The HPLC methods developed and presented were aimed at developing a chromatographic system capable of resolving the drug peak from all of its degradation product peaks.

The preliminary investigations were directed towards the effect of various variables on the system suitability of the method. The parameters assessed include the detection wavelength, the type and quantity of organic modifier, the column, concentration of buffer, flow rate and the pH of the mobile phase. The analytical wavelength was finalized by taking into consideration of low interference of solvent, placebo and optimum response of the drug and degradation products at various wavelength. Column for the method was finalized by having maximum no. of theoretical plates, least tailing, best peak shape, good separation and less runtime. In choosing mobile phase importance is given to achieve baseline separation of the drug and its degradation products. pH effect on buffer was also studied to achieve good separation between peaks and does not effect the separation even if there is slight deviation in the pH. Above all due importance is given on the total cost involved, as the methods are to be used for routine analysis, stability analysis where large number of samples are to analysed. Priority is given to choose the materials which are easily available and low cost, where as quality of the analyis is not affected.
Results and Discussion

Consequently, the optimum condition mentioned under section experiment were applied and the method were subjected to validations according to the ICH guidelines. The validation data obtained for each drug have been discussed.

5.1 Candesartan

The retention time of candesartan peak was about 11.3 min. The relative standard deviation of the area of the candesartan peak for replicate injections was found to be less than 0.18%. The column efficiency was measured by no. of theoretical plates which was found to be greater than 8000 and the tailing factor of 1.17. Typical chromatograph of candesartan is shown in figure 5.1F1.

Figure 5.1F1 : Typical chromatograph of Candesartan

Method precision shows a mean of 93.64% label claim with a RSD of 0.59% was obtained. The mean recovery data for each level is within accepted values (100.22, 99.03 and 98.26 % label claim for 80,100 and 120%level respectively). Therefore, these results indicated a good accuracy of the method for Candesartan. The mean recovery was 99.17 % label claim and % RSD was 0.99.

The method was shown to be linear from 25.04 to 75.12 μg/ml of candesartan concentration. A calibration curve was constructed using characteristic
parameters for regression equation \( Y = a + bx \) and coefficient of correlation \( r^2 \) was found to be 1.000. The linearity graph is shown in figure 5.1F2.

**Figure 5.1F2 : Linearity graph of Candesartan**

![Linearity graph of Candesartan](image)

All the degradation peaks generated in the forced degradation studies were well separated and peak purity of candesartan peak was always greater than 99.0 % proving the stability indicating nature of the method. Major degradation was observed under acidic, alkaline and oxidative degradation conditions. Typical chromatographs obtained in degradation study are shown in figure 5.1F3.

**Figure 5.1F3 : Specificity study chromatographs of Candesartan**

![Specificity study chromatographs of Candesartan](image)
Results and Discussion

Alkali degradation

Peroxide degradation

Thermal degradation

Bipin Bihari P.G College, Bundelkhand University, Jhansi, U.P
Results and Discussion

Sun-light degradation
The result obtained from degradation study shows peak purity of Candesertan was 100 % as calculated by PDA detector, proving that no degradation product is interfering with the main peak. The % residual drug was calculated in comparison with the standard, which is 55.71, 62.16, 79.89, 91.15 and 86.87% for Acid, Alkali, Peroxide, Thermal and sunlight degradation respectively.

The result obtained during robustness shows that by changing deliberately, some internal and external parameters of the method does not influence the results. solution stability of 14 hrs was observed.

Table 5.1.1 : Summary of the performance parameters of the HPLC procedure for Candesertan Tablets

<table>
<thead>
<tr>
<th>S.no</th>
<th>Parameters</th>
<th>Observed value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>System Suitability</td>
<td></td>
</tr>
<tr>
<td></td>
<td>a. Theoretical Plates</td>
<td>8155</td>
</tr>
<tr>
<td></td>
<td>b. Tailing Factor</td>
<td>1.17</td>
</tr>
<tr>
<td>2.</td>
<td>Instrument Precision</td>
<td>RSD 0.18 %</td>
</tr>
<tr>
<td>3.</td>
<td>Method Precision</td>
<td>Label claim 93.64 %</td>
</tr>
<tr>
<td>4.</td>
<td>Linearity and range</td>
<td>Correlation coefficient(r²) = 1.0000</td>
</tr>
<tr>
<td>5.</td>
<td>Accuracy</td>
<td>Mean recovery 99.17%</td>
</tr>
<tr>
<td>6.</td>
<td>Specificity</td>
<td>Peak Purity of candesertan peak</td>
</tr>
<tr>
<td></td>
<td></td>
<td>after degradation was 100.0 %</td>
</tr>
<tr>
<td>7.</td>
<td>Robustness</td>
<td>Difference from original condition</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.44%</td>
</tr>
<tr>
<td>8.</td>
<td>Solution stability</td>
<td>14 hrs</td>
</tr>
</tbody>
</table>
5.2 Captopril

The retention time of Captopril peak was about 6.4 min. The relative standard deviation of the area of the Captopril peak for replicate injections was found to be less than 0.61%. The column efficiency was measured by no. of theoretical plates which was found to be greater than 5400 and the tailing factor of 0.99. Typical chromatograph of Captopril is shown in figure 5.2F1.

Figure 5.2 F1: Typical chromatograph of Captopril

Method precision shows a mean of 100.19 % label claim with a RSD of 0.43% was obtained. The mean recovery data for each level is within accepted values (99.93, 99.17 and 99.20 % label claim for 80,100 and 120% level respectively). Therefore, these results indicated a good accuracy of the method for Captopril. The mean recovery was 99.43 % label claim and % RSD was 0.43.

The method was shown to be linear from 25.12 to 75.36 μg/ml of Captopril concentration. A calibration curve was constructed using characteristic parameters for regression equation \( Y= a + bx \) and coefficient of correlation \( r^2 \) was found to be 1.000. The linearity graph is shown in figure 5.2F2.
Results and Discussion

Figure 5.2F2: Linearity graph of Captopril

All the degradation peaks generated in the forced degradation studies were well separated and peak purity of Captopril peak was always greater than 99.0% proving the stability indicating nature of the method. Major degradation was observed under acidic, alkaline and oxidative degradation conditions. Typical chromatographs obtained in degradation study are shown in figure 5.2F3.

Figure 5.2F3: Specificity study chromatographs of Captopril

Acid degradation
Results and Discussion

Alkali degradation

Peroxide degradation

Thermal degradation

Bipin Bihari P.G College, Bundelkhand University, Jhansi, U.P
Results and Discussion

Sun-light degradation

The result obtained from degradation study shows peak purity of Captopril was 100 % as calculated by PDA detector, proving that no degradation product is interfering with the main peak. The % residual drug was calculated in comparison with the standard, which is 79.91, 17.30, 30.91, 95.86 and 97.06% for Acid, Alkali, Peroxide, Thermal and sunlight degradation respectively.

The result obtained during robustness shows that by changing deliberately, some internal and external parameters of the method does not influence the results. Solution stability of 19 hrs was observed.

Table 5.2.1: Summary of the performance parameters of the HPLC procedure for Captopril Tablets

<table>
<thead>
<tr>
<th>S.no</th>
<th>Parameters</th>
<th>Observed value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>System Suitability</td>
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</tr>
<tr>
<td></td>
<td>a. Theoretical Plates</td>
<td></td>
</tr>
<tr>
<td></td>
<td>b. Tailing Factor</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5400</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.99</td>
</tr>
<tr>
<td>2.</td>
<td>Instrument Precision</td>
<td>RSD 0.61 %</td>
</tr>
<tr>
<td>3.</td>
<td>Method Precision</td>
<td>Label claim 100.19 %</td>
</tr>
<tr>
<td>4.</td>
<td>Linearity and range</td>
<td>Correlation coefficient($r^2$) = 1.0000</td>
</tr>
<tr>
<td>5.</td>
<td>Accuracy</td>
<td>Mean recovery 99.43%</td>
</tr>
<tr>
<td>6.</td>
<td>Specificity</td>
<td>Peak Purity of Captopril peak after degradation was 100.0 %</td>
</tr>
<tr>
<td>7.</td>
<td>Robustness</td>
<td>Difference from original condition 0.44%</td>
</tr>
<tr>
<td>8.</td>
<td>Solution stability</td>
<td>19 hrs</td>
</tr>
</tbody>
</table>
Results and Discussion

5.3 Propranolol

The retention time of Propranolol peak was about 8.5 min. The relative standard deviation of the area of the Propranolol peak for replicate injections was found to be less than 0.19%. The column efficiency was measured by no. of theoretical plates which was found to be greater than 8000 and the tailing factor of 1.31. Typical chromatograph of Propranolol is shown in figure 5.3F1.

Figure 5.3F1: Typical chromatograph of Propranolol

Method precision shows a mean of 100.43% label claim with a RSD of 0.48% was obtained. The mean recovery data for each level is within accepted values (101.4, 100.7 and 99.4% label claim for 80, 100 and 120% level respectively). Therefore, these results indicated a good accuracy of the method for Propranolol. The mean recovery was 100.5% label claim and % RSD was 1.03.

The method was shown to be linear from 50.1 to 150.2 µg/ml of Propranolol concentration. A calibration curve was constructed using characteristic parameters for regression equation \( Y = a + bx \) and coefficient of correlation \( r^2 \) was found to be 1.000. The linearity graph is shown in figure 5.3F2.
Results and Discussion

Figure 5.3F2: Linearity graph of Propranolol

All the degradation peaks generated in the forced degradation studies were well separated and peak purity of Propranolol peak was always greater than 99.0% proving the stability indicating nature of the method. Major degradation was observed under all studied degradation conditions. Typical chromatographs obtained in degradation study are shown in figure 5.3F3.

Figure 5.3F3: Specificity study chromatographs of Propranolol

Acid degradation

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Results and Discussion

Alkali degradation

Peroxide degradation

Thermal degradation
Results and Discussion

Sun-light degradation

The result obtained from degradation study shows peak purity of Propranolol was 100 % as calculated by PDA detector, proving that no degradation product is interfering with the main peak. The % residual drug was calculated in comparison with the standard, which is 88.6, 61.1, 86.4, 85.0 and 86.9% for Acid, Alkali, Peroxide, Thermal and sunlight degradation respectively.

The result obtained during robustness shows that by changing deliberately, some internal and external parameters of the method does not influence the results. Solution stability of 27 hrs was observed.

Table 5.3.1: Summary of the performance parameters of the HPLC procedure for Propranolol Tablets

<table>
<thead>
<tr>
<th>S.no</th>
<th>Parameters</th>
<th>Observed value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>System Suitability</td>
<td></td>
</tr>
<tr>
<td></td>
<td>a. Theoretical Plates</td>
<td></td>
</tr>
<tr>
<td></td>
<td>b. Tailing Factor</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Instrument Precision</td>
<td>RSD 0.19 %</td>
</tr>
<tr>
<td>3.</td>
<td>Method Precision</td>
<td>Label claim 100.43 %</td>
</tr>
<tr>
<td>4.</td>
<td>Linearity and range</td>
<td>Correlation coefficient((r^2)) = 1.0000</td>
</tr>
<tr>
<td>5.</td>
<td>Accuracy</td>
<td>Mean recovery 100.51 %</td>
</tr>
<tr>
<td>6.</td>
<td>Specificity</td>
<td>Peak Purity of propranolol peak after degradation was 100.0 %</td>
</tr>
<tr>
<td>7.</td>
<td>Robustness</td>
<td>Difference from original condition 0.15%</td>
</tr>
<tr>
<td>8.</td>
<td>Solution stability</td>
<td>27 hrs</td>
</tr>
</tbody>
</table>
5.4 Terazosin

The retention time of Terazosin peak was about 7.0 min. The relative standard deviation of the area of the Terazosin peak for replicate injections was found to be less than 0.64%. The column efficiency was measured by no. of theoretical plates which was found to be greater than 7000 and the tailing factor of 1.22. Typical chromatograph of Terazosin is shown in figure 5.4F1.

Figure 5.4F1: Typical chromatograph of Terazosin

Method precision shows a mean of 102.00% label claim with a RSD of 0.62% was obtained. The mean recovery data for each level is within accepted values (100.2, 98.6 and 99.8% label claim for 80, 100 and 120% level respectively). Therefore, these results indicated a good accuracy of the method for Terazosin. The mean recovery was 99.5% label claim and % RSD was 0.81.

The method was shown to be linear from 5.1 to 15.3 µg/ml of Terazosin concentration. A calibration curve was constructed using characteristic parameters for regression equation (Y = a + bx) and coefficient of correlation $r^2$ was found to be 1.000. The linearity graph is shown in figure 5.4F2.
Results and Discussion

Figure 5.4F2: Linearity graph of Terazosin

All the degradation peaks generated in the forced degradation studies were well separated and peak purity of Terazosin peak was always greater than 99.0% proving the stability indicating nature of the method. Major degradation was observed under all studied degradation conditions. Typical chromatographs obtained in degradation study are shown in figure 5.4F3.

Figure 5.4F3: Specificity study chromatographs of Terazosin

Acid degradation

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Results and Discussion

Alkali degradation

PDA-245 nm
ALKALI DEGRADATION
ISRA.14
Retention Time

Minutes

0 5 10 15 20 25 30

Peroxide degradation

PDA-245 nm
PEROXIDE DEGRADATION
ISRA.17
Retention Time

Minutes

0 5 10 15 20 25 30

Thermal degradation

PDA-245 nm
THERMAL DEGRADATION
ISRA.19
Retention Time

Minutes

0 5 10 15 20 25 30

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Results and Discussion

Sun-light degradation
The result obtained from degradation study shows peak purity of Terazosin was 100 % as calculated by PDA detector, proving that no degradation product is interfering with the main peak. The % residual drug was calculated in comparison with the standard, which is 78.1, 75.9, 94.6, 94.9 and 97.5% for Acid, Alkali, Peroxide, Thermal and sunlight degradation respectively.

The result obtained during robustness shows that by changing deliberately, some internal and external parameters of the method does not influence the results. solution stability of 18 hrs was observed.

Table 5.4.1 : Summary of the performance parameters of the HPLC procedure for Terazosin Tablets

<table>
<thead>
<tr>
<th>S.no</th>
<th>Parameters</th>
<th>Observed value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>System Suitability</td>
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</tr>
<tr>
<td></td>
<td>a. Theoretical Plates</td>
<td></td>
</tr>
<tr>
<td></td>
<td>b. Tailing Factor</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>7339</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.22</td>
</tr>
<tr>
<td>2.</td>
<td>Instrument Precision</td>
<td>RSD 0.64 %</td>
</tr>
<tr>
<td>3.</td>
<td>Method Precision</td>
<td>Label claim 102.00 %</td>
</tr>
<tr>
<td>4.</td>
<td>Linearity and range</td>
<td>Correlation coefficient($r^2$) = 1.0000</td>
</tr>
<tr>
<td>5.</td>
<td>Accuracy</td>
<td>Mean recovery 99.52%</td>
</tr>
<tr>
<td>6.</td>
<td>Specificity</td>
<td>Peak Purity of Terazosin peak after degradation was 100.0 %</td>
</tr>
<tr>
<td>7.</td>
<td>Robustness</td>
<td>Difference from original condition 0.20%</td>
</tr>
<tr>
<td>8.</td>
<td>Solution stability</td>
<td>18 hrs</td>
</tr>
</tbody>
</table>
Results and Discussion

5.5 Verapamil

The retention time of Verapamil peak was about 13.2 min. The relative standard deviation of the area of the Verapamil peak for replicate injections was found to be less than 0.27%. The column efficiency was measured by no. of theoretical plates which was found to be greater than 8000 and the tailing factor of 1.55. Typical chromatograph of Verapamil is shown in figure 5.5F1.

Figure 5.5F1: Typical chromatograph of Verapamil

![Chromatogram of Verapamil](image)

Method precision shows a mean of 103.3% label claim with a RSD of 0.24% was obtained. The mean recovery data for each level is within accepted values (101.0, 101.3 and 98.6% label claim for 80, 100 and 120% level respectively). Therefore, these results indicated a good accuracy of the method for Verapamil. The mean recovery was 100.3% label claim and % RSD was 1.5.

The method was shown to be linear from 100.5 to 301.4 μg/ml of Verapamil concentration. A calibration curve was constructed using characteristic parameters for regression equation (Y = a + bx) and coefficient of correlation $r^2$ was found to be 1.000. The linearity graph is shown in figure 5.5F2.
Results and Discussion

Figure 5.4F2 : Linearity graph of Verapamil

All the degradation peaks generated in the forced degradation studies were well separated and peak purity of Verapamil peak was always greater than 99.0 % proving the stability indicating nature of the method. Major degradation was observed under Alkali and Peroxide degradation conditions. Typical chromatographs obtained in degradation study are shown in figure 5.5F3.

Figure 5.5F3 : Specificity study chromatographs of Verapamil

Acid degradation
Results and Discussion

Alkali degradation

Peroxide degradation

Thermal degradation

Bipin Bihari P.G College, Bundelkhand University, Jhansi, U.P
Results and Discussion

Sun-light degradation

The result obtained from degradation study shows peak purity of Verapamil was 100 % as calculated by PDA detector, proving that no degradation product is interfering with the main peak. The % residual drug was calculated in comparison with the standard, which is 97.8, 80.9, 69.4, 99.3 and 100.6% for Acid, Alkali, Peroxide, Thermal and sunlight degradation respectively.

The result obtained during robustness shows that by changing deliberately, some internal and external parameters of the method does not influence the results. solution stability of 25 hrs was observed.

Table 5.5.1: Summary of the performance parameters of the HPLC procedure for Verapamil Tablets

<table>
<thead>
<tr>
<th>S.no</th>
<th>Parameters</th>
<th>Observed value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>System Suitability</td>
<td></td>
</tr>
<tr>
<td></td>
<td>a. Theoretical Plates</td>
<td>8155</td>
</tr>
<tr>
<td></td>
<td>b. Tailing Factor</td>
<td>1.55</td>
</tr>
<tr>
<td>2.</td>
<td>Instrument Precision</td>
<td>RSD 0.27 %</td>
</tr>
<tr>
<td>3.</td>
<td>Method Precision</td>
<td>Label claim 103.3 %</td>
</tr>
<tr>
<td>4.</td>
<td>Linearity and range</td>
<td>Correlation coefficient($r^2$) = 1.0000</td>
</tr>
<tr>
<td>5.</td>
<td>Accuracy</td>
<td>Mean recovery 100.3%</td>
</tr>
<tr>
<td>6.</td>
<td>Specificity</td>
<td>Peak Purity of Verapamil peak after degradation was 100.0 %</td>
</tr>
<tr>
<td>7.</td>
<td>Robustness</td>
<td>Difference from original condition 0.15%</td>
</tr>
<tr>
<td>8.</td>
<td>Solution stability</td>
<td>25 hrs</td>
</tr>
</tbody>
</table>

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Results and Discussion

5.6 Citalopram

The retention time of Citalopram peak was about 7.1 min. The system suitability is determined by obtaining the resolution factor between citalopram and impurity 5, which is found to be 10.2. Typical chromatograph of Citalopram (1000 ppm) along with spiked impurities at 0.2 % level is shown in figure 5.6F1.

Figure 5.6F1 : Typical chromatograph of Citalopram

![Chromatograph](image)

The relative retention time of all the impurities are summarized under table 5.6.1

Table 5.6.1 : The Relative retention time for Citalopram and its impurities

<table>
<thead>
<tr>
<th>Components</th>
<th>Retention time(min)</th>
<th>Relative retention time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citalopram</td>
<td>7.1</td>
<td>1.00</td>
</tr>
<tr>
<td>Impurity 1</td>
<td>3.4</td>
<td>0.48</td>
</tr>
<tr>
<td>Impurity 2</td>
<td>4.0</td>
<td>0.56</td>
</tr>
<tr>
<td>Impurity 3</td>
<td>4.8</td>
<td>0.68</td>
</tr>
<tr>
<td>Impurity 4</td>
<td>6.3</td>
<td>0.89</td>
</tr>
<tr>
<td>Impurity 5</td>
<td>10.4</td>
<td>1.60</td>
</tr>
<tr>
<td>Impurity 6</td>
<td>21.0</td>
<td>3.23</td>
</tr>
<tr>
<td>Impurity 7</td>
<td>24.7</td>
<td>3.80</td>
</tr>
</tbody>
</table>
Results and Discussion

The relative standard deviation of the area of the Citalopram and its impurities peak for replicate injections was found to be less than 5.0%. Table 5.6.2 shows detailed of instrument precision data.

Table 5.6.2: Results for Instrument precision of Citalopram and its impurities

<table>
<thead>
<tr>
<th>Injection</th>
<th>Detector response (area counts)</th>
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<tbody>
<tr>
<td></td>
<td>Imp 1</td>
</tr>
<tr>
<td>1</td>
<td>46180</td>
</tr>
<tr>
<td>2</td>
<td>46057</td>
</tr>
<tr>
<td>3</td>
<td>46148</td>
</tr>
<tr>
<td>4</td>
<td>45891</td>
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<tr>
<td>5</td>
<td>46031</td>
</tr>
<tr>
<td>6</td>
<td>45950</td>
</tr>
<tr>
<td>Mean</td>
<td>46043</td>
</tr>
<tr>
<td>% RSD</td>
<td>0.24</td>
</tr>
</tbody>
</table>

Method precision shows a relative standard deviation of less than 5.0% for all impurities, therefore the method can be said as precise. Details of method precision study is tabulated under table no. 5.6.3

Table 5.6.3: Result of Method Precision for Citalopram impurities

<table>
<thead>
<tr>
<th>Set #</th>
<th>Detector response (area counts)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Imp 1</td>
</tr>
<tr>
<td>1</td>
<td>0.1943</td>
</tr>
<tr>
<td>2</td>
<td>0.1928</td>
</tr>
<tr>
<td>3</td>
<td>0.1940</td>
</tr>
<tr>
<td>4</td>
<td>0.1929</td>
</tr>
<tr>
<td>5</td>
<td>0.1949</td>
</tr>
<tr>
<td>6</td>
<td>0.1943</td>
</tr>
<tr>
<td>Mean</td>
<td>0.1939</td>
</tr>
<tr>
<td>% RSD</td>
<td>0.43</td>
</tr>
</tbody>
</table>

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Results and Discussion

The method was shown to be linear from 50 – 150 % of Citalopram limit concentration. A calibration curve was constructed using characteristic parameters for regression equation \( Y = a + bx \) and coefficient of correlation \( r^2 \) was found to be not less than 0.99. The details of linearity data is shown in table no. 5.6.4

**Table 5.6.4 : Details for Citalopram and impurities of Linearity study**

<table>
<thead>
<tr>
<th>Set #</th>
<th>Impurities</th>
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<th></th>
<th></th>
<th></th>
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<tbody>
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<td></td>
<td></td>
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<td>Mean</td>
<td>Conc</td>
<td>Mean</td>
<td>Conc</td>
<td>Mean</td>
<td>Conc</td>
<td>Mean</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Area</td>
<td>Area</td>
<td>Area</td>
<td>Area</td>
<td>Area</td>
<td>Area</td>
<td>Area</td>
<td>Area</td>
</tr>
<tr>
<td>1</td>
<td>Imp 1</td>
<td>1.14</td>
<td>27459</td>
<td>1.22</td>
<td>11253</td>
<td>1.20</td>
<td>11871</td>
<td>1.06</td>
<td>13800</td>
</tr>
<tr>
<td>2</td>
<td>Imp 2</td>
<td>1.71</td>
<td>37636</td>
<td>1.83</td>
<td>17182</td>
<td>1.80</td>
<td>17703</td>
<td>1.59</td>
<td>21380</td>
</tr>
<tr>
<td>3</td>
<td>Imp 3</td>
<td>2.28</td>
<td>45962</td>
<td>2.44</td>
<td>22555</td>
<td>2.40</td>
<td>23562</td>
<td>2.12</td>
<td>27981</td>
</tr>
<tr>
<td>4</td>
<td>Imp 4</td>
<td>2.85</td>
<td>55472</td>
<td>3.05</td>
<td>28076</td>
<td>3.00</td>
<td>29493</td>
<td>2.65</td>
<td>34871</td>
</tr>
<tr>
<td>5</td>
<td>Imp 5</td>
<td>3.42</td>
<td>66219</td>
<td>3.66</td>
<td>34172</td>
<td>3.60</td>
<td>35905</td>
<td>3.18</td>
<td>42280</td>
</tr>
<tr>
<td></td>
<td>Slope</td>
<td>16729.06</td>
<td>9300.55</td>
<td>9976.56</td>
<td>13292.64</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Intercept</td>
<td>8407.40</td>
<td>-45.93</td>
<td>-236.73</td>
<td>-118.33</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Correlation coefficient</td>
<td>0.9992</td>
<td>0.9998</td>
<td>0.9998</td>
<td>0.9998</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Set #</th>
<th>Impurities</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>CITALOPRAM</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Conc</td>
<td>Mean</td>
<td>Conc</td>
<td>Mean</td>
<td>Conc</td>
<td>Mean</td>
<td>Conc</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Area</td>
<td>Area</td>
<td>Area</td>
<td>Area</td>
<td>Area</td>
<td>Area</td>
<td>Area</td>
</tr>
<tr>
<td>1</td>
<td>Imp 5</td>
<td>1.22</td>
<td>11361</td>
<td>1.22</td>
<td>11244</td>
<td>1.12</td>
<td>12037</td>
<td>0.50</td>
</tr>
<tr>
<td>2</td>
<td>Imp 6</td>
<td>1.83</td>
<td>18333</td>
<td>1.83</td>
<td>18760</td>
<td>1.68</td>
<td>19295</td>
<td>0.76</td>
</tr>
<tr>
<td>3</td>
<td>Imp 7</td>
<td>2.44</td>
<td>23496</td>
<td>2.44</td>
<td>27635</td>
<td>2.24</td>
<td>26124</td>
<td>1.01</td>
</tr>
<tr>
<td>4</td>
<td>CITALOPRAM</td>
<td>3.05</td>
<td>28339</td>
<td>3.05</td>
<td>31085</td>
<td>2.80</td>
<td>31885</td>
<td>1.26</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>3.66</td>
<td>34955</td>
<td>3.66</td>
<td>40718</td>
<td>3.36</td>
<td>38309</td>
<td>1.51</td>
</tr>
<tr>
<td></td>
<td>Slope</td>
<td>9458.14</td>
<td>11684.10</td>
<td>11631.19</td>
<td>14554.89</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Intercept</td>
<td>319.07</td>
<td>-2620.60</td>
<td>-523.93</td>
<td>872.80</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Correlation coefficient</td>
<td>0.9987</td>
<td>0.9929</td>
<td>0.9991</td>
<td>0.9990</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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Figure 5.6F2: Linearity graphs of Citalopram and impurities
Results and Discussion

Figure 5.6F2: Linearity graphs of Citalopram and impurities

IMPURITY 6

IMPURITY 7

CITALOPRAM
Results and Discussion

The mean recovery data for each level is within accepted values (95-105 % recovery) for 70,85,100,115 and 130 % of label claim. Therefore, these results indicated a good accuracy of the method for citalopram impurity profile. The details of recovery data is shown in table no. 5.6.5

**Table 5.6.5 : Details for recovery study for Citalopram impurities**

<table>
<thead>
<tr>
<th>% level of standard</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Imp 1</td>
</tr>
<tr>
<td>70</td>
<td>101.73</td>
</tr>
<tr>
<td>85</td>
<td>97.72</td>
</tr>
<tr>
<td>100</td>
<td>99.42</td>
</tr>
<tr>
<td>115</td>
<td>100.18</td>
</tr>
<tr>
<td>130</td>
<td>95.90</td>
</tr>
<tr>
<td>mean</td>
<td>98.99</td>
</tr>
<tr>
<td>% RSD</td>
<td>2.28</td>
</tr>
</tbody>
</table>

All the degradation peaks generated in the forced degradation studies were well separated and 3 point peak purity of Citalopram peak was always greater than 0.99, proving the stability indicating nature of the method. Major degradation was observed under Alkaline and Peroxide degradation conditions. Typical chromatographs obtained in degradation study are shown in figure 5.6F3.

**Figure 5.6F3 : Specificity study chromatographs of Citalopram**

Acid degradation

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Results and Discussion

Alkali degradation

Peroxide degradation

Thermal degradation

Sun-light degradation

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Results and Discussion

For determining the limit of detection (LOD) and limit of quantitation (LOQ), the method based on the residual standard deviation of a regression line and slope was adopted, a specific calibration curve was constructed. The results are tabulated below under Table no. 5.6.6

**Table 5.6.6: Details for LOD and LOQ for Citalopram impurities**

<table>
<thead>
<tr>
<th>Components</th>
<th>Limit of quantitation (µg/ml)</th>
<th>Limit of detection (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(%)</td>
<td>(%)</td>
</tr>
<tr>
<td>Impurity 1</td>
<td>0.068</td>
<td>0.068</td>
</tr>
<tr>
<td>Impurity 2</td>
<td>0.073</td>
<td>0.073</td>
</tr>
<tr>
<td>Impurity 3</td>
<td>0.144</td>
<td>0.072</td>
</tr>
<tr>
<td>Impurity 4</td>
<td>0.127</td>
<td>0.062</td>
</tr>
<tr>
<td>Impurity 5</td>
<td>0.305</td>
<td>0.146</td>
</tr>
<tr>
<td>Impurity 6</td>
<td>0.610</td>
<td>0.305</td>
</tr>
<tr>
<td>Impurity 7</td>
<td>0.560</td>
<td>0.280</td>
</tr>
<tr>
<td>Citalopram</td>
<td>0.250</td>
<td>0.060</td>
</tr>
<tr>
<td></td>
<td>0.025</td>
<td>0.006</td>
</tr>
</tbody>
</table>

The result obtained during robustness shows that by changing deliberately, some internal and external parameters of the method does not influence the results. The results are tabulated below under table no. 5.6.7

**Table 5.6.7: Results for Robustness of method**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Imp 1</th>
<th>Imp 2</th>
<th>Imp 3</th>
<th>Imp 4</th>
<th>Imp 5</th>
<th>Imp 6</th>
<th>Imp 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original</td>
<td>0.231</td>
<td>0.214</td>
<td>0.335</td>
<td>0.210</td>
<td>0.238</td>
<td>0.232</td>
<td>0.332</td>
</tr>
<tr>
<td>Analyst change</td>
<td>0.229</td>
<td>0.212</td>
<td>0.348</td>
<td>0.211</td>
<td>0.234</td>
<td>0.238</td>
<td>0.340</td>
</tr>
<tr>
<td>Column Change</td>
<td>0.236</td>
<td>0.204</td>
<td>0.337</td>
<td>0.223</td>
<td>0.245</td>
<td>0.241</td>
<td>0.343</td>
</tr>
<tr>
<td>Column temp change</td>
<td>0.228</td>
<td>0.212</td>
<td>0.342</td>
<td>0.221</td>
<td>0.233</td>
<td>0.235</td>
<td>0.337</td>
</tr>
<tr>
<td>Mobile phase comp</td>
<td>0.232</td>
<td>0.214</td>
<td>0.348</td>
<td>0.210</td>
<td>0.236</td>
<td>0.241</td>
<td>0.330</td>
</tr>
<tr>
<td>Instrument change</td>
<td>0.244</td>
<td>0.213</td>
<td>0.351</td>
<td>0.196</td>
<td>0.235</td>
<td>0.244</td>
<td>0.337</td>
</tr>
</tbody>
</table>

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Results and Discussion

Solution stability of 54 hrs was observed by periodically injecting stability solution. The details of peak area at different time intervals are tabulated under table 5.6.8. The result indicates that % deviation from mean initial area counts are not more than 5.0 % proving that solution is not needed to be freshly prepared.

Table 5.6.8 : Results for Solution stability of method

<table>
<thead>
<tr>
<th>Time Hrs</th>
<th>Impurities</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Imp 1</td>
</tr>
<tr>
<td></td>
<td>Mean Area counts</td>
</tr>
<tr>
<td>Initial</td>
<td>200724</td>
</tr>
<tr>
<td>11</td>
<td>200997</td>
</tr>
<tr>
<td>18</td>
<td>200614</td>
</tr>
<tr>
<td>30</td>
<td>200484</td>
</tr>
<tr>
<td>47</td>
<td>201621</td>
</tr>
<tr>
<td>54</td>
<td>201392</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time Hrs</th>
<th>Impurities</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Imp 5</td>
</tr>
<tr>
<td></td>
<td>Mean Area counts</td>
</tr>
<tr>
<td>Initial</td>
<td>117126</td>
</tr>
<tr>
<td>11</td>
<td>116595</td>
</tr>
<tr>
<td>18</td>
<td>116228</td>
</tr>
<tr>
<td>30</td>
<td>116592</td>
</tr>
<tr>
<td>47</td>
<td>115959</td>
</tr>
<tr>
<td>54</td>
<td>116085</td>
</tr>
</tbody>
</table>
### Table 5.6.9: Summary of the performance parameters of the HPLC procedure for Citalopram bulk drug

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Observed Results</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>System suitability Resolution</strong></td>
<td></td>
</tr>
<tr>
<td>Imp 1</td>
<td>10.2</td>
</tr>
<tr>
<td>Imp 2</td>
<td></td>
</tr>
<tr>
<td>Imp 3</td>
<td></td>
</tr>
<tr>
<td>Imp 4</td>
<td></td>
</tr>
<tr>
<td>Imp 5</td>
<td></td>
</tr>
<tr>
<td>Imp 6</td>
<td></td>
</tr>
<tr>
<td>Imp 7</td>
<td></td>
</tr>
<tr>
<td>Citalopram</td>
<td></td>
</tr>
<tr>
<td><strong>Instrument Precision</strong></td>
<td></td>
</tr>
<tr>
<td>% RSD Limit NMT 5.0%</td>
<td>0.24 0.41 0.24 0.33 0.38 1.59 1.20 0.36</td>
</tr>
<tr>
<td><strong>Method Precision</strong></td>
<td></td>
</tr>
<tr>
<td>% RSD Limit NMT 5.0%</td>
<td>0.194 0.196 0.200 0.196 0.202 0.198 0.202 -</td>
</tr>
<tr>
<td><strong>Linearity and Range</strong></td>
<td></td>
</tr>
<tr>
<td>Coefficient of correlation Limit NLT 0.99</td>
<td>0.9992 0.9998 0.9998 0.9998 0.9987 0.9929 0.9991 0.9990</td>
</tr>
<tr>
<td><strong>Accuracy</strong></td>
<td></td>
</tr>
<tr>
<td>% recovery Limit 95-105 %</td>
<td>98.99 98.08 100.49 99.03 101.19 103.03 101.37 -</td>
</tr>
<tr>
<td><strong>Minimum quantitation level (%)</strong></td>
<td></td>
</tr>
<tr>
<td>0.007</td>
<td></td>
</tr>
<tr>
<td><strong>Minimum detection level (%)</strong></td>
<td></td>
</tr>
<tr>
<td>0.007</td>
<td></td>
</tr>
<tr>
<td><strong>Robustness</strong></td>
<td></td>
</tr>
<tr>
<td>Difference NMT 10.0% of impurity limit in original condition</td>
<td></td>
</tr>
<tr>
<td>Original</td>
<td>0.231 0.214 0.335 0.210 0.238 0.232 0.332 -</td>
</tr>
<tr>
<td>Analyst</td>
<td>0.229 0.212 0.348 0.211 0.234 0.238 0.340 -</td>
</tr>
<tr>
<td>Column</td>
<td>0.236 0.204 0.337 0.223 0.245 0.241 0.343 -</td>
</tr>
<tr>
<td>Column temp</td>
<td>0.228 0.212 0.342 0.221 0.233 0.235 0.337 -</td>
</tr>
<tr>
<td>Mobile Phase composition</td>
<td>0.232 0.214 0.348 0.210 0.236 0.241 0.330 -</td>
</tr>
<tr>
<td>Instrument</td>
<td>0.244 0.213 0.351 0.196 0.235 0.244 0.337 -</td>
</tr>
<tr>
<td><strong>Specificity</strong></td>
<td></td>
</tr>
<tr>
<td>3 point peak purity not less than 0.99 in all degradation condition</td>
<td></td>
</tr>
<tr>
<td><strong>Solution stability</strong></td>
<td></td>
</tr>
<tr>
<td>Upto 54 hrs</td>
<td></td>
</tr>
</tbody>
</table>
5.7 Metaxalone

The retention time of Metaxalone peak was about 5.6 min. The system suitability is determined by obtaining the resolution factor between Metaxalone and impurity 4, which is found to be 4.8. Typical chromatograph of Metaxalone (1000 ppm) along with spiked impurities at 0.2 % level, is shown in figure 5.7F1.

Figure 5.7F1 : Typical chromatograph of Metaxalone

The relative retention time of all the impurities are summarized under table 5.7.1

Table 5.7.1 : The Relative retention time for Metaxalone and its impurities

<table>
<thead>
<tr>
<th>Components</th>
<th>Retention time(min)</th>
<th>Relative retention time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metaxalone</td>
<td>5.6</td>
<td>1.00</td>
</tr>
<tr>
<td>Impurity 1</td>
<td>3.2</td>
<td>0.6</td>
</tr>
<tr>
<td>Impurity 2</td>
<td>6.7</td>
<td>1.2</td>
</tr>
<tr>
<td>Impurity 3</td>
<td>19.2</td>
<td>3.4</td>
</tr>
<tr>
<td>Impurity 4</td>
<td>25.6</td>
<td>4.6</td>
</tr>
<tr>
<td>Impurity 5</td>
<td>35.2</td>
<td>6.3</td>
</tr>
</tbody>
</table>

The relative standard deviation of the area of the Metaxalone and its impurities peak for replicate injections was found to be less than 5.0%. Table 5.7.2 shows detailed of instrument precision data.
Results and Discussion

Table 5.7.2: Results for Instrument precision of Metaxalone and its impurities

<table>
<thead>
<tr>
<th>Injection</th>
<th>Detector response (area counts)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Imp 1</td>
</tr>
<tr>
<td>1</td>
<td>66757</td>
</tr>
<tr>
<td>2</td>
<td>66968</td>
</tr>
<tr>
<td>3</td>
<td>66831</td>
</tr>
<tr>
<td>4</td>
<td>66199</td>
</tr>
<tr>
<td>5</td>
<td>66876</td>
</tr>
<tr>
<td>6</td>
<td>66837</td>
</tr>
<tr>
<td>Mean</td>
<td>66745</td>
</tr>
<tr>
<td>% RSD</td>
<td>0.41</td>
</tr>
</tbody>
</table>

Method precision shows a relative standard deviation of less than 5.0 % for all impurities, therefore the method can be said as precise. Details of method precision study is tabulated under table no. 5.7.3

Table 5.7.3: Result of Method Precision for Metaxalone impurities

<table>
<thead>
<tr>
<th>Set #</th>
<th>Detector response (area counts)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Imp 1</td>
</tr>
<tr>
<td>1</td>
<td>0.2049</td>
</tr>
<tr>
<td>2</td>
<td>0.2052</td>
</tr>
<tr>
<td>3</td>
<td>0.2051</td>
</tr>
<tr>
<td>4</td>
<td>0.2052</td>
</tr>
<tr>
<td>5</td>
<td>0.2051</td>
</tr>
<tr>
<td>6</td>
<td>0.2053</td>
</tr>
<tr>
<td>Mean</td>
<td>0.2051</td>
</tr>
<tr>
<td>% RSD</td>
<td>0.07</td>
</tr>
</tbody>
</table>

The method was shown to be linear from 50 – 150 % of Metaxalone limit concentration. A calibration curve was constructed using characteristic parameters for regression equation (Y= a + bx) and coefficient of correlation $r^2$ was found to be not less then 0.99. The details of linearity data is shown in table no. 5.7.4

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## Results and Discussion

### Table 5.7.4: Details for Metaxalone and impurities of Linearity study

<table>
<thead>
<tr>
<th>Set #</th>
<th>Impurities</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Imp 1</td>
</tr>
<tr>
<td>Conc</td>
<td>Mean Area</td>
</tr>
<tr>
<td>1</td>
<td>0.97</td>
</tr>
<tr>
<td>2</td>
<td>1.46</td>
</tr>
<tr>
<td>3</td>
<td>1.94</td>
</tr>
<tr>
<td>4</td>
<td>2.43</td>
</tr>
<tr>
<td>5</td>
<td>2.92</td>
</tr>
</tbody>
</table>

Slope 34230.86 30159.79 36548.72
Intercept -177.00 -351.53 -1276.93
Correlation coefficient 1.000 1.000 1.000

### Set #

<table>
<thead>
<tr>
<th>Impurities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imp 4</td>
</tr>
<tr>
<td>Conc</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>5</td>
</tr>
</tbody>
</table>

Slope 34426.48 49702.25 40881.18
Intercept -1518.00 -265.87 -674.20
Correlation coefficient 1.000 0.9999 0.9999

---

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Figure 5.7F2: Linearity graphs of Metaxalone and impurities

**IMPURITY 1**

**IMPURITY 2**

**IMPURITY 3**

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Figure 5.7F2: Linearity graphs of Metaxalone and impurities

IMPURITY 4

IMPURITY 5

METAXALONE

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Results and Discussion

The mean recovery data for each level is within accepted values (95-105 % recovery) for 70, 85, 100, 115 and 130 % of label claim, Therefore, these results indicated a good accuracy of the method for Metaxalone impurity profile. The details of recovery data is shown in table no. 5.7.5

Table 5.7.5 : Details for recovery study for Metaxalone impurities

<table>
<thead>
<tr>
<th>% level of standard</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Imp 1</td>
</tr>
<tr>
<td>70</td>
<td>100.92</td>
</tr>
<tr>
<td>85</td>
<td>100.43</td>
</tr>
<tr>
<td>100</td>
<td>97.96</td>
</tr>
<tr>
<td>115</td>
<td>99.65</td>
</tr>
<tr>
<td>130</td>
<td>100.96</td>
</tr>
<tr>
<td>mean</td>
<td>99.98</td>
</tr>
<tr>
<td>% RSD</td>
<td>1.25</td>
</tr>
</tbody>
</table>

All the degradation peaks generated in the forced degradation studies were well separated and 3 point peak purity of Metaxalone peak was always greater than 0.99, proving the stability indicating nature of the method. Major degradation was observed under Alkali and Peroxide degradation conditions. Typical chromatographs obtained in degradation study are shown in figure 5.7F3.

Figure 5.7F3 : Specificity study chromatographs of Metaxalone

Acid degradation

Bipin Bihari P.G College, Bundelkhand University, Jhansi, U.P
Results and Discussion

Alkali degradation

Peroxide degradation

Thermal degradation

Sun-light degradation

Bipin Bihari P.G College, Bundelkhand University, Jhansi, U.P
Results and Discussion

For determining the limit of detection (LOD) and limit of quantitation (LOQ), the method based on the residual standard deviation of a regression line and slope was adopted, a specific calibration curve was constructed. The results are tabulated below under table no. 5.7.6

<table>
<thead>
<tr>
<th>Components</th>
<th>Limit of quantitation (µg/ml)</th>
<th>Limit of detection (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Impurity 1</td>
<td>0.115</td>
<td>0.029</td>
</tr>
<tr>
<td>Impurity 2</td>
<td>0.118</td>
<td>0.029</td>
</tr>
<tr>
<td>Impurity 3</td>
<td>0.120</td>
<td>0.030</td>
</tr>
<tr>
<td>Impurity 4</td>
<td>0.245</td>
<td>0.029</td>
</tr>
<tr>
<td>Impurity 5</td>
<td>0.250</td>
<td>0.030</td>
</tr>
<tr>
<td>Metaxalone</td>
<td>0.059</td>
<td>0.015</td>
</tr>
</tbody>
</table>

The result obtained during robustness shows that by changing deliberately, some internal and external parameters of the method does not influence the results. The results are tabulated below under table no. 5.7.7

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Imp 1</th>
<th>Imp 2</th>
<th>Imp 3</th>
<th>Imp 4</th>
<th>Imp 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original</td>
<td>0.2049</td>
<td>0.1993</td>
<td>0.1997</td>
<td>0.1992</td>
<td>0.2016</td>
</tr>
<tr>
<td>Analyst change</td>
<td>0.2012</td>
<td>0.2009</td>
<td>0.2006</td>
<td>0.1994</td>
<td>0.2010</td>
</tr>
<tr>
<td>Column Change</td>
<td>0.2038</td>
<td>0.1996</td>
<td>0.2011</td>
<td>0.2006</td>
<td>0.2015</td>
</tr>
<tr>
<td>Column temp change</td>
<td>0.2023</td>
<td>0.1964</td>
<td>0.2078</td>
<td>0.2073</td>
<td>0.2103</td>
</tr>
<tr>
<td>Mobile phase composition</td>
<td>0.1993</td>
<td>0.2003</td>
<td>0.2007</td>
<td>0.2004</td>
<td>0.2011</td>
</tr>
<tr>
<td>Instrument change</td>
<td>0.2002</td>
<td>0.2014</td>
<td>0.2031</td>
<td>0.1986</td>
<td>0.2016</td>
</tr>
</tbody>
</table>
Results and Discussion

Solution stability of 43 hrs was observed by periodically injecting stability solution.

The details of peak area at different time intervals are tabulated under table 5.7.8.

The result indicates that % deviation from mean initial area counts are not more than 5.0 % proving that solution is not needed to be freshly prepared.

Table 5.7.8 : Results for Solution stability of method

<table>
<thead>
<tr>
<th>Time Hrs</th>
<th>Impurities</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Imp 1</td>
<td>Imp 2</td>
<td>Imp 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean Area counts</td>
<td>% deviation from mean initial area</td>
<td>Mean Area counts</td>
<td>% deviation from mean initial area</td>
<td>Mean Area counts</td>
</tr>
<tr>
<td>Initial</td>
<td>810479</td>
<td>0.00</td>
<td>734701</td>
<td>0.00</td>
<td>907498</td>
</tr>
<tr>
<td>4</td>
<td>816131</td>
<td>-0.70</td>
<td>738899</td>
<td>-0.57</td>
<td>914974</td>
</tr>
<tr>
<td>11</td>
<td>816358</td>
<td>-0.73</td>
<td>739356</td>
<td>-0.63</td>
<td>914170</td>
</tr>
<tr>
<td>17</td>
<td>807582</td>
<td>0.36</td>
<td>733996</td>
<td>0.10</td>
<td>905236</td>
</tr>
<tr>
<td>43</td>
<td>806728</td>
<td>0.46</td>
<td>733413</td>
<td>0.18</td>
<td>903432</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time Hrs</th>
<th>Impurities</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Imp 4</td>
<td>Imp 5</td>
<td>METAXALONE</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean Area counts</td>
<td>% deviation from mean initial area</td>
<td>Mean Area counts</td>
<td>% deviation from mean initial area</td>
<td>Mean Area counts</td>
</tr>
<tr>
<td>Initial</td>
<td>841136</td>
<td>0.00</td>
<td>1246782</td>
<td>0.00</td>
<td>456531</td>
</tr>
<tr>
<td>4</td>
<td>845986</td>
<td>-0.58</td>
<td>1255771</td>
<td>-0.72</td>
<td>459604</td>
</tr>
<tr>
<td>11</td>
<td>844529</td>
<td>-0.40</td>
<td>1256237</td>
<td>-0.76</td>
<td>459453</td>
</tr>
<tr>
<td>17</td>
<td>836756</td>
<td>0.52</td>
<td>1247703</td>
<td>-0.007</td>
<td>455775</td>
</tr>
<tr>
<td>43</td>
<td>834961</td>
<td>0.73</td>
<td>1249581</td>
<td>-0.22</td>
<td>455338</td>
</tr>
</tbody>
</table>

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Table 5.7.9: Summary of the performance parameters of the HPLC procedure for Metaxalone bulk drug

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Observed Results</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>System suitability</strong></td>
<td></td>
</tr>
<tr>
<td>Resolution</td>
<td>4.8</td>
</tr>
<tr>
<td><strong>Instrument Precision</strong></td>
<td></td>
</tr>
<tr>
<td>% RSD</td>
<td></td>
</tr>
<tr>
<td>Limit NMT 5.0 %</td>
<td></td>
</tr>
<tr>
<td>Imp 1</td>
<td>0.41</td>
</tr>
<tr>
<td>Imp 2</td>
<td>0.23</td>
</tr>
<tr>
<td>Imp 3</td>
<td>0.33</td>
</tr>
<tr>
<td>Imp 4</td>
<td>0.23</td>
</tr>
<tr>
<td>Imp 5</td>
<td>0.36</td>
</tr>
<tr>
<td>Metaxalone</td>
<td>1.25</td>
</tr>
<tr>
<td><strong>Method Precision</strong></td>
<td></td>
</tr>
<tr>
<td>mean</td>
<td></td>
</tr>
<tr>
<td>% RSD</td>
<td></td>
</tr>
<tr>
<td>Limit NMT 5.0 %</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.2051</td>
</tr>
<tr>
<td>Imp 1</td>
<td>0.1998</td>
</tr>
<tr>
<td>Imp 2</td>
<td>0.1997</td>
</tr>
<tr>
<td>Imp 3</td>
<td>0.1997</td>
</tr>
<tr>
<td>Imp 4</td>
<td>0.2018</td>
</tr>
<tr>
<td>Metaxalone</td>
<td>-</td>
</tr>
<tr>
<td><strong>Linearity and Range</strong></td>
<td></td>
</tr>
<tr>
<td>Coefficient of correlation</td>
<td></td>
</tr>
<tr>
<td>Limit NLT 0.99</td>
<td></td>
</tr>
<tr>
<td>1.0000</td>
<td>1.0000</td>
</tr>
<tr>
<td>1.0000</td>
<td>1.0000</td>
</tr>
<tr>
<td>1.0000</td>
<td>0.9999</td>
</tr>
<tr>
<td>0.9999</td>
<td></td>
</tr>
<tr>
<td><strong>Accuracy</strong></td>
<td></td>
</tr>
<tr>
<td>% recovery</td>
<td></td>
</tr>
<tr>
<td>Limit 95-105 %</td>
<td></td>
</tr>
<tr>
<td>99.98</td>
<td>100.51</td>
</tr>
<tr>
<td>100.57</td>
<td>99.85</td>
</tr>
<tr>
<td>100.93</td>
<td></td>
</tr>
<tr>
<td><strong>Minimum quantitation level (%)</strong></td>
<td></td>
</tr>
<tr>
<td>0.012</td>
<td>0.012</td>
</tr>
<tr>
<td>0.012</td>
<td>0.025</td>
</tr>
<tr>
<td>0.025</td>
<td></td>
</tr>
<tr>
<td>0.006</td>
<td></td>
</tr>
<tr>
<td><strong>Minimum detection level (%)</strong></td>
<td></td>
</tr>
<tr>
<td>0.003</td>
<td>0.003</td>
</tr>
<tr>
<td>0.003</td>
<td>0.003</td>
</tr>
<tr>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td><strong>Robustness</strong></td>
<td></td>
</tr>
<tr>
<td>Difference NMT 10.0 % of impurity limit in original condition</td>
<td></td>
</tr>
<tr>
<td>Original</td>
<td>0.2049</td>
</tr>
<tr>
<td>Analyst</td>
<td>0.2012</td>
</tr>
<tr>
<td>Column</td>
<td>0.2038</td>
</tr>
<tr>
<td>Column temp</td>
<td>0.2023</td>
</tr>
<tr>
<td>Mobile Phase composition</td>
<td>0.1993</td>
</tr>
<tr>
<td>Instrument</td>
<td>0.2002</td>
</tr>
<tr>
<td><strong>Specificity</strong></td>
<td></td>
</tr>
<tr>
<td>3 point peak purity not less than 0.99 in all degradation condition</td>
<td></td>
</tr>
<tr>
<td><strong>Solution stability</strong></td>
<td>Upto 43 hrs</td>
</tr>
</tbody>
</table>

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Results and Discussion

5.8 Ondansetron

The retention time of Ondansetron peak was about 6.5 min. The system suitability is determined by obtaining the resolution factor between Ondansetron and impurity A, which is found to be 7.5. Typical chromatograph of Ondansetron (500 ppm) along with spiked impurities at 0.2 % level A, C and 0.1 % of impurity D is shown in figure 5.8F1.

Figure 5.8F1 : Typical chromatograph of Ondansetron

![Chromatograph](image)

The relative retention time of all the impurites are summarized under table 5.8.1

<table>
<thead>
<tr>
<th>Components</th>
<th>Retention time(min)</th>
<th>Relative retention time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ondansetron</td>
<td>6.5</td>
<td>1.00</td>
</tr>
<tr>
<td>Impurity A</td>
<td>4.5</td>
<td>0.7</td>
</tr>
<tr>
<td>Impurity C</td>
<td>17.5</td>
<td>2.7</td>
</tr>
<tr>
<td>Impurity D</td>
<td>27.6</td>
<td>4.2</td>
</tr>
</tbody>
</table>

The relative standard deviation of the area of the Ondansetron and its impurities peak for replicate injections was found to be less than 5.0%. Table 5.8.2 shows detailed of instrument precision data.
Results and Discussion

Table 5.8.2: Results for Instrument precision of Ondansetron and its impurities

<table>
<thead>
<tr>
<th>Injection</th>
<th>Detector response(area counts)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Imp A</td>
</tr>
<tr>
<td>1</td>
<td>82585</td>
</tr>
<tr>
<td>2</td>
<td>81873</td>
</tr>
<tr>
<td>3</td>
<td>81663</td>
</tr>
<tr>
<td>4</td>
<td>82332</td>
</tr>
<tr>
<td>5</td>
<td>81297</td>
</tr>
<tr>
<td>6</td>
<td>81837</td>
</tr>
<tr>
<td>Mean</td>
<td>81931</td>
</tr>
<tr>
<td>% RSD</td>
<td>0.57</td>
</tr>
</tbody>
</table>

Method precision shows a relative standard deviation of less than 5.0 % for all impurities, therefore the method can be said as precise. Details of method precision study is tabulated under table no. 5.8.3

Table 5.8.3: Result of Method Precision for Ondansetron impurities

<table>
<thead>
<tr>
<th>Set #</th>
<th>Detector response(area counts)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Imp A</td>
</tr>
<tr>
<td>1</td>
<td>0.1997</td>
</tr>
<tr>
<td>2</td>
<td>0.1982</td>
</tr>
<tr>
<td>3</td>
<td>0.1968</td>
</tr>
<tr>
<td>4</td>
<td>0.1987</td>
</tr>
<tr>
<td>5</td>
<td>0.2003</td>
</tr>
<tr>
<td>6</td>
<td>0.2004</td>
</tr>
<tr>
<td>Mean</td>
<td>0.1990</td>
</tr>
<tr>
<td>% RSD</td>
<td>0.70</td>
</tr>
</tbody>
</table>

The method was shown to be linear from 50 – 150 % of Ondansetron limit concentration. A calibration curve was constructed using characteristic parameters.
for regression equation \((Y = a + bx)\) and coefficient of correlation \(r^2\) was found to be not less than 0.99. The details of linearity data is shown in table no. 5.8.4

**Table 5.8.4 : Details for Ondansetron and impurities of Linearity study**

<table>
<thead>
<tr>
<th>Set #</th>
<th>Impurities</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Imp A</td>
<td>Imp C</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Conc</td>
<td>Mean Area</td>
<td>Conc</td>
</tr>
<tr>
<td>1</td>
<td>0.50</td>
<td>41470</td>
<td>0.50</td>
</tr>
<tr>
<td>2</td>
<td>0.75</td>
<td>59049</td>
<td>0.75</td>
</tr>
<tr>
<td>3</td>
<td>1.00</td>
<td>83335</td>
<td>0.99</td>
</tr>
<tr>
<td>4</td>
<td>1.25</td>
<td>100500</td>
<td>1.24</td>
</tr>
<tr>
<td>5</td>
<td>1.50</td>
<td>125392</td>
<td>1.49</td>
</tr>
<tr>
<td>Slope</td>
<td></td>
<td>83650.81</td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>-1768.47</td>
<td></td>
<td>-1203.60</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.9982</td>
<td></td>
<td>0.9982</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Set #</th>
<th>Impurities</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Imp D</td>
<td>ONDARSETRON</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Conc</td>
<td>Mean Area</td>
<td>Conc</td>
</tr>
<tr>
<td>1</td>
<td>0.25</td>
<td>27459</td>
<td>0.26</td>
</tr>
<tr>
<td>2</td>
<td>0.38</td>
<td>39660</td>
<td>1.03</td>
</tr>
<tr>
<td>3</td>
<td>0.50</td>
<td>56104</td>
<td>10.30</td>
</tr>
<tr>
<td>4</td>
<td>0.63</td>
<td>67805</td>
<td>103.00</td>
</tr>
<tr>
<td>5</td>
<td>0.75</td>
<td>83715</td>
<td>515.00</td>
</tr>
<tr>
<td>Slope</td>
<td></td>
<td>112077.82</td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>-1314.20</td>
<td></td>
<td>567191.13</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.9987</td>
<td></td>
<td>0.9952</td>
</tr>
</tbody>
</table>
Figure 5.8F2: Linearity graphs of Ondansetron and impurities

- **Impurity A**
  - Y-axis: 0 to 140000
  - X-axis: 0 to 2.00

- **Impurity C**
  - Y-axis: 0 to 180000
  - X-axis: 0 to 2.00

- **Impurity D**
  - Y-axis: 0 to 90000
  - X-axis: 0 to 0.60

- **Ondansetron**
  - Y-axis: 0 to 30000000
  - X-axis: 0 to 800.00

---

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Results and Discussion

The mean recovery data for each level is within accepted values (95-105 % recovery) for 70, 85, 100, 115 and 130 % of label claim. Therefore, these results indicated a good accuracy of the method for Ondansetron impurity profile. The details of recovery data is shown in table no. 5.8.5

Table 5.8.5 : Details for recovery study for Ondansetron impurities

<table>
<thead>
<tr>
<th>% level of STD</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Imp A</td>
</tr>
<tr>
<td>70</td>
<td>99.89</td>
</tr>
<tr>
<td>85</td>
<td>100.23</td>
</tr>
<tr>
<td>100</td>
<td>98.13</td>
</tr>
<tr>
<td>115</td>
<td>99.14</td>
</tr>
<tr>
<td>130</td>
<td>100.75</td>
</tr>
<tr>
<td>mean</td>
<td>99.63</td>
</tr>
<tr>
<td>% RSD</td>
<td>1.02</td>
</tr>
</tbody>
</table>

All the degradation peaks generated in the forced degradation studies were well separated and 3 point peak purity of Ondansetron peak was always greater than 0.99. proving the stability indicating nature of the method. Major degradation was observed under Alkali and Peroxide degradation conditions. Typical chromatographs obtained in degradation study are shown in figure 5.8F3.

Figure 5.8F3 : Specificity study chromatographs of Ondansetron

Acid degradation

Bipin Bihari P.G College, Bundelkhand University, Jhansi, U.P
Results and Discussion

Alkali degradation

Peroxide degradation

Thermal degradation

For determining the limit of detection (LOD) and limit of quantitation (LOQ), the method based on the residual standard deviation of a regression line and slope was adopted, a specific calibration curve was constructed. The results are tabulated below under table no. 5.8.6

Bipin Bihari P.G College, Bundelkhand University, Jhansi, U.P
Results and Discussion

Table 5.8.6: Details for LOD and LOQ for Ondansetron impurities

<table>
<thead>
<tr>
<th>Components</th>
<th>Limit of quantitation</th>
<th>Limit of detection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(µg/ml)</td>
<td>(%)</td>
</tr>
<tr>
<td>Impurity A</td>
<td>0.125</td>
<td>0.030</td>
</tr>
<tr>
<td>Impurity C</td>
<td>0.124</td>
<td>0.030</td>
</tr>
<tr>
<td>Impurity D</td>
<td>0.126</td>
<td>0.030</td>
</tr>
</tbody>
</table>

The result obtained during robustness shows that by changing deliberately, some internal and external parameters of the method does not influence the results. The results are tabulated below under table no. 5.8.7

Table 5.8.7: Results for Robustness of method

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Imp A</th>
<th>Imp C</th>
<th>Imp D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original</td>
<td>0.196</td>
<td>0.195</td>
<td>0.093</td>
</tr>
<tr>
<td>Analyst change</td>
<td>0.197</td>
<td>0.193</td>
<td>0.098</td>
</tr>
<tr>
<td>Column Change</td>
<td>0.202</td>
<td>0.210</td>
<td>1.00</td>
</tr>
<tr>
<td>Flow rate change</td>
<td>0.198</td>
<td>0.200</td>
<td>0.097</td>
</tr>
<tr>
<td>Mobile phase composition</td>
<td>0.210</td>
<td>0.197</td>
<td>0.100</td>
</tr>
<tr>
<td>Instrument change</td>
<td>0.203</td>
<td>0.199</td>
<td>0.097</td>
</tr>
</tbody>
</table>

Solution stability of 43 hrs was observed by periodically injecting stability solution. The details of peak area at different time intervals are tabulated under table 5.8.8. The result indicates that % deviation from mean initial area counts are not more than 5.0 % proving that solution is not needed to be freshly prepared.

Bipin Bihari P.G College, Bundelkhand University, Jhansi, U.P
### Table 5.8.8: Results for Solution stability of method

<table>
<thead>
<tr>
<th>Time Hrs</th>
<th>Impurities</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Imp A</td>
<td>Imp C</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean Area counts</td>
<td>% deviation from mean initial area</td>
<td>Mean Area counts</td>
</tr>
<tr>
<td>Initial</td>
<td>7537840</td>
<td>-</td>
<td>10338525</td>
</tr>
<tr>
<td>4</td>
<td>7450931</td>
<td>1.15</td>
<td>10261906</td>
</tr>
<tr>
<td>30</td>
<td>7431283</td>
<td>1.41</td>
<td>10323326</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time Hrs</th>
<th>Impurities</th>
<th>Ondansetron</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Imp D</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean Area counts</td>
<td>% deviation from mean initial area</td>
</tr>
<tr>
<td>Initial</td>
<td>10403485</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>10283815</td>
<td>1.15</td>
</tr>
<tr>
<td>30</td>
<td>10696541</td>
<td>-2.82</td>
</tr>
</tbody>
</table>

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Table 5.8.9: Summary of the performance parameters of the HPLC procedure for Ondansetron bulk drug

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Observed Results</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>System suitability</strong></td>
<td></td>
</tr>
<tr>
<td>Resolution</td>
<td>4.8</td>
</tr>
<tr>
<td><strong>Instrument Precision</strong></td>
<td></td>
</tr>
<tr>
<td>% RSD</td>
<td></td>
</tr>
<tr>
<td>Limit NMT 5.0 %</td>
<td></td>
</tr>
<tr>
<td>Imp A</td>
<td>0.57</td>
</tr>
<tr>
<td>Imp C</td>
<td>1.40</td>
</tr>
<tr>
<td>Imp D</td>
<td>2.67</td>
</tr>
<tr>
<td>Ondansetron</td>
<td>0.66</td>
</tr>
<tr>
<td><strong>Method Precision</strong></td>
<td></td>
</tr>
<tr>
<td>mean</td>
<td></td>
</tr>
<tr>
<td>% RSD</td>
<td></td>
</tr>
<tr>
<td>Limit NMT 5.0 %</td>
<td></td>
</tr>
<tr>
<td>Imp A</td>
<td>0.1990</td>
</tr>
<tr>
<td>Imp C</td>
<td>0.1964</td>
</tr>
<tr>
<td>Imp D</td>
<td>0.0993</td>
</tr>
<tr>
<td>Ondansetron</td>
<td>-</td>
</tr>
<tr>
<td><strong>Linearity and Range</strong></td>
<td></td>
</tr>
<tr>
<td>Coefficient of correlation</td>
<td></td>
</tr>
<tr>
<td>Limit NLT 0.99</td>
<td></td>
</tr>
<tr>
<td>Imp A</td>
<td>0.9982</td>
</tr>
<tr>
<td>Imp C</td>
<td>0.9982</td>
</tr>
<tr>
<td>Imp D</td>
<td>0.9987</td>
</tr>
<tr>
<td>Ondansetron</td>
<td>0.9952</td>
</tr>
<tr>
<td><strong>Accuracy</strong></td>
<td></td>
</tr>
<tr>
<td>% recovery</td>
<td></td>
</tr>
<tr>
<td>Limit 95-105 %</td>
<td></td>
</tr>
<tr>
<td>Imp A</td>
<td>99.63</td>
</tr>
<tr>
<td>Imp C</td>
<td>99.77</td>
</tr>
<tr>
<td>Imp D</td>
<td>99.44</td>
</tr>
<tr>
<td>Ondansetron</td>
<td>-</td>
</tr>
<tr>
<td><strong>Minimum quantitation level (%)</strong></td>
<td></td>
</tr>
<tr>
<td>Imp A</td>
<td>0.03</td>
</tr>
<tr>
<td>Imp C</td>
<td>0.03</td>
</tr>
<tr>
<td>Imp D</td>
<td>0.03</td>
</tr>
<tr>
<td>Ondansetron</td>
<td>-</td>
</tr>
<tr>
<td><strong>Minimum detection level (%)</strong></td>
<td></td>
</tr>
<tr>
<td>Imp A</td>
<td>0.006</td>
</tr>
<tr>
<td>Imp C</td>
<td>0.006</td>
</tr>
<tr>
<td>Imp D</td>
<td>0.006</td>
</tr>
<tr>
<td>Ondansetron</td>
<td>-</td>
</tr>
<tr>
<td><strong>Robustness</strong></td>
<td></td>
</tr>
<tr>
<td>Difference NMT 10.0 % of impurity limit in original condition</td>
<td></td>
</tr>
<tr>
<td>Original</td>
<td>0.196</td>
</tr>
<tr>
<td>Analyst</td>
<td>0.195</td>
</tr>
<tr>
<td>Column</td>
<td>0.093</td>
</tr>
<tr>
<td>Flow rate</td>
<td>-</td>
</tr>
<tr>
<td>Mobile Phase composition</td>
<td></td>
</tr>
<tr>
<td>Instrument</td>
<td></td>
</tr>
<tr>
<td>Imp A</td>
<td>0.198</td>
</tr>
<tr>
<td>Imp C</td>
<td>0.200</td>
</tr>
<tr>
<td>Imp D</td>
<td>0.097</td>
</tr>
<tr>
<td>Ondansetron</td>
<td>-</td>
</tr>
<tr>
<td><strong>Specificity</strong></td>
<td></td>
</tr>
<tr>
<td>3 point peak purity not less than 0.99 in all degradation condition</td>
<td>Upto 30 hrs</td>
</tr>
<tr>
<td><strong>Solution stability</strong></td>
<td></td>
</tr>
</tbody>
</table>