CHAPTER 4

FORCED DEGRADATION STUDY

TEMZOLOMIDE CAPSULES 20 MG AND ANASTROZOLE TABLETS 1 MG

FOR

ASSAY AND RELATED SUBSTANCE
The forced degradation will be performed using Acid Degradation, Base Degradation, Oxidative Degradation, Photochemical Degradation, and Thermal Degradation.

**Control Sample Preparation**

**For Anastrozole:**

A Sample about 1.0 gm. was weighed and transferred into a 10 ml volumetric flask equivalent to 20 mg of Anastrozole, to this flask added 7 mL of equal volumes of Acetonitrile and water respectively and the mixture was subjected to vigorous shaking for 10 min and diluted up to volume with same solvent. The solution was filtered through 0.45µ nylon filter before analysis. 10 µl of this solution were injected into the HPLC system.

**For Temozolomide**

Weighed 0.290 mg of powdered Sample of Temozolomide and transferred to a 10 mL volumetric flask. 7 mL of equal volumes of Acetonitrile and water respectively and the mixture was subjected to vigorous shaking for 10 min and diluted up to volume with same solvent & filtered this solution with 0.45-µ nylon filter, 10 µl of these solutions were injected into the HPLC system.
Acid Degradation Sample Preparation

Prepared 1 N HCl solution in water: Acetonitrile as described below: To 1 mL of 12 N HCl added 5 mL of water, followed by 6 mL of Acetonitrile. This will generate 1 N HCl in 1:1 water- Acetonitrile. Used this solution for acid degradation

For Anastrozole:

A Sample about 1.0 gm. was weighed and transferred in to 10 ml volumetric flask equivalent 20 mg of Anastrozole, Carefully added 1 mL of 1 N HCl (prepared above) and mix well. Allow the mixture to stand at room temperature for 2 hrs. Added 1 mL of 1 N NaOH (prepared in 1:1 water: Acetonitrile) to neutralize the sample and mix well. further diluted this solution with equal volume of water- Acetonitrile and the mixture was subjected to vigorous shaking for 10 min and diluted up to volume with same solvent.. The solution was filtered through 0.45µ nylon filter before analysis. 10 µl of this solution were injected in to the HPLC system.

For Temozolomide

Weighed 0.290 mg of powdered Sample of Temozolomide and transferred to 10 mL volumetric flask carefully added 1 mL of 1 N HCl (prepared above) and mix well. Allow the mixture to stand at room temperature for 2 hrs. Added 1 mL of 1 N NaOH (prepared in 1:1 water: Acetonitrile) to neutralize the sample and mix well. further diluted this solution with equal volumes of Acetonitrile and water respectively and the mixture was subjected to vigorous shaking for 10 min and diluted up to volume with same solvent & filtered this solution with 0.45-µ nylon filter, 10 µl of these solutions were injected in to the HPLC system.
Base Degradation Sample Preparation

Prepared 1 N NaOH solution in water: Acetonitrile as described below: To 4.0 gm. of NaOH added 50 mL of water, followed by 50 mL of Acetonitrile. This will generate 1 N NaOH in 1:1 water- Acetonitrile. Used this solution for acid degradation

For Anastrozole:

A Sample about 1.0 gm. was weighed and transferred in to 10 ml volumetric flask equivalent 20 mg of Anastrozole, Carefully added 1 mL of 1 N NaOH (prepared above) and mix well. Allow the mixture to stand at room temperature for 2 hrs. Added 1 mL of 1 N HCl (prepared in 1:1 water: Acetonitrile) to neutralize the sample and mix well. Diluted the solution with equal volume of water- Acetonitrile and the mixture was subjected to vigorous shaking for 10 min and diluted up to volume with same solvent.. The solution was filtered through 0.45µ nylon filter before analysis. 10 µl of this solution were injected in to the HPLC system.

For Temozolomide

Weighed 0.290 mg of powdered Sample of Temozolomide and transferred to 10 mL volumetric flask carefully added 1 mL of 1 N NaOH (prepared above) and mix well. Allow the mixture to stand at room temperature for 2 hrs. Added 1 mL of 1 N HCL (prepared in 1:1 water: Acetonitrile) to neutralize the sample and mix well. further diluted this solution with equal volumes of Acetonitrile and water respectively and the mixture was subjected to vigorous shaking for 10 min and diluted up to volume with same solvent & filtered this solution with 0.45-µ nylon filter, 10 µl of these solutions were injected in to the HPLC system.
Oxidation Degradation Sample Preparation

For Anastrozole:
A Sample about 1.0 gm. was weighed and transferred in to 10 ml volumetric flask equivalent 20 mg of Anastrozole, Carefully added 1 mL of 3% hydrogen peroxide and mix well . Allowed the mixture stand at room temperature for 10 hours to this flask Added 200 mg of solid sodium sulfite and mix well to decompose excess hydrogen peroxide Further diluted up to volume with equal volumes of Acetonitrile and water respectively and the mixture was subjected to vigorous shaking for 10 min and diluted up to volume with same solvent.. The solution was filtered through 0.45µ nylon filter before analysis. 10 µl of this solution were injected in to the HPLC system.

For Temozolomide
Weighed 0.290 mg of powdered Sample of Temozolomide and transferred to 10 mL volumetric flask. Carefully added 1 mL of 3% hydrogen peroxide and mix well. Allowed the mixture stand at room temperature for 10 hours to this flask Added 200 mg of solid sodium sulfite and mix well to decompose excess hydrogen peroxide Further diluted up to volume with equal volumes of Acetonitrile and water respectively and the mixture was subjected to vigorous shaking for 10 min and diluted up to volume with same solvent & filtered this solution with 0.45-µ nylon filter, 10 µl of these solutions were injected in to the HPLC system.
Thermal Degradation Sample Preparation

**For Anastrozole:**

Heat the sample at 80°C for 18 hours

A Sample about 1.0 gm. was weighed and transferred in to 10 ml volumetric flask equivalent 20 mg of Anastrozole, to this flask added 7 mL of equal volumes of Acetonitrile and water respectively and the mixture was subjected to vigorous shaking for 10 min and diluted up to volume with same solvent. The solution was filtered through 0.45µ nylon filter before analysis. 10 µl of this solution were injected in to the HPLC system.

**For Temozolomide**

Heat the sample at 80°C for 18 hours

Weighed 0.290 mg of powdered Sample of Temozolomide and transferred to 10 mL volumetric flask. 7 mL of equal volumes of Acetonitrile and water respectively and the mixture was subjected to vigorous shaking for 10 min and diluted up to volume with same solvent & filtered this solution with 0.45-µ nylon filter, 10 µl of these solutions were injected in to the HPLC system.
Photochemical Degradation Sample Preparation

For Anastrozole:

Place one sample into a light box and expose the sample to an accumulated dose of visible light of about 0.4 million lux hours

Place one sample in a light box and expose the sample to an accumulated dose of ultraviolet light of about 75 Watt-hours

A Sample about 1.0 gm. was weighed and transferred into a 10 ml volumetric flask equivalent to 20 mg of Anastrozole, to this flask added 7 mL of equal volumes of Acetonitrile and water respectively and the mixture was subjected to vigorous shaking for 10 min and diluted up to volume with same solvent. The solution was filtered through 0.45µ nylon filter before analysis. 10 µl of this solution were injected into the HPLC system.

For Temozolomide

Place one sample into a light box and expose the sample to an accumulated dose of visible light of about 0.4 million lux hours

Place one sample in a light box and expose the sample to an accumulated dose of ultraviolet light of about 75 Watt-hours

Weighed 0.290 mg of powdered Sample of Temozolomide and transferred to 10 mL volumetric flask. 7 mL of equal volumes of Acetonitrile and water respectively and the mixture was subjected to vigorous shaking for 10 min and diluted up to volume with same solvent & filtered this solution with 0.45-µ nylon filter, 10 µl of these solutions were injected in to the HPLC system.
Observations for Anastrozole

In Acidic degradation study it was observed that Anastrozole had degraded less than 30%, hence the study was repeated using the stronger condition i.e. the sample was exposed to 1 mL of 1 N HCL for 6 hrs. In this study it was observed that Anastrozole had degraded within specified limit i.e. within 10% to 30%.

In basic degradation study it was observed that Anastrozole had degraded more than 30% hence the study was repeated using milder condition i.e. sample was exposed to 1 mL of 1N NaOH for 30 mins. In this study it was observed that Anastrozole had degraded within specified limit i.e. within 10% to 30%.

In Oxidative degradation study it was observed that Anastrozole had degraded less than 10%. Hence the study was repeated using stronger condition i.e. 30% H₂O₂ for 6 hr. In this study it was observed that Anastrozole had degraded within the specified limit i.e. within 10% to 30%.

In Thermal degradation study it was observed that Anastrozole had degraded within specified limit. i.e. within 10% to 30%.

In Photochemical UV degradation study it was observed that peak of Anastrozole had degraded more than 30%. Hence the study was repeated using milder condition i.e. sample was exposed to 0.5 hrs. In this study it was observed that Temozolomide had degraded within the specified limit i.e. within 10% to 30%

In Photochemical Visible degradation study it was observed that peak of Anastrozole had degraded more than 30%. Hence the study was repeated using milder condition i.e. sample was exposed to 4 hrs. In this study it was observed that Temozolomide had degraded within the specified limit i.e. within 10% to 30%
Observations for Temozolomide

In Acidic degradation study it was observed that Temozolomide had degraded more than 30%, hence the study was repeated using the milder condition i.e. the sample was exposed to 1 mL of 0.1 N HCl for 2 hrs. In this study it was observed that Temozolomide had degraded within specified limit. i.e. within 10% to 30%.

In basic degradation study it was observed that Temozolomide had degraded less than 30% hence the study was repeated using stronger condition i.e. sample was exposed to 1 mL of 2 N NaOH for 2 Hrs. In this study it was observed that Temozolomide had degraded within specified limit. i.e. within 10% to 30%.

In Oxidative degradation study it was observed that Temozolomide had degraded less than 10%. Hence the study was repeated using stronger condition i.e. 30% H₂O₂ for 2 hr. In this study it was observed that Temozolomide had degraded within the specified limit i.e. within 10% to 30%.

In Thermal degradation study it was observed that Temozolomide had degraded within specified limit. i.e. within 10% to 30%.

In Photochemical UV degradation study it was observed that peak of Temozolomide had degraded less than 30%. Hence the study was repeated using milder condition i.e. sample was exposed to 2 hrs. in this study it was observed that Temozolomide had degraded within the specified limit i.e. within 10% to 30%.

In Photochemical Visible degradation study it was observed that peak of Temozolomide had degraded more than 30%. Hence the study was repeated using milder condition i.e. sample was exposed to 2 hrs. In this study it was observed that Temozolomide had degraded within the specified limit i.e. within 10% to 30%.
Summary of force degradation study

For Anastrozole

Table No. 101: Result for assay and related substance in force degradation study.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Degradent used</th>
<th>Drug from</th>
<th>% Total impurities</th>
<th>% Assay</th>
<th>Mass balance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control Sample</td>
<td>Drug product</td>
<td>0.08</td>
<td>101.6</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>1 N HCl 2 HR</td>
<td>Drug product</td>
<td>0.02</td>
<td>103.5</td>
<td>103.6</td>
</tr>
<tr>
<td>3</td>
<td>1 N HCl 6 HR</td>
<td>Drug product</td>
<td>1.0</td>
<td>87.3</td>
<td>87.9</td>
</tr>
<tr>
<td>4</td>
<td>1 N NaOH 2 HR</td>
<td>Drug product</td>
<td>0.10</td>
<td>77.0</td>
<td>76.2</td>
</tr>
<tr>
<td>5</td>
<td>1 N NaOH 0.5 HR</td>
<td>Drug product</td>
<td>0.12</td>
<td>87.8</td>
<td>86.5</td>
</tr>
<tr>
<td>6</td>
<td>3% H2O2 10 HR</td>
<td>Drug product</td>
<td>3.5</td>
<td>103.4</td>
<td>107.0</td>
</tr>
<tr>
<td>7</td>
<td>30% H2O2 6 HR</td>
<td>Drug product</td>
<td>3.6</td>
<td>90.3</td>
<td>109.3</td>
</tr>
<tr>
<td>8</td>
<td>Visible light 10 HR</td>
<td>Drug product</td>
<td>0.03</td>
<td>77.4</td>
<td>80.5</td>
</tr>
<tr>
<td>9</td>
<td>Visible light 4 HR</td>
<td>Drug product</td>
<td>0.19</td>
<td>105.5</td>
<td>103.9</td>
</tr>
<tr>
<td>10</td>
<td>Ultraviolet light 5 HR</td>
<td>Drug product</td>
<td>0.02</td>
<td>69.2</td>
<td>78.3</td>
</tr>
<tr>
<td>11</td>
<td>Ultraviolet light 0.5 HR</td>
<td>Drug product</td>
<td>0.11</td>
<td>86.9</td>
<td>84.2</td>
</tr>
<tr>
<td>12</td>
<td>80°C</td>
<td>Drug product</td>
<td>0.00</td>
<td>101.3</td>
<td>102.4</td>
</tr>
</tbody>
</table>
For Temozolomide

**Table No. 102:** Result for assay and related substance in force degradation study.

<table>
<thead>
<tr>
<th>Degradant used</th>
<th>Drug</th>
<th>% Total impurities</th>
<th>% Assay</th>
<th>Mass balance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Sample</td>
<td>Drug product</td>
<td>0.08</td>
<td>101.6</td>
<td>-</td>
</tr>
<tr>
<td>2 1 N HCl 2 HR</td>
<td>Drug product</td>
<td>0.02</td>
<td>77.5</td>
<td>78.6</td>
</tr>
<tr>
<td>3 0.1 N HCl 2 HR</td>
<td>Drug product</td>
<td>1.0</td>
<td>90.7</td>
<td>89.9</td>
</tr>
<tr>
<td>4 1 N NaOH 2 HR</td>
<td>Drug product</td>
<td>0.10</td>
<td>67.0</td>
<td>67.2</td>
</tr>
<tr>
<td>5 2 N NaOH 2 HR</td>
<td>Drug product</td>
<td>0.12</td>
<td>87.8</td>
<td>86.5</td>
</tr>
<tr>
<td>6 3% H2O2 10 HR</td>
<td>Drug product</td>
<td>3.5</td>
<td>78.4</td>
<td>75.0</td>
</tr>
<tr>
<td>7 30% H2O2 2 HR</td>
<td>Drug product</td>
<td>3.6</td>
<td>90.5</td>
<td>109.3</td>
</tr>
<tr>
<td>8 Visible light 10 HR</td>
<td>Drug product</td>
<td>0.03</td>
<td>68.4</td>
<td>60.5</td>
</tr>
<tr>
<td>9 Visible light 2 HR</td>
<td>Drug product</td>
<td>0.19</td>
<td>87.5</td>
<td>89.9</td>
</tr>
<tr>
<td>10 Ultraviolet light 5 HR</td>
<td>Drug product</td>
<td>0.02</td>
<td>76.2</td>
<td>77.3</td>
</tr>
<tr>
<td>11 Ultraviolet light 2 HR</td>
<td>Drug product</td>
<td>0.11</td>
<td>100.9</td>
<td>100.2</td>
</tr>
<tr>
<td>12 80°C</td>
<td>Drug product</td>
<td>0.00</td>
<td>103.3</td>
<td>103.4</td>
</tr>
</tbody>
</table>

**Conclusion for Forced degradation study:**

1. Peak purity of main peak in all conditions of force degradation passes.
2. PDA Scan for degraded drug substance and drug product is comparable to that of untreated drug substance and drug product.
3. All peaks due to degradation are well separated from each other and from main peak. So there is no interference of blank, placebo and degradant at retention time of main peak.