# CHAPTER – 6

# SIMULTANEOUS DETERMINATION OF DRUG RELEASE DURING DISSOLUTION OF TELMISARTAN AND HYDROCHLOROTHIAZIDE IN TABLET DOSAGE FORM USING REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

# CHAPTER – 6

## Simultaneous determination of drug release during dissolution of Telmisartan and Hydrochlorothiazide in tablet dosage form using reverse phase high performance liquid chromatography

### 6.0 INTRODUCTION

The objective was to develop a single method for simultaneous determination of percentage drug release of the actives in Telmisartan and Hydrochlorothiazide tablet dosage form. The method was validated as per ICH guidelines Q2 (R1).

The target for this research work was to present comprehensive methods of critical tests for each drug product. Thus this work can be treated as part of a monograph for the drug product.

The main aim for this current study was to develop the analytical determination/ parameters of drug release rather than the dissolution parameters.

A brief introduction of each molecule is has already been included in chapter 4.

### 6.1 LITERATURE SURVEY

The literature survey reveals that, TE and HCTZ are reported in British Pharmacopoeia [6, 7]. There have been several publications describing analytical methods for the determination of HCTZ and TE individually or with other drugs as combination.

Although there are a few papers published on simultaneous determination of TE and HCTZ in formulation most of them deal with the assay of each constituent. Several methods are reported for the determination of TE like Spectrophotometric [7] and HPLC. [9-11] The other methods available in the literature are based on Linear Sweep polarography, [12] LC–MS. [13] Articles on the determination of HCTZ in combination with other drugs by HPLC are also reported in literature. [14, 15]

However the exhaustive literature survey revealed that none of the most recognized pharmacopoeias or any journals includes these drugs in combination for the simultaneous determination of drug release of TE and HCTZ is not available[@]. So the aim of this work was to develop a liquid chromatographic procedure which will serve a reliable, accurate, sensitive HPLC method for the simultaneous determination of drug release of TE and HCTZ in TE + HCTZ tablets.

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[@] This search was performed at the start and during the course of the study. A draft monograph has been published in USP Forum Vol 36(3). The Monograph is now official as per USP 34, from December 2011. A comparison of methods is given in the next section.

**Table 6.1.1: Dissolution – Pharmacopeia methods for <u>Drug Product</u>**

| | Telmisartan | | HCTZ | | Telmisartan-HCTZ |
|---|---|---|---|---|---|
| | BP 2011 | USP 34 | BP 2011 | USP 34 | USP 35 |
| Method | Not Available | UV Spectrophotometer | Not Mentioned | UV Spectrophotometer | HPLC |
| Column | Not Applicable | Not Applicable | Not Applicable | Not Applicable | C8<br>3mm x 6cm; 5µm |
| Column Temp | Not Applicable | Not Applicable | Not Applicable | Not Applicable | 40°C |
| Mobile Phase | Not Applicable | Not Applicable | Not Applicable | Not Applicable | Solution A: 5.0 *g/L* of ammonium dihydrogen phosphate was prepared in water. The pH was adjusted with phosphoric acid to 3.0<br>Solution B: Acetonitrile |
| Gradient | Not Applicable | Not Applicable | Not Applicable | Not Applicable | Time (min) / %A / %B<br>0 / 85 / 15<br>3.50 / 85 / 15 |

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<tr>
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<tr><td>3.51</td><td>45</td><td>55</td></tr>
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</td>
</tr>
<tr>
<td>Flow Rate</td>
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<td>Not Applicable</td>
<td>Not Applicable</td>
<td>Not Applicable</td>
<td>Flow gradient
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<tr><td>Time (min)</td><td>Flow</td></tr>
<tr><td>0</td><td>0.6</td></tr>
<tr><td>5</td><td>0.6</td></tr>
<tr><td>5.01</td><td>1</td></tr>
<tr><td>6.2</td><td>1</td></tr>
<tr><td>6.21</td><td>0.6</td></tr>
<tr><td>9.7</td><td>0.6</td></tr>
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</td>
</tr>
<tr>
<td>Wavelength</td>
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<td>296nm</td>
<td>Not Applicable</td>
<td>270nm</td>
<td>270-Telmisartan<br>298- Hydrochlorothiazide</td>
</tr>
<tr>
<td>Inj Vol (µL)</td>
<td>Not Applicable</td>
<td>Not Applicable</td>
<td>Not Applicable</td>
<td>Not Applicable</td>
<td>4</td>
</tr>
</table>

## 6.2 PRESENT WORK AND DISCUSSION

### 6.2.1 Selection of Chromatographic Method

Reverse Phase chromatography is the natural choice for method development because of its ease of handling and robust nature. All development was conducted using reverse phase methods. Official methods and methods published in literature for Telmisartan and Hydrochlorothiazide are based on reverse phase chromatographic (RPC) separation. Moreover since drug release is being studied in aqueous medium there is no option to go for normal phase HPLC separations.

### 6.2.2 Selection of Stationary Phase

USP, EP monograph and reported HPLC methods of Telmisartan or hydrochlorothiazide recommended use of C18 column for the purpose of determination of related impurities and assay. So C18 columns were preferred as stationary phase. As described in the previous chapter, C8 column was used in assay to reduce the run time to 15 minutes. However for dissolution, 15 minutes is considered a long run time, especially when one is considering dissolution profiles. Typically a six unit dissolution profile would involve about 60 injections. Thus, a 15 minute run time for injection would result in an exercise that would last for 15 hours which is not practical. Therefore, the shorter the run time, the quicker the experiment can be planned for subsequent formulation development trial batches. The usage of ion pairing agent was avoided since that would retain the Telmisartan peak extensively. From the experience of the method development on C18 and C8 column, a highly polar Cyano (CN) column was preferred since the target was to achieve respectable retention and separation of the two actives within an isocratic run. The biggest challenge in using a cyano phase is the column to column variation. Thus column quality and manufacturing reproducibility is of utmost importance. In this regard ACE Cyano column was chosen for trials.

### 6.2.3 Selection of Wavelength for Analysis

The optimum wavelength selected was 270 nm which represents the wavelength where both actives have suitable responses in order to permit simultaneous determination of Telmisartan and HCTZ. 270nm is also the wavelength which has absorption maxima for hydrochlorothiazide and absorption minima for Telmisartan. Thus, at this wavelength, minor changes will not affect peak areas and consequently, the final result. This will, in effect, produce a robust method with respect to wavelength.

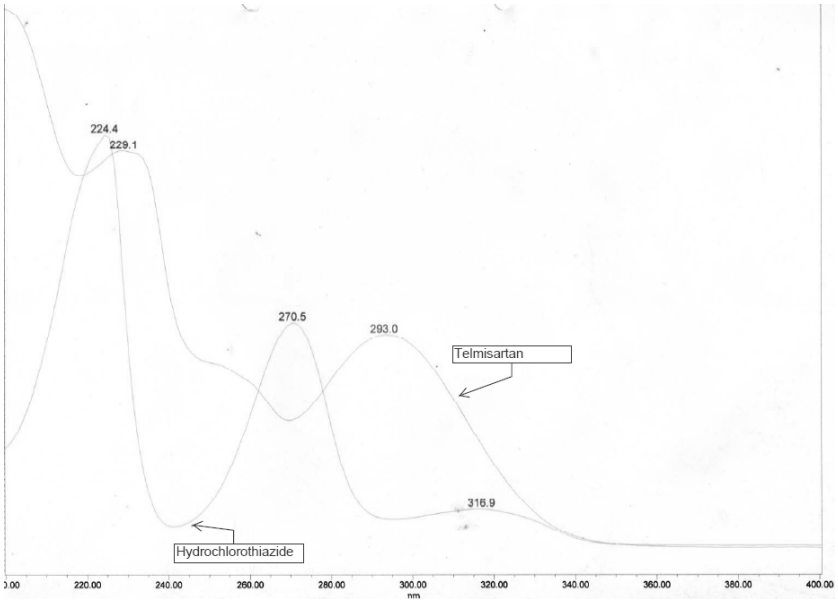



**Figure: 6.2.3.1 Overlaid UV spectrum of Telmisartan and Hydrochlorothiazide**

### 6.2.4 Selection and Optimization of Mobile Phase

Isocratic method was the target and it was achieved using the cyano column. Although the dissolution method needs not be stability indicating, the known impurities (essentially degradants from Hydrochlorothiazide) were separated from main peak. The other concern was the peak shape since the injection will be of purely aqueous samples.

BCS solubility study was performed on each molecule. The results are given in Table 6.1

Table 6.1: BCS Solubility

| Medium | % dissolved (HCTZ) | % dissolved (Telmisartan) |
|---|---|---|
| **Water** | 93.3 | 0.2 |
| **0.1 N HCl** | 85.1 | 100.2 |
| **0.01 N HCl** | 95.8 | 30.9 |
| **0.001 N HCl** | 95.0 | 0.7 |
| **Citrate pH 3.0 buffer** | 98.1 | 1.5 |
| **Acetate pH 4.5 buffer** | 97.8 | 0.1 |
| **Acetate pH 5.0 buffer** | 94.1 | 0.0 |
| **Phosphate pH 6.8 buffer** | 97.7 | 0.3 |
| **Phosphate pH 7.4 buffer** | 74.8 | 1.1 |

All the samples were analysed by the HPLC method and peak shapes were acceptable proving that this method is capable to accept fully aqueous injections and thus suitable for dissolution study.

**Optimized Chromatographic Conditions:**

Dissolution Conditions;

| | | |
|---|---|---|
| Instruments/Equipment | : | Dissolution Apparatus, Make-Electrolab, Model-TDT-08L with fraction collector, or equivalent |
| Apparatus | : | USP Apparatus II (Paddle) |
| Dissolution medium | : | Phosphate Buffer pH 7.5 |
| Volume | : | 900 ml |
| Temperature | : | 37°C ± 0.5°C |
| Speed | : | 75 rpm |
| Time | : | 60 minutes |

Preparation of Phosphate Buffer pH 7.5:
Weigh accurately and transfer 6.8 gm of Potassium Dihydrogen Phosphate and 0.9 gm of Sodium Hydroxide in 1000 ml of water. Adjust the pH 7.5 with Sodium Hydroxide solution.

The method employed for Telmisartan + Hydrochlorothiazide tablets is separation using isocratic HPLC with detection by UV.
Chromatographic conditions:

| | | |
|---|---|---|
| Instruments/Equipment | : | HPLC, Make – Waters, Alliance, 2695 Separation Module, (UV/PDA), or equivalent.<br>Analytical Balance, Make –Mettler Toledo, Model-XS205DU, or equivalent. |
| Column | : | ACE Cyano, 150 x 4.6 mm, 5µm or equivalent |
| Flow rate | : | 2.0 ml/minute |
| Column temperature | : | 35°C |
| Wavelength | : | 270 nm |
| Sample temperature | : | 25°C |
| Injection volume | : | 20 µl |
| Retention time | : | approx. 1.52 minutes for Hydrochlorothiazide<br>approx. 7.56 minutes for Telmisartan |

Diluent:

0.1N Methanolic NaOH:

Weigh accurately and transfer 4 gm of Sodium hydroxide in 1000 ml volumetric flask add 40 ml water sonnicate to dissolve and make up to mark with methanol.

Buffer:

Mix 2 ml of Triethylamine in 1000 ml water. Adjust pH 3.0 with ortho-phosphoric acid.

Preparation of Mobile Phase:

Prepare a mixture of Buffer: Acetonitrile: Methanol in the ratio 80:15:5 v/v/v. Mix and degas.

## 6.3 EXPERIMENTAL WORK

### 6.3.1 Instrumentation

| Equipment | Make | Model |
|---|---|---|
| HPLC | Waters | 2695Alliance Separation Module, (PDA/UV Detector) 2996/2487 |
| Column | ACE | ACE Cyano, 150 x 4.6 mm, 5µm |
| Dissolution apparatus | Electrolab | TDT-08L TDT-14L |
| pH meter | Thermo Electron Corp. | Orion-4star 1117000 |
| Analytical Balance | Mettler Toledo | XS205DU |

### 6.3.2 Chemicals and Reagents

| Name | Grade | Manufacturer |
|---|---|---|
| Triethylamine | HPLC grade | Merck |
| Methanol | Gradient grade | Merck |
| Acetonitrile | Gradient grade | Rankem |
| Sodium Hydroxide | GR | Merck |
| Ortho-phosphoric acid | GR | Merck |
| Water | HPLC milli-Q | In-house |

### 6.3.3 Working Standard

Working Standard:

| Standard | Lot .No. | Potency (as is) |
|---|---|---|
| Telmisartan | TE0010108 | 98.7 |
| Hydrochlorothiazide | HCT/60914 | 99.5 |

Test Sample:

| Batch. No. | Label claim (mg/tablets) | |
|---|---|---|
| | Telmisartan | Hydrochlorothiazide |
| THT/80-12.5/034 | 80 | 12.5 |
| THT/40-12.5/032 | 40 | 12.5 |

Placebo

| Batch. No. |
|---|
| THT/80-12.5/034 P |
| THT/80-12.5/034 PH |
| THT/80-12.5/034 PT |

### 6.3.4 Solution Preparation

Preparation of Standard solution:

Standard Stock solution- Telmisartan:

Accurately weigh and transfer about 90.0 mg of Telmisartan standard a to a 50 ml volumetric flask, add 30ml of diluent, sonnicate to dissolve and make up the volume with diluent.

Standard Stock solution- Hydrochlorothiazide:

Accurately weigh and transfer about 55.0 mg of Hydrochlorothiazide standard to a 100 ml volumetric flask, add 70ml of Acetonitrile, sonnicate to dissolve and make up the volume with Acetonitrile.

<u>For 40-12.5 mg</u>

Take 5 ml of Standard Stock solution of Telmisartan and 5 ml of Standard Stock solution of Hydrochlorothiazide in 200 ml volumetric flask; make the volume with dissolution media.

For 80-12.5 mg

Take 10 ml of Standard Stock solution of Telmisartan and 5 ml of Standard Stock solution of Hydrochlorothiazide in 200 ml volumetric flask; make the volume with dissolution media.

Preparation of Sample solution:

Accurately weigh one tablet and transfer in the dissolution vessel. Run the Dissolution as per the set parameters. Withdraw about 10 ml of the sample after 60 minutes. Filter through on line SS filter, discarding first 2-3 ml of filtrate inject the filtrate into the HPLC.

Preparation of Placebo solution:

Weigh accurately placebo (without Telmisartan and Hydrochlorothiazide), placebo with Telmisartan and Placebo with Hydrochlorothiazide same equivalent to one tablet and transfer in the dissolution vessel. Run the Dissolution as per the set parameters. Withdraw about 10 ml of the sample after 60 minutes. Filter through on line SS filter, discarding first 2-3 ml of filtrate inject the filtrate into the HPLC. (Only for validation)

Evaluation of System suitability:

Inject the Telmisartan Standard five times; the relative standard deviation of five replicate injections should not be more than 2.0%. The USP tailing factor for Telmisartan and Hydrochlorothiazide peak should not be more than 2.0. The USP plates for Telmisartan and Hydrochlorothiazide peak should not be less than 2000.

Procedure:

Inject equal volumes of Blank (diluent), Standard (5 replicate) and sample solutions.

Calculation:

Calculate the amount of Telmisartan present in the tablets as per give formula.

For 80 mg

$$\% \text{ Release} = \frac{AT}{AS} \times \frac{WS}{50} \times \frac{10}{200} \times \frac{900}{1 \text{ Tab}} \times \frac{P}{LC}$$

For 40 mg

$$\% \text{ Release} = \frac{AT}{AS} \times \frac{WS}{50} \times \frac{5}{200} \times \frac{900}{1 \text{ Tab}} \times \frac{P}{LC}$$

Calculate the amount of Hydrochlorothiazide present in the tablets as per give formula.

For 12.5 mg

$$\% \text{ Release} = \frac{AT}{AS} \times \frac{WS}{100} \times \frac{5}{200} \times \frac{900}{1 \text{ Tab}} \times \frac{P}{LC}$$

Where,

AT = Area of peak due to Active Ingredient sample preparation.

AS = Area of peak due to Active Ingredient in standard preparation.

WS = Weight of Active Ingredient standard in mg.

LC = Label claim of Active Ingredient per tablet in mg.

P = Potency of Active Ingredient standard on as is basis.

## 6.4 VALIDATION OF THE DEVELOPED METHOD

### 6.4.1 Validation parameters and acceptance criteria

The Table 6.4.1.1 summarizes the validation acceptance criteria along with the obtained results.

Table 6.4.1.1: Validation Summary

<table>
<tr><th>Sr.No.</th><th>Parameters</th><th>Acceptance criteria</th><th colspan="3">Result obtained</th></tr>
<tr><td rowspan="2">1.0</td><td rowspan="2">System suitability<br>% RSD for Standard solution<br>USP Tailing<br>USP Plate count</td><td rowspan="2">NMT 2.0%<br>NMT 2.0<br>NLT 2000.</td><td>Telmisartan</td><td colspan="2">HCTZ</td></tr>
<tr><td>0.08<br>1.01<br>3602</td><td colspan="2">0.12<br>1.06<br>5155</td></tr>
<tr><td rowspan="3">2.0<br>2.1</td><td rowspan="3">Specificity<br>Identification</td><td rowspan="3">Results should be comparable with respect to the retention time.</td><td colspan="3">Retention time (min)</td></tr>
<tr><td>Std-8.281</td><td colspan="2">Std-1.580</td></tr>
<tr><td>Sample-8.274</td><td colspan="2">Sample-1.578</td></tr>
<tr><td>2.2</td><td>Interference</td><td>No interference from blank and placebo to main component.</td><td colspan="3">Complies</td></tr>
<tr><td rowspan="7">2.3</td><td rowspan="7">Peak purity</td><td rowspan="7">Purity angle should be less than purity threshold.<br>Standard peak should be pure for working concentration level.</td><td>Sample</td><td>Purity angle</td><td>Purity Threshold</td></tr>
<tr><td colspan="3">Telmisartan</td></tr>
<tr><td>Standard</td><td>0.056</td><td>1.060</td></tr>
<tr><td>Sample</td><td>0.072</td><td>1.078</td></tr>
<tr><td colspan="3">HCTZ</td></tr>
<tr><td>Standard</td><td>0.168</td><td>1.097</td></tr>
<tr><td>Sample</td><td>0.188</td><td>1.117</td></tr>
</table>

Table 6.4.1.1: Validation Summary (Continued)

<table>
<tr><th>Sr. No.</th><th>Parameters</th><th>Acceptance criteria</th><th colspan="3">Result obtained</th></tr>
<tr><td rowspan="4">3.0</td><td rowspan="4">Linearity</td><td>Response should be Linear</td><td colspan="3">Response is linear</td></tr>
<tr><td rowspan="2">Correlation coefficient should not be less than 0.999.</td><td colspan="2">Telmisartan</td><td rowspan="2">HCTZ</td></tr>
<tr><td>80 mg</td><td>40 mg</td></tr>
<tr><td>1.0000</td><td>1.0000</td><td>0.9998</td></tr>
<tr><td>% Limit of Y- Intercept should be within ± 5.0% of the corresponding Y-co-ordinate of the working level.</td><td>-0.39</td><td>0.34</td><td>0.76</td></tr>
<tr><td rowspan="12">4.0</td><td rowspan="12">Accuracy (Recovery)</td><td rowspan="12">Mean recovery should be in the range of 95.0%- 105.0%.</td><td>Level %</td><td colspan="2">% Mean Recovery</td></tr>
<tr><td colspan="3">Telmisartan</td></tr>
<tr><td></td><td>80 mg</td><td>40 mg</td></tr>
<tr><td>10</td><td>97.3</td><td>96.5</td></tr>
<tr><td>50</td><td>98.7</td><td>99.2</td></tr>
<tr><td>100</td><td>99.3</td><td>98.4</td></tr>
<tr><td>150</td><td>101.2</td><td>99.2</td></tr>
<tr><td colspan="3">HCTZ</td></tr>
<tr><td>10</td><td>100.1</td><td>96.0</td></tr>
<tr><td>50</td><td>98.7</td><td>97.2</td></tr>
<tr><td>100</td><td>103.7</td><td>103.1</td></tr>
<tr><td>150</td><td>100.8</td><td>98.4</td></tr>
</table>

Table 6.4.1.1: Validation Summary (Continued)

| Sr. No. | Parameters | Acceptance criteria | Result obtained | | | |
|---|---|---|---|---|---|---|
| | | | Telmisartan | | HCTZ | |
| | | | 80 mg | 40 mg | 80 mg | 40 mg |
| 5.0 | System Precision<br>% RSD for Standard solution | NMT 2.0% | 0.08 | 0.19 | 0.12 | 0.14 |
| | USP Tailing | NMT 2.0 | 1.01 | 0.99 | 1.06 | 1.07 |
| | USP Plate count | NLT 2000 | 3602 | 3621 | 5155 | 5432 |
| 5.1 | Method Precision % RSD of six determination | NMT 5.0%. | 1.14 | 1.88 | 1.39 | 1.62 |
| 5.2 | Intermediate Precision (Ruggedness)<br>% RSD for Standard solution | NMT 2.0% | 0.30 | 0.48 | 0.45 | 0.59 |
| | USP Tailing | NMT 2.0 | 1.03 | 1.00 | 1.04 | 1.04 |
| | USP Plate count | NLT 2500. | 3108 | 3123 | 5532 | 5590 |
| | RSD for % release | NMT 5.0%. | 2.49 | 1.55 | 1.38 | 1.59 |
| | Difference for pooled result ( Analyst-I and II ) | The difference in the mean should not be more than ± 5. | 2.0 | 5.0 | 1.0 | 0.0 |

Table 6.4.1.1: Validation Summary (Continued)

| Sr. No. | Parameters | Acceptance criteria | Result obtained | |
|---|---|---|---|---|
| 6.0 | Stability in analytical solution | The difference should not be more than ± 5. | Telmisartan<br>Sample stable for at least 24 hours at 25°C | HCTZ<br>Sample stable for at least 24 hours at 25°C |
| 7.0 | Filter compatibility | The difference between centrifuged sample and filtered sample should not be more than ± 5. | complies | complies |
| 8.0 | Robustness<br>Change in Flow rate<br>(± 0.2 ml/min) | No significant change should be in System suitability parameters.<br>% RSD should be less than 5%. | No significant change.<br>Compiles | |
| | Change in wavelength<br>(± 5nm) | No significant change should be in System suitability parameters.<br>% RSD should be less than 5%. | No significant change.<br>Compiles | |
| | Column oven temperature (± 5°C) | No significant change should be in System suitability parameters.<br>% RSD should be less than 5%. | No significant change.<br>Compiles | |
| | Change in Mobile phase composition<br>(± 2 % absolute) | No significant change should be in System suitability parameters.<br>% RSD should be less than 5%. | No significant change.<br>Compiles | |

| | | | |
|---|---|---|---|
| | Change in Buffer pH (± 0.2) | No significant change should be in System suitability parameters. % RSD should be less than 5%. | No significant change. Compiles |
| | Change in Speed of rotation. (± 4% rpm) | No significant change should be in System suitability parameters. % RSD should be less than 5%. | No significant change. Compiles |

### 6.4.2 System suitability:

Single injection of Blank (Diluent) and five replicate Standard solution were made on the system. The data obtained is summarized in Table 6.4.2.1. The data demonstrate that the system suitability is within the acceptance criteria, thus the system is suitable

Table 6.4.2.1: System suitability

| **Standard solution** | | |
|---|---|---|
| | **Telmisartan** | **Hydrochlorothiazide** |
| **USP Tailing** | **1.01** | **1.06** |
| **USP Plates** | **3602** | **5155** |
| **Area** | **Standard solution** | |
| | 1590248 | 510817 |
| | 1588324 | 511411 |
| | 1589088 | 511427 |
| | 1590342 | 510766 |
| | 1587588 | 509953 |
| **Mean** | **1589118** | **510875** |
| **SD** | **1198.68** | **603.999** |
| **%RSD** | **0.08** | **0.12** |

### 6.4.3 Specificity:

The Specificity study included Identification of the main peak, Interference study and Peak Purity

Injections of Blank and standard solutions were made as directed in the method; sample solution and placebo preparation were made and injected into the HPLC. The data obtained is summarized in Table 6.4.3.1. Purity angle is less than purity threshold for all components. The data demonstrate that there is no interference in blank and placebo with Telmisartan and Hydrochlorothiazide peaks.

Table 6.4.3.1: Specificity (Identification and Interference)

| Component | Retention time (min) | USP Plates | USP Tailing | Purity angle | Purity threshold |
|---|---|---|---|---|---|
| **Standard Solution** | | | | | |
| Telmisartan | 8.281 | 3630 | 1.01 | 0.056 | 1.060 |
| Hydrochlorothiazide | 1.580 | 5348 | 0.99 | 0.168 | 1.097 |
| **Sample Solution** | | | | | |
| Telmisartan | 8.274 | 3648 | 1.01 | 0.074 | 1.086 |
| Hydrochlorothiazide | 1.578 | 5711 | 0.98 | 0.188 | 1.117 |

Purity angle is less than purity threshold for all components.

Chromatograms of Blank (diluent), Placebo without Telmisartan and Hydrochlorothiazide, Placebo with Telmisartan, Placebo with Hydrochlorothiazide, Standard solution and Sample solution are given below under figure 6.4.3.1, 6.4.3.2, 6.4.3.3, 6.4.3.4, 6.4.3.5 and 6.4.3.6 respectively

Figure-6.4.3.1: Chromatogram of Blank.

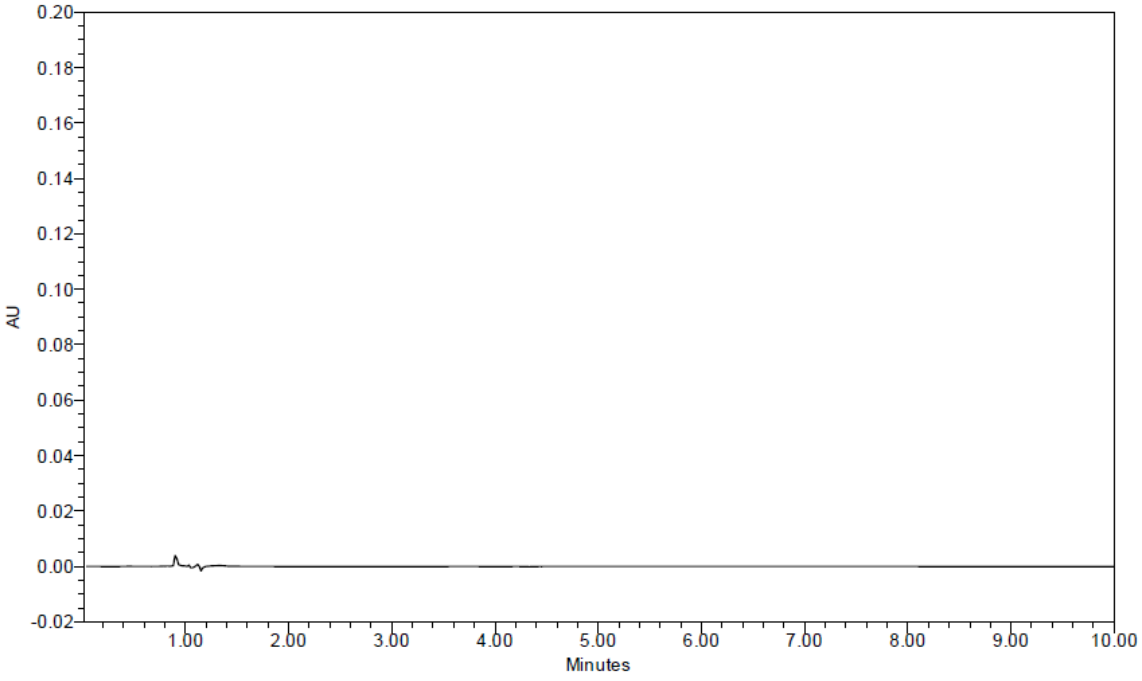


Figure-6.4.3.2: Chromatogram of Placebo without Telmisartan and Hydrochlorothiazide.

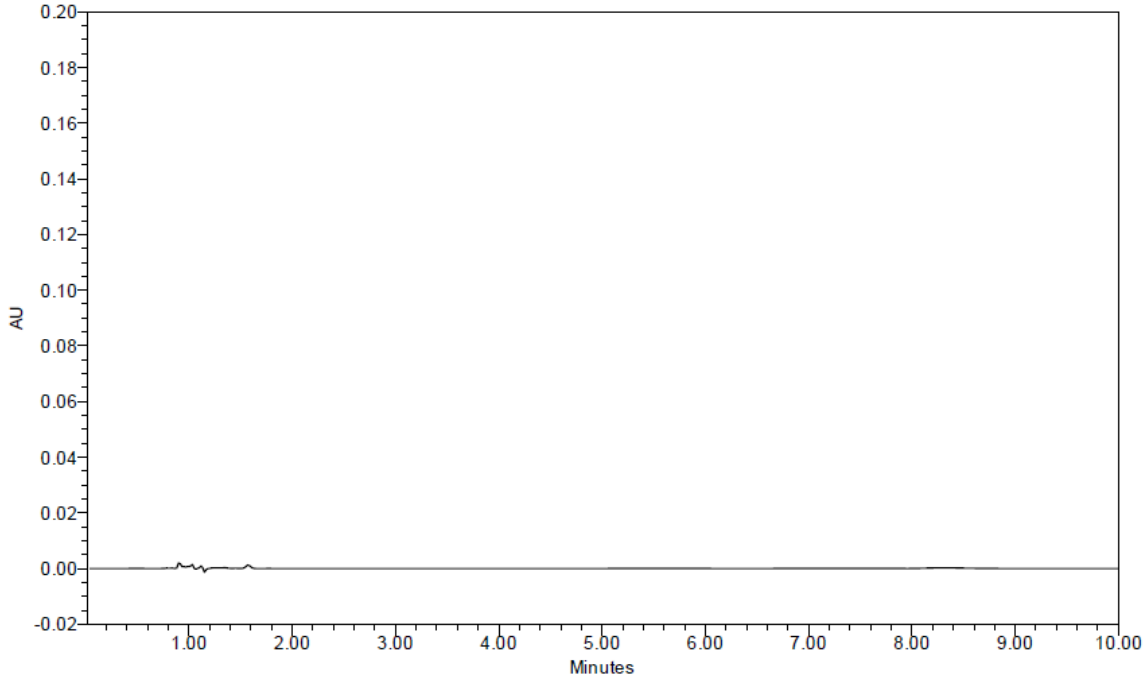



Figure-6.4.3.3: Chromatogram of Placebo with Telmisartan.

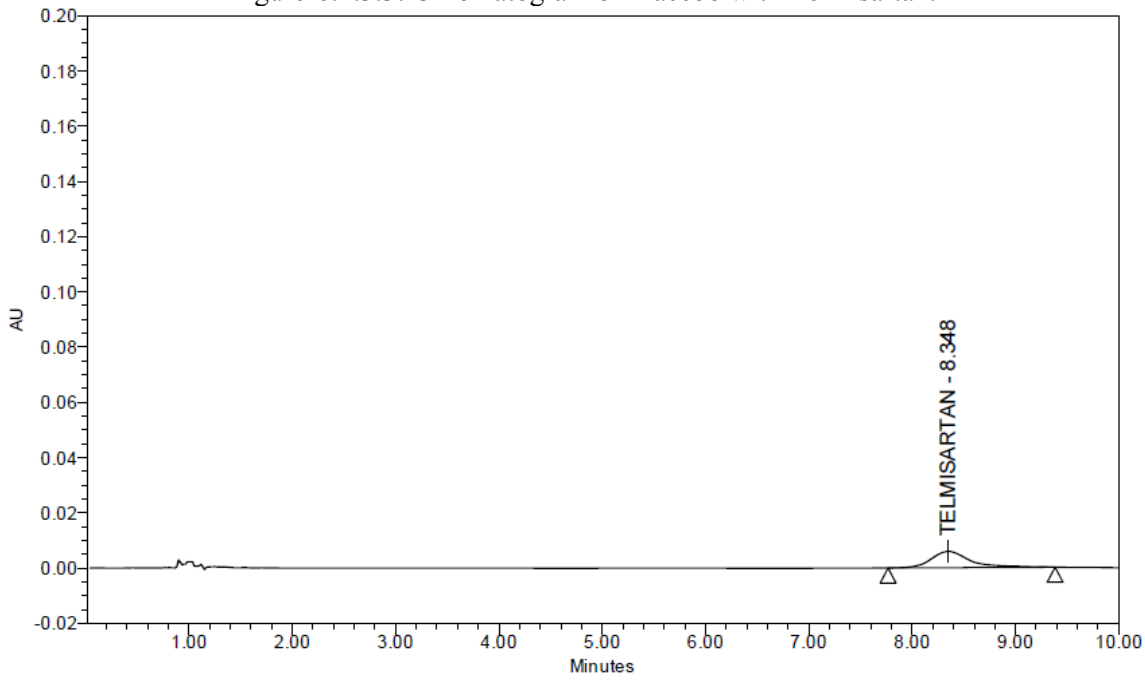


Figure-6.4.3.4: Chromatogram of Placebo with Hydrochlorothiazide

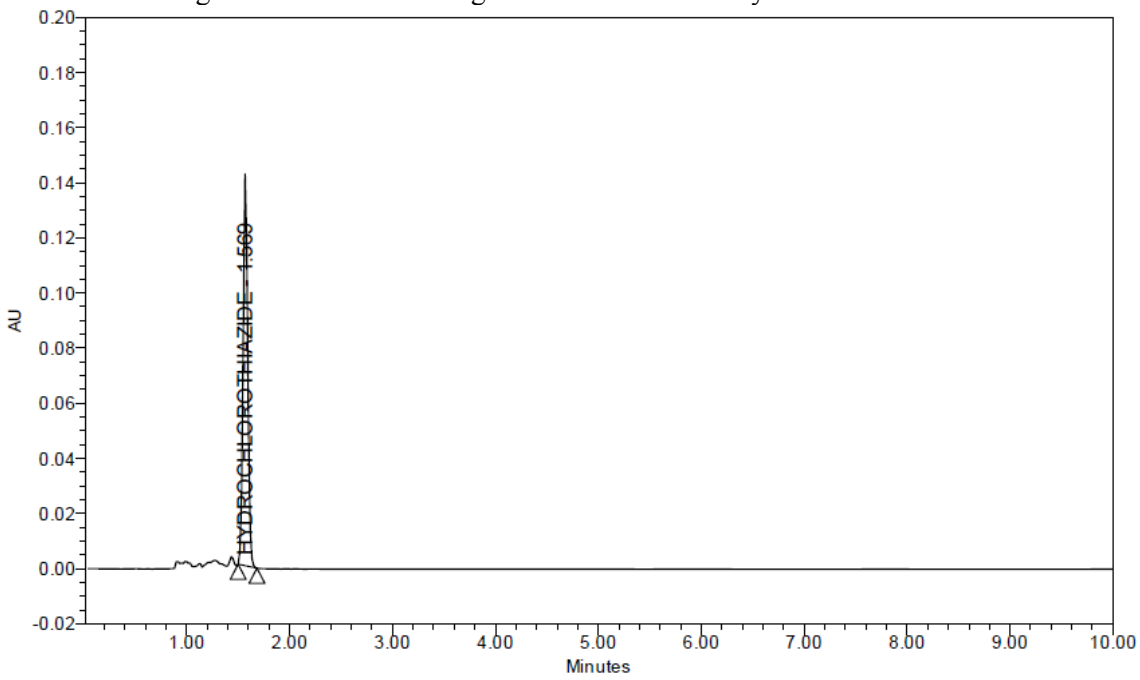



Figure-6.4.3.5: Chromatogram of Standard solution.

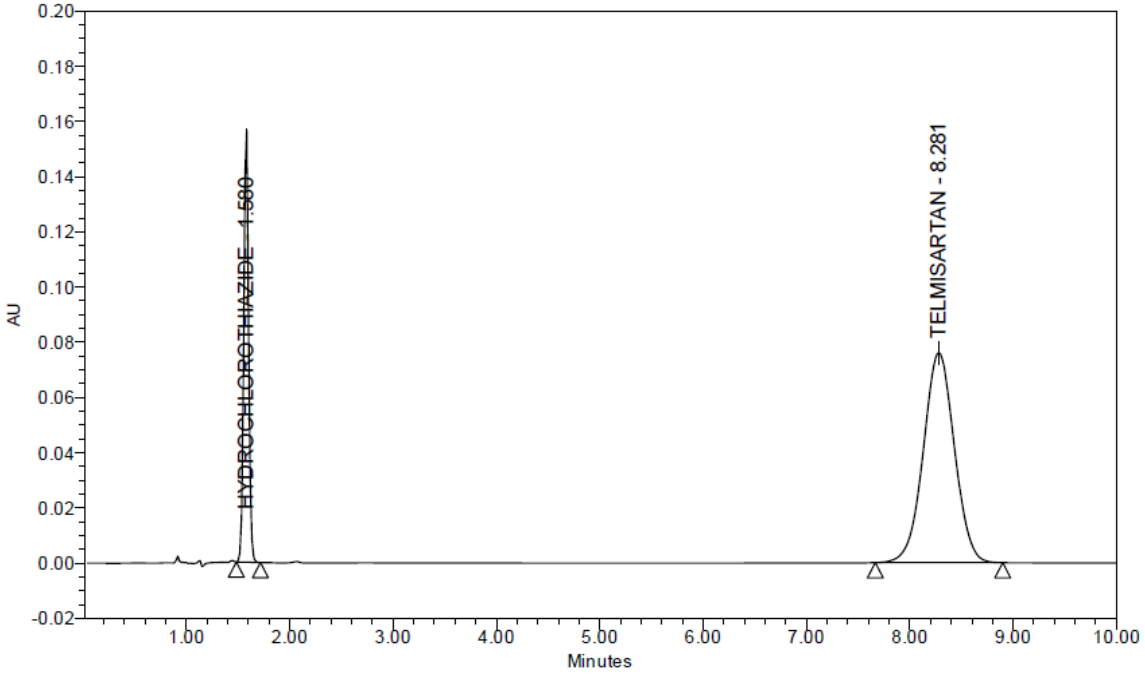
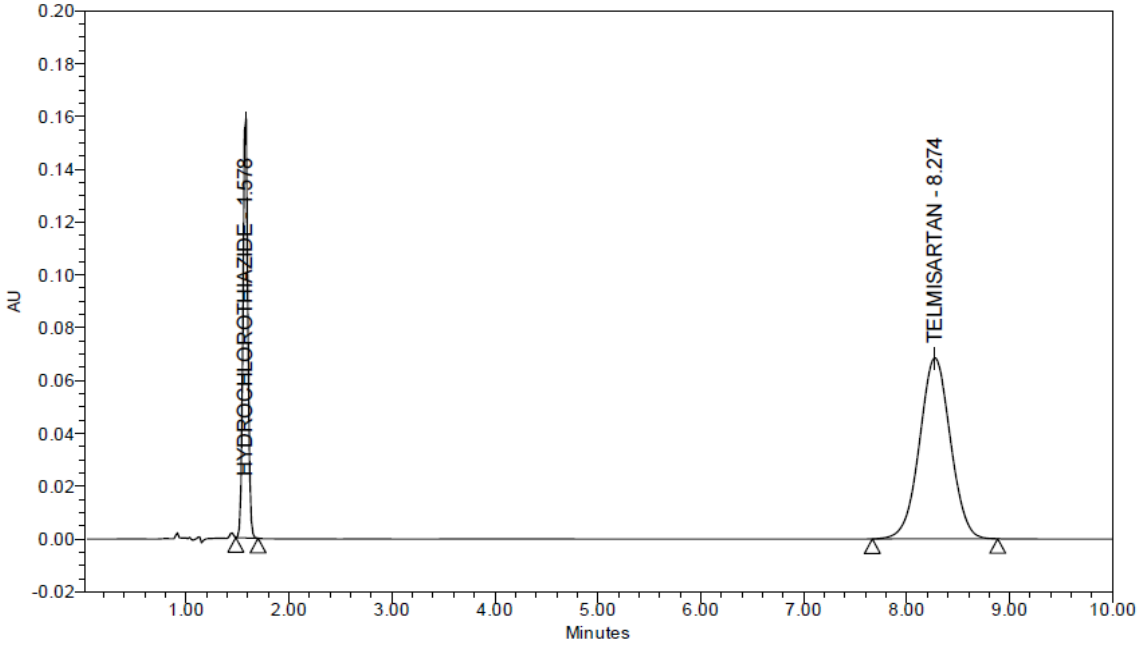


Figure-6.4.3.6: Chromatogram of Control Sample solution.




### 6.4.4 Linearity and Range:

The Linearity of response was determined by preparing different concentrations of standard stock solution ranging from 10% to 150% of the working concentration of tablets (80/12.5 mg and 40/12.5 mg). The data summarized in Table 6.4.4.1, 6.4.4.2 and 6.4.4.2. The data shows that the response is found to be linear for all constituents; Correlation coefficient is more than 0.999. The Y-intercept is also within the set criterion

Table 6.4.4.1: Linearity of Telmisartan (80 mg Tablets)

| Level | Concentration (%) | Response | | |
|---|---|---|---|---|
| | | 1 | 2 | Mean |
| 1 | 10.01 | 159520 | 158085 | 158803 |
| 2 | 50.04 | 795499 | 794949 | 795224 |
| 3 | 55.60 | 883971 | 882956 | 883464 |
| 4 | 66.72 | 1061820 | 1058702 | 1060261 |
| 5 | 77.84 | 1230513 | 1231606 | 1231060 |
| 6 | 100.08 | 1596956 | 1597363 | 1597160 |
| 7 | 133.44 | 2129389 | 2128318 | 2128854 |
| 8 | 155.68 | 2482805 | 2483224 | 2483015 |
| **CORRELATION COEFFICIENT (r)** | | | | **1.0000** |
| **SLOPE** | | | | **15973** |
| **Y-INTERCEPT** | | | | **-4423** |
| **MEDIAN (AREA)** | | | | **1145660** |
| **% LIMIT OF Y-INTERCEPT ± 5% OF MEDIAN** | | | | **-0.39** |

Table 6.4.4.2: Linearity of Telmisartan (40 mg Tablets)

| Level | Concentration (%) | Response | | |
|---|---|---|---|---|
| | | 1 | 2 | Mean |
| 1 | 10.01 | 79262 | 78778 | 79020 |
| 2 | 44.48 | 359804 | 358912 | 359359 |
| 3 | 55.60 | 437282 | 437910 | 437596 |
| 4 | 66.72 | 532535 | 530750 | 531643 |
| 5 | 100.08 | 795499 | 794949 | 795224 |
| 6 | 111.20 | 883971 | 882956 | 883464 |
| 7 | 133.44 | 1061820 | 1058702 | 1060261 |
| 8 | 155.68 | 1230513 | 1231606 | 1231060 |
| **CORRELATION COEFFICIENT (r)** | | | | **1.0000** |
| **SLOPE** | | | | **7914** |
| **Y-INTERCEPT** | | | | **2277** |
| **MEDIAN (AREA)** | | | | **663433** |
| **% LIMIT OF Y-INTERCEPT ± 5% OF MEDIAN** | | | | **0.34** |

Table 6.4.4.3: Linearity of Hydrochlorothiazide.

| Level | Concentration (%) | Response | | |
|---|---|---|---|---|
| | | 1 | 2 | Mean |
| 1 | 11.30 | 53956 | 53803 | 53884 |
| 2 | 56.51 | 269135 | 269157 | 269146 |
| 3 | 98.90 | 467804 | 467674 | 467739 |
| 4 | 113.02 | 524989 | 524755 | 524872 |
| 5 | 127.15 | 584189 | 584178 | 584184 |
| 6 | 141.28 | 659175 | 659528 | 659352 |
| 7 | 169.54 | 790175 | 791378 | 790777 |
| **CORRELATION COEFFICIENT (r)** | | | | **0.9998** |
| **SLOPE** | | | | **4629** |
| **Y-INTERCEPT** | | | | **4004** |
| **MEDIAN (AREA)** | | | | **524872** |
| **% LIMIT OF Y-INTERCEPT ± 5% OF MEDIAN** | | | | **0.76** |

The graphical depiction is included in Figures 6.4.4.1, 6.4.4.2, 6.4.4.3

Figure-6.4.4.1: Linearity for Telmisartan (80 mg Tablets).

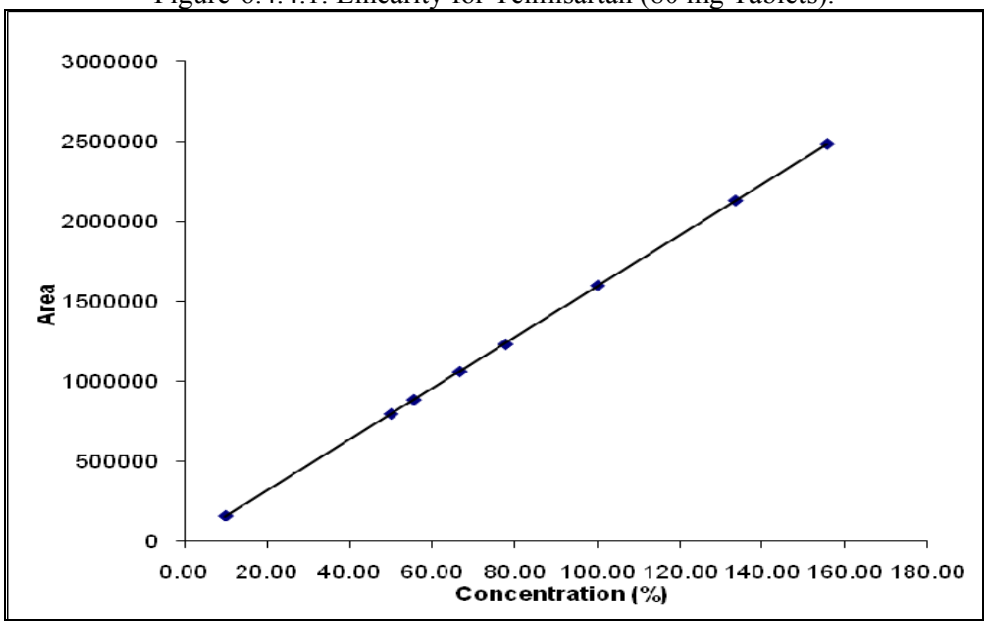



Figure-6.4.4.2: Linearity for Telmisartan (40 mg Tablets).

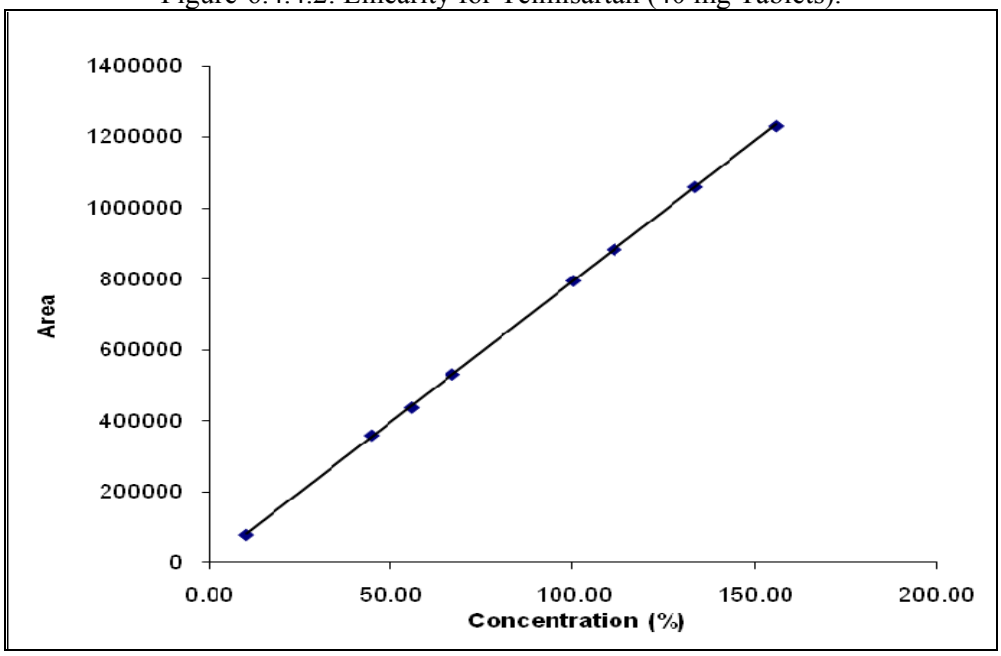
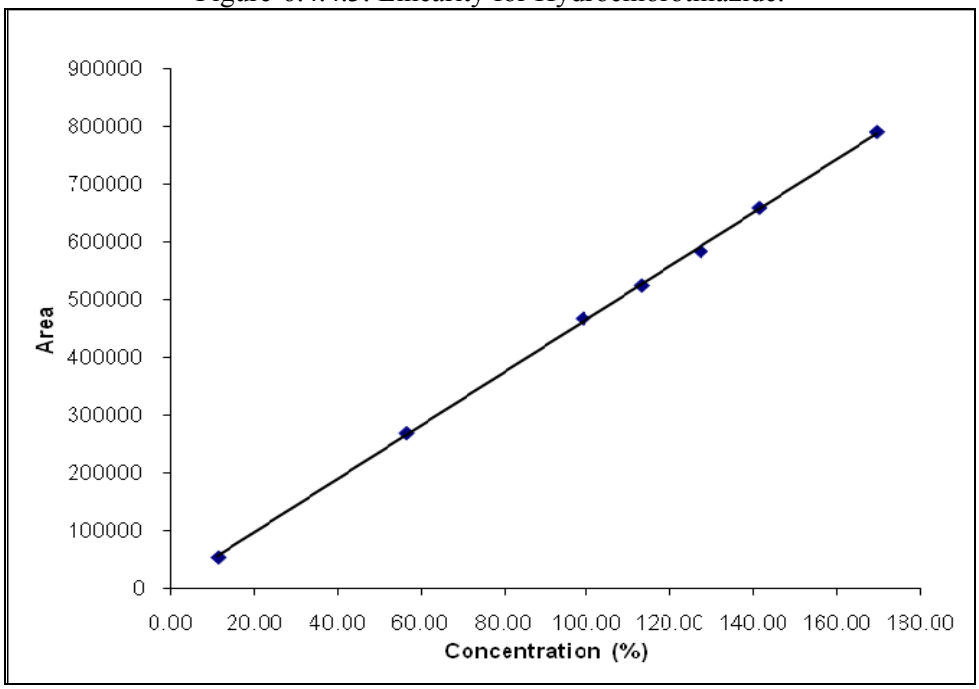


Figure-6.4.4.3: Linearity for Hydrochlorothiazide.



### 6.4.5 Accuracy:

The standard solution was spiked into the placebo at four different levels, 10%,50%, 100% and 150% from three different standard stock solutions and each level in duplicate were injected. This was performed for both strengths and the results are summarized. From the amount added and the amount found, the percentage recovery was calculated. The mean recovery was calculated. The results obtained were summarized from Table 6.4.5.1 to 6.4.5.6. The data shows that the percentage mean recovery at each level is within the acceptance criteria.

Table 6.4.5.1: % Recovery for Telmisartan (For 80/12.5mg Tablets)

| **Level %** | **Response** | **% Recovery** | **Mean recovery %** |
|---|---|---|---|
| 10 % | 160623 | 95.3 | 97.3 |
| | 159139 | 96.5 | |
| | 158146 | 100.0 | |
| 50 % | 778564 | 99.0 | 98.7 |
| | 777928 | 98.1 | |
| | 782521 | 99.0 | |
| 100 % | 1577903 | 99.8 | 99.3 |
| | 1569893 | 98.9 | |
| | 1570228 | 99.1 | |
| 150 % | 2379112 | 100.3 | 101.2 |
| | 2405902 | 101.8 | |
| | 2412083 | 101.6 | |

Table 6.4.5.2: % Recovery Hydrochlorothiazide (For 80/12.5mg Tablets)

| Level % | Response | % Recovery | Mean recovery % |
|---|---|---|---|
| 10 % | 49707 | 97.2 | 100.1 |
| | 51792 | 100.7 | |
| | 52829 | 102.5 | |
| 50 % | 252342 | 98.7 | 98.7 |
| | 253555 | 98.6 | |
| | 254865 | 98.9 | |
| 100 % | 529097 | 103.4 | 103.7 |
| | 532293 | 103.5 | |
| | 536431 | 104.1 | |
| 150 % | 778896 | 101.5 | 100.8 |
| | 770861 | 99.9 | |
| | 781205 | 101.0 | |

Table 6.4.5.3: % Recovery for Telmisartan (For 40/12.5mg Tablets)

| Level % | Response | % Recovery | Mean recovery % |
|---|---|---|---|
| 10 % | 76901 | 97.0 | 96.5 |
| | 80293 | 97.2 | |
| | 80715 | 95.5 | |
| 50 % | 399263 | 99.3 | 99.2 |
| | 397340 | 97.8 | |
| | 402408 | 100.6 | |
| 100 % | 780794 | 97.5 | 98.4 |
| | 789970 | 98.4 | |
| | 788937 | 99.2 | |
| 150 % | 1187221 | 99.3 | 99.2 |
| | 1182715 | 99.2 | |
| | 1174468 | 99.0 | |

Table 6.4.5.4: % Recovery Hydrochlorothiazide (For 40/12.5mg Tablets)

| **Level %** | **Response** | **% Recovery** | **Mean recovery %** |
|---|---|---|---|
| 10 % | 48860 | 95.2 | 96.0 |
| | 50036 | 96.9 | |
| | 49655 | 96.0 | |
| 50 % | 246541 | 96.1 | 97.2 |
| | 250312 | 97.0 | |
| | 254765 | 98.5 | |
| 100 % | 522466 | 101.8 | 103.1 |
| | 532310 | 103.1 | |
| | 539995 | 104.4 | |
| 150 % | 759734 | 98.7 | 98.4 |
| | 758767 | 98.0 | |
| | 765570 | 98.7 | |

### 6.4.6 Precision

#### 6.4.6.1 System Precision:

Single injection of Blank (Diluent) and five replicate injections of standard solution were made on the system. Please refer to Table 6.4.2.1 for system suitability of 80-12.5mg strength. Table 6.4.6.1.1 depicts the system suitability for 40-12.5 strength. All the data were acceptable as per the system suitability requirements.

Table 6.4.6.1.1: System precision (For 40/12.5 mg Tablets)

| **Standard solution** | | |
|---|---|---|
| | **Telmisartan** | **Hydrochlorothiazide** |
| **USP Tailing** | **0.99** | **1.07** |
| **USP Plates** | **3621** | **5432** |
| **Area** | **Standard solution** | |
| | 794947 | 503794 |
| | 793062 | 504032 |
| | 793848 | 504781 |
| | 797008 | 504752 |
| | 795511 | 503147 |
| **Mean** | **794875** | **504101** |
| **SD** | **1524.88** | **688.35** |
| **%RSD** | **0.19** | **0.14** |

### 6.4.6.2 Method Precision:

Six independent sample solutions were prepared and injected on the HPLC. The data obtained is summarized in Table 6.4.6.2.1 and 6.4.6.2.2 The data shows that % RSD is within the acceptance criteria.

Table 6.4.6.2.1: Method precision (80/12.5 mg Tablets)

| Tablet No. | % Release | |
|---|---|---|
| | Telmisartan | Hydrochlorothiazide |
| 1 | 91 | 100 |
| 2 | 89 | 99 |
| 3 | 91 | 99 |
| 4 | 90 | 98 |
| 5 | 91 | 96 |
| 6 | 92 | 98 |
| **Mean** | **91** | **98** |
| **SD** | **1.033** | **1.366** |
| **% RSD** | **1.14** | **1.39** |

Table 6.4.6.2.2: Method precision (40/12.5 mg Tablets)

| Tablet No. | % Release | |
|---|---|---|
| | Telmisartan | Hydrochlorothiazide |
| 1 | 93 | 103 |
| 2 | 92 | 100 |
| 3 | 90 | 99 |
| 4 | 94 | 102 |
| 5 | 95 | 103 |
| 6 | 92 | 101 |
| **Mean** | **93** | **101** |
| **SD** | **1.751** | **1.633** |
| **% RSD** | **1.88** | **1.62** |

### 6.4.6.3 Intermediate Precision (Ruggedness):

Same procedure of system precision and method precision was followed by another Analyst on different instrument and on different day. The data demonstrate that the system complied with system suitability requirements. The data obtained from Analyst-II are summarized from Table 6.4.6.3.1 to 6.4.6.3.4. The data shows that percentage RSD is within the acceptance criteria for system suitability as well as for intermediate precision.

Table 6.4.6.3.1: Intermediate precision – System Suitability (For 80/12.5 mg Tablets)

| **Standard solution** | | |
|---|---|---|
| | **Telmisartan** | **Hydrochlorothiazide** |
| **USP Tailing** | **1.03** | **1.04** |
| **USP Plates** | **3108** | **5532** |
| **Area** | **Standard solution** | |
| | 1663210 | 526817 |
| | 1665038 | 527459 |
| | 1665648 | 527090 |
| | 1671229 | 531085 |
| | 1657249 | 524510 |
| **Mean** | **1664475** | **527392** |
| **SD** | **5028.039** | **2364.87** |
| **%RSD** | **0.30** | **0.45** |

Table 6.4.6.3.2: Intermediate precision – System Suitability (For 40/12.5 mg Tablets)

| Standard solution | | |
|---|---|---|
| | **Telmisartan** | **Hydrochlorothiazide** |
| **USP Tailing** | **1.00** | **1.04** |
| **USP Plates** | **3123** | **5590** |
| **Area** | **Standard solution** | |
| | 853613 | 513824 |
| | 861737 | 519757 |
| | 860066 | 518801 |
| | 862997 | 521692 |
| | 863898 | 520646 |
| **Mean** | **860462** | **518944** |
| **SD** | **4090.34** | **3055.492** |
| **%RSD** | **0.48** | **0.59** |

Table 6.4.6.3.3: Ruggedness (80/12.5 mg Tablets)

| **Tablet No.** | **% Release** | |
|---|---|---|
| | **Telmisartan** | **Hydrochlorothiazide** |
| 1 | 91 | 101 |
| 2 | 96 | 99 |
| 3 | 93 | 98 |
| 4 | 91 | 101 |
| 5 | 96 | 99 |
| 6 | 92 | 98 |
| **Mean** | **93** | **99** |
| **SD** | **2.317** | **1.366** |
| **% RSD** | **2.49** | **1.38** |

Table 6.4.6.3.4: Ruggedness (40/12.5 mg Tablets)

| Tablet No. | % Release | |
|---|---|---|
| | Telmisartan | Hydrochlorothiazide |
| 1 | 88 | 101 |
| 2 | 90 | 102 |
| 3 | 87 | 99 |
| 4 | 88 | 101 |
| 5 | 90 | 103 |
| 6 | 87 | 99 |
| **Mean** | **88** | **101** |
| **SD** | **1.366** | **1.602** |
| **% RSD** | **1.55** | **1.59** |

The pooled data obtained from Analyst-I and Analyst-II is summarized in Table 6.4.6.3.5 and 6.4.6.3.6. The data shows that % difference is not more than ± 5.

Table 6.4.6.3.5: Pooled data (80/12.5 mg Tablets)

| Analyst | % Release | |
|---|---|---|
| | Telmisartan | Hydrochlorothiazide |
| I | 91 | 100 |
| | 89 | 99 |
| | 91 | 99 |
| | 90 | 98 |
| | 91 | 96 |
| | 92 | 98 |
| **Mean** | **91** | **98** |
| II | 91 | 101 |
| | 96 | 99 |
| | 93 | 98 |
| | 91 | 101 |
| | 96 | 99 |
| | 92 | 98 |
| **Mean** | **93** | **99** |
| **% Difference between two means** | **2.0** | **1.0** |

Table 6.4.6.3.6: Pooled data (40/12.5 mg Tablets)

| Analyst | % Release | |
|---|---|---|
| | **Telmisartan** | **Hydrochlorothiazide** |
| I | 93 | 103 |
| | 92 | 100 |
| | 90 | 99 |
| | 94 | 102 |
| | 95 | 103 |
| | 92 | 101 |
| **Mean** | **93** | **101** |
| II | 88 | 101 |
| | 90 | 102 |
| | 87 | 99 |
| | 88 | 101 |
| | 90 | 103 |
| | 87 | 99 |
| **Mean** | **88** | **101** |
| **% Difference between two means** | **5.0** | **0.0** |

### 6.4.7 Stability in Analytical solution:

The Sample solution was kept at sample temperature for 24 hours were injected on to the HPLC time to time. The data obtained are summarized in Table 6.4.7.1 and 6.4.7.2. The data shows that for Telmisartan, both the sample and standard solution were stable for (at least) upto 24 hours at 25°C.

The observation was similar for Hydrochlorothiazide.

Table 6.4.7.1: Stability in analytical solution (Telmisartan)

| Time | Standard Area | Cumulative RSD of Standard area | % Release | Cumulative % Difference |
|---|---|---|---|---|
| Initial (control) | 1588324 | - | 91 | - |
| 3 hrs | 1589401 | 0.05 | 90 | 1 |
| 6 hrs | 1594321 | 0.27 | 91 | 0 |
| 9 hrs | 1589904 | 0.07 | 90 | 1 |
| 12 hrs | 1593050 | 0.21 | 91 | 0 |
| 20 hrs | 1593050 | 0.21 | 90 | 1 |
| 24hrs | 1601447 | 0.58 | 90 | 1 |

Table 6.4.7.2: Stability in analytical solution (Hydrochlorothiazide)

| Time | Standard Area | Cumulative RSD of Standard area | % Release | Cumulative % Difference |
|---|---|---|---|---|
| Initial (control) | 511411 | - | 100 | - |
| 3 hrs | 510253 | 0.16 | 101 | 1 |
| 6 hrs | 508835 | 0.36 | 101 | 1 |
| 9 hrs | 507830 | 0.50 | 101 | 1 |
| 12 hrs | 505523 | 0.82 | 101 | 1 |
| 20 hrs | 503509 | 1.10 | 99 | 1 |
| 24hrs | 498312 | 1.83 | 98 | 2 |

### 6.4.8 Filter compatibility:

The Sample solution was centrifuged and used as control for this study. Samples filtered through different filter were also injected on to the HPLC.

The data shows that % difference is not more than ± 5. Thus all filters tested were compatible with the sample. The data obtained are summarized in Table 6.4.8.1.

Table 6.4.8.1: Filter compatibility

| Filter | Telmisartan | | Hydrochlorothiazide | |
|---|---|---|---|---|
| | % Release | Cumulative % Difference | % Release | Cumulative % Difference |
| Centrifuged | 95 | - | 102 | - |
| Glass filter | 95 | 0 | 102 | 0 |
| On line SS filter | 91 | 4 | 101 | 1 |
| Nylon filter | 93 | 2 | 101 | 1 |
| Teflon filter | 95 | 0 | 102 | 0 |
| PVDF | 97 | 2 | 102 | 0 |

### 6.4.9 Robustness:

The changes in system suitability parameters and results, when deliberate controlled changes were made to the method, were studied in robustness. No significant changes in system suitability parameters or results were observed during robustness study proving the method to be considerable robust The data obtained are summarized in Table 6.4.9.1 and 6.4.9.2.

Table 6.4.9.1: Robustness (Telmisartan).

| Changes in parameters | Values | Retention time of Telmisartan | USP Plates | USP Tailing | % RSD of standard area | % Release | % Difference |
|---|---|---|---|---|---|---|---|
| Control | As per method | 8.269 | 3602 | 1.01 | 0.08 | 91 | - |
| Flow rate (ml/min) | 1.8 | 9.193 | 3722 | 1.01 | 0.17 | 90 | 1 |
| | 2.2 | 7.527 | 3293 | 1.01 | 0.23 | 91 | 0 |
| Wavelength (nm) | 265 | 8.281 | 3611 | 1.01 | 0.05 | 91 | 0 |
| | 275 | 8.281 | 3579 | 1.00 | 0.18 | 90 | 1 |
| Mobile phase composition (Buffer: ACN+MeOH) | 78:22 | 5.867 | 3706 | 1.02 | 0.06 | 93 | 2 |
| | 82:18 | 8.501 | 3330 | 1.04 | 0.05 | 93 | 2 |
| Column temperature | 30°C | 8.130 | 3408 | 1.05 | 0.12 | 93 | 2 |
| | 40°C | 7.036 | 3616 | 1.00 | 0.07 | 93 | 2 |
| Buffer pH | 2.8 | 7.791 | 2923 | 1.05 | 0.42 | 93 | 2 |
| | 3.2 | 6.78 | 2854 | 1.03 | 0.03 | 93 | 2 |
| Speed of rotation - RPM | 72 | 8.269 | 3602 | 1.01 | 0.08 | 91 | 0 |
| | 78 | 8.269 | 3602 | 1.01 | 0.08 | 91 | 0 |

Table 6.4.9.2: Robustness (Hydrochlorothiazide).

| Changes in parameters | Values | Retention time of HCTZ | USP Plates | USP Tailing | % RSD of standard area | % Release | % Difference |
|---|---|---|---|---|---|---|---|
| Control | As per method | 1.567 | 5155 | 1.06 | 0.12 | 100 | - |
| Flow rate (ml/min) | 1.8 | 1.752 | 5651 | 1.06 | 0.05 | 100 | 0 |
| | 2.2 | 1.437 | 4933 | 1.08 | 0.22 | 100 | 0 |
| Wavelength (nm) | 265 | 1.580 | 5320 | 0.99 | 0.18 | 100 | 0 |
| | 275 | 1.580 | 5310 | 0.99 | 0.09 | 100 | 0 |
| Mobile phase composition (Buffer: ACN+MeOH) | 78:22 | 1.490 | 5646 | 1.10 | 0.10 | 100 | 0 |
| | 82:18 | 1.601 | 5075 | 1.06 | 0.06 | 100 | 0 |
| Column temperature | 30°C | 1.607 | 5136 | 1.08 | 0.18 | 99 | 1 |
| | 40°C | 1.490 | 5563 | 1.10 | 0.15 | 99 | 1 |
| Buffer pH | 2.8 | 1.553 | 5373 | 1.04 | 0.40 | 100 | 0 |
| | 3.2 | 1.512 | 5274 | 1.04 | 0.06 | 100 | 0 |
| Speed of rotation | 72 | 1.567 | 5155 | 1.06 | 0.12 | 99 | 1 |
| | 78 | 1.567 | 5155 | 1.06 | 0.12 | 98 | 2 |

### 6.4.10 Conclusions

- The method has been shown to be specific for Telmisartan and Hydrochlorothiazide tablets.
- The method has been shown to be Linear, precise and accurate across the suitable analytical range and stability indicating.
- Solution has been shown to be stable for at least 24 hours when stored at 25°C.
- The method has been shown to be robust towards deliberate minor changes in the method parameters of both HPLC and Dissolution.
- The method can be used in quality control laboratory for release of production batches.