Chapter – 5

Summary and Conclusion
8.1.0. Summary and Conclusion for Lopinavir & Ritonavir Assay Method.

1. Specificity

Interference:

The Chromatogram Obtained From blank injection preparation and placebo (Excipient blend preparation) preparation does not show any peak at the elution time (RT) of Both drug peaks in chromatogram obtained with Standard Preparation.

Resolution:
In all chromatograms it is found that Lopinavir & Ritonavir peaks is completely separated from each other. The Re-solution between Lopinavir & Ritonavir is 3.30.

Resolution:
In all chromatogram obtained With Standard Sample preparation the Theoretical plates for Lopinavir & Ritonavir peak found 4301 and 4124 for these both peaks which passed as per required criteria.

Tailing Factor:
In all chromatogram obtained With Standard Sample preparation the Tailing factor for Lopinavir & Ritonavir peak found 1.24 and 1.05 respectively and it passed as per required criteria.

% RSD:
In all chromatogram obtained With Standard Sample preparation the % RSD for Lopinavir & Ritonavir peak found are as follow
Lopinavir- 0.16
Ritonavir- 0.58
Conclusion:
All the injections were processed at the wavelength provided in the method and found that no disturbance observed due to diluent blank Sample, placebo, known impurities with Lopinavir & Ritonavir peak from all this results it is conclude that the developed method for assay of Lopinavir & Ritonavir was selective. As this method was selective it is useful in routine analysis an any analysis centre which is very sensitively worked for results.
2. Forced Degradation:

% Degradation:
The degradation peaks are well separated from the peak due to Lopinavir & Ritonavir peaks. Approximately 15.60% degradation achieve when sample treated with base. The angle of purity of Lopinavir & Ritonavir peaks was not exceeding value of purity threshold of peak in each degradation.

Resolution:
In all chromatograms it is found that Lopinavir & Ritonavir peaks is completely separated from each other. The Re-solution between Lopinavir & Ritonavir is 3.10

USP plate counts:
In all chromatogram obtained With Standard Sample preparation the Theoretical plates for Lopinavir & Ritonavir peak found are as follow
Lopinavir- 3987
Ritonavir- 3765

Tailing factor:
In all chromatogram obtained With Standard Sample preparation the Tailing factor for Lopinavir & Ritonavir peak found are 1.24 and 1.05 respectively, criteria passed for both peaks.

% RSD:
In all chromatogram obtained With Standard Sample preparation the above parameter ie % RSD for Lopinavir & Ritonavir peak found 0.10, 0.37 respectively and test passed for this parameter.

Conclusion:
All the injections were processed at the wavelength provided in the method. No disturbance found due to diluent blank Sample, placebo, known impurities with Lopinavir & Ritonavir peak. The peak purity angle of Lopinavir & Ritonavir peaks was less than peak purity threshold in each degradation. Also from calculation it is found that 15.60 % degradation observed and no degrading peak interfered to Lopinavir & Ritonavir peak hence from all this results it is conclude that the developed method for Lopinavir & Ritonavir (assay) was also useful for stability analysis.
3. Linearity:

Correlation Coefficient:
The observed value for Linearity Correlation Coefficient as Lopinavir: 0.9999
Ritonavir: 0.9999
The Y intercept as obtained in linearity as Lopinavir: -0.50, Ritonavir: -0.22

Resolution:
In all chromatograms it is found that Lopinavir & Ritonavir peaks is completely
separated from each other. The Resolution between Lopinavir & Ritonavir is 3.24

USP plate counts:
In chromatogram of Standard Sample preparation the Theoretical plates for Lopinavir
& Ritonavir peak found 4045, 3978 respectively for both peaks

Tailing Factor:
In all chromatogram obtained With Standard Sample preparation the Tailing factor
for Lopinavir & Ritonavir peak found are1.30, 1.19

% RSD:
In all chromatogram obtained With Standard Sample preparation the % RSD for
Lopinavir & Ritonavir peak found 0.64, 0.56 respectively.

Conclusion:
As all results found passed for linearity, based on results it is found method is linear.
All the injections were processed at the wavelength provided in the method.
The Linearity Correlation Coefficient was NMT 0.999. The Y intercept as obtained in
linearity found between ± 2. Hence developed method for Lopinavir & Ritonavir was
Linear and Precise and ready to used any recommendation results.
4. Accuracy:

Accuracy Results:
The % Recovery summarized as follow:

For 50 %
- Peak of Lopinavir - 100.58 %
- Peak of Ritonavir - 100.88 %

For 100 %
- Peak of Lopinavir – 99.17 %
- Peak of Ritonavir - 99.47 %

For 150 %
- Peak of Lopinavir – 99.44 %
- Peak of Ritonavir - 99.73 %

Resolution (Separation between Two peaks):
When Chromatogram was processed it is found that Lopinavir & Ritonavir peaks are completely separated from each other. The Re-solution between Lopinavir & Ritonavir is 2.98 which were between our acceptances criteria.

Theoretical plates:
By processing the chromatogram obtained With Standard Sample preparation the Theoretical plates for Lopinavir & Ritonavir peak found are as 3658, 3785 respectively for both peaks and found passed as per acceptance criteria.

Tailing Factor:
After processing chromatogram obtained With Standard Sample preparation the Tailing factor for both peaks found as 1.20, 1.16 respectably for both peaks and found passed as per acceptance criteria.

% RSD:
In all chromatogram obtained With Standard Sample preparation the % RSD for Lopinavir & Ritonavir peak found are as 1.13, 1.54 and found passed as per acceptance criteria.
Conclusion:
From all above results of accuracy it is conclude that method is accurate. All the injections were processed at the wavelength provided in the method. The % recovery for this instrumental method found within Limit. From all this results it is conclude that the diluent selected for instrumental method suitable and hence developed method for assay of Lopinavir & Ritonavir was Accurate and Précised.
5. Method Precision:

Method precision Results:

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>% Assay of Lopinavir</th>
<th>% Assay of Ritonavir</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100.68</td>
<td>99.42</td>
</tr>
<tr>
<td>2</td>
<td>101.31</td>
<td>98.19</td>
</tr>
<tr>
<td>3</td>
<td>100.66</td>
<td>98.32</td>
</tr>
<tr>
<td>4</td>
<td>101.35</td>
<td>99.43</td>
</tr>
<tr>
<td>5</td>
<td>100.90</td>
<td>98.35</td>
</tr>
</tbody>
</table>

All above results found within acceptance criteria i.e. 90% to 110%

The observed % RSD value for sample of Method Precision as 0.33%, 0.62% respectively for both peaks.

Resolution (Separation between Two peaks):

When Chromatogram was processed it is found that Lopinavir & Ritonavir peaks are completely separated from each other. The Re-solution between Lopinavir & Ritonavir is 30.9 which were between our acceptance criteria.

By processing the chromatogram obtained With Standard Sample preparation the Theoretical plates for Lopinavir & Ritonavir peak found are as 4045, 3978 respectively for both peaks and found passed as per acceptance criteria.

Tailing Factor:

After processing chromatogram obtained With Standard Sample preparation the Tailing factor for both peaks found as 1.35, 1.24 respectively for both peaks and found passed as per acceptance criteria.

% RSD:

In all chromatogram obtained With Standard Sample preparation the % RSD for Lopinavir & Ritonavir peak found are as 0.13, 0.66 and found passed as per acceptance criteria.
Conclusion:
From all above results, all the injections were processed at the wavelength provided in the method. The % RSD for Different sample Preparation was calculated for injected sample of Precision of Method and it is found within the acceptance criteria hence it is proved that developed method for Lopinavir & Ritonavir Soft Gelatin Capsules is précised and method used full for regular analysis.
6. Robustness

Table- 49….Cont. Summary

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Theoretical plates</th>
<th>Taling factor</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>High Flow</td>
<td>3956</td>
<td>1.20</td>
<td>0.44</td>
</tr>
<tr>
<td>Low Flow</td>
<td>3987</td>
<td>1.18</td>
<td>1.9</td>
</tr>
<tr>
<td>High pH</td>
<td>4014</td>
<td>1.24</td>
<td>0.12</td>
</tr>
<tr>
<td>Low pH</td>
<td>4025</td>
<td>1.19</td>
<td>0.28</td>
</tr>
</tbody>
</table>

Table- 49….Cont. Summary

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Theoretical plates</th>
<th>Taling factor</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>High Flow</td>
<td>4015</td>
<td>1.08</td>
<td>0.65</td>
</tr>
<tr>
<td>Low Flow</td>
<td>4036</td>
<td>1.13</td>
<td>0.68</td>
</tr>
<tr>
<td>High pH</td>
<td>4125</td>
<td>1.01</td>
<td>0.76</td>
</tr>
<tr>
<td>Low pH</td>
<td>4098</td>
<td>1.05</td>
<td>0.14</td>
</tr>
</tbody>
</table>

All above result reported found within limit

Resolution (Separation between Two peaks):

When Chromatogram was processed it is found that Lopinavir & Ritonavir peaks is completely separated from each other. The Re-solution for both peak passed as per acceptance criteria.

Theoretical plates:

By processing the chromatogram obtained With Standard Sample preparation the Theoretical plates found within limit hence method robust for this parameter.
**Tailing Factor:**
After processing chromatogram obtained with Standard Sample preparation the Tailing factor for both peaks found within limit and passed as per acceptance criteria.

**% RSD:**
In all chromatogram obtained with Standard Sample preparation the % RSD for Lopinavir & Ritonavir peak passed as per acceptance criteria.

**Conclusion:**
Based on data of robustness when all the injections were processed at the wavelength provided in the method. The % RSD for system suitability injection in all robustness parameter found within Limit hence it is proved that developed method for Lopinavir & Ritonavir Soft Gelatin Capsules is robust to pH and flow and from all result of robustness it was proved that method was not sensitive to pH or any other condition hence method is useful for routine analysis in any sector with precise result.
7. Solution Stability:

Conclusion:
Based on data for solution stability all the injections were processed at the wavelength provided in the method all results are found within limits. In all chromatograms it is found that Lopinavir & Ritonavir peaks is completely separated from each other. The Re-solution between Lopinavir & Ritonavir is in acceptance Criteria. The Theoretical plates, Factor for tailing and % RSD for Lopinavir & Ritonavirpeak found are within the Limit. Hence from all above it is proved that the Samples were Stable up to 24 Hours hence sample use up to 24 Hrs at the time of analysis.
## 9.1.0. SUMMARY AND CONCLUSION: MONTAIR TABLETS

Table- 50.... Summary

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Parameter</th>
<th>Result</th>
<th>Acceptance Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Specificity: Selectivity</td>
<td>The Montelukast peak is well resolved from Diluent blank Sample, excipient blend Sample and known impurities. Diluent blank Sample, excipient blend does not show any peak at the RT of the Montelukast and its known impurities.</td>
<td>The Montelukast Would be completely separated from any other peaks. The placebo, diluent blank and known Impurity Would not show peak at the RT of the Montelukast.</td>
</tr>
<tr>
<td></td>
<td>Specificity: Forced Degradation</td>
<td>1] The peaks due to degradation products do not show any peak at the RT of Montelukast at each condition of degradation. 1] The peaks due to degradation products are well separated from the peak due to Montelukast. 2] Approximately 12.13% degradation was observed when the tablet powder was subjected to oxidation degradation and 17.00 % degradation was observed when tablet powder was subjected to photolytic degradation. 3] The purity angle for Montelukast peak was less than purity threshold for same peak at each condition of degradation.</td>
<td>Degradant peaks Would be completely separated from the Montelukast peak. 10% – 30% degradation in at least one stress condition shall be achieved. Peak purity angle of peak due to Montelukast would not be more than the peak purity threshold in untreated and treated samples which will states that peak purity passes.</td>
</tr>
</tbody>
</table>

(Montelukast Sodium Chewable Tablets 5 mg)
### Table- 50….Cont. Summary

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Parameter</th>
<th>Result</th>
<th>Acceptance Criteria</th>
</tr>
</thead>
</table>
| 2.      | Linearity and Range        | Correlation coefficient = 0.9999  
Range = 49.00 ppm to 147.00 ppm | Correlation coefficient between concentration and area of peak for 50 percent to 150 percent sample would not be less than 0.999.                      |
| 3.      | Precision                  | System precision  
% RSD = 0.15  
Factor for tailing = 1.45  
Theoretical Plates = 6300 | % RSD of Main peak areas of five standards Sample (Reference Sample) would not be more than 2.0.                                                   |
|         |                            | Method precision  
% RSD = 1.16  
Factor for tailing = 1.49  
Theoretical Plates = 6363 | Results obtained with % RSD for six different sample would not exceed 2.0 % value                                                              |
| 4.      | Recovery ie.accuracy (Recovery) | ![Table](#)  
| Level   | %Recovery  | % RSD  | The % Recovery would be between 98.0 % to 102.0 %  
% RSD for Accuracy would not exceed value 2.0. |
| 50% Level | 98.96  | 0.74  |  
| 100% Level | 98.56  | 0.78  |
| 150% Level | 99.37  | 0.47  |  
| 5.      | Filter validation          | The difference between filtered, unfiltered Sample is less than 2.0. | The difference between filtered, unfiltered Sample would not be Exceed 2.0% value                                                                    |
| 6.      | Robustness                 | Robustness Change in column oven temperature (± 5°C)  
% RSD between results obtained with changed condition and six results of method precision are less than 2.0. | % RSD between results obtained with changed condition and six results of method precision would not be more than 2.0. |
<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Parameter</th>
<th>Result</th>
<th>Acceptance Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Robustness – change in pH - buffer (± 0.2 unit)</td>
<td>% RSD between results obtained with changed condition and six results of method precision are less than 2.0.</td>
<td>% RSD between results obtained with changed condition and six results of method precision would not be more than 2.0.</td>
</tr>
<tr>
<td></td>
<td>Robustness Change in wavelength (± 2 Nanometer)</td>
<td>% RSD between results obtained with changed condition and six results of method precision are less than 2.0.</td>
<td>% RSD between results obtained with changed condition and six results of method precision would not be more than 2.0.</td>
</tr>
<tr>
<td>7.</td>
<td>Stability of analytical Sample</td>
<td>The % RSD of the stored and initial prepared system suitability and test Sample are less than 2.0 up to 3 days. Hence the Sample is found stable up to 3 days at RT (room temp)</td>
<td>Solutions (Sample) considered stable, till the time point where the % RSD of the stored and initial prepared test and standard Sample (Reference Sample) is NMT 2.0.</td>
</tr>
</tbody>
</table>
9.1.1. Conclusion:

1. Selectivity:
The Montelukast peak is well resolved from any other peak.
The diluent blank Sample, excipient blend Sample and known impurities do not show any peak at the RT of the Montelukast. Hence method is established as selective.

2. Forced Degradation:
From all above forced degradation data it is proved that method useful in stability analysis. Degrading peaks are separated from main peak in the degradation samples of API and tablets at all degradation conditions. The peak purity of Montelukast is found to meet the pre-established acceptance criteria. The peaks due to degradation products of excipient blend do not show any peak at the RT of Montelukast at each condition of degradation. Based on the above observations it is concluded that the method is specific and used in routine analysis.

3. Linearity and Range:
Linearly plot from lower level to higher level in that it is observed that linearity graph of the average area at each level against the concentration (%) is plotted and is found a straight line graph. The correlation coefficient is found more than 0.999. Hence it is concluded that, the method is found to be linear in given working concentration of the analytical method is 49.00 ppm to 147.00 ppm.

4. Method precision:
The % RSD of the assay results of six test Samples are found 0.40 and meet the pre-established acceptance criteria. Hence, it is concluded that the method is précised.

5. Recovery:
Recover is most important in validation, the percentage recovery for Montelukast at each level lies between 98.0% and 102.0% and % RSD is less than 2.0. The analytical method meets the pre-established acceptance criteria for recovery study as validation criteria hence, it is proved that accurate method established.
6. Filter validation:
This parameter carried out on same sample by using different filter based on data the difference between filtered, unfiltered sample is less than 2.0%. The analytical method meets the pre-established acceptance criteria for filter validation study as per protocol. Hence Zero point Forty five (0.4 micro meter) µm nylon size filters can be used, and it is recommended to use the filtered test sample in the routine analysis.

7. Robustness:
The analysis of the same lot of Montelukast Sodium Chewable Tablets 5 mg was carried out at different conditions of column oven temperature, pH -buffer and wavelength. The system suitability was found to meet the pre-established criteria at all the conditions and the % RSD between results obtained with changed condition and six result of method precision, is NMT 2.0. The analytical method meets the pre-established acceptance criteria for robustness study as per protocol. Thus the method is robust.

8. Stability of analytical Sample
Solution stability carried out and based on data the system suitability was found to within criteria and the % RSD between assay results obtained for freshly prepared test sample and the stored test samples is less than 2.0 up to 3 days. There is no significant change in assay level observed up to 3 days for system suitability, test samples at room temperature. Thus, it can be concluded that the sample is stable up to 3 days at room temperature.
**Recommendation**

- It is possible to reduce more retention time by using UPLC i.e. ultra performance liquid chromatography which is newly form technique by using small particle size silica columns.

- During analysis it is possible to prepare impurities in such solvents as impurities stable for long time to reduce cost of impurities.

- It is also possible to demonstrate relative retention time for known impurities, as it is not necessary to prepare and inject impurities every time.

- It is also possible to prepare high ppm concentration stock of known impurity in non degradable solvents and use during analysis up to its validity.

- In feature it might be possibilities of new formulation which forms new unknown impurities with different combination hence needs to develop regarding new ones.
Feature Scope

- The analytical method for related substances of Lopinavir & Ritonavir are chance to develop by reducing run time of method by using new UPLC Methods.

- The analytical method for related substances of Lopinavir & Ritonavir are chance to develop in one common method as it is more critical.

- The analytical method also chance to reduces cost and time by using new generation aquity columns of smaller particle size for immediate elution pattern.

- It is great scope to develop common analytical method for assay and related substance for both molecules.

- In Feature it might be possibilities of developing new formulation with another combination of this drug hence it scope to develop method accordingly of this method basis.
Limitations

- Solution stability needs more time to establish its stability as there is no need to develop method for solution stability more than 72 Hours.

- As chemicals are very costly it does not possible to done mobile phase stability for more time.

- As impurities are rearly available from industries or pharmacopeial bodies due to new regulation it very critical to develop and validate method with small quantities.

- Small particle size columns are not easily available and they are costly hence not reduces run time for related substances methods.

- Some small particle size column available but HPLC does not bare the pressure.

- Required some costly solvents to achieve more resolution as it observed in respective method.