Development and validation of reverse phase high performance liquid chromatographic method for the estimation of metoprolol tartrate tablets 50, 100 mg

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ABSTRACT:
A new simple, rapid, specific, precise, accurate and reproducible reverse phase high performance liquid chromatographic stability indicating method had been developed and validated for quantitative determination of Metoprolol in Metoprolol tartrate tablets. The separation method developed produce acceptable values of recovery. The chromatogram developed has well resolved peak of Metoprolol without any interference. Sample preparation was assured by powdered method. Separation occurred on symmetry C₁₈ RP column (150 x 4.6 mm) 5 µm with a mobile phase of phosphate buffer pH- 3.0 and Acetonitrile (80:20% v/v) and detection at 275 nm in 0.1N HCl as diluents. The standard curve was linear over the concentration range of 100.52-603.11 ppm. All the degradation peaks were resolved effectively using developed method with different retention times. The developed method was validated according to ICH Q2-R1 guidelines. The lower limit of quantification for Metoprolol tartrate was 186ppm. As the method could effectively separates the degradation products from active ingredients, it can be used for industrial analysis purpose for the analysis of Metoprolol tartrate tablet BP 50, 100 mg.

Key words: Metoprolol Tartrate, Analytical Method development, Analytical method validation.

INTRODUCTION
Metoprolol tartrate is available as 50- and 100-mg tablets for oral administration. Metoprolol tartrate USP is a white, practically odorless, crystalline powder with a molecular weight of 684.82. It is very soluble in water; freely soluble in methylene chloride, in chloroform, and in alcohol; slightly soluble in acetone; and insoluble in ether.

Fig. 1.1 Structure of Metoprolol tartrate

MATERIALS AND METHODS

MATERIALS
Metoprolol tartrate WRS (potency-99.7%), Milli-Q water, Methanol HPLC grade, Acetonitrile HPLC grade, Hydrochloric acid, Ortho phosphoric acid, Potassium dihydrogen phosphate AR, Ammonium acetate AR, Acetic acid.

METHOD DEVELOPMENT AND OPTIMIZATION TRAILS
Trail-1: Metoprolol tartrate tablets HPLC method development by using RS-methodology of Metoprolol tartrate tablets given in BP.
Parameters:
1. Column: Symmetry C$_{18}$ (150×3.9) mm,5µ
2. Flow rate: 1.0 mL/min
3. Injection volume: 25µL
4. Wave length: 275nm
5. Column temperature: 25°C
6. Mobile phase: 50ml of 1M monobasic sodium phosphate (NaH$_2$Po$_4$) + 8.0ml of phosphoric acid in 900ml of water. Adjust pH 3.0 with 1M OPA or 1M NaH$_2$Po$_4$. Make up to 1000ml with water.
7. Mix buffer and acetonitrile in ratio 75:25 v/v
8. Diluent 1: mobile phase
9. Diluent 2: 0.1% OPA and methanol in ratio 75:25 v/v

**Standard preparation:**
50.66 → 100ml, 5ml → 50ml with diluent-1
50.83 → 100ml, 5ml → 50ml with diluent-2

**Test preparation:**
Placebo: 339.6mg placebo powder of 50mg tablets is taken in 500ml volumetric flask. 350ml diluent -1 is added and sonicate for 30min with intermittent swirling. Make up to the volume with diluent-1. Filter the solution through 0.45µ PVDF membrane filter.
Sample: 444.04mg sample powder is taken in 500ml volumetric flask. 350ml diluent -1 is added and sonicate for 30min with intermittent swirling. Make up to the volume with diluent-1. Filter the solution through 0.45µ PVDF membrane filter.

**Preparation of impurities**
Impurities are prepared as mentioned in earlier and all the impurities are injected individually at spec level to check the retention time.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>NAME</th>
<th>RT</th>
<th>Amount present</th>
<th>Percentage purity</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Metoprolol sample-50mg</td>
<td>3.02</td>
<td>50.58</td>
<td>101.2</td>
<td>1250890</td>
</tr>
<tr>
<td>2</td>
<td>Metoprolol sample-1’00mg</td>
<td>3.02</td>
<td>101.74</td>
<td>101.7</td>
<td>1254136</td>
</tr>
</tbody>
</table>

**Impurity specificity checking**
Inference:
In this mobile phase recovery is better and all impurities were specific with main peak except impurity J. So mobile phase has to be modified to attain specificity with respect to impurity J.

**Trial – 2:** Modification mobile phase ration to attain specificity towards impurity J.

**Mobile phase:** 50ml of 1M monobasic sodium phosphate (NaH$_2$PO$_4$) + 8.0ml of phosphoric acid in 900ml of water. Adjust pH 3.0 with 1M OPA or 1M NaH$_2$PO$_4$. Make up to 1000ml with water.

Mix buffer and acetonitrile in ratio 80:20v/v

Inference:
Metoprolol tartrate peak is eluted at 5.4min and impurity J is eluted at 6.2min. Hence by changing the proportion from 75:25 to 80:20 of inorganic (buffer) and organic (ACN) ratio,
Metoprolol peak and impurity J separation is achieved. And recovery seems to be better in 0.1N HCl than in mobile phase. Hence 0.1N HCl is finalized as diluent for Metoprolol tartrate tablets assay.

Finalized Parameters for Metoprolol Tartrate Tablets Assay:
1. Column: Symmetry C18 (150×3.9)mm,5µ
2. Flow rate:1.0 mL/min
3. Injection volume: 15µL
4. Wave length: 275nm
5. Column temperature: 25°C
6. Mobile phase: 50ml of 1M monobasic sodium phosphate (NaH₂PO₄) + 8.0ml of phosphoric acid in 900ml of water. Adjust pH 3.0 with 1M OPA or 1M NaH₂PO₄. Make up to 1000ml with water.
7. Mix buffer and acetonitrile in ratio 80:20v/v
8. Diluent 1: 0.1 N HCl

Further validation has done with the finalized method parameters.

METHOD VALIDATION
Accuracy:
Sample solutions were prepared using an equivalent amount of placebo and API present in Metoprolol tartrate tablets and analyzed as per methodology. The recovery levels include 100% of test concentration in duplicate preparation and injected into HPLC.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>% Assay</th>
<th>Statistical analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>101.0</td>
<td>Mean</td>
</tr>
<tr>
<td>2</td>
<td>101.4</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>101.2</td>
<td>SD</td>
</tr>
<tr>
<td>4</td>
<td>101.4</td>
<td>0.24</td>
</tr>
<tr>
<td>5</td>
<td>101.5</td>
<td>%RSD</td>
</tr>
<tr>
<td>6</td>
<td>101.7</td>
<td>0.24</td>
</tr>
</tbody>
</table>

Method precision for 50mg tablets

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>% Assay</th>
<th>Statistical analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>101.4</td>
<td>Mean</td>
</tr>
<tr>
<td>2</td>
<td>101.6</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>101.5</td>
<td>SD</td>
</tr>
<tr>
<td>4</td>
<td>101.4</td>
<td>0.15</td>
</tr>
<tr>
<td>5</td>
<td>101.6</td>
<td>%RSD</td>
</tr>
<tr>
<td>6</td>
<td>101.2</td>
<td>0.15</td>
</tr>
</tbody>
</table>

Method precision for 50mg tablets

Acceptance criteria:
RSD of assay result should be not more than 2%

Linearity
The linearity is determined from 50% of the ICH reporting level to 150 % of the proposed shelf life specifications of the related substance as a minimum.

<table>
<thead>
<tr>
<th>Range</th>
<th>Dilution in mL</th>
<th>Concentration (ppm)</th>
<th>Avg Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>25%</td>
<td>1.00</td>
<td>20</td>
<td>100.52</td>
</tr>
<tr>
<td>50%</td>
<td>2.00</td>
<td>20</td>
<td>201.04</td>
</tr>
<tr>
<td>75%</td>
<td>3.00</td>
<td>20</td>
<td>301.55</td>
</tr>
<tr>
<td>100%</td>
<td>4.00</td>
<td>20</td>
<td>402.07</td>
</tr>
<tr>
<td>125%</td>
<td>5.00</td>
<td>20</td>
<td>502.59</td>
</tr>
<tr>
<td>150%</td>
<td>6.00</td>
<td>20</td>
<td>603.11</td>
</tr>
</tbody>
</table>

Acceptance Criteria:
Recovery should be in the range of 95.0% to 105.0%

Precision:
Assay was performed for six units of Metoprolol tartrate tablets as per test method for both 100 and 50mg tablets and injected each solution into HPLC.

![Linearity Graph](image)

**Fig. 1.17 Linearity Graph**

**Limit of Detection**
From the above Linearity calibration curve Limit of Detection and Limit of Quantitation were established.
The detection limit (DL) may be expressed as:
\[
DL = 3.3 \frac{s}{S}
\]
Where \(s\) = the standard deviation of y-intercepts of regression lines
\(S\) = the slope of the calibration curve
By the above calibration curve,
Standard deviation (s) = 658353.789
Slope (S) = 35231
DL = 3.3×658353.789/35231 = 61.67 ppm

Limit of Quantitation
The Quantitation limit (QL) may be expressed as:
QL = 10 s/S
Where s = the standard deviation of the response
S = the slope of the calibration curve
Standard deviation (s) = 658353.789
Slope (S) = 35231
QL = 10×658353.789/3523 = 186 ppm

Specificity
Placebo interference: Placebo solution was prepared in actual concentration of sample and injected into HPLC as per test procedure.
Impurity interference: Injected individual impurities for retention time check. Prepared all impurity mixture at spec level and injected into HPLC as per test procedure.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Variation</th>
<th>Impurity E</th>
<th>Impurity F</th>
<th>Impurity G</th>
<th>Impurity H</th>
<th>Impurity J</th>
</tr>
</thead>
<tbody>
<tr>
<td>As such</td>
<td>-</td>
<td>8.34</td>
<td>4.21</td>
<td>3.17</td>
<td>2.13</td>
<td>6.36</td>
</tr>
<tr>
<td>Organic</td>
<td>(-)20ml</td>
<td>11.44</td>
<td>5.32</td>
<td>3.50</td>
<td>2.36</td>
<td>8.70</td>
</tr>
<tr>
<td></td>
<td>(+)20ml</td>
<td>5.89</td>
<td>3.38</td>
<td>2.84</td>
<td>1.70</td>
<td>4.58</td>
</tr>
<tr>
<td>flow</td>
<td>(-)0.2ml</td>
<td>10.41</td>
<td>5.25</td>
<td>3.94</td>
<td>2.64</td>
<td>7.92</td>
</tr>
<tr>
<td></td>
<td>(+)0.2ml</td>
<td>7.01</td>
<td>3.55</td>
<td>2.96</td>
<td>1.80</td>
<td>5.36</td>
</tr>
</tbody>
</table>

SUMMARY AND CONCLUSION
A rapid, sensitive and selective RP-HPLC method for the determination of Metoprolol tartrate in tablets was developed and validated. Sample preparation was assured by powdered method. Separation occurred on symmetry C18 RP column (150 x 4.6 mm) 5µm with a mobile phase of phosphate buffer pH 3.0 and Acetonitrile (80:20% v/v) and detection at 275nm in 0.1N HCl as diluents. The standard curve was linear over the concentration range of 100.52-603.11ppm. The lower limit of quantification for Metoprolol tartrate was 186ppm. This method was successfully applied to the industrial analysis purpose for the analysis of Metoprolol tartrate tablet BP 50, 100mg. The analytical method developed is simple and shows good accuracy, specificity and reproducible. It can be used for the estimation of Metoprolol in Metoprolol tartrate tablets. The separation method developed produce acceptable values of recovery. The chromatogram developed has well resolved peak of Metoprolol without any interference.
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