CHAPTER 4

STABILITY INDICATING HPTLC METHOD DEVELOPMENT AND VALIDATION OF RUFINAMIDE IN BULK AND ITS PHARMACEUTICAL DOSAGE FORM
4.1: Experimental

4.1.1 Apparatus and Instruments

- Camag Linomat IV (Semiautomatic Spotting device)
- Camag Twin trough Chamber (10 × 10 cm²)
- Camag TLC Scanner-3
- Camag CATS4 Software
- Hamilton Syringe (100 μL)
- Shimadzu Libror AEG – 220 balance
- Volumetric flask (Borosil glass) – 10, 25, 100 mL
- Pipettes (Borosil glass) – 1, 2, 5, 10 mL
- Beaker, measuring cylinder, conical flask etc used are of borosil glass
- Sonicator (Frontline FS-4)

4.1.2 Reagents & Materials

- Rufinamide (Gift sample from Torrent research centre, Ahmedabad)
- TLC Aluminium sheet pre coated with silica gel G 60 F²₅₄ (20×20 cm², E. Merck, Germany)
- Distilled water (AR Grade)
- Chloroform (S.d. Fine Chemicals, Ahmedabad) (AR Grade)
- Methanol (S.d. Fine Chemicals, Ahmedabad) (AR Grade)
- Glacial acetic acid (S.d. Fine Chemicals, Ahmedabad) (AR Grade)

4.1.3 Formulation:
Rufinamide tablets were procured from local market (Brand name: Banzel®, Label claim: 200 mg)
4.1.4 Chromatographic conditions

<table>
<thead>
<tr>
<th>Stationary phase</th>
<th>Pre coated Silica gel G 60 F&lt;sub&gt;254&lt;/sub&gt; aluminium Sheets, Layer thickness 0.2 mm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activation</td>
<td>TLC plates prewashed with methanol and activated in oven at 60°C for 15 min.</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>Chloroform: Methanol: Glacial Acetic Acid (9:1:0.1, v/v/v)</td>
</tr>
<tr>
<td>Chamber saturation time</td>
<td>30 min</td>
</tr>
<tr>
<td>Temperature</td>
<td>Ambient</td>
</tr>
<tr>
<td>Migration distance</td>
<td>80 mm</td>
</tr>
<tr>
<td>Spotting parameters</td>
<td>Band width: 6 mm</td>
</tr>
<tr>
<td></td>
<td>Space between two bands: 5 mm</td>
</tr>
<tr>
<td></td>
<td>Distance between two tracks: 11 mm</td>
</tr>
<tr>
<td></td>
<td>Spraying rate: 15 sec/µL</td>
</tr>
<tr>
<td>Scanning parameters</td>
<td>Slit dimension: 5 × 0.45 mm</td>
</tr>
<tr>
<td></td>
<td>Wavelength of detection: 210 nm</td>
</tr>
<tr>
<td></td>
<td>Lamp: Deuterium</td>
</tr>
<tr>
<td></td>
<td>Measurement mode: Absorption/Reflection</td>
</tr>
<tr>
<td></td>
<td>Monochromator band width: 20 nm</td>
</tr>
<tr>
<td></td>
<td>Scanning speed: 100 mm/sec</td>
</tr>
<tr>
<td>Integration parameters</td>
<td>Baseline correction: Yes</td>
</tr>
<tr>
<td></td>
<td>Peak threshold, height: 50 AU</td>
</tr>
<tr>
<td></td>
<td>Peak threshold, area: 50</td>
</tr>
<tr>
<td></td>
<td>Peak threshold, slope: 5</td>
</tr>
</tbody>
</table>

4.1.5 Preparation of standard solutions

4.1.5.1 Stock solution of Rufinamide (1000 µg/mL):
Accurately weighed Rufinamide (10 mg) was transferred into 10 mL volumetric flask, dissolved in and diluted upto the mark with methanol.
4.1.5.2 Preparation of Hydrochloric acid (1 N)
Hydrochloric acid (8.5 mL, 36 %) was accurately measured and transferred to a 100 mL volumetric flask and diluted up to the mark with distilled water.

4.1.5.3 Preparation of Sodium hydroxide solution (1 N)
Sodium hydroxide (4 gm) was accurately weighed and transferred to a 100 mL volumetric flask, dissolved in and diluted up to the mark with distilled water.

4.1.5.4 Preparation of mobile phase
The mobile phase constituted of 9.0 mL chloroform, 1.0 mL methanol and 0.1 mL of glacial acetic acid. The mobile phase was transferred into a twin-trough chamber covered with lid and allowed to saturate for 30 min.

4.1.5.5 Pre-treatment of pre coated plates
TLC plate of required size was cut and prewashed with methanol. For prewashing, the plate was placed in twin-trough glass chamber containing methanol as a mobile phase. Methanol was allowed to run up to the upper edge of plate (ascending method). The plate was allowed to air dry and activated in oven at 60°C for 15 minutes. Plate was allowed to cool at room temperature and used immediately.

4.1.5.6 Selection of wavelength
The UV spectrum of Rufinamide and its degradation product were recorded over range of 200-400 nm using HPTLC. The wavelength 210 nm was selected for analysis as at this wavelength both, Rufinamide and its degradation product showed better absorbance.

4.1.6 Calibration curve for Rufinamide:
Stock solutions of Rufinamide (10-35 µL), were spotted on pre coated TLC plate to obtain concentration 1000-35000 ng with the use of micro liter syringe under nitrogen stream, using semi-automated spotter. The TLC plate was developed, dried and analyzed photometrically. The calibration curves were obtained by plotting peak area verses concentration (ng/spot).
4.1.7 Chromatographic separation:

- Appropriate volumes of the standard or sample were spotted on the TLC plate 1 cm from bottom edge using Camag Linomat IV semiautomatic spotting device under nitrogen stream.

- TLC plate was developed in ascending mode in twin-trough chamber previously saturated for 30 min with mobile phase Chloroform: Methanol: Glacial acetic acid (9: 1: 0.1 v/v/v) upto 80 mm at 28°C.

- The plate was removed from the chamber, dried in air and scanned in absorbance / reflectance mode using Camag TLC Scanner 3 at 210 nm.

- All the analysis was carried out in a laboratory at ambient temperature control. Densitometry scanning was done in absorbance mode at 210 nm using a deuterium lamp.

- Data of peak area was recorded using Camag CATS 4 software.

4.1.8 HPTLC method validation

Validation of analytical method is the process by which it is established during the laboratory studies, that performance characteristic of method meet the requirement for intended analytical application.

4.1.8.1 Linearity

The linearity of analytical method is its ability to elicit test results that are directly proportional to concentration of analyte in sample within given range. The linearity range of analytical method is interval between upper and lower level of analyte including level that been demonstrated to be determining with precision and accuracy using method. The linearity is expressed in term of correlation co-efficient of linear regression analysis. The linearity of response for Rufinamide was assessed by analysis of six independent levels of calibration curve in range of 1000-3500 ng/spot in terms of slope, intercept and correlation coefficient values.

4.1.8.2 Precision

The precision is measure of either the degree of reproducibility or repeatability of analytical method. It is indication of random error. The precision of analytical method is
usually expressed as a standard deviation (S.D.), relative standard deviation (R.S.D.) or co-efficient of variance (% R.S.D.) of series of measurement.

4.1.8.2.1 Repeatability / Replication

Repeatability / Replication are a precision under the same condition (same analyte, same apparatus, same identical reagent, short interval of time). It includes following two parameters.

4.1.8.2.1 (a) Repeatability of measurement of peak area or peak height: (%R.S.D. < 1 %)

Stock solution of Rufinamide (20 μL) was spotted on pre coated TLC plate. The plate was developed, dried and photometrically analyzed as described previously. Area and height of spot was measured six times without changing the position of plate and % R.S.D. of obtained data was calculated.

4.1.8.2.1 (b) Repeatability of sample application: (%R.S.D. < 2 %)

Stock solution of Rufinamide (20 μL) was spotted on pre coated TLC plate six times. The plate was developed, dried and photometrically analyzed. The areas of six spots were measured and % R.S.D. was calculated.

4.1.8.2.2 Precision

Variations of results within same day and amongst days are called as precision. It includes following parameter.

4.1.8.2.2 (a) Intraday precision

A variation of results within same day is called intraday variation. It was determined by repeating calibration curve 3 times on same day.

4.1.8.2.2 (b) Interday precision

Variation of results amongst day is called interday variation. It was determined by repeating calibration curve daily for 3 different days.

4.1.8.3 Limit of detection (LOD)

It is the lowest concentration of an analyte in a sample that can be detected but not necessarily quantified under the stated analytical conditions. LOD is commonly used to substantiate that an analyte concentration is above or below a certain level.

The limit of detection (LOD) was calculated using the following equation as per the ICH guidelines.⁹⁷
LOD = $3.3 \times \sigma/S$

Where $\sigma$ is the standard deviation of the response,

$S$ is the standard deviation of $y$-intercept of regression lines.

### 4.1.8.4 Limit of quantification (LOQ)

It is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental condition.

Limit of quantitation (LOQ) of the drugs were calculated using the following equations as per ICH guidelines.$^{97,98}$

LOQ = $10 \times \sigma/S$

Where $\sigma$ is the standard deviation of the response,

$S$ is the standard deviation of $y$-intercept of regression lines.

### 4.1.8.5 Accuracy (% Recovery)

Accuracy of an analysis is determined by calculating systemic error involved. It was determined by calculating recovery of Rufinamide by standard addition method at three different concentration levels of drug. 5, 10 and 15 $\mu$L of standard solution were added on succeeding spots to obtain final concentration range of 1500, 2000, 2500 ng /spot for Rufinamide under nitrogen atmosphere. The plate was developed, dried and photometrically analyzed. The amount of drug was calculated by employing corresponding calibration curve equations. Average recovery obtained at all 3 levels was reported as % recovery.

### 4.1.8.6 Specificity

The specificity of the method was ascertained by analyzing standard drug and sample. The spot for Rufinamide in sample was confirmed by comparing the $R_f$ and spectra of the spot with those of standard. The peak purity of sample was assessed by comparing the spectra at peak start, peak apex and peak end positions of the spot.
4.1.8.7 Solution Stability
Rufinamide solution (200 μg/mL) was prepared and stored at room temperature for 2, 4, 6, 10, 12, 24 and 48 h. At every time interval, 10 μL was spotted and plate was developed and analyzed and the peak areas were noted.

4.1.9 Assay of marketed formulation
To determine the content of Rufinamide in Banzel® tablets (Label claim: 200 mg per tablet) twenty tablets were powdered and powder equivalent to 200 mg of Rufinamide was weighed and transferred to 100 mL volumetric flask. Methanol (50 mL) was added and the flask was sonicated for 30 min. The solution was filtered through Whatman filter paper no.41, the residue washed thoroughly with methanol. Filtrate and washings were combined and volume was made up to the mark with methanol. Aliquot (1 mL) was further transferred in a 10 mL volumetric flask and diluted with methanol up to the mark. The resultant solution (10 μL) was spotted onto the plate followed by development and scanning. The analysis was performed in triplicate. The possibilities of excipients interference in the analysis were studied.

4.1.10 Accelerated degradation of Rufinamide
4.1.10.1 Acid induced degradation
Accurately weighed Rufinamide (100 mg) was transferred into 100 mL volumetric flask, 50 mL methanol and 50 mL 1N HCl was added to it. The drug was subjected to accelerated degradation at 80° C for a period of 3 hours. The accelerated degradation in acidic media was performed in the dark in order to exclude the possible effects of light on the drug. From the resultant solution, 10 mL was transferred into 50 mL flask, neutralized with 1 N NaOH and diluted up to the mark with methanol. 10 μL of the sample was spotted on TLC plate and the plate was developed and analyzed.

4.1.10.2 Base induced degradation
Accurately weighed Rufinamide (100 mg) was transferred into 100 mL volumetric flask, 50 mL methanol and 50 mL 1N NaOH was added to it. The drug was subjected to accelerated degradation at 80° C for a period of 3 hours. The accelerated degradation in basic media was performed in the dark in order to exclude the possible effects of light on
the drug. From the resultant solution, 10 mL was transferred into 50 mL flask, neutralized with 1 N HCl and diluted upto the mark with methanol. 10 μL of the sample was spotted on TLC plate and the plate was developed and analyzed.

4.1.10.3 Hydrogen peroxide induced degradation (Oxidation)
Accurately weighed Rufinamide (100 mg) was transferred into 100 mL volumetric flask, 50 mL methanol and 50 mL 3 % v/v hydrogen peroxide was added to it. The solution was kept in dark for 24 h at room temperature. The solution was heated after 24 h in boiling water bath for 1 h till the removal of excess hydrogen peroxide. From the resultant solution, 10 mL was transferred into 50 mL flask, diluted upto the mark with methanol and 10 μL of the sample was spotted on TLC plate and the plate was developed and analyzed.

4.1.10.4 Photolytic degradation
Accurately weighed Rufinamide (100 mg) was taken into 100 mL volumetric flask and dissolved and diluted upto the 100 mL with methanol. The photochemical stability of the drug was studied by exposing the drug solution to direct sunlight for 48 hours. Aliquot (10 mL) was transferred into 50 mL volumetric flask and diluted with methanol up to the mark. 10 μL solution was spotted on TLC plate and plate was developed and analyzed.

4.1.10.5 Thermal degradation
Rufinamide (100 mg) was stored at 100 °C for 12 hours under dry heat condition. After 12 hours the drug sample (100 mg) was transferred in 100 mL volumetric flask. Dissolved & diluted upto the mark with methanol. Aliquot (10 mL) was transferred 50 mL volumetric flask and diluted with methanol. 10 μL solution was spotted on TLC plate and plate was developed and analyzed.
4.2: Results and Discussion

4.2.1 Selection of wavelength

210 nm was found to be wavelength of detection with highest sensitivity for Rufinamide and its degradation product.

Figure 4.1: HPTLC-UV spectrum of Rufinamide
Figure 4.2: HPTLC- UV spectrum of degraded sample of Rufinamide
4.2.2 Selection of mobile phase

Various mobile phases were tried and tested for resolution of Rufinamide & its degradation products. Some mobile phases like hexane: ethylacetate (7:3, 3:7 v/v), chloroform: methanol (9:1 v/v) and chloroform: methanol: acetic acid (9: 1: 0.1). The chromatographic conditions were optimized with mobile phase containing chloroform: methanol: glacial acetic acid (9:1:0.1 v/v/v), which was found satisfactory to obtain sharp, well defined Rufinamide peak with better reproducibility and repeatability.

![HPTLC chromatogram of Rufinamide](image)

Figure 4.3 HPTLC chromatogram of Rufinamide ($R_f = 0.68 \pm 0.02$)
4.2.2 Method validation

4.2.2.1 Linearity

Linear correlation was obtained between peak area and concentration of Rufinamide in the range of 1000- 3500 ng/spot. The linearity of the calibration curve was validated by the value of correlation coefficients of the regression (r). The optical and regression characteristics are listed in Table 4.1.

Figure 4.4: Chromatogram for calibration of Rufinamide.
Table 4.1 Calibration curve data for Rufinamide (n=3)

<table>
<thead>
<tr>
<th>Concentration (ng/spot)</th>
<th>Area1</th>
<th>Area2</th>
<th>Area3</th>
<th>Average Area</th>
<th>S.D.</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>6550.50</td>
<td>6643.00</td>
<td>6485.80</td>
<td>6559.77</td>
<td>79.01</td>
<td>1.20</td>
</tr>
<tr>
<td>1500</td>
<td>7403.90</td>
<td>7421.80</td>
<td>7470.60</td>
<td>7432.10</td>
<td>34.52</td>
<td>0.46</td>
</tr>
<tr>
<td>2000</td>
<td>8277.50</td>
<td>8426.70</td>
<td>8516.90</td>
<td>8407.03</td>
<td>120.91</td>
<td>1.44</td>
</tr>
<tr>
<td>2500</td>
<td>9129.50</td>
<td>9182.60</td>
<td>9407.30</td>
<td>9239.80</td>
<td>147.47</td>
<td>1.60</td>
</tr>
<tr>
<td>3000</td>
<td>9927.40</td>
<td>10028.30</td>
<td>10107.00</td>
<td>10020.90</td>
<td>90.03</td>
<td>0.90</td>
</tr>
<tr>
<td>3500</td>
<td>10890.70</td>
<td>10685.90</td>
<td>10956.40</td>
<td>10844.33</td>
<td>141.08</td>
<td>1.30</td>
</tr>
</tbody>
</table>

Figure 4.5 Calibration curve of Rufinamide
4.2.2.2 Repeatability

The % R.S.D. of the repeatability of measurement of peak area was found to be 0.12; while of the repeatability of sample application was found to be 1.06 for Rufinamide. The % R.S.D. for intra-day precision was found to be in the range of 1.20-1.44 %; while inter-day precision was found to be in the range of 0.58 – 0.79 % for Rufinamide, which indicated that the method was precise.

Table 4.2: Results of repeatability (n=6)

<table>
<thead>
<tr>
<th>Repeatability of scanner and Spotter</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sr No</strong></td>
<td><strong>Scanner</strong></td>
<td><strong>Spotter</strong></td>
</tr>
<tr>
<td>1</td>
<td>8426.70</td>
<td>8498.30</td>
</tr>
<tr>
<td>2</td>
<td>8432.80</td>
<td>8527.20</td>
</tr>
<tr>
<td>3</td>
<td>8421.80</td>
<td>8710.40</td>
</tr>
<tr>
<td>4</td>
<td>8439.40</td>
<td>8449.30</td>
</tr>
<tr>
<td>5</td>
<td>8412.10</td>
<td>8563.80</td>
</tr>
<tr>
<td>6</td>
<td>8435.50</td>
<td>8601.10</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>8428.05</td>
<td>8558.35</td>
</tr>
<tr>
<td><strong>S.D.</strong></td>
<td>10.02</td>
<td>91.04</td>
</tr>
<tr>
<td><strong>% R.S.D.</strong></td>
<td>0.12</td>
<td>1.06</td>
</tr>
</tbody>
</table>

4.2.2.3 Precision

Table 4.3: Intraday precision

<table>
<thead>
<tr>
<th>Concentration (ng/spot)</th>
<th>Area1</th>
<th>Area2</th>
<th>Area3</th>
<th>Average area</th>
<th>S.D.</th>
<th>% R.S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>6437.80</td>
<td>6512.50</td>
<td>6477.30</td>
<td>6475.87</td>
<td>37.37</td>
<td>0.58</td>
</tr>
<tr>
<td>2000</td>
<td>8178.50</td>
<td>8226.70</td>
<td>8098.90</td>
<td>8168.03</td>
<td>64.54</td>
<td>0.79</td>
</tr>
<tr>
<td>3500</td>
<td>11040.20</td>
<td>11082.10</td>
<td>10918.60</td>
<td>11013.63</td>
<td>84.93</td>
<td>0.77</td>
</tr>
</tbody>
</table>
### 4.2.2.4 Limit of detection and limit of quantification

The Limit of detection (LOD) was found to be 196.5 ng/spot while the Limit of quantification (LOQ) was found to be 595.7 ng/spot for Rufinamide.

### 4.2.2.5 Accuracy (% Recovery)

Accuracy of the developed method was accessed by the standard addition techniques to pre-analyzed sample of market formulation with three concentrations of drug corresponding to 80, 100, 120 % and determining the recovery of added drug. At each level of the amount, three determinations were performed. The percent recovery was found in the range of 98.25 – 99.43 % for Rufinamide, which indicated accuracy of the method.

<table>
<thead>
<tr>
<th>Level of Addition</th>
<th>Amount Taken (ng/spot)</th>
<th>Amount added (ng/spot)</th>
<th>Amount Found (ng/spot)</th>
<th>% Recovery ± S.D.</th>
<th>% RS.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 %</td>
<td>1000</td>
<td>0</td>
<td>982.5</td>
<td>98.25 ± 0.61</td>
<td>0.06</td>
</tr>
<tr>
<td>50 %</td>
<td>1000</td>
<td>500</td>
<td>1474.1</td>
<td>99.43 ± 0.72</td>
<td>0.14</td>
</tr>
<tr>
<td>100 %</td>
<td>1000</td>
<td>1000</td>
<td>1967.9</td>
<td>99.26 ± 0.79</td>
<td>0.08</td>
</tr>
<tr>
<td>150 %</td>
<td>1000</td>
<td>1500</td>
<td>2451.3</td>
<td>98.74 ± 1.17</td>
<td>0.07</td>
</tr>
</tbody>
</table>

### 4.2.2.6 Specificity

The peak purity of Rufinamide was assessed by comprising its respective spectra at the peak start, apex, and peak end positions of the spot, i.e., $r (S, M) = 0.9997$ and $r (M, E) = 0.9998$. The proposed method was found to be specific as no interference of excipients or
impurities was inferred. The peak purity and correlation > 0.99 indicated that the method is specific.

### 4.2.2.7 Solution Stability

The areas of Rufinamide spot obtained at different time interval did not showed more than 2% deviation concluding that the sample remains stable for a period of 48h.

<table>
<thead>
<tr>
<th>Sr No</th>
<th>Time (hour)</th>
<th>Concentration (ng)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>1999</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>1994</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>1993</td>
</tr>
<tr>
<td>4</td>
<td>12</td>
<td>1990</td>
</tr>
<tr>
<td>5</td>
<td>24</td>
<td>1984</td>
</tr>
<tr>
<td>6</td>
<td>48</td>
<td>1982</td>
</tr>
</tbody>
</table>
4.2.3 Analysis of Tablet Dosage Form

The proposed HPTLC method was successfully applied for determination of Rufinamide from tablet dosage form. The percentage of Rufinamide was found to be satisfactory, which was comparable with the corresponding label claim.

Table 4.7: Analysis results of tablet dosage form (n=3)

<table>
<thead>
<tr>
<th>Brand Name</th>
<th>Formulation</th>
<th>Labeled amount (mg)</th>
<th>Amount Found (mg)</th>
<th>Assay % ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Banzel</td>
<td>Tablet</td>
<td>200</td>
<td>196.30</td>
<td>98.15 ± 1.42</td>
</tr>
</tbody>
</table>

Figure 4.6: Chromatogram of tablet sample
Table 4.8: Summary of validation parameter

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity range</td>
<td>1000-3500 ng/spot</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.9989</td>
</tr>
<tr>
<td>LOD (ng/spot)</td>
<td>196.59</td>
</tr>
<tr>
<td>LOQ (ng/spot)</td>
<td>595.74</td>
</tr>
<tr>
<td>Accuracy (n=3)</td>
<td>98.91 ± 0.53</td>
</tr>
<tr>
<td>Intra-day precision (n=3)</td>
<td>0.58-0.79</td>
</tr>
<tr>
<td>Inter-day precision (n=3)</td>
<td>1.20-1.44</td>
</tr>
<tr>
<td>Repeatability (n=6)</td>
<td></td>
</tr>
<tr>
<td>Scanner</td>
<td>0.12</td>
</tr>
<tr>
<td>Spotter</td>
<td>1.06</td>
</tr>
<tr>
<td>Specificity</td>
<td>Specific</td>
</tr>
</tbody>
</table>
4.2.4 Results of Degradation Study

4.2.4.1 Acid and Base induced degradation

The Chromatogram of the acid (Figure 4.7) and base (Figure 4.8) degraded samples for Rufinamide showed an additional peak at R_f value of 0.23. The concentration of the drug was found to be changing from the initial concentration, indicating that Rufinamide undergoes degradation under both the conditions. The rate of alkaline degradation was higher when compared with that of acidic condition.

Figure 4.7 Chromatogram for acid induced degradation of Rufinamide
Figure 4.8: Chromatogram for base induced degradation of Rufinamide
4.2.4.2 Hydrogen peroxide induced degradation

The sample did not show any other peak except that of standard Rufinamide. The result indicates that Rufinamide does not degrade under oxidative stress condition. (Figure 4.9)

Figure 4.9: Chromatogram for hydrogen peroxide (3% v/v) induced degradation of Rufinamide
4.2.4.3 Photolytic degradation:
The photo degraded sample showed no additional peak. No significant degradation was observed in standard that was left in day light for 48 h (Figure 4.10)

![Chromatogram](image)

Figure 4.10: Chromatogram for photolytic degradation of Rufinamide
4.2.4.4 Thermal degradation

The sample showed no additional peak. No significant degradation was observed in standard even after exposure at 100°C for 12 h (Figure 4.11)

![Chromatogram for thermal degradation of Rufinamide](image)

Figure 4.11: Chromatogram for thermal degradation of Rufinamide

<table>
<thead>
<tr>
<th>Condition</th>
<th>Solvent</th>
<th>Temperature (°C)</th>
<th>Time (h)</th>
<th>% Degradation</th>
</tr>
</thead>
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<td>Acidic</td>
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<tr>
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<td>Methanol</td>
<td>Sunlight</td>
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<td>1.0</td>
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<tr>
<td>Thermal</td>
<td>-</td>
<td>100</td>
<td>12</td>
<td>0.28</td>
</tr>
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</table>
4.2.5 Isolation and characterization of degradation product

TLC analysis of acid and base induced degraded sample suggested that the degradation product resolved in both the cases is same which was further authenticated by mass, IR and UV analysis. To isolate the degradation product, 1 gm of Rufinamide was dissolved in 100 mL of methanol and 250 mL of 1 N NaOH was added to it and the resultant solution was refluxed for 6 h. Complete hydrolysis was confirmed by TLC, and the resultant solution was extracted with chloroform (ethyl-acetate and other non-halogenated organic solvents were tried for extraction, but showed lower recovery values and poor solubility for degradant). Chloroform was evaporated to dryness. Residue was crystallized using chloroform: methanol (9:1). The degradation product was characterized using spectrometric analysis. The Rf of degradant obtained at 0.23 and maximum wavelength was 210 nm (Figure 4.1). The doublet in IR of standard close to 3178 cm⁻¹, characteristic of –NH₂ group (Figure 1.1) and that was missing in case of IR spectra of degradation product (Figure 4.13) Mass Spectra showed the molecular ion peak (m/z) at 239 for the standard (Figure 4.12), while for degradant molecular ion peak (m/z) at 240 (Figure 4.14). The NMR spectra for degradant further confirmed its structure (Figure 4.15). Based on spectral data, the structure was identified as 2- (2, 6- diflorobenzyl) - 2H-1, 2, 3- triazole-4-carboxylic acid. The Structure was supported by appropriate mechanistic explanation (Figure 4.16)
Figure 4.12 Mass spectrum of standard Rufinamide
Figure 4.13 IR spectrum of degradation product of Rufinamide
Figure 4.14 Mass spectrum of degradation product of Rufinamide
Figure 4.15 NMR spectrum of degradation product of Rufinamide

Figure 4.16 Conversion of Rufinamide to its degradation product
4.3 Conclusion:
A high performance thin layer chromatographic method has been developed and validated for the determination of Rufinamide from tablet dosage form. The results were comparable with label claim. The method was found to be specific as there was no interference of excipients and impurity. The proposed method was found to be simple, accurate, precise and sensitive. As the method could effectively separate the drug from their degradation products, it can be employed as a stability indicating one. Hence, it can be used successfully for the routine analysis of Rufinamide and its degradants in bulk and pharmaceutical dosage forms.