ANALYTICAL SECTION
11.0 ANALYTICAL METHODS

11.1 Determination of $\lambda_{\text{max}}$

The wavelength scan of Acitretin and Telmisartan were determined by UV spectrophotometer method. 250 $\mu$L of the standard solution containing 10ppm of the active material was transferred into multi quartz. The spectrum was recorded in the wavelengths between 200-400nm at the interval of 5nm to get the $\lambda_{\text{max}}$ against blank solutions prepared in the same manner without the drug. The obtained $\lambda_{\text{max}}$ values for the two drugs under study is given below table-7

<table>
<thead>
<tr>
<th>S.No</th>
<th>Compound</th>
<th>$\lambda_{\text{Max}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Acitretin</td>
<td>360 nm</td>
</tr>
<tr>
<td>2</td>
<td>Telmisartan</td>
<td>240 nm</td>
</tr>
</tbody>
</table>

11.2 Analytical Procedure for Telmisartan soft gelatin capsules

Reverse phase HPLC analysis was carried out using waters 2695-module pump, waters 2465 dual $\lambda$ absorbance detectors. Waters empower Software was used for the data processing and peak analysis. Optimization of chromatographic conditions was carried out in two methods (1.isocratic; 2.gradient). In the case of isocratic method of analysis, various mobile phase compositions (acetonitrile and 0.1% acetic acid or pH 10 buffer) were tried with constant flow rate (1ml/min); Column C18 and Column temperature (25±1°C), with an initial system pressure of approximately 1500 psi. Sample (standard drug samples) was injected (volume of 25 $\mu$L) and the full spectrum was recorded from which data processing and peak analysis were performed by using water empower software.

In the case of gradient method of analysis, mobile phase consisting of acetonitrile (HLPC grade, RFCL ltd., new Delhi, India) in pump A, and 0.1% acetic acid in pump B was employed. The flow rate was maintained at 1mL/min with an initial system pressure of approximately 1500 psi. Sample was injected (volume of 25 $\mu$L) was used and full spectrum was recorded from which data
processing and peak analysis was performed by using water empower software. 50% acetonitrile and 50% water was used as wash solution for column maintenance. The below table-8 shows the gradient program employed for the optimization study of chromatographic conditions.

HPLC gradient program

**Table-11**

<table>
<thead>
<tr>
<th>Time</th>
<th>Flow ml</th>
<th>A%</th>
<th>B%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>30</td>
<td>70</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>70</td>
<td>30</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>70</td>
<td>30</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>60</td>
<td>40</td>
</tr>
</tbody>
</table>

11.3 Preparation of mobile phase

11.3.1 Buffer (0.1% acetic acid buffer)

Accurately measure 1 ml of glacial acetic acid was added to 999ml of ultra pure water, mixed well and sonicated. pH was measured and found to be around 2.

11.3.2 Buffer (pH 10)

Accurately measure 1.2 ml of glacial acetic acid and add 3.5 ml triethylamine and added to 950ml of water, then made up to 1000ml with ultra pure water. Mixed well and sonicated. pH was measured and found to be around 10.
11.3.4 Standard Solution Preparation
Accurately weighed 5 mg of reference standard transferred to a 50 ml volumetric flask and added 25 ml of Acetonitrile dissolved by sonication and make up with water up to the mark to get the stock solution of 100μg/ml. From this serial dilution were made to get the desired concentration of 0.5, 5, 10, 25, 50μg/ml. This standard solution was injected to ensure the linearity with minimum $R^2$ value of 0.9999.

11.4 Analytical method For Acitretin Capsules

11.4.1 Reagents
- Ethanol    AR Grade
- Glacial acetic acid AR Grade
- Sodium lauryl sulphate AR Grade
- Tetra hydro furan AR Grade

11.4.2 Chromatographic condition
- Column : 155 cm x 4.6mm packed with LI; octadecyl silane chemically bonded to silica packed with or ceramic microparticles 5.0 μ
- Wavelength : 360 nm
- Flow rate : 1.0 ml/minute
- Injection volume : 10μl
- Column temperature : 35°C
- Sample cool temperature : 10 °C
- Run time : 6 minutes
11.4.3 Mobile phase preparation:
Preparation a mixture of 850 volumes of ethanol, 150 volumes of water, and 3 volumes of glacial acetic acid. Filter through 0.45 µm and degas.

11.4.4 Calculation:
Calculate the % of Acitretin by using the formula:

\[
\% \text{ dissolved} = \frac{\text{AT}}{\text{AS}} \times \frac{\text{WS}}{\text{DS}} \times \frac{\text{DT}}{1} \times \frac{\text{D}}{100} \times \frac{100}{\text{LC}}
\]

Where,
AT – Area response of the Acitretin peak from sample preparation chromatogram
AS – Average area response of Acitretin peak from the standard preparation chromatogram
WS- Weight of the Acitretin standard taken in mg
DT – Dilution of the test solution
DS - Dilution of the standard solution
D- Purity of Acitretin standard in % on as is basis
LC- Label claim in mg

11.5 Calibration curve of Telmisartan and Acitretin
The calibration curves were generated for different concentrations of Telmisartan and Acitretin. The concentrations were chosen based on the sensitivity of the method for each compound. Accordingly, the concentrations from 5.0µg/ml to 50 µg/ml were chosen. These standard solutions were measured and the values are shown in table-12 Calibration curves were obtained by a linear regression of the peak area ratio of Telmisartan and Acitretin.

Exactly 1mL of the sample make up with 25 ml of 1:1 ratio of acetonitrile and water and vigorously shaken. Then this sample solution was filter in a 0.2µm membrane filters. After filtrations, 25 µL of supernatant solution was injected into the HPLC system. Calculation was done based on the standard plot method and the linearity was shown in the figure.
11.5.1 Linearity Graph

Table-12

<table>
<thead>
<tr>
<th>Concentration in mcg/ml</th>
<th>Telmisartan Area</th>
<th>Acitretin Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>33689</td>
<td>80703</td>
</tr>
<tr>
<td>5</td>
<td>392800</td>
<td>806723</td>
</tr>
<tr>
<td>10</td>
<td>786343</td>
<td>1601235</td>
</tr>
<tr>
<td>25</td>
<td>1965010</td>
<td>4035115</td>
</tr>
<tr>
<td>50</td>
<td>3881064</td>
<td>8070236</td>
</tr>
</tbody>
</table>

Figure 2: Linearity Graph of Telmisartan and Acitretin
Drug content evaluation

11.6.1 Telmisartan

The drug content of Telmisartan Nanoemulsion formulations was determined by HPLC analysis. The HPLC system consisted of waters 2695 module pump, waters 2465 dual λ absorbance detector and waters empower Software was used for the data processing and peak analysis. The Telmisartan was detected at 240 nm. Chromatographic separations were achieved using an Agilent ODS-C18 column (250 × 4.6 mm) (GL Science, Tokyo, Japan). The mobile phase used for the sample was 40% of 0.1% acetic acid buffer with 60% of acetonitrile. Filtration of the buffer was done using 0.2µm membrane filters, and it was degassed by sonication. The sample chromatograms are represented below

![Telmisartan representative chromatogram](image-url)
11.6.2 Acitretin:

Acitretin drug concentration in aqueous phase was determined by separating two phases using ultracentrifugation. 5 ml of the formulation was diluted up to 25 ml suitable solvent and centrifuged at 10000 rpm for 30 minutes using centrifuge. Supernatant was separated and filtered and analysed by HPLC with the above mentioned analytical method.

Then this diluted sample solution was filter in a 0.2µm membrane filters. After filtrations, 25 µL of supernatant solution was injected into the HPLC system. Calculation was done based on the standard plot method and the linearity was found to have $R^2$ value of 1.

The Chromatograms of representative samples are captured below.