2.8 Chromatographic Conditions:

Table 10: Summery Table of Chromatographic Conditions and Mass Spectrometric Conditions

<table>
<tr><td rowspan="2">HPLC Conditions</td><td>Pump Flow Rate</td><td colspan="3">Autosampler Injection Volume</td><td>Autosampler Temp</td><td>Column Oven Temp</td></tr>
<tr><td>1.0 ml/min</td><td colspan="3">5 µl</td><td>4˚C</td><td>30˚C</td></tr>
<tr><td rowspan="9">Chromatography</td><td rowspan="7">Mobile phase Gradient Elution</td><td>Step</td><td>Total Time(min)</td><td>Flow Rate (µl/min)</td><td>A (%) Methanol</td><td>B (%) 0.2% F.A</td></tr>
<tr><td>0</td><td>0.00</td><td>1000</td><td>50.0</td><td>50.0</td></tr>
<tr><td>1</td><td>0.01</td><td>1000</td><td>50.0</td><td>50.0</td></tr>
<tr><td>2</td><td>0.02</td><td>1000</td><td>100.0</td><td>0.0</td></tr>
<tr><td>3</td><td>0.70</td><td>1000</td><td>100.0</td><td>0.0</td></tr>
<tr><td>4</td><td>0.71</td><td>1000</td><td>50.0</td><td>50.0</td></tr>
<tr><td>5</td><td>2.00</td><td>1000</td><td>50.0</td><td>50.0</td></tr>
<tr><td>Column type</td><td colspan="5">ACE 5 C18 Column (50 X 2.1 mm), 5µ</td></tr>
<tr><td rowspan="2">Expected Retention times(minutes)</td><td colspan="2">Candesartan</td><td colspan="3">Irbesartan (I.S)</td></tr>
<tr><td colspan="2">1.4</td><td colspan="3">1.0</td></tr>
<tr><td rowspan="3">MRM Detection Conditions</td><td>Analytes</td><td>Q1 Mass</td><td>Q3 Mass</td><td>Dwell</td><td>FP</td><td>DP</td><td>EP</td><td>CE</td><td>CXP</td></tr>
<tr><td>Candesartan</td><td>441.200</td><td>263.200</td><td>150</td><td>70</td><td>81</td><td>10</td><td>19</td><td>22</td></tr>
<tr><td>Irbesartan (IS)</td><td>429.453</td><td>207.300</td><td>150</td><td>70</td><td>26</td><td>10</td><td>8</td><td>22</td></tr>
<tr><td rowspan="2">MS Conditions</td><td>CUR</td><td>CAD</td><td>IS</td><td colspan="2">TEM</td><td colspan="3">NEB</td></tr>
<tr><td>10</td><td>6</td><td>5500</td><td colspan="2">400</td><td colspan="3">5</td></tr>
</table>

2.9 Irbiartan as internal standard

Irbesartan as internal standard is used to determine the concentration of candesartan by calculating response factor. irbesartan is similar to candesartan and have a similar retention time. It is stable and does not interfere with the sample components.