± 0.2254</td>
<td>0.2819</td>
</tr>
<tr>
<td>Day II</td>
<td>9.81 ± 0.5412</td>
<td>0.6780</td>
</tr>
<tr>
<td>Instrument I</td>
<td>9.85 ± 0.1184</td>
<td>0.1483</td>
</tr>
<tr>
<td>Instrument II</td>
<td>9.86 ± 0.1228</td>
<td>0.1538</td>
</tr>
</tbody>
</table>

The Ruggedness study of CIL and TEL are carried out, results are found to be fairly accurate by statistical manner and obeys ICH guidelines.

**LOD:** Limit of detection of CIL and TEL were found to be 0.626233 µg and 2.061672 µg respectively.

**LOQ:** Limit of Quantitation of CIL and TEL were found to be 1.897675 µg and 6.247491 µg respectively.

**RESULT AND DISCUSSION**

The spectrophotometric study of both drugs were carried out in methanol and 30% methanolic phosphoric acid buffer pH 8.0 as it was found to be suitable solvent for estimation of both CIL and TEL. During preliminary studies, both the drugs dissolved in methanol and it was observed that both standard stock solution were stable. Aliquots from working standard solution were further diluted with 80% aqueous methanol for determination of spectral characteristics of both drugs. On the basis of overlay spectra of both drugs having concentrations 10 µg/mL CIL and 40 µg/mL TEL was recorded and two wavelengths were selected for estimation of both drugs by simultaneous equation method, λ max of Cilnidipine at 243 nm and Telmisartan at 295 nm respectively were selected. The relation between concentration and absorbance for individual drug was studied. CIL and TEL solution individually followed the Beer-Lambert’s law over concentration range 2-10 µg/ml and 8-40 µg/ml respectively.

Validation of proposed method was done using ICH guidelines.26,27 Accuracy of method is ascertained by recovery studies performed at different levels of concentrations (80%, 100% and 120%). Mean % recovery were found to be within 99.55 to 99.86% for CIL 99.60 to 99.96 % for TEL for absorption ratio method with % RSD less than 2.

The study of linearity CIL and TEL marketed formulation was found to be linear in the range of 2-10 µg/ml (R²=0.9961) and 8-40 µg/ml (R²=0.9996) respectively with a limit of detection of CIL and TEL was found to be 0.626233 µg and 2.061672 µg and limit of quantitation found to be 1.897675 µg and 6.247491 µg respectively.

**CONCLUSION**

The proposed simultaneous UV Spectrophotometric Estimation method presented in this paper has advantages of simplicity, accuracy, precision and convenience for quantitative estimation of CIL and TEL. The proposed method can be used for the quality control of CIL and TEL in typical laboratories.

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