METHOD DEVELOPMENT AND VALIDATION OF SIMULTANEOUS ESTIMATION OF CILOSTAZOL AND TELMISARTAN

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ABSTRACT

Three rapid, sensitive and specific UV-Visible methods were developed and validated for the estimation of Cilostazol and Telmisartan in synthetic mixture. All method was validated according to ICH guideline in terms of linearity, accuracy, precision, and specificity, limit of detection and limit of quantification. The first method is simultaneous equation method. Method I is based on simultaneous equation. Cilostazol and Telmisartan show absorbance maximum at 258 and 296 nm respectively, so absorbance was measured at the same wavelength for estimation of Cilostazol and Telmisartan. Method II is based on determination of Q-value. Absorbance is measured at 237.5 nm (Isoabsorptive point) and 258 nm (λmax of Cilostazol). Method III is dual wavelength method, in which two were selected for each drug in a way so that the difference in absorbance is zero for another drug. Both drugs obey the Beer Lambert’s law in the concentration range of 1-40 μg/ml for Cilostazol and 1-25 μg/ml for Telmisartan.

KEYWORDS

Cilostazol, Telmisartan, Simultaneous equation, Q-Absorbance ratio, Dual wavelength.

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INTRODUCTION

Cilostazol, whose chemical name is 6-[4-(1-cyclohexyl-1H-tetrazol-5-y1) butoxy]-3, 4-dihydro-2 (1H) – quinolinone (see Fig 1), is aquinolinone derivative that inhibits cellular phosphodiesterase III, and is used for the inhibition of platelet aggregation and as a vasodilator.

![Figure 1: Chemical structure of Cilostazol.](image1)

Telmisartan is 4' - [(4-methyl-6-(1-methyl-1H-benzimidazol-2yl)-2-propyl-1H-benzimidazol-1-yl)methyl]-2- biphenylcarboxylic acid. Telmisartan is a new angiotensin II receptor antagonist for the treatment of essential hypertension usually given in combination with hydrochlorothiazide. The combination is useful in the treatment of mild to moderate hypertension, well tolerated with a lower incidence of cough than ACE inhibitors.

![Figure 2: Chemical structure of Telmisartan.](image2)

Present paper represents three method for the simultaneous estimation Cilostazol and Telmisartan in synthetic mixture.

MATERIALS AND METHODS

**Instruments**

A Shimadzu model 1700 double beam UV-Visible spectrophotometer with spectral width of 1 nm, wavelength accuracy of ± 0.1 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV-Probe system software (Ver.2.1). The samples were weighed on electronic analytical balance (A×120, shimadzu).

**Materials**

All chemicals and reagents were used of AR grade(Thermo Fisher scientific IND PVT. LTD Mumbai). Telmisartan was kindly gifted from Alembic pharmaceutical, Vadodara and cilostazol was purchased from Swapnroop drug Pvt. Ltd Bombay.
Selection of common solvent

After checking the solubility of drugs in different solvents methanol has been selected as common solvent for developing spectral characteristics.

Selection of detection wavelength

Solutions of drug were scanned over the range of 200-400 nm. It was observed that the drugs showed maximum absorbance at 258 nm for Cilostazol and 296 nm for Telmisartan were selected as the wavelength for detection.

Preparation of standard stock solution

The standard stock solutions of cilostazol and telmisartan were prepared by dissolving 25 mg of each drug in methanol and final volume was adjusted with same solvent in 25 mL of volumetric flask to get a solution containing 1000 μg/mL of each drug.

Preparation of Working Standard Solution

From the above solution, further dilute 10 ml of stock solution up to 100 ml in volumetric flask to get second stock of 100 μg/mL.

Preparation of Calibration Curve of Standard

From working std. solution of CILO (100 μg/ml) 0.4, 0.8, 1.2, 1.6,2.0 and 2.4 ml were transferred to 10 ml volumetric flasks and volume were made up to the mark with methanol. This gives 4 to 24 μg/ml of CILO. From working std. solution of TELMI (100 μg/ml) 0.1, 0.2, 0.3,0.4, 0.5and 0.6 ml were transferred to 10 ml volumetric flasks and volume were made up to the mark with methanol. This gives 1 to 6 μg/ml of TELMI.

Method I (Simultaneous equation method)

If a sample contain two absorbing drug each of which absorbs at the λmax of the other, it may be possible to determine both drugs by the technique of simultaneous equation. Two wavelengths selected for the development of the simultaneous equations are 258 nm and 296 nm (fig 3). The absorptivity values determined for cilostazol at 258 and 296 are (ax1), and (ax2). And for telmisartan are (ay1), and (ay2) at 258 nm and 296 nm respectively. These values are means of six estimations. The absorbance and absorptivity at these wavelengths were substituted in equation 1 and 2 to obtain the concentration of both drugs.

\[ C_x (\text{CILO}) = \frac{(A_2 ay_1 - A_1 ay_2)}{(ax_2 ay_1 - ax_1 ay_2)} \] ……(1)

\[ C_y (\text{TELMI}) = \frac{(A_1 ax_2 - A_2 ax_1)}{(ax_2 ay_1 - ax_1 ay_2)} \] …… (2)

A1 and A2 = Absorbance of sample at λ1 and λ2
Cx and Cy = Concentrations of cilostazol and telmisartan in sample matrix.
ax1 and ax2 = Absorptivities of cilostazol at λ1 and λ2
ay1 and ay2 = Absorptivities of telmisartan at λ1 and λ2
Figure 3: Zero Ordered Overlay Spectra For Cilo (4-20 µg/ml RED)& TELMI (1-5 µg/ml BLACK).

**Method II (Absorbance ratio method)**

Absorbance ratio method of analysis is based on the absorbance at two selected wavelengths, one of which is an isoabsorptive point and the other being the wavelength of maximum absorption of one drug. From overlain spectra of cilostazol and telmisartan (isoabsorptive point) (237.5) are selected for the formation of Q absorbance equation (Fig.5). The absorptivity values determined for cilostazol at 258 nm are (ax1), and 237.5 nm are (ax2) and for telmisartan at 258 nm are (ay1), at 237.5 nm are (ay2). These values are means of six estimations. The absorbance and absorptivity at these wavelengths were substituted in equation 3 and 4 to obtain the concentration of drugs.
CX = \[(QM - QY) / (QX - QY)\] × A1/ax1........... (3)

CY = \[(QM - QX) / (QY - QX)\] × A1/ay1 ........... (4)

Where, A1 and A2 are absorbances of mixture at 258 nm and 296 nm;
ax1 and ay1 are absorptivities of cilostazol and telmisartan at 258 nm;
ax2 and ay2 are absorptivities of cilostazol and telmisartan respectively at 296 nm;
QM = A2 / A1, QX = ax2 / ax1 and QY = ay2 / ay1.

Figure 5: Zero Ordered Overlay Spectra for Cilo (4-20 µg/ml RED) & TELMI (1-5 µg/ml) BLACK.

Figure 6.1: Calibration Curve for CILO 4-20 µg/ml

Figure 6.2: Calibration Curve for TELMI 1-5 µg/ml.
Method III Dual wavelength method

From the overlain spectra, four wavelengths 284 nm, 264.9 nm, 243.4 nm and 270.6 nm were selected (Fig. 7) for quantitation of both the drugs by dual wavelength spectrophotometric method. The quantitative determination of cilostazol is carried out by measuring the absorbance difference value at between 284 nm and 264.9 nm where telmisartan has the same absorbance at both the wavelength. The difference between 243.4 nm and 270.6 nm where cilostazol has the same absorbance at both wavelengths. This difference between these wavelengths is directly proportional to concentration.

Figure 7: Zero Ordered Overlay Spectra for Cilo (4-20 µg/ml RED) & MET (1-5µg/ml BLACK).

Figure 8.1: Calibration Curve for CILO 4-20 µg/ml

Figure 8.2: Calibration Curve for TELMI 1-5 µg/ml.
APPLICABILITY OF THE DEVELOPED UV SPECTROPHOTOMETRIC METHODS

All methods were successfully applied for the estimation of cilostazol and telmisartan in synthetic mixture.

Preparation of synthetic mixture:

Synthetic mixture was prepared using various the excipients in the pharmaceutical oral synthetic mixture (CILO 40 mg & TELMI 10 mg). Inactive ingredients of the formulation include MCC, copovidone, SSG, Mg stearate, cornstarch & Talc.

Analysis of synthetic mixture:

From synthetic mixture an amount equivalent to 4 mg CILO and 16 mg TELMI was weighed and dissolved in 100 ml methanol. Solutions were filtered using whatmann filter paper grade 1. Appropriate dilutions were prepared in methanol using aliquots of the clear filtrates and subjected to analysis using all the three methods described above (Table 1).

Table 1: Results of Simultaneous Estimation of Synthetic Mixture for Method I, II, And III.

<table>
<thead>
<tr>
<th>Formulation:- Synthetic Mixture</th>
<th>Labelled Claim :- CILO : TELMI (40 mg: 10 mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method</td>
<td>CILO±SD</td>
</tr>
<tr>
<td>I</td>
<td>102.5±0.1473</td>
</tr>
<tr>
<td>II</td>
<td>102.5±0.1674</td>
</tr>
<tr>
<td>III</td>
<td>100.75±0.122</td>
</tr>
</tbody>
</table>

*mean value of three determination

VALIDATION OF THE DEVELOPED METHODS

Linearity

For each drug, appropriate dilutions of standard stock solutions were assayed as per the developed methods. For method I and II, and III the Beer- Lambert’s concentration range was found to be 1-40 μg/mL cilostazol and 1-25 μg/mL for telmisartan.

Accuracy

To check the accuracy of the proposed method, recovery studies were carried out 80, 100 and 120% of the test concentration as per ICH guidelines. The recovery study was performed threetimes at each level. The result of the recovery studies are reported in table.
**Precision**

**Interday and Intraday precision**

The interday and intraday precision was determined by assay of the sample solution on the same day and on different days at different time intervals respectively. The results of the same are presented in Table.

**Limit of Detection and Limit of Quantification**

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations designated by International Conference on Harmonization (ICH) guidelines.

\[
\text{LOD} = 3.3 \times \frac{\sigma}{S}
\]

\[
\text{LOQ} = 10 \times \frac{\sigma}{S}
\]

Where,

\[\sigma = \text{the standard deviation of the response and}\]

\[S = \text{slope of the calibration curve.}\]

**RESULTS AND DISCUSSION**

Developed spectrophotometric methods for the simultaneous estimation of CILO and TELMI were validated according to ICH guidelines and data complying with the standards were obtained. The results of validation parameters for all the three developed methods are reported (Table 2 and 3).

**Table 2: Summary of Validation Parameter by Developed Method.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method I CILO</th>
<th>Method I TELMI</th>
<th>Method II CILO</th>
<th>Method II TELMI</th>
<th>Method III CILO</th>
<th>Method III TELMI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analytical wavelength (nm)</td>
<td>258</td>
<td>296</td>
<td>258, 237.5 (isoabsorptive)</td>
<td>284, 264.9</td>
<td>243.4, 270.6</td>
<td></td>
</tr>
<tr>
<td>Beer’s range (µg/ml)</td>
<td>4.0-20.0</td>
<td>1.0-5.0</td>
<td>4.0-20.0</td>
<td>1.0-5.0</td>
<td>4.0-20.0</td>
<td>1.0-5.0</td>
</tr>
<tr>
<td>Slope</td>
<td>0.0413</td>
<td>0.0479</td>
<td>0.0178</td>
<td>0.0708</td>
<td>0.0262</td>
<td>0.0134</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.0052</td>
<td>0.0015</td>
<td>-0.0059</td>
<td>-0.0003</td>
<td>0.0085</td>
<td>-0.0022</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.9977</td>
<td>0.9989</td>
<td>0.9971</td>
<td>0.9991</td>
<td>0.9982</td>
<td>0.9982</td>
</tr>
</tbody>
</table>
CONCLUSION

Three Spectrophotometric methods (Simultaneous equation method, absorbance ratio method, dual wavelength method) were developed for simultaneous estimation of CILO and TELMI in their combined formulation without prior separation. Methods were found to be precise and accurate as can be reflected from validation data. Developed methods were successfully applied for estimation of CILO and TELMI in synthetic mixture and there for method is can be extended for the analysis of formulation.

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