DEVELOPMENT AND VALIDATION OF STABILITY INDICATING
RP-HPLC METHOD FOR THE ESTIMATION OF METOPROLOL
AND IVABRADINE IN SOLID DOSAGE FORM

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ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the Metoprolol and Ivabradine in Tablet dosage form. Chromatogram was run through Agilent C18 150 x 4.6 mm, 5μ. Mobile phase containing Buffer 0.01N KH2PO4 (3.75pH): Acetonitrile taken in the ratio 50:50 was pumped through column at a flow rate of 0.8 ml/min. Buffer used in this method was 0.01N KH2PO4. Temperature was maintained at 30°C. Optimized wavelength selected was 260.0nm. Retention time of Metoprolol and Ivabradine were found to be 2.461min and 3.309min. %RSD of the Metoprolol and Ivabradine were found to be 0.7 and 0.7 respectively. % Recovery was obtained as 100.14% and 100.01% for Metoprolol and Ivabradine respectively. LOD, LOQ values obtained from regression equations of Metoprolol and Ivabradine were 0.41, 1.23 and 0.28, 0.85 respectively. Regression equation of Metoprolol is y = 12344x + 11645. And y = 23564x + 1992.2 of Ivabradine. Retention times were decreased and run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

KEYWORDS: Metoprolol, Ivabradine, RP-HPLC, Excipients, Validation.

INTRODUCTION

Ivabradine: Ivabradine is a novel pulse bringing down medication for the symptomatic administration of stable angina pectoralis and symptomatic perpetual heart disappointment. Ivabradine acts by specifically hindering the "amusing" channel pacemaker current (If) in the
sinoatrial hub in a portion subordinate design, bringing about a lower pulse and in this manner more blood to stream to the myocardium.\textsuperscript{[11]} Despite the fact that non-dihydropyridine calcium channel blockers and beta blockers additionally adequately bring down pulse, they show antagonistic occasions because of their negative inotropic impacts.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{IvabradineStructure.png}
\caption{Structure of Ivabradine.}
\end{figure}

\begin{table}
\centering
\caption{Drug profile of Ivabradine.}
\begin{tabular}{|l|l|}
\hline
CAS Number & 155974-00-8 \\
\hline
IUPAC Name & 3-[[([7S]-3,4-dimethoxybicyclo[4.2.0]octa-1,3,5-trien-7-yl)methyl]amino)propyl]-7,8-dimethoxy-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one \\
\hline
Molecular Weigh & 468.594 \\
\hline
Molecular Formula & C\textsubscript{27}H\textsubscript{36}N\textsubscript{2}O\textsubscript{5} \\
\hline
Appearance & Powder \\
\hline
Physical State & Solid \\
\hline
Solubility & ethanol, DMSO, and dimethyl formamide \\
\hline
Melting Point & $>190^\circ$C \\
\hline
pK Values & 9.37 \\
\hline
\end{tabular}
\end{table}

\textbf{Metoprolol}

Metoprolol is a cardio selective $\beta_1$-adrenergic blocking agent used for acute myocardial infarction (MI), heart failure, angina pectoris and mild to moderate hypertension. It may also be used for supraventricular and tachyarrhythmias and prophylaxis for migraine headaches. At low doses, metoprolol selectively blocks cardiac $\beta_1$-adrenergic receptors with little activity against $\beta_2$-adrenergic receptors of the lungs and vascular smooth muscle.\textsuperscript{[12]}
Fig 2: Chemical structure of Metoprolol.

Table no 2: Drug profile of Metoprolol.

<table>
<thead>
<tr>
<th>CAS Number</th>
<th>51384-51-1</th>
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<tr>
<td>IUPAC Name</td>
<td>1-[4-(2-methoxyethyl)phenoxy]-3-[(propan-2-yl)amino]propan-2-ol</td>
</tr>
<tr>
<td>Molecular Weigh</td>
<td>267.3639</td>
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<tr>
<td>Molecular Formula</td>
<td>C15H25NO3</td>
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<tr>
<td>Appearance</td>
<td>white crystalline powder</td>
</tr>
<tr>
<td>Physical State</td>
<td>Solid</td>
</tr>
<tr>
<td>Solubility</td>
<td>water, methanol &amp; sparingly soluble in ethanol</td>
</tr>
<tr>
<td>Melting Point</td>
<td>120 °C</td>
</tr>
<tr>
<td>pK Values</td>
<td>14.09</td>
</tr>
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</table>

MATERIALS AND METHODS

Instrumentation

- Electronics Balance-Denver
- pH meter -BVK enterprises, India
- Ultrasonicator-BVK enterprises
- WATERS HPLC 2695 SYSTEM equipped with quaternary pumps, Photo Diode Array detector and Auto sampler integrated with Empower 2 Software.
- UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2 mm and 10mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbance of Ivabradine and Metoprolol solutions.

Preparation of solution

Preparation of mobile phase

Based up on the solubility of the drugs, diluent was selected, Acetonitrile and 0.01N KH₂PO₄ taken in the ratio of 50:50.
Preparation of Diluent
The mobile phase itself is used as a diluent.

Preparation of Standard stock solutions: Accurately weighed 5 mg of Ivabradine, 25 mg of Metoprolol and transferred to individual 25 ml volumetric flasks separately. 3/4 Th of diluents was added to both of these flasks and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution 1 and 2. (200µg/ml of Ivabradine and 1000µg/ml of Metoprolol).

Preparation of Standard working solutions (100% solution): 1 ml from each stock solution was pipetted out and taken into a 10 ml volumetric flask and made up with diluent. (20µg/ml Ivabradine and 100µg/ml of Metoprolol).

Preparation of Sample stock solutions: 10 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 50 ml volumetric flask, 25 ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (100µg/ml of Ivabradine and 500µg/ml of Metoprolol).

Preparation of Sample working solutions (100% solution): 2 ml of filtered sample stock solution was transferred to 10 ml volumetric flask and made up with diluent. (20µg/ml of Ivabradine and 100µg/ml of Metoprolol).

Preparation of buffer
0.1% OPA Buffer: 1 ml of Conc Ortho Phosphoric acid was diluted to 1000 ml with water.
0.01N KH₂PO₄ Buffer: Accurately weighed 1.36 gm of Potassium dihyrogen Ortho phosphate in a 1000 ml of Volumetric flask add about 900 ml of milli-Q water added and degas to sonicate and finally make up the volume with water then PH adjusted to 3.48 with dil. Orthophosphoric acid solution.

Solution preparation for System suitability parameters
The system suitability parameters were determined by preparing standard solutions of Ivabradine (20 ppm) and Metoprolol (100 ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.

(The % RSD for the area of six standard injections results should not be more than 2%)
Solution preparation for Precision

Preparation of Standard stock solutions: Accurately weighed 5 mg of Ivabradine, 25 mg of Metoprolol and transferred to individual 25 ml volumetric flasks separately. 3/4 th of diluents was added to both of these flasks and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution 1 and 2. (200µg/ml of Ivabradine and 100µg/ml of Metoprolol).

Preparation of Standard working solutions (100% solution): 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (20µg/ml of Ivabradine and 100µg/ml of Metoprolol).

Preparation of Sample stock solutions: 10 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 50 ml volumetric flask, 25ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (100µg/ml of Ivabradine and 500µg/ml of Metoprolol).

Preparation of Sample working solutions (100% solution): 2ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (20µg/ml of Ivabradine and 100µg/ml of Metoprolol).

Solution preparation for Accuracy

Preparation of Standard stock solutions: Accurately weighed 5 mg of Ivabradine, 25 mg of Metoprolol and transferred to individual 25 ml volumetric flasks separately. 3/4 th of diluents was added to both of these flasks and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution 1 and 2. (200µg/ml of Ivabradine and 1000µg/ml of Metoprolol).

Preparation of 50% Spiked Solution: 0.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 100% Spiked Solution: 1.0ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.
**Preparation of 150% Spiked Solution:** 1.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

**LOD sample Preparation:** 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flasks and made up with diluents. From the above solutions 0.1ml each of Ivabradine, Metoprolol, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents.

**LOQ sample Preparation:** 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flask and made up with diluent. From the above solutions 0.3ml each of Ivabradine, Metoprolol, and solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluent.

**Optimized chromatographic conditions**
After systematic and detailed study of the various parameters involved in the method, the following conditions were employed.

- **Mobile phase**: 50% 0.01N KH2PO4 buffer: 50% Acetonitrile v/v
- **Flow rate**: 0.8 ml/min
- **Column**: Agilent C18 (4.6 x 150mm, 5µm)
- **Detector wave length**: 260.0nm
- **Column temperature**: 30°C
- **Injection volume**: 10µL
- **Run time**: 6min
- **Diluent**: 0.01N KH2PO4 and Acetonitrile in the ratio 50:50 v/v

**Procedure**
Column was equilibrated for at least 60 minutes with the mobile phase flowing through the system at a rate of 0.8ml/min. Detector was set at a wavelength of 260nm. Separately Injected 10µL of diluent, placebo, standard solution, test solutions into the system and the chromatograms were recorded. The percent assay values of the Ivabradine and Metoprolol were calculated by using the following formula.
% Assay

\[
\begin{array}{cccccc}
\text{AT} & \text{WS} & \text{DT} & \text{P} & \text{Avg. Wt} \\
\text{AS} & \text{DS} & \text{WT} & 100 & \text{Label Claim}
\end{array}
\]

Where
AT = Peak Area of Metoprolol/Ivabradine obtained with test preparation
AS = Peak Area of Metoprolol/Ivabradine obtained with standard preparation
WS = Weight of working standard taken in mg
WT = Weight of sample taken in mg
DS = Dilution of Standard solution
DT = Dilution of sample solution
P = Percentage purity of working standard

Figure 3: Chromatogram of blank.

Figure 4: Chromatogram of placebo.
Analytical method validation

System suitability
To ascertain its effectiveness 10μL of freshly prepared standard solution containing Ivabradine 20μg/ml and Metoprolol 100 μg/ml was injected 6 times into the HPLC system by using optimized chromatographic conditions and System suitability results were calculated. All the results were tabulated in the below table.

Table 4: System suitability parameters for Metoprolol and Ivabradine.

<table>
<thead>
<tr>
<th>Sr no</th>
<th>Inj</th>
<th>Metoprolol</th>
<th></th>
<th></th>
<th>Ivabradine</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>RT(min)</td>
<td>USP Plate Count</td>
<td>Tailing</td>
<td>RT(min)</td>
<td>USP Plate Count</td>
<td>Tailing</td>
<td>Resolution</td>
</tr>
<tr>
<td>1</td>
<td>2.458</td>
<td>8018</td>
<td>1.32</td>
<td>3.309</td>
<td>8050</td>
<td>1.32</td>
<td>6.4</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2.458</td>
<td>6973</td>
<td>1.37</td>
<td>3.311</td>
<td>8931</td>
<td>1.34</td>
<td>6.5</td>
<td></td>
</tr>
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<td>3</td>
<td>2.461</td>
<td>7931</td>
<td>1.31</td>
<td>3.311</td>
<td>8592</td>
<td>1.31</td>
<td>6.4</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2.462</td>
<td>7483</td>
<td>1.29</td>
<td>3.313</td>
<td>8366</td>
<td>1.30</td>
<td>6.5</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>2.463</td>
<td>7630</td>
<td>1.29</td>
<td>3.314</td>
<td>8476</td>
<td>1.30</td>
<td>6.5</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>2.463</td>
<td>7533</td>
<td>1.28</td>
<td>3.315</td>
<td>8534</td>
<td>1.30</td>
<td>6.5</td>
<td></td>
</tr>
</tbody>
</table>

Fig 6: System suitability Chromatogram.
Interference from degradation product

Preparation of degradation sample

Preparation of sample for acid degradation: Metoprolol and Ivabradine sample was refluxed with the 1M HCl at 60°C for 1 hour and then neutralized with 1N NaOH. The sample was prepared as per the test method and then further diluted up to the required concentration with the diluent.

Preparation of sample for alkaline degradation: Metoprolol and Ivabradine sample was refluxed with the 1M NaOH at 60°C for 1 hour and then neutralized with 1N HCl. The sample was prepared as per the test method and then further diluted up to the required concentration with the diluents.

Preparation of sample for peroxide degradation: Metoprolol and Ivabradine sample was refluxed with the 10% H2O2 by heating on water bath at 60°C for 1 hour. The sample was prepared as per the test method and then further diluted up to the required concentration with the diluent.

Preparation of sample for UV degradation: Metoprolol and Ivabradine sample was exposed to UV (200 watt-hr/m2) and visible (1.2 million lux hrs) The sample was prepared as per the test method and then further diluted up to the required concentration with the diluent.

Preparation of sample for thermal degradation: Metoprolol and Ivabradine sample was exposed to temperature at 105°C for 24 hrs. The sample was prepared as per the test method and then further diluted up to the required concentration with the diluent.

Preparation of sample for humidity degradation: Metoprolol and Ivabradine sample was exposed to 85% humidity for 24 hrs. The sample was prepared as per the test method and then further diluted up to the required concentration with the diluent.

All the stressed samples were injected into the HPLC system by using optimized chromatographic conditions and the chromatograms were recorded. The chromatograms of the stressed samples were evaluated for peak purity of the drug using PDA detector and Empower software. In all forced degradation samples all the three drugs passed the peak purity (purity angle is less than purity threshold). All the degradant peaks were observed for the drug. Thus the method can be used for estimation of lornoxicam in bulk and pharmaceutical formulations and also the method is stability indicating.
Table 3: Degradation data.

<table>
<thead>
<tr>
<th>Type of degradation</th>
<th>Metoprolol</th>
<th>Ivabradine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AREA</td>
<td>%RECOVERED</td>
</tr>
<tr>
<td>Acid</td>
<td>1180726</td>
<td>94.64</td>
</tr>
<tr>
<td>Alkaline</td>
<td>1165907</td>
<td>93.45</td>
</tr>
<tr>
<td>Peroxide</td>
<td>1206990</td>
<td>96.74</td>
</tr>
<tr>
<td>Thermal</td>
<td>1212360</td>
<td>97.17</td>
</tr>
<tr>
<td>Uv</td>
<td>1231187</td>
<td>98.68</td>
</tr>
<tr>
<td>Humidity</td>
<td>1239663</td>
<td>98.68</td>
</tr>
</tbody>
</table>

Degradation chromatograms

Acid degradation chromatogram

Fig 7. Acid.

Base degradation chromatogram

Fig8. base.
Peroxide degradation chromatogram

Fig 9: peroxide.

Thermal degradation chromatogram

Fig 10: thermal.

Uv degradation chromatogram

Fig 11: UV.
Water degradation chromatogram

Method precision
From a single volumetric flask of working standard solution six injections were given and the obtained areas were mentioned in table. Average area, standard deviation and % RSD were calculated for two drugs. % RSD obtained as 0.5% and 0.6% respectively for Metoprolol and Ivabradine. As the limit of Precision was less than “2” the system precision was passed in this method.

Table 4: System precision table of Metoprolol and Ivabradine.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Area of Metoprolol</th>
<th>Area of Ivabradine</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>1239246</td>
<td>476109</td>
</tr>
<tr>
<td>2.</td>
<td>1252215</td>
<td>469516</td>
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<tr>
<td>3.</td>
<td>1242319</td>
<td>470850</td>
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<td>4.</td>
<td>1246866</td>
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<td>5.</td>
<td>1233067</td>
<td>472074</td>
</tr>
<tr>
<td>6.</td>
<td>1242272</td>
<td>477400</td>
</tr>
<tr>
<td>Mean</td>
<td>1242664</td>
<td>473192</td>
</tr>
<tr>
<td>S.D</td>
<td>6525.9</td>
<td>3048.4</td>
</tr>
<tr>
<td>%RSD</td>
<td>0.5</td>
<td>0.6</td>
</tr>
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</table>

LOD AND LOQ
A study to establish the limit of detection and limit of quantification of Metoprolol and Ivabradine was conducted. Limit of detection and limit and quantification were established based on signal to noise ratio. A series of dilutions of the test solution were injected. Limit of detection was established by identifying the concentration which gives signal to noise ratio of about 3. Limit of quantification was established by identifying the concentration which gives signal to noise ratio of about 10.
Table 5: Sensitivity table of Metoprolol and Ivabradine.

<table>
<thead>
<tr>
<th>Molecule</th>
<th>LOD</th>
<th>LOQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metoprolol</td>
<td>0.41</td>
<td>1.23</td>
</tr>
<tr>
<td>Ivabradine</td>
<td>0.28</td>
<td>0.85</td>
</tr>
</tbody>
</table>

Fig13. LOD Chromatogram of Standard.

Fig14. LOQ Chromatogram of Standard
Accuracy

Three levels of Accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean % Recovery was obtained as 100.14% and 100.01% for Metoprolol and Ivabradine respectively.

Table 6: Accuracy table of Metoprolol.

<table>
<thead>
<tr>
<th>% Level</th>
<th>Amount Spiked (μg/mL)</th>
<th>Amount recovered (μg/mL)</th>
<th>% Recovery</th>
<th>Mean % Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>50%</td>
<td>50</td>
<td>49.56</td>
<td>99.13</td>
<td></td>
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<tr>
<td></td>
<td>50</td>
<td>49.67</td>
<td>99.34</td>
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<td></td>
<td>50</td>
<td>50.03</td>
<td>100.06</td>
<td></td>
</tr>
<tr>
<td>100%</td>
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<td>150</td>
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<td></td>
<td>150</td>
<td>148.83</td>
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</tr>
</tbody>
</table>

Table 7: Accuracy table of Ivabradine.

<table>
<thead>
<tr>
<th>% Level</th>
<th>Amount Spiked (μg/mL)</th>
<th>Amount recovered (μg/mL)</th>
<th>% Recovery</th>
<th>Mean % Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>50%</td>
<td>10</td>
<td>10.05</td>
<td>100.46</td>
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<td></td>
<td>10</td>
<td>9.92</td>
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<tr>
<td>100%</td>
<td>20</td>
<td>20.19</td>
<td>100.94</td>
<td>100.01%</td>
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<td>150%</td>
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<td></td>
<td>30</td>
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</table>

Chromatogram of Accuracy
Fig No15. Accuracy 50% Chromatogram of Metoprolol and Ivabradine

Fig. no. 16: Accuracy 100% chromatogram of Metoprolol & Ivabradine
Fig No17. Accuracy 150% Chromatogram of Metoprolol and Ivabradine.

**Linearity and Range**

Linearity of the detector response was established by plotting a graph of concentration versus peak area. A series of solutions of standard were prepared by appropriate dilutions of linearity standard stock solution.

**Solution preparation for Linearity**

**Preparation of Standard stock solutions:** Accurately weighed 5 mg of Ivabradine, 25 mg of Metoprolol and transferred to individual 25 ml volumetric flasks separately. 3/4 th of diluents was added to both of these flasks and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution 1 and 2. (200µg/ml of Ivabradine and 1000µg/ml of Metoprolol).

**25% Standard solution:** 0.25ml each from two standard stock solutions was pipetted out and made up to 10ml. 5µg/ml of Ivabradine and 25 µg/ml of Metoprolol).
50% **Standard solution:** 0.5ml each from two standard stock solutions was pipetted out and made up to 10ml. (10µg/ml of Ivabradine and 50µg/ml of Metoprolol).

75% **Standard solution:** 0.75ml each from two standard stock solutions was pipetted out and made up to 10ml. (15µg/ml of Ivabradine and 75µg/ml of Metoprolol).

100% **Standard solution:** 1.0ml each from two standard stock solutions was pipetted out and made up to 10ml. (20µg/ml of Ivabradine and 100µg/ml of Metoprolol).

125% **Standard solution:** 1.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (25µg/ml of Ivabradine and 125µg/ml of Metoprolol).

150% **Standard solution:** 1.5ml each from two standard stock solutions was pipetted out and made up to 10ml (30µg/ml of Ivabradine and 150µg/ml of Metoprolol).

**Table 8: Linearity table for Metoprolol and Ivabradine.**

<table>
<thead>
<tr>
<th></th>
<th>Metoprolol</th>
<th></th>
<th>Ivabradine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conc (µg/mL)</td>
<td>Peak area</td>
<td>Conc (µg/mL)</td>
<td>Peak area</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
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![Fig No18. Calibration curve of Metoprolol.](image-url)
Chromatogram of Linearity

Fig. No19. Calibration curve of Ivabradine

Fig. No20. Linearity 25% Chromatogram of Metoprolol and Ivabradine.
Fig No. 6.18 Linearity 50% Chromatogram of Metoprolol and Ivabradine.

Fig No. 21. Linearity 75% Chromatogram of Metoprolol and Ivabradine.

Fig No. 22: Linearity 100% chromatogram of Metoprolol & Ivabradine
Robustness

Robustness conditions like Flow minus (0.7ml/min), Flow plus (0.9ml/min), mobile phase minus (55B:45A), mobile phase plus (45B:55A), temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.
Table 9: Robustness data for Metoprolol and Ivabradine.

<table>
<thead>
<tr>
<th>S.no</th>
<th>Condition</th>
<th>% RSD of Metoprolol</th>
<th>% RSD of Ivabradine</th>
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<tr>
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<td>Flow rate (-) 0.7ml/min</td>
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<tr>
<td>2</td>
<td>Flow rate (+) 0.9ml/min</td>
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<td>3</td>
<td>Mobile phase (-) 55B:45A</td>
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<tr>
<td>4</td>
<td>Mobile phase (+) 45B:55A</td>
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<tr>
<td>5</td>
<td>Temperature (-) 25°C</td>
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<tr>
<td>6</td>
<td>Temperature (+) 35°C</td>
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<td>0.6</td>
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</table>

Chromatogram of Robustness

Fig No24. Flow minus Chromatogram of Metoprolol and Ivabradine.
Fig No25. Flow plus Chromatogram of Metoprolol and Ivabradine.

Fig No26. Mobile phase minus Chromatogram of Metoprolol and Ivabradine.
Fig No. 27. Mobile phase Plus Chromatogram of Metoprolol and Ivabradine.
RESULT AND DISCUSSION

The drug solution was scanned from 200-400 nm, it was observed that the drug show appreciable absorbance at 260nm., hence detection was set at 260nm for method development. Attempts were made to get good separation of the drug by varying parameters like, flow rate, pH, buffer molarity, buffer components, type of organic modifier, gradient times, and buffer: organic modifier ratio and could get good elution time of Metoprolol and Ivabradine in isocratic mode. To achieve this, experiments were conducted by changing the columns and
run time, flow rate but unsuccessful in getting good peaks with less run time. Then method was optimized to separate the main peak.

The satisfactory chromatographic separation, with good peak shapes were achieved on Agilent C18 (4.6 x 150mm, 5µm) column and mobile phase 0.01N KH2PO4 buffer: Acetonitrile (50:50) with a flow rate of 0.8 ml/min. All the System Suitability parameters are within the acceptance limits. The calibration curve for Metoprolol and Ivabradine was obtained by plotting the respective peak areas against their concentration. The graph was found to be linear over the range 25-150µg/ml and 5-30µg/ml for Metoprolol and Ivabradine with the correlation coefficient 0.999 for two drugs which shows that the good correlation exists between peak area and concentration of the drug. This is precise. The high % recovery values obtained for these drugs show that the method is accurate. The LOD value of Metoprolol and Ivabradine was found to be 0.41µg/ml and 0.28µg/ml respectively. The LOQ was 1.23µg/ml and 0.85µg/ml respectively. The low values of LOD and LOQ show that the method is sensitive and can estimate at micro gram level. The absence of additional peaks indicates the method is specific and the drug was stable in the diluents for 24 hours which is sufficient to complete the work. Also robustness values found within the limit. The stability indicating studies were performed for the above mentioned drug viz…. acid, alkali, thermal, humidity, UV, peroxide and the percentage degradation of Metoprolol was found 5.36%, 6.55%, 2.83%,1.32%,1.32%,3.26% And the percentage degradation of Ivabradine was found 8.31%, 7.16%, 2.41%, 0.58%, 1.23%, 4.28% respectively.

CONCLUSION
The proposed RP-high-performance liquid chromatographic method has been evaluated for the accuracy, precision and linearity. The method was found to be precise, accurate and linear over the linear concentration range. In this method, there was no interference from matrix sources. Moreover, the lower solvent consumption along with the short analytical run time of 8 minutes that allows the analysis of a large number of samples in a short period of time. Therefore, this HPLC method can be used as a routine analysis of the drugs in, pharmaceutical formulations and also for stability studies.
REFERENCE

16. https://www.drugbank.ca/drugs/DB09083
18. https://www.drugbank.ca/drugs/DB00264


