HPLC METHOD DEVELOPMENT AND ITS VALIDATION FOR SIMULTANEOUS ESTIMATION OF TIMOLOL MALEATE AND HYDROCHLOROTHIAZIDE IN THEIR COMBINED TABLET DOSAGE FORM

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ABSTRACT

High-performance liquid chromatography (HPLC) is a form of liquid chromatography to separate compounds that are dissolved in solution. HPLC is able to separate macromolecules and ionic species, labile natural products, a wide variety of other high molecular-weight poly functional groups and polymeric materials. Prepare a degassed mixture of methanol, water and TEA in the Ratio (85:15:0.25v/v). Drugs showed maximum absorbance both Timolol Maleate and Hydrochlorothiazide at 225nm. Timolol maleate is a beta1 and beta 2 (non-selective) adrenergic receptor blocking agent that does not have significant intrinsic sympathomimetic, direct myocardial depressant, or local anesthetic activity. Hydrochlorothiazide is a diuretic and antihypertensive agent. It affects the renal tubular mechanism of electrolyte reabsorption. Hydrochlorothiazide increases excretion of sodium and chloride in approximately equivalent amounts. The mechanism of the antihypertensive effect of thiazides may be related to the excretion and redistribution of body sodium. Hydrochlorothiazide usually does not cause clinically important changes in normal blood pressure. The validation protocols usually followed is that defined by the International Conference on Harmonization (ICH) under their Validation for analytical procedures methodology.
1. INTRODUCTION

High-performance liquid chromatography (HPLC) is a form of liquid chromatography to separate compounds that are dissolved in solution. HPLC is able to separate macromolecules and ionic species, labile natural products, a wide variety of other high molecular-weight poly functional groups and polymeric materials. HPLC is based on the mechanism of adsorption, mass distribution, ion exchange & stereo chemical interaction. Where, Normal phase HPLC consist a polar stationary phase and non-polar mobile phase, and polar mobile phase and non-polar stationary phase in case of reversed phase HPLC. RP-HPLC is most widely used technique due to Simplicity, Versatility and able to handle compound of diverse polarity and molecular mass. The proposed analytical method development and simultaneous estimation of Timolol maleate and Hydrochlorothiazide and its validation by RP-HPLC is accurate, precise, simple, selective, sensitive and rapid can be applied successfully for routine analysis in quality control. The validation protocols usually followed is that defined by the International Conference on Harmonization (ICH) under their Validation for analytical procedures methodology.

Introduction to drugs

1.1 TIMOLOL MALEATE:- Timolol maleate is a beta 1 and beta 2 (non-selective) adrenergic receptor blocking agent that does not have significant intrinsic sympathomimetic, direct myocardial depressant, or local anesthetic activity.

1.1.1 Description of drug

![Figure no-1 Structure of Timolol Maleate](image-url)

1.1.2 EMPIRICAL FORMULA-C$_{13}$H$_{24}$N$_{2}$O$_{6}$SC$_{4}$H$_{4}$O$_{4}$

1.2 HYDROCHLOROTHIAZIDE- Hydrochlorothiazide is a diuretic and antihypertensive agent. It affects the renal tubular mechanism of electrolyte reabsorption. Hydrochlorothiazide increases excretion of sodium and chloride in approximately equivalent amounts. The mechanism of the antihypertensive effect of thiazides may be related to the excretion and redistribution of body sodium. Hydrochlorothiazide usually does not cause clinically important changes in normal blood pressure.
1.2.1 Description of drug

![Structure of Hydrochlorothiazide](image)

**Figure no-II Structure of Hydrochlorothiazide**

1.2.2 **EMPIRICAL FORMULA-C$_7$H$_8$ClN$_3$O$_4$S$_2$**

2. **MATERIALS AND METHODS**

Timolol maleate and Hydrochlorothiazide Supplied by Switzer Life sciences Pvt. Ltd., Ahmedabad.

2.1 Methods:-

2.1.1 Estimated Methodology for Timolol Maleate and Hydrochlorothiazide

2.1.1.2 Preparation of Mobile Phase

15ml of mill-Q water and 85ml of Methanol adjust the to pH 3.0

2.1.1.1 Preparation of sample solution:

Timolol Maleate and Hydrochlorothiazide are slightly soluble and freely soluble in water and methanol, so water methanol mixture was used.

3. **RESULT AND DISCUSSION**

3.1 HPLC method development, optimization and validation of Timolol Maleate and Hydrochlorothiazide

3.1.1 Selection of detection wavelength:

Drugs showed maximum absorbance both Timolol Maleate and Hydrochlorothiazide at 225nm.

3.1.2 Selection of Column Temperature

An inclusion of column temperature (40°C) has minimized day-to-day variation of retention time due to fluctuations in the ambient temperature; along with this peak sharpening and shortening of run time were observed.

![Typical Chromatogram for Timolol Maleate and Hydrochlorothiazide](image)

**Fig. 1. Typical Chromatogram for Timolol Maleate and Hydrochlorothiazide**
Table no. I Optimized condition for Timolol Maleate and Hydrochlorothiazide

<table>
<thead>
<tr>
<th>Column</th>
<th>Thermoscientific, Syneronics C_{18}, 250 mm × 4.60 mm, 5µ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow rate</td>
<td>1.0 ml/minute</td>
</tr>
<tr>
<td>Detection</td>
<td>AT 225nm</td>
</tr>
<tr>
<td>Injection Volume</td>
<td>10 µl</td>
</tr>
<tr>
<td>Run time</td>
<td>10 minutes</td>
</tr>
<tr>
<td>Diluent</td>
<td>Mobile Phase</td>
</tr>
<tr>
<td>Mobile phase Ratio</td>
<td>Methanol : Water : TEA (85:15:0.25v/v)</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>Isocratic</td>
</tr>
<tr>
<td>Retention Time</td>
<td>2.993 (TIMO) and 5.620 (HCTZ)</td>
</tr>
</tbody>
</table>

3.2 Method validation:

3.2.1 Accuracy:- The % Accuracy is within limit (98.0 – 102.0 %) with %RSD less than 2%. So the method is accurate.

### Table No. II Accuracy…For Timolol

<table>
<thead>
<tr>
<th>Level</th>
<th>Amount of Drug Added (ppm)</th>
<th>Amount of drug recovered (ppm)</th>
<th>% Recovery</th>
<th>% Mean</th>
<th>% RSD</th>
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<tbody>
<tr>
<td>80</td>
<td>8</td>
<td>8.09</td>
<td>80.99</td>
<td>100.19</td>
<td>0.87</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>7.91</td>
<td>79.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>8.04</td>
<td>80.42</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>10</td>
<td>10.09</td>
<td>100.91</td>
<td>100.19</td>
<td>0.80</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>9.94</td>
<td>99.40</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>9.90</td>
<td>99.05</td>
<td></td>
<td></td>
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<tr>
<td>120</td>
<td>12</td>
<td>12.05</td>
<td>100.46</td>
<td>100.33</td>
<td>0.62</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>11.95</td>
<td>99.66</td>
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<td></td>
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<td></td>
<td>12</td>
<td>12.04</td>
<td>100.77</td>
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### Table No. III Accuracy…For HCTZ

<table>
<thead>
<tr>
<th>Level</th>
<th>Amount of Drug Added (ppm)</th>
<th>Amount of drug recovered (ppm)</th>
<th>% Recovery</th>
<th>% Mean</th>
<th>% RSD</th>
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<tbody>
<tr>
<td>80</td>
<td>20</td>
<td>20.24</td>
<td>80.97</td>
<td>100.230</td>
<td>0.84</td>
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<td></td>
<td>20</td>
<td>19.80</td>
<td>79.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>20.10</td>
<td>80.43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>25</td>
<td>25.24</td>
<td>100.90</td>
<td>100.051</td>
<td>0.78</td>
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<tr>
<td></td>
<td>25</td>
<td>24.84</td>
<td>99.39</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>25</td>
<td>24.77</td>
<td>99.10</td>
<td></td>
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</tr>
<tr>
<td>120</td>
<td>30</td>
<td>29.90</td>
<td>119.60</td>
<td>100.32</td>
<td>0.60</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>30.22</td>
<td>120.88</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>30</td>
<td>29.90</td>
<td>119.60</td>
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3.4.2 Precision:

### Table No. IV Precision…Intraday

<table>
<thead>
<tr>
<th>Set no.</th>
<th>Conc.ppm</th>
<th>Mean TIMO</th>
<th>SD TIMO</th>
<th>% RSD TIMO</th>
<th>Mean HCTZ</th>
<th>SD HCTZ</th>
<th>% RSD HCTZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<td>25</td>
<td>654.676</td>
<td>6.5949</td>
<td>18.7156</td>
<td>1.0</td>
<td>0.9</td>
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<td>2</td>
<td>10</td>
<td>25</td>
<td>2058.326</td>
<td>18.7156</td>
<td>18.7156</td>
<td>1.0</td>
<td>0.9</td>
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<tr>
<td>3</td>
<td>10</td>
<td>25</td>
<td>2090.683</td>
<td>28.0462</td>
<td>28.0462</td>
<td>1.29</td>
<td>1.34</td>
</tr>
</tbody>
</table>

### Table No. V Precision…Inter day

<table>
<thead>
<tr>
<th>Set no.</th>
<th>Conc.ppm</th>
<th>Mean TIMO</th>
<th>SD TIMO</th>
<th>% RSD TIMO</th>
<th>Mean HCTZ</th>
<th>SD HCTZ</th>
<th>% RSD HCTZ</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>25</td>
<td>664.772</td>
<td>8.6022</td>
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<td>1.29</td>
<td>1.34</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>25</td>
<td>2090.683</td>
<td>28.0462</td>
<td>28.0462</td>
<td>1.29</td>
<td>1.34</td>
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<tr>
<td>3</td>
<td>10</td>
<td>25</td>
<td>2090.683</td>
<td>28.0462</td>
<td>28.0462</td>
<td>1.29</td>
<td>1.34</td>
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3.4.3 Linearity and Range

Table No. VI Linearity and Range Studies

<table>
<thead>
<tr>
<th>Linearity Range</th>
<th>Stock solution to be taken in mL Stock A</th>
<th>Stock B</th>
<th>Dilute to volume (mL) with diluent</th>
<th>Final concentration in µg/mL (TIMOLOL)</th>
<th>Final concentration in µg/mL (HCTZ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50%</td>
<td>5</td>
<td>5</td>
<td>100</td>
<td>5</td>
<td>12.5</td>
</tr>
<tr>
<td>75%</td>
<td>10</td>
<td>10</td>
<td>100</td>
<td>7.5</td>
<td>18.75</td>
</tr>
<tr>
<td>100%</td>
<td>15</td>
<td>15</td>
<td>100</td>
<td>10</td>
<td>25</td>
</tr>
<tr>
<td>125%</td>
<td>20</td>
<td>20</td>
<td>100</td>
<td>12.5</td>
<td>31.25</td>
</tr>
<tr>
<td>150%</td>
<td>25</td>
<td>25</td>
<td>100</td>
<td>15</td>
<td>37.5</td>
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</table>

Fig. 2 Graphs of Linearity and Range Studies

3.4.4 LOD and LOQ

The LOD and LOQ were estimated from the set of 5 calibration curves used to determine method linearity. It may be calculated as

\[
\text{LOD} = 3.3 \times \left( \frac{SD}{\text{Slope}} \right) \\
\text{LOD} = 10 \times \left( \frac{SD}{\text{Slope}} \right)
\]

Where, SD = the standard deviation of Y-intercept of 5 calibration curves.
Slope = the mean slope of the 5 calibration curves.
5. CONCLUSION

A novel RP-HPLC method has been developed for the simultaneous estimation of TIMO and HCTZ in marketed formulations. The method gave good resolution for both the drugs with a short analysis time of 10 minutes. The developed method was validated. It was found to be simple, precise and accurate. The good % recovery in tablet forms suggests that the excipients present in the dosage forms have no interference in the determination. The %RSD was also less than 2% showing high degree of precision of the proposed method. The proposed method can be used for routine analysis of HCTZ and TIMO in combined dosage form. It can be also used in the quality control in bulk manufacturing.

REFERENCES


<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>TIMO</th>
<th>HCTZ</th>
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</thead>
<tbody>
<tr>
<td>S.D. of Intercept</td>
<td>2.563</td>
<td>33.696</td>
</tr>
<tr>
<td>Slope of Calibration Curve</td>
<td>65.11</td>
<td>79.78</td>
</tr>
<tr>
<td>LOD (ppm)</td>
<td>0.129</td>
<td>1.393</td>
</tr>
<tr>
<td>LOQ (ppm)</td>
<td>0.393</td>
<td>4.223</td>
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</tbody>
</table>

Table No. VII LOD and LOQ