DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF METOPROLOL SUCCINATE AND CHLORTHALIDONE IN BULK AND TABLET DOSAGE FORM

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Abstract:
A simple stability indicating high performance liquid chromatographic method has been developed for the simultaneous determination of Metoprolol succinate and Chlorthalidone in bulk and pharmaceutical tablet dosage form using reverse phase Zorbax Eclipse Plus C8 column (250mm×4.6mm), with mobile phase phosphate buffer (0.05M KH₂PO₄): acetonitrile (60:40v/v) pH 4.5 adjusted with ortho-phosphoric acid, the flow rate was 1.0 mL/min and the detection was carried at 220 nm. The retention times of Metoprolol and Chlorthalidone were 3.103 and 3.407 min respectively. The correlation coefficient of Metoprolol and Chlorthalidone was found to be 0.999 and 0.997. Calibration plots were linear over the concentration ranges 10-60µg/mL and 2.5-15 µg/mL for Metoprolol and Chlorthalidone. The LOD and LOQ of Metoprolol were 1.694 µg/mL and 5.133 µg/mL while for Chlorthalidone was 0.808µg/mL and 2.44µg/mL. The accuracy of the proposed method was determined by recovery studies and found to be 100.03% for Metoprolol and 100.02% for Chlorthalidone respectively. The method was validated for accuracy, linearity, sensitivity, precision, robustness, system suitability. The proposed method could be utilized for routine analysis of Metoprolol succinate and Chlorthalidone in bulk and pharmaceutical tablet dosage form.

Keywords: Metoprolol Succinate and Chlorthalidone, Method development and validation, RP-HPLC, Stability Indicating Assay.

Introduction: Metoprolol is chemically described as: (±) 1-(isopropyl amino)-3-[p-(2-methoxyethyl) phenoxy] 2-propanol Succinate with molecular formula (C₁₅H₂₅NO₃)₂·C₄H₆O₄ and molecular weight is 652.81 gm/moL. The structure is given in figure 1.1. It is a beta -adrenergic receptor blocking agent mainly used in treatment of several diseases of the cardiovascular system, especially hypertension. The literature survey revealed several analytical
methods developed for estimation of Metoprolol Succinate by UV spectrophotometric \cite{3-7}, HPTLC \cite{8-10} and RP-HPLC \cite{11-30} methods.

The combination tablet of Metoprolol (50 mg) and Chlorthalidone (12.5 mg) available in market (Vinicor-D). The present drug combination has promising effect to control hypertension and other heart diseases.

Chlorthalidone is chemically described as: 2-chloro-5-(1-hydroxy-3-oxo-2, 3-dihydro-1H-isoindol-1-yl) benzene sulfonamide with molecular formula C$_{14}$H$_{11}$N$_2$O$_4$ScI and molecular weight is 338.76 gm/moL \cite{31-32}. The structure is given in figure 1.2. It is used as thiazide diuretic drug that has antihypertensive action. Literature survey revealed developed bioanalytical method as LC-MS-MS \cite{33} for detection of Chlorthalidone in human serum and blood, few UV spectrophotometric \cite{7, 34-39}, HPTLC \cite{40} and RP-HPLC \cite{41-51} methods for the quantitative estimation of Chlorthalidone in bulk and pharmaceutical formulations.

![Figure 1.1: Structure of Metoprolol Succinate.](image)

![Figure 1.2: Structure of Chlorthalidone.](image)

From the literature survey it was clear that no stability indicating method have been developed and validated to access stability of MET and CLT in bulk and tablet dosage form. To establish stability indicating nature of the RP-HPLC method, forced degradation of drug substances were performed under stress conditions \cite{52} (oxidation, acid and base hydrolysis, thermal, neutral and photolytic). The proposed method was optimized and validated as per ICH guidelines \cite{53-56}.

The present research work describes a rapid, accurate, sensitive and reproducible stability indicating RP-HPLC method for simultaneous estimation of Metoprolol and Chlorthalidone from bulk and tablet formulation.

**Experimental:**

1. **Chemicals:** The pure drug sample of Metoprolol Succinate and Chlorthalidone were obtained as gift sample from, Trichem Life Sciences Ltd., Bangalore. Double distilled water (HPLC grade), Methanol (HPLC grade), Acetonitrile
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(HPLC grade), orthophosphoric acid and Potassium dihydrogen ortho phosphate were purchased from Research–Lab Fine Chem Industries, Mumbai. The tablet containing Metoprolol and Chlorthalidone (Vinicor-D) having 50mg of MET and 12.5 mg CLT was purchased from local market (Manufactured by IPCA Laboratories Ltd., Mumbai).

2. Instrumentation and Equipments:
The HPLC analysis was accomplished on Agilent 1220 high pressure liquid chromatography System with an Infinity Isocratic LC Manual Injector, Zorbax Eclipse Plus C8 column (250mm×4.6mm) analytical column reversed-phase material of 5µ size with Variable wavelength detector. All the parameters of HPLC were controlled by Ezchrom Elite software. Other instruments used were UV –Vis Double Beam Spectrophotometer Schimadzu 1800 with UV probe Software, Contech electronic balance, pH meter (Elico India) and sonicator (Bio Technics India).

3. Analytical Method Development:

3.1. Selection of Detection Wavelength:
The Detection of wavelength was done in UV Schimadzu 1800 instrument. An ideal wavelength is the one that gives good response for the drugs that are to be detected. In the present study standard drug solutions of 100 µg/mL Metoprolol Succinate and 100 µg/mL Chlorthalidone were, therefore, prepared in solvent mixtures of phosphate buffer (pH 4.5): acetonitrile (60:40). This drug solution was scanned in the UV region of 200-400 nm. Four isobestic points were observed in the overlain spectra of MET and CLT. From these points the wavelength selected for the HPLC analysis was 220 nm to which both the drugs showed significant absorbance and very good resolution. The overlain UV spectra of MET and CLT are as shown in figure 2.

![Figure 2: Isobestic Point of Metoprolol and Chlorthalidone (220 nm).](image-url)
3.2. Selection of Mobile Phase:

After assessing the solubility of drugs in different solvents as well on the basis of literature survey, the standard solution of Metoprolol Succinate and Chlorthalidone were injected into the HPLC system by using different solvent systems. Different mobile phases were tried in order to find the best conditions for the separation of both the drugs. It was found that of potassium dihydrogen orthophosphate buffer (pH adjusted to 4.5 with orthophosphoric acid) and acetonitrile in the ratio of 60:40%v/v showed satisfactory results as compared to other mobile phases. Prior to use, the mobile phase was degassed by an ultrasonic bath and filtered through 0.45μm filter paper. Final optimized chromatographic parameters are shown in table 1.

Table 1: Final Optimized Chromatographic Parameter.

<table>
<thead>
<tr>
<th>Column</th>
<th>Zorbax Eclipse Plus C8 (4.6 × 250 mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobile Phase</td>
<td>Phosphate buffer: Acetonitrile (60:40) pH adjusted to 4.5 with ortho phosphoric acid (OPA).</td>
</tr>
<tr>
<td>Flow Rate</td>
<td>1 mL/min.</td>
</tr>
<tr>
<td>Detection Wavelength</td>
<td>220 nm</td>
</tr>
<tr>
<td>Injection Volume</td>
<td>20 μL</td>
</tr>
<tr>
<td>Run Time</td>
<td>10 min</td>
</tr>
<tr>
<td>Column Temperature</td>
<td>Ambient Temperature</td>
</tr>
</tbody>
</table>

3.3. Preparation of mobile phase:

600 mL of Buffer (pH 4.5), 400 mL Acetonitrile (HPLC grade) were mixed and filtered through 0.45μm filter paper, sonicated for 10 minutes to degas and used as mobile phase.

3.4. Preparation of Standard Solution:

Standard stock solution of MET and CLT in combination was prepared by dissolving 40 mg of MET and 10 mg of CLT in 100 mL volumetric flask with 70 mL mobile phase. It was sonicated to dissolve completely and made volume up to the mark with the same diluents to get concentration of 400 & 100 μg/mL respectively (Stock solution). From this, 1.25 mL of the solution was pipette out into another 10 mL volumetric flask and diluted up to the mark with diluents to get a working standard solution of 50 & 12.5 μg/mL concentration.

3.5. Preparation of Sample Solution:

Twenty tablets were accurately weighed and transferred powder equivalent to 10 mg of Chlorthalidone into a 100 mL clean dry volumetric flask containing about 70 mL of mobile phase, sonicated it for 30 min to dissolve the contents completely and volume was made up to the mark with the mobile phase. Filtered this solution through 0.45 μm
Whatmann filter paper, this stock solution contains 100 µg/mL of chlorthalidone (and 400 µg/mL metoprolol succinate). The above stock solution (1.25mL) was diluted to get the sample solutions of concentrations of 12.5 µg/mL of chlorthalidone (and 50 µg/mL of metoprolol succinate) respectively.

3.6. Assay Procedure:

20 µL of the standard and sample solutions were injected into the chromatographic system and areas for the Metoprolol Succinate and Chlorthalidone peaks were measured. % Assay was calculated by using the formula. Result of assay of both MET and CLT are shown in table 2.

Table 2: Results of Assay.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Standard (API)</th>
<th>Sample (Tablet)</th>
<th>Standard (API)</th>
<th>Sample (Tablet)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>MET</td>
<td>MET</td>
<td>CLT</td>
<td>CLT</td>
</tr>
<tr>
<td>Peak Area</td>
<td>11729389</td>
<td>11842324</td>
<td>7958492</td>
<td>8087030</td>
</tr>
<tr>
<td>RT</td>
<td>3.110</td>
<td>3.109</td>
<td>4.063</td>
<td>4.060</td>
</tr>
<tr>
<td>Resolution (R_s)</td>
<td>-</td>
<td>-</td>
<td>7.32</td>
<td>7.28</td>
</tr>
<tr>
<td>% Assay</td>
<td>100.05</td>
<td>100.93</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\[
\% \text{Assay} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{\text{Avg.Wt}}{LC} \times 100
\]

Where,

AT : Average area from the Sample solution chromatogram.
AS : Average area from the Standard solution chromatogram.
WS : Weight of MET or CLT standard in mg.
DT : Dilution of sample in mL.
DS : Dilution of standard in mL.
WT : Volume of sample taken in mL.
P : Potency of MET or CLT reference standard (%).
LC : Label claim in (mg).

4. Analytical Method Validation:

To develop a precise, accurate and reproducible HPLC method for the estimation of MET and CLT various mobile phases, stationary phases and sample preparation methods were employed and the proposed chromatographic condition was found to be appropriate for the quantitative determination. After optimization of the analytical
conditions, the evaluation of the fundamental parameters, such as system suitability test, linearity, precision, accuracy, recovery selectivity, and stability were performed for the method validation.

4.1. System Suitability Test:
To verify the analytical system is working properly and can give accurate and precise results, the system suitability parameters are to be set. The standard solution which is prepared as per the procedure is injected six injections of diluted drug in the linear region of the calibration curve and measuring the relative standard deviation in percentage (%RSD) to check the instrument is giving consistent results.

4.2. Accuracy:
Accuracy was carried out by % recovery studies at three different concentration levels. To the pre-analyzed sample solution of MET and CLT a known amount of standard concentration of drug MET and CLT were added at 50, 100 and 150 % level.

4.3. Precision:
The system precision of the method was verified by six replicate injections of standard solution containing MET and CLT. The precision for repeatability (intra-day and Inter-days precision) was carried out the analyte six times in a day in order to record any intra-day variations in the results. For Inter-days variations studies, analysis was carried out on three different days with same concentrations of MET and CLT.

4.4. Linearity and Range:
The linearity was determined separately for MET and CLT. Linearity of the method was studied by injecting six concentrations of both drugs prepared in methanol and calibration curves were constructed by plotting peak area against the respective concentrations. Concentration range will be selected for MET 10-60 µg/mL and for CLT 2.5-15 µg/mL.

4.5. Limit of Detection and Limit of Quantitation:
Sensitivity of the proposed method was estimated in terms of limit of detection (LOD) and limit of quantitation (LOQ). The LOD and LOQ were calculated according to the formula given by the ICH guidelines as described below,

\[
LOD = \frac{3.3\sigma}{S}
\]
LOQ is calculated from the formula: -

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

Where,

\( \sigma \) = Standard deviation of the response of calibration curve.

\( S \) = Slope of the calibration curve.

4.6. Robustness:
Robustness was evaluated by making deliberate variations in method parameters such as variation of wavelength; flow rate and change in pH of mobile phase. The robustness of the method was studied for MET and CLT.

4.7. Table Top Stability of Solution
Evaluate the stability in analytical solution by injecting the standard preparation and sample preparation at regular interval. The table top stability of solution is carried out at 0, 3, 6, 12, 24, 48 hrs.

5. Stability Indicating Assay Method (Forced Degradation Study):
Sample Stock solutions containing 400\( \mu \)g/mL Metoprolol Succinate and 100\( \mu \)g/mL Chlorthalidone were prepared in diluents. These solutions were used for forced degradation to provide an indication of the stability indicating property of proposed method. In all degradation studies the average peak area of MET and CLT samples after analysis were recorded in order to study the degradation products of both the drugs.

1. **Acidic and Basic degradation:** To 5 mL of sample stock solution of drugs, 5 mL of each of 5 N HCl and 5 N NaOH were added. Acidic and Basic mixture of drug was placed in the water bath for 30 min. at 80 \( ^\circ \)C. The solutions were neutralized as needed (5 N NaOH or 5 N HCl).

2. **Hydrogen peroxide degradation:** To 5 mL of sample solution of drugs, 5 mL of each 3% w/v of hydrogen peroxide (H\( _2 \)O\( _2 \)) was added. The solutions were placed in the water bath for 3 hr at 80 \( ^\circ \)C.

3. **Neutral hydrolysis:** To 5 mL of sample solution of drugs, 5 mL of distilled water was added. The solutions were placed in the water bath for 4 hr at 80 \( ^\circ \)C to study the degradation under neutral conditions.

4. **Thermal degradation:** To carry out thermal degradation of sample solution, take 5 mL of sample solutions of drug and placed in the hot air oven for 4 hr at 80 \( ^\circ \)C.

5. **Photolytic degradation:** As per ICH guidelines for photo-stability testing of new drug substances and products, 5 mL samples should be exposed to direct sunlight for 4 hrs.
From the above solutions, each sample was taken in 50 mL volumetric flask, cooled to room temperature and dilute up to with diluents. Filter and sonicate for 10 min. and analyze this solution in HPLC.

Results and Discussion:

1. Selection of Chromatographic Conditions and Optimization of Mobile Phase:

Mobile phase was optimized to separate MET and CLT using reverse phase Zorbax Eclipse Plus C8 column (250mm×4.6mm), with mobile phase consist of phosphate buffer (0.05M KH$_2$PO$_4$): acetonitrile (60:40v/v) at pH 4.5 adjusted with ortho-phosphoric acid, the flow rate was 1.0 mL/min and the detection was carried at 220 nm. A typical chromatogram of blank solution is as shown in figure 3. Blank sample solution was screened and interference of endogenous substances was not observed at retention time of MET and CLT which represented the selectivity of the method. Under optimum chromatographic conditions, the retention time for MET and CLT standard stock solution was found to be 3.109 and 4.060 min, respectively. A typical chromatogram of two drugs in standard and formulation is shown in figure 4 and 5. The method is simple, accurate, and reproducible and can be used for simultaneous analysis of metoprolol succinate and chlorthalidone.

![Figure 3: Chromatogram for Blank.](image1)

![Figure 4: Chromatogram of Standard MET and CLT.](image2)
Figure 5: Chromatogram of MET and CLT formulation (Tablet).

All of the analytical validation parameters for this proposed method were determined according to ICH guidelines \[53\].

2. System Suitability Test:

The results of system suitability test are shown in table 3. The % RSD calculated for the method was found to be less than 2%, which revealed the suitability of the developed method and the optimized chromatographic conditions.

Table 3: Results of System Suitability Test.

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Std. Sample (µg/mL)</th>
<th>Retention Time (Min.)</th>
<th>Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(MET)</td>
<td>(CLT)</td>
<td>(MET)</td>
</tr>
<tr>
<td>1</td>
<td>50</td>
<td>12.5</td>
<td>3.023</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>12.5</td>
<td>3.020</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>12.5</td>
<td>3.023</td>
</tr>
<tr>
<td>4</td>
<td>50</td>
<td>12.5</td>
<td>3.023</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
<td>12.5</td>
<td>3.023</td>
</tr>
<tr>
<td>6</td>
<td>50</td>
<td>12.5</td>
<td>3.023</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
<td>3.0225</td>
</tr>
<tr>
<td>SD</td>
<td>0.001225</td>
<td>0.011862</td>
<td>236337.7</td>
</tr>
<tr>
<td>% RSD</td>
<td>0.0405</td>
<td>0.301</td>
<td>0.883</td>
</tr>
<tr>
<td>Asymmetry Factor</td>
<td></td>
<td></td>
<td>1.29</td>
</tr>
<tr>
<td>Theoretical plates</td>
<td></td>
<td></td>
<td>9692.8</td>
</tr>
</tbody>
</table>

3. Recovery studies:

The accuracy of the method in term of recovery was studied at three different concentration levels i.e. 50%, 100 % and 150 % showed acceptable % recoveries in the range of 100.03% for MET and 100.02% for CLT. The results are shown in table 4 and 5.
Table 4: Results of % Recovery of MET.

<table>
<thead>
<tr>
<th>Spike Level in %</th>
<th>Peak Area</th>
<th>Theoretical content (µg/mL)</th>
<th>Amt. Added (Sample)</th>
<th>Amt. Added (Std.)</th>
<th>Total Amt.</th>
<th>Amt. Found (µg/mL)</th>
<th>% Recovery</th>
<th>Mean</th>
<th>SD</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>50%</td>
<td>8113596</td>
<td>40</td>
<td>20</td>
<td>60</td>
<td>60.40</td>
<td>100.68</td>
<td></td>
<td>100.65</td>
<td>0.344</td>
<td>0.341</td>
</tr>
<tr>
<td></td>
<td>7787859</td>
<td>40</td>
<td>20</td>
<td>60</td>
<td>60.18</td>
<td>100.30</td>
<td></td>
<td>100.65</td>
<td>0.344</td>
<td>0.341</td>
</tr>
<tr>
<td></td>
<td>8207154</td>
<td>40</td>
<td>20</td>
<td>60</td>
<td>60.59</td>
<td>100.99</td>
<td></td>
<td>99.03</td>
<td>0.528</td>
<td>0.533</td>
</tr>
<tr>
<td>100%</td>
<td>12337451</td>
<td>40</td>
<td>40</td>
<td>80</td>
<td>79.22</td>
<td>99.02</td>
<td></td>
<td>99.03</td>
<td>0.528</td>
<td>0.533</td>
</tr>
<tr>
<td></td>
<td>12582129</td>
<td>40</td>
<td>40</td>
<td>80</td>
<td>79.65</td>
<td>99.56</td>
<td></td>
<td>99.03</td>
<td>0.528</td>
<td>0.533</td>
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<tr>
<td></td>
<td>12500481</td>
<td>40</td>
<td>40</td>
<td>80</td>
<td>78.80</td>
<td>98.51</td>
<td></td>
<td>99.03</td>
<td>0.528</td>
<td>0.533</td>
</tr>
<tr>
<td>150%</td>
<td>17095766</td>
<td>40</td>
<td>60</td>
<td>100</td>
<td>100.41</td>
<td>100.41</td>
<td></td>
<td>100.39</td>
<td>0.204</td>
<td>0.203</td>
</tr>
<tr>
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<td>17637160</td>
<td>40</td>
<td>60</td>
<td>100</td>
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<td>100.39</td>
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<tr>
<td></td>
<td>17635148</td>
<td>40</td>
<td>60</td>
<td>100</td>
<td>100.59</td>
<td>100.59</td>
<td></td>
<td>100.39</td>
<td>0.204</td>
<td>0.203</td>
</tr>
</tbody>
</table>

Table 5: Results of % Recovery of CLT.

<table>
<thead>
<tr>
<th>Spike Level in %</th>
<th>Peak Area</th>
<th>Theoretical content (µg/mL)</th>
<th>Amt. Added (Sample)</th>
<th>Amt. Added (Std.)</th>
<th>Total Amt.</th>
<th>Amt. Found (µg/mL)</th>
<th>% Recovery</th>
<th>Mean</th>
<th>SD</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>50%</td>
<td>6401263</td>
<td>10</td>
<td>5</td>
<td>15</td>
<td>15.10</td>
<td>100.73</td>
<td></td>
<td>100.83</td>
<td>0.352</td>
<td>0.350</td>
</tr>
<tr>
<td></td>
<td>6582229</td>
<td>10</td>
<td>5</td>
<td>15</td>
<td>15.18</td>
<td>101.23</td>
<td></td>
<td>100.83</td>
<td>0.352</td>
<td>0.350</td>
</tr>
<tr>
<td></td>
<td>6510055</td>
<td>10</td>
<td>5</td>
<td>15</td>
<td>15.08</td>
<td>100.55</td>
<td></td>
<td>98.73</td>
<td>0.531</td>
<td>0.538</td>
</tr>
<tr>
<td>100%</td>
<td>9551078</td>
<td>10</td>
<td>10</td>
<td>20</td>
<td>19.77</td>
<td>98.88</td>
<td></td>
<td>98.73</td>
<td>0.531</td>
<td>0.538</td>
</tr>
<tr>
<td></td>
<td>9552019</td>
<td>10</td>
<td>10</td>
<td>20</td>
<td>19.62</td>
<td>98.14</td>
<td></td>
<td>98.73</td>
<td>0.531</td>
<td>0.538</td>
</tr>
<tr>
<td></td>
<td>9703118</td>
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<td>10</td>
<td>20</td>
<td>19.83</td>
<td>99.17</td>
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<td>98.73</td>
<td>0.531</td>
<td>0.538</td>
</tr>
<tr>
<td>150%</td>
<td>13149641</td>
<td>10</td>
<td>15</td>
<td>25</td>
<td>25.10</td>
<td>100.43</td>
<td></td>
<td>100.50</td>
<td>0.211</td>
<td>0.210</td>
</tr>
<tr>
<td></td>
<td>13265229</td>
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<td>15</td>
<td>25</td>
<td>25.18</td>
<td>100.73</td>
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<td>100.50</td>
<td>0.211</td>
<td>0.210</td>
</tr>
<tr>
<td></td>
<td>13229620</td>
<td>10</td>
<td>15</td>
<td>25</td>
<td>25.08</td>
<td>100.33</td>
<td></td>
<td>100.50</td>
<td>0.211</td>
<td>0.210</td>
</tr>
</tbody>
</table>

4. Precision:

The precision of the method was measured by the percentage relative standard deviation (% RSD) over the concentration range of high, middle and low QC samples respectively of drug during course of validation. The precision study was evaluated on the basis of % RSD value was found to be within limit. The % RSD values were
found to be 1.08 and 0.699 for MET, CLT stating that the developed method is precise. Results of precision study are shown in table 6. Intra-day precision of the method ranging from 0.778 to 1.078 %RSD (table 7). Inter-days precision of the method was found to be 0.839 to 1.191 % RSD (table 8).

Table 6: Results of System Precision.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Peak Area (MET)</th>
<th>Peak Area (CLT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>26643032</td>
<td>19848210</td>
</tr>
<tr>
<td>2</td>
<td>26481468</td>
<td>19883811</td>
</tr>
<tr>
<td>3</td>
<td>26605582</td>
<td>19536892</td>
</tr>
<tr>
<td>4</td>
<td>26885887</td>
<td>19842893</td>
</tr>
<tr>
<td>5</td>
<td>26297546</td>
<td>19896924</td>
</tr>
<tr>
<td>6</td>
<td>26058335</td>
<td>19716625</td>
</tr>
<tr>
<td>Average</td>
<td>26495308</td>
<td>19787559.17</td>
</tr>
<tr>
<td>SD</td>
<td>288714.89</td>
<td>138442.13</td>
</tr>
<tr>
<td>% RSD</td>
<td>1.08</td>
<td>0.699</td>
</tr>
<tr>
<td>Asymmetry Factor</td>
<td>1.12</td>
<td>1.08</td>
</tr>
<tr>
<td>Theoretical plates</td>
<td>9774.1</td>
<td>11914.8</td>
</tr>
</tbody>
</table>

Table 7: Intra-day Precision of MET and CLT.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Conc. (µg/mL)</th>
<th>Peak Area Mean (n=6)</th>
<th>Mean</th>
<th>SD</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>MET</td>
<td>20</td>
<td>10672914 10782914 10552914</td>
<td>10669580.67</td>
<td>115036.22</td>
<td>1.078</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>20552040 20352040 20753040</td>
<td>20552373.33</td>
<td>200500.20</td>
<td>0.975</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>29504062 29984062 29804062</td>
<td>29764062</td>
<td>242487.11</td>
<td>0.814</td>
</tr>
<tr>
<td>CLT</td>
<td>5</td>
<td>7578382 7592382 7696382</td>
<td>7622382</td>
<td>64467.04</td>
<td>0.845</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>15663556 15884556 15603556</td>
<td>15717222.67</td>
<td>147987.61</td>
<td>0.941</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>22460955 22520955 22790955</td>
<td>22590955</td>
<td>175783.95</td>
<td>0.778</td>
</tr>
</tbody>
</table>

Table 8: Inter-days Precision of MET and CLT.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Conc. (µg/mL)</th>
<th>Peak Area Mean (n=6)</th>
<th>Mean</th>
<th>SD</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>MET</td>
<td>20</td>
<td>10672914 20552040 29504062</td>
<td>10684025.33</td>
<td>103949.33</td>
<td>0.972</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>10694581 20547040 29487396</td>
<td>20590955</td>
<td>175783.95</td>
<td>0.778</td>
</tr>
</tbody>
</table>
5. Linearity and Range:

The linearity was determined separately for MET and CLT. Linearity of the method was studied by injecting six concentrations of both drugs prepared in mobile phase and calibration curves were constructed by plotting peak area against the respective concentrations. The MET and CLT followed linearity in the concentration range of 10-60 µg/mL and 2.5-15 g µg/mL respectively (table 9 and figure 6 & 7).

Table 9: Results of Linearity Study of MET and CLT.

<table>
<thead>
<tr>
<th>Concentration (µg/mL)</th>
<th>Peak Area (MET)</th>
<th>Concentration (µg/mL)</th>
<th>Peak Area (CLT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>5393277</td>
<td>2.5</td>
<td>4165635</td>
</tr>
<tr>
<td>20</td>
<td>10691450</td>
<td>5.0</td>
<td>7909406</td>
</tr>
<tr>
<td>30</td>
<td>15971804</td>
<td>7.5</td>
<td>12671298</td>
</tr>
<tr>
<td>40</td>
<td>20656274</td>
<td>10</td>
<td>15618974</td>
</tr>
<tr>
<td>50</td>
<td>26628423</td>
<td>12.5</td>
<td>19555379</td>
</tr>
<tr>
<td>60</td>
<td>31338216</td>
<td>15</td>
<td>23233873</td>
</tr>
<tr>
<td>Intercept</td>
<td>22456</td>
<td>Intercept</td>
<td>53641</td>
</tr>
<tr>
<td>Slope</td>
<td>520628</td>
<td>Slope</td>
<td>1522592</td>
</tr>
<tr>
<td>Correlation Coefficient ($r^2$)</td>
<td>0.999</td>
<td>Correlation Coefficient ($r^2$)</td>
<td>0.997</td>
</tr>
</tbody>
</table>

**Figure 6: Results of Linearity Study of MET.**

\[ y = 52062x + 22456 \]
\[ R^2 = 0.999 \]
6. Limit of Detection (LOD) and Limit of Quantitation (LOQ):

The LOD for MET and CLT was found to be 1.69 and 0.808 µg/mL respectively. The LOQ for MET and CLT was found to be 5.13 and 2.44 µg/mL respectively. The low values of LOD and LOQ indicates high sensitivity of the method (table 10).

**Table 10: LOD and LOQ of MET and CLT.**

<table>
<thead>
<tr>
<th>Drug</th>
<th>LOD</th>
<th>LOQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>MET</td>
<td>1.69 µg/mL</td>
<td>5.13 µg/mL</td>
</tr>
<tr>
<td>CLT</td>
<td>0.808 µg/mL</td>
<td>2.44 µg/mL</td>
</tr>
</tbody>
</table>

7. Robustness study:

Robustness of the method was studied by making deliberate changes in the chromatographic conditions (change in flow rate, wavelength and pH) and the effects on the results were examined. The low value changes of theoretical plates, tailing factor indicating robustness of the method (table 11).

**Table 11: Results of Robustness of MET and CLT.**

<table>
<thead>
<tr>
<th>Std. Sample (µg/mL)</th>
<th>Results of Robustness-Change in Flow Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.8mL/min MET</td>
</tr>
<tr>
<td>MET</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>12.5</td>
</tr>
<tr>
<td>CLT</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>12.5</td>
</tr>
</tbody>
</table>
Table 12: Table Top Stability of MET and CLT in Sample Solution (n=6).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HPLC data</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MET</td>
</tr>
<tr>
<td>0</td>
<td>26759344</td>
</tr>
<tr>
<td>3</td>
<td>26628423</td>
</tr>
<tr>
<td>6</td>
<td>26808456</td>
</tr>
</tbody>
</table>

8. Table Top Stability of Solution:

Short-term stability study indicated that sample solutions remained stable for 24 h even at room temperature (25°C).

The results of table top stability of solution are shown in table 12.

In acidic conditions it was found that around 33.42 and 26.39 % of the drugs contents were degraded (figure 8-9) whereas in alkaline condition it was found to be around 7.75 and 15.95 % of the drugs contents were degraded and impurity peak was found at 2.114, 2.750 min (figure 10-11). Major degradation was observed in oxidative condition in which drug products were degraded up to 67.83 and 46.02 % (figure 12-13). Drug contents were found to be degraded around 2.041 & 3.53 % in neutral condition while slightly degradation was observed under the thermal condition (figure 14-17). In photolytic condition where the drugs were directly exposed to sunlight the degradation was found to be 10.48 & 3.13 % (figure 18-19). The results of forced degradation study are shown in table 13.

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>26897223</td>
<td>19571014</td>
</tr>
<tr>
<td>24</td>
<td>25575831</td>
<td>19173707</td>
</tr>
<tr>
<td>48</td>
<td>26628423</td>
<td>19555379</td>
</tr>
<tr>
<td>Mean Peak Area</td>
<td>26599616.67</td>
<td>19458589.17</td>
</tr>
<tr>
<td>SD</td>
<td>369654.30</td>
<td>150181.58</td>
</tr>
<tr>
<td>%RSD</td>
<td>1.389</td>
<td>0.771</td>
</tr>
</tbody>
</table>

Figure 8: Chromatogram for standard drug in 5 N HCl.
Figure 9: Chromatogram for formulation in 5 N HCl.

Figure 10: Chromatogram for standard drug in 5 N NaOH.

Figure 11: Chromatogram for formulation in 5 N NaOH.

Figure 12: Chromatogram for standard in 3% H$_2$O$_2$.
Figure 13: Chromatogram for formulation in 3% $\text{H}_2\text{O}_2$.

Figure 14: Chromatogram for standard in Neutral degradation.

Figure 15: Chromatogram for formulation in Neutral degradation.

Figure 16: Chromatogram for standard in hot air oven.
Figure 17: Chromatogram for formulation in hot air oven.

Figure 18: Chromatogram for standard in direct sunlight.

Figure 19: Chromatogram for formulation in direct sunlight.

Table 13: Results of Forced Degradation Study of Sample Solution.

<table>
<thead>
<tr>
<th>Stress Condition</th>
<th>Degradation Peaks at ($t_R$ in min.)</th>
<th>% of Active Drug Present after Degradation</th>
<th>% Degradation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MET</td>
<td>CLT</td>
</tr>
<tr>
<td>Acidic Degradation</td>
<td>2.092</td>
<td>66.57</td>
<td>73.60</td>
</tr>
<tr>
<td>Alkali Degradation</td>
<td>2.114, 2.750</td>
<td>92.24</td>
<td>84.05</td>
</tr>
<tr>
<td>Peroxide Degradation</td>
<td>2.630</td>
<td>32.16</td>
<td>53.97</td>
</tr>
<tr>
<td>Neutral Degradation</td>
<td>4.113, 5.107</td>
<td>97.95</td>
<td>96.46</td>
</tr>
<tr>
<td>Thermal Degradation</td>
<td>4.117</td>
<td>99.14</td>
<td>98.54</td>
</tr>
<tr>
<td>Photolytic Degradation</td>
<td>4.113, 5.107</td>
<td>89.51</td>
<td>96.86</td>
</tr>
</tbody>
</table>

6. Conclusion

The study represents a simple and validated stability indicating HPLC method for simultaneous estimation of Metoprolol Succinate and Chlorthalidone in the presence of degradation products. The developed method is specific, accurate, precise and robust. The method was linear response in stated range and is accurate and precise. All the
degradation products formed during forced decomposition studies were well separated from the analyte peaks demonstrating that the developed method is specific and stability indicating. The method could be applied with success even to the analysis of marketed products of MET and CLT combined tablet formulation, as no interference was observed due to excipients along with the degradants present therein.

7. Acknowledgement

Authors would like to thank Trichem Life Science Ltd, Bangalore, India for providing the gift samples of Metoprolol Succinate and Chlorthalidone. Authors would also like to thank the Management of the college for providing necessary facility for the research work.

References:


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