3.3 Reverse Phase-HPLC Method creation and Validation for the Estimation of Trandolapril in Tablet unit dose Form

3.3.1 Drug Profile – Trandolapril

3.3.1.1 Description:

It is an ACE inhibitor, for the treatment high blood pressure.

3.3.1.2 IUPAC -Name:

\[(2S,3aR,7a S)-1-[(2 S)-2-{[(2 S)-1-ethoxy-1-oxo-4-phenylbutan-2yl] amino propanoy l}]-octahydro-1H-indole-2-carboxylic acid.\]

3.3.1.3 Structure:

![Structure of Trandolapril](image)

3.3.1.4 Molecular Formula:

\[C_{24}H_{34}N_{2}O_{5}\]

3.3.1.5 Percentage composition:

Carbon 66.95%, Hydrogen 7.96%, Nitrogen 6.51%, Oxygen 18.58%
3.3.1 Molecular Weight:
430.54

3.3.1.7 Melting Point:
125°C

3.3.1.8 Route of Administration:
Oral

3.3.1.9 Mechanism of Action:
Trandolapril predominantly acts by aggressive hindrance of angiotensin changing over compound, is a key protein in renin-angiotensin framework which regulates blood pressure.

3.3.2 Aim and Objective
To evolve a new RP-high performance fluid chromatography procedure for assessment of Trandolapril in tablet formulation & its validation according to ICH rules.

3.3.3 Materials and Methods

3.3.3.1 Instrumentation
Chromatography was performed by utilizing Alliance waters 2695 HPLC gave auto sampler, section broiler, degasser and 2996 PDA finder to give class Empower-2 software.

3.3.3.2 Chemicals and Reagents:
The standard Trandolapril drug, a gift sample was given from SPRS, Hyderabad. The chromatographic chemicals purchased from Bombay. Good grade quality used for the entire work. Water is of Milli-Q framework. Tablets available in the market (MAVIK – 2mg) were acquired from nearby drug store.

3.3.3.3 Condition of chromatography:
Phosphate buffer and acetonitrile present in the ratio of thirty five: sixty five were used as motile phase. Used a stream rate of one milli litre per minute. Column instituted
for study is Altima, C18, it’s particle size is 4.6 x150mm, 5μ particle size. Wave length chosen as 220nm for detection.

3.3.3.4 Preparation of standard stock solution

Precisely measured 20 mg of Trandolapril as working Standard and moved into a 100 ml dry volumetric carafe, then include 70ml of diluent, subject to ultrasonic vibration for thirty minutes and made up to last volume with diluent.

5.3.5 Preparation of Working Standard Solutions

From the stock solution pipette out an aliquot of 0.3, 0.6, 0.9, 1.2, 1.5 & 1.8 mL, then transferred into ten mL volumetric carafe and made the volume of solution up to 10 mL with dilutant. This results in the formation of 6, 12, 18, 24, 30 and 36 micro gram/mL solutions for Trandolapril.

3.3.3.6 Preparation of phosphate Buffer

Weigh about 1.36 g of Potassium dihydrogen Orthophosphate and put it to a thousand ml of volumetric carafe, add milli-Q water 900 ml & add one ml of tri-ethylamine, degas, subject to ultrasonic vibration and made up the amount with water, finally adjust the hydrogen ion concentration to 3.6 with dilute solution of ortho phosphoric acid.

3.3.3.7 Specimen preparation

Twenty tablets were weighed and figure the typical weight then the weight practically identical to one tablet was moved into a 100 mL volumetric carafe, to this 70mL of dilutant included and subject to ultrasound vibration for thirty minutes, then volume made up with dilutant and sifted. From this arrangement 1.2ml was pipetted out into a 10 ml volumetric carafe and make upto 10ml with dissolvable.

3.3.3.8 Validation method.

Parameters, for example, systems fitness, Linearity, precision, specificity, LOD and LOQ and power were performed by ICH rules.

3.3.4 Results and Discussion

Method development

At first switch eliminate chromatographic partition was conveyed to create utilizing
different dissolvable proportions of Acetonitrile and Water, Methanol and Water as versatile stages, in that tranquilize did not reacted legitimately. Natural substance of portable stage was additionally researched to improve the movement of the medication. For enhancing tailing variable, hydrogen ion concentration of portable stage gets to be essential element. For that point, phosphate buffer and acetonitrile is taking in isocratic proportion, 35:65 and along with stream rate of one mL/minute is considering. Column Altima C18 having particle size 4.6 x 150mm, 5μ was chosen as the permanent stage to lessen the tailing at the top & maintained two hundred and twenty nanometer as wavelength for PDA finder. The maintenance times were found to around 2.9 min and the outcomes are shown in table 3.3.4.1.

Table 3.3.4.1: Effective conditions of chromatography

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parameter</th>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Motile phase</td>
<td>Acetonitrile: Phosphate Buffer: (65:35)</td>
</tr>
<tr>
<td>2</td>
<td>H ion concentration</td>
<td>3.6(+/-0.5)</td>
</tr>
<tr>
<td>3</td>
<td>Column</td>
<td>C18 (150 x 4.6 mm, 5μ), Altima</td>
</tr>
<tr>
<td>4</td>
<td>Temperature of Column</td>
<td>30°C</td>
</tr>
<tr>
<td>5</td>
<td>Wave length</td>
<td>220nm</td>
</tr>
<tr>
<td>6</td>
<td>Injection volume</td>
<td>10ul</td>
</tr>
<tr>
<td>7</td>
<td>Flow rate</td>
<td>1.0ml/min</td>
</tr>
<tr>
<td>8</td>
<td>Run time</td>
<td>5mins</td>
</tr>
<tr>
<td></td>
<td>Retention time</td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>----------------</td>
<td>---</td>
</tr>
<tr>
<td>9</td>
<td>2.9mins</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 3.3.4.1: Standard Chromatogram of Trandolapril**

![Chromatogram](image1)

**Figure 3.3.4.2: Blank chromatogram**

![Chromatogram](image2)
Figure 3.3.4.3: Placebo Chromatogram

Method Validation:

System suitability

The assay procedure development and validation is done through ICH rules. This test were conducted to assess the chromatographic parameters (tailing of the peak, number of theoretical plates) before the validation runs.

Linearity

The linearity performed was at range of 6 - 36μg/mL. The medication reaction was straight and the relapse condition was y = 34750x - 792.38 & the correlation coefficient were observed as 0.9999 and outcomes are as below in table 3.3.4.2

Table 3.3.4.2: Linearity results

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Concentration in μg/mL</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>201012</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>416462</td>
</tr>
<tr>
<td>3</td>
<td>18</td>
<td>628462</td>
</tr>
<tr>
<td>4</td>
<td>24</td>
<td>828288</td>
</tr>
<tr>
<td>5</td>
<td>30</td>
<td>1030119</td>
</tr>
</tbody>
</table>
Figure 3.3.4.4: Linearity 25% chromatogram of Trandolapril

![Figure 3.3.4.4](image1)

Figure 3.3.4.5: Linearity 50% chromatogram of Trandolapril

![Figure 3.3.4.5](image2)
Figure 3.3.4.6: Linearity 75% chromatogram of Trandolapril

Figure 3.3.4.7: Linearity 100% chromatogram of Trandolapril
Figure 3.3.4.8: Linearity 125% chromatogram of Trandolapril

Figure 3.3.4.9: Linearity 150% chromatogram of Trandolapril
Precision

It is calculated as the level of test-retest reliability of a systematic strategy in a typical operative conditions. Accuracy technique was executed as test occurring within one day and in between the days precision.

Intra-day precision

Six repeat solution standards of Trandolapril were infused for within one day exactness. The percent relative standard deviation computed and observed to be 0.45 that are well inside the adequate criteria of not greater than 2.0.
Inter-day precision

To consider the inter day accuracy, 6 replicates solution standards of Trandolapril were infused. The % relative standard deviation computed and observed to be 0.52 that are well inside the satisfactory criteria of not greater than two.

Specificity

The impact of extensive variety of excipients and different added substances normally show in the dosage form in the determinations in the ideal conditions were explored. The parameters of the chromatography are particular for Testing drug.

LOD AND LOQ

A calibration curve was readied utilizing focuses as a part of the linear range. The standard deviation of Y-captures of relapse line was resolved. The limit of detection and Limit of quantification of Trandolapril were individually 0.28 and 0.85 µg/mL.

Accuracy

The exactness in the technique were dictated by std. expansion strategy. A known measure of std. medication was mixed to the altered measure of already analysed solution standard. The addition method is performing at 50%, 100% and 150% level of standard ppm. The solutions are analysed in triplicate at every time as with the suggested procedure. The %RSD & % restoration is 1.39. Agreeable recuperations running from 98% to 102% were gotten by the suggested strategy. This shows the strategy was precise.

Figure 3.3.4.11: Intra-day precision chromatogram of Trandolapril
Table 3.3.4.3: Results of intermediate precision of Trandolapril

<table>
<thead>
<tr>
<th></th>
<th>Set 1(n=6)</th>
<th>Set 2(n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean assay (%)</td>
<td>100.16</td>
<td>100.92</td>
</tr>
<tr>
<td>%RSD</td>
<td>0.93</td>
<td>0.90</td>
</tr>
</tbody>
</table>

Figure 3.3.4.13: Accuracy 50% chromatogram of Trandolapril
Figure 3.3.4.14: Accuracy 100% chromatogram of Trandolapril
Robustness

Vigor of the technique was controlled by rolling out slight improvements in the conditions of chromatography. As there is no stamped alterations with the chromatograms, rp-hplc strategy created is powerful.

Tablet Analysis

The ingredients of Trandolapril in the unit dose was analysed by the proposed technique. RSD values are observed and it is 0.46.

Figure 3.3.4.16: Assay chromatogram of Trandolapril
Table 3.3.4.4: Assay results

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Formulation</th>
<th>Label claim</th>
<th>Amount found</th>
<th>%Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MAVIK</td>
<td>2mg</td>
<td>2.002mg</td>
<td>100.13%</td>
</tr>
</tbody>
</table>

### 3.3.5 Conclusion

Another exact precise and basic HPLC technique was produced and approved for the evaluation of Trandolapril in tablet form. This technique is quick, exact and delicate subsequently it can be utilized for normal quality control of Trandolapril tablets in Quality Control research facilities and commercial enterprises.