CHAPTER – 3
Development and Validation of a RP-HPLC method for Dutasteride and its impurities in bulk drug
3.1. INTRODUCTION

This chapter describes the method development and validation of a RP-HPLC method for Dutasteride and its impurities in bulk drug. Active ingredients therapeutic activity, review of literature, materials and methods, development trials, validation results and summary and conclusion were covered.

Dutasteride has the chemical name (5α, 17β)-N-{2,5 bis(trifluoromethyl)phenyl}-3-oxo-4-azaandrost-1-ene-17-carboxamide. It is a white solid substance with a molecular formula of C_{27}H_{30}N_{2}F_{6}O_{2}. Dutasteride is a dual 5-α reductase inhibitor that inhibits conversion of testosterone to dihydrotestosterone (DHT). Dutasteride is FDA approved for treatment of benign prostatic hyperplasia (BPH) and is also prescribed off-label for treatment of male pattern baldness (MPB). Dutasteride is approved for the treatment of benign prostatic hyperplasia (BPH) (also known as enlarged prostate).

The teratogenic effect (abnormalities of physiological development) from dutasteride is harmful to male children. Women who are pregnant should not handle the capsules, as inadvertent consumption, such as skin contact, could cause birth defects of the male fetus. The adverse effects would be similar to 5-alpha-reductase deficiency, where a developing male child is naturally deficient in 5-alpha reductase type II, and thus unable to synthesize it. As Dutasteride blocks the same process, developing males would have a DHT deficiency with its adverse effects as a result of the drug. Men who are taking dutasteride should not donate blood, and due to its long half-life, should also not donate blood for at least 6 months after the cessation of treatment. These precautions are to be taken in order to prevent the potential risk of causing birth defects in a pregnant woman who receives a transfusion with blood that contains Dutasteride.

Analytical HPLC method has been developed for Dutasteride \(^1\-^7\) and impurities. Many HPLC, LC-MS and HPTLC methods are observed in publications.
during method development, some of the methods are mentioned by LC-MS for blood samples and Serum samples. An HPLC method was developed for Dutasteride and impurities.

![Figure-3.1: Structure of Dutasteride.](image)

Chemical name : \((5\alpha, 17\beta)-N\-{2,5 \text{ bis(trifluoromethyl) phenyl}}-3\text{-oxo-4-azaandrost-1-ene-17-carboxamide}

CAS Registry Number : 164656-23-9

Molecular formula : \(C_{27}H_{30}N_{2}F_6O_2\)

Molecular weight : 528.58

Therapeutic category : benign prostatic hyperplasia (BPH)

<table>
<thead>
<tr>
<th>S. No</th>
<th>Impurity structure</th>
<th>Chemical name</th>
<th>Molecular weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Impurity-1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
3.2. REVIEW OF LITERATURE

A simple, rapid, sensitive and specific liquid chromatography–tandem mass spectrometry method was developed and validated for quantification of dutasteride (I), a potent and the first specific dual inhibitor of 5α-reductase, in human plasma. The analyte and internal standard (finasteride [II]) were extracted by liquid–liquid extraction with diethyl ether/ dichloromethane (70/30, v/v) using a Glas-Col Multi-Pulse Vortexer. The Chromatographic separation was performed on a reverse phase Xterra MS C18 column with a mobile phase of 10mM ammonium formate/acetonitrile (15/85, v/v, pH adjusted to 3.0 with formic acid). The protonated analyte was quantitated in positive ionization by multiple reaction
monitoring with a mass spectrometer. The mass transitions m/z 529.5 to 461.5 and m/z 373.3 to 317.4 were used to measure I and II, respectively. The assay exhibited a linear dynamic range of 0.1–25.0 ng/mL (Nano grams/milliliter) for dutasteride in human plasma. The lower limit of quantitation was 100 pg/mL (Pico grams/milliliter) with a relative standard deviation less than 15%. Acceptable precision and accuracy were obtained for concentrations over the standard curve ranges. A run time of 1.2 min for each sample made it possible to analyze a throughput of more than 400 human plasma samples/day. The validated method has been successfully used to analyze human plasma samples for application in pharmacokinetic, bioavailability or bioequivalence studies. The chromatography was on Waters Xterra MS C18 column (3.5µm, 50mm × 3mm I.D.) at 30°C temperature. The mobile phase composition was a mixture of 10mM ammonium formate buffer/acetonitrile (15/85, v/v, pH adjusted to 3.0 with formic acid), which was pumped at a flow-rate of 0.6 mL/min.

A simple, sensitive and precise RP-HPLC method was developed for the determination of dutasteride in tablet dosage form. The RP-HPLC separation was achieved on phenomenex C18 column (250 mm, ID 4.6 mm, 5 µm) using mobile phase methanol: water (90:10 v/v) at a flow rate of 1 ml/min at an ambient temperature. Quantification was achieved with photodiode array detection at 235 nm over the concentration range 1-12 μg/mL. The method was validated statistically and was applied successfully for the determination of dutasteride in tablets. Drug was retained in mobile phase consisting of acetonitrile: water (60: 40, v/v) and methanol: water (60: 40, v/v). In acetonitrile: water (90: 10, v/v) tailing in the peak was observed. Good peak symmetry and satisfactory retention time was obtained with mobile phase consisting of methanol: water (90: 10 v/v). Quantification was achieved with PDA detection at 235 nm based on peak area. The retention time of DTS obtained was 5.24±0.112. The system suitability tests for HPLC were carried out on freshly prepared solution of DTS (10 μg/mL).

A simple, rapid, specific and sensitive Reverse Phase-HPLC method has been developed and validated for the simultaneous estimation of alfuzosin hydrochloride
(ALF) and dutasteride (DUTA) in bulk powder and pharmaceutical dosage form. The RP-HPLC separation was performed on HiQ Sil C18HS column (4.6mm I.D. X 250 mm) using mobile phase methanol: water (90:10 v/v) at a flow rate of 1 mL/min at an ambient temperature. Quantitation by HPLC was achieved with UV detection at 244 nm based on peak area with linear calibration curves at concentration ranges 1-5 μg/mL and 4-20 μg/mL for ALF and DUTA, respectively. The limit of detections (LOD) was 0.2 μg/mL and 1 μg/mL for ALF and DUTA, respectively. The method was validated for linearity, accuracy, specificity, precision, robustness and recovery as per ICH guidelines. The method was applied for analysis of pharmaceutical dosage form and no chromatographic interference from the tablet excipients was found. Statistical data reveals that the method is accurate, repeatable and selective for the simultaneous quantitation of the above drugs in pharmaceutical dosage form and for routine analysis of raw material of drugs in quality control laboratories. Different ratios of methanol and water were tried but it was found that methanol: water in the ratio 90: 10 (v/v) at flow rate of 1 mL/min gives acceptable retention time (t<sub>R</sub>) of 6.2 min and 4.8 min for ALF and DUTA respectively. The mixed standard stock solution (10 μg/mL each of ALF and DUTA) was injected in system. The detection wavelength, 244 nm, was selected from overlain spectra of the drugs acquired form UV spectrophotometer.

### 3.3. OBJECTIVE

The main objective of this research work is to develop simple, accurate and stability indicating RP-HPLC method for the quantification of impurities in Dutasteride. Review of literature reveals that the reported methods were qualitative and instrument methods with high analysis time and no quantification of impurities. Development and Validation of a RP-HPLC method for Dutasteride and its impurities in bulk drug.

### 3.4. MATERIALS AND METHODS

#### 3.4.1. Reagents & Chemicals.
a. Water : Rankem
b. Acetonitrile HPLC GRADE : Rankem
c. Potassium dihydrogen ortho phoshate : Rankem
d. Potassium hydroxide : Rankem

3.4.2. Drug Substances:

Dutasteride, impurity-1, impurity-2 and impurity-3 are gift samples received from Lupin Ltd., India.

3.4.3. Instrument details:

The High Performance Liquid Chromatography using waters HPLC instrument having quaternary pumps including auto injector. This HPLC connected with PDA detector, make Waters instrument. All the components are controlled with Empower2 software.

3.4.4. Method development:

Development trials were performed with all neutral buffer salts and different make HPLC columns but finally the chromatographic conditions were optimized with the potassium di potassium orthophoshate, acetonitrile, and water with simple isocratic method.

3.4.4.1 Wave length Selection:

The UV spectrums were generated for Dutasteride, impurity-1, impurity-2 and impurity-3 using with Photo diode array detector (PDA). Dutasteride and its impurities were found to have varying absorption maxima over a range of wavelength. But it was found that at about 210 nm, Dutasteride and its impurities were found to have optimum UV absorption. Therefore, 210 nm was selected for the study and Quantification of Dutasteride and it’s related impurities.
Figure- 3.2: UV Spectra of Dutasteride.

Figure- 3.3: UV Spectra of impurity-1.

Figure- 3.4: UV Spectra of impurity-2.
3.4.4.2. Selection of mobile phase and stationary phase:

Dutasteride, impurity-1, impurity-2 and impurity-3 were found that different functional groups, shows different affinities with mobile phases and stationary phase. A different column with different selectivity provides good separation for method development. Two parameters were chosen to get required resolutions, separations and symmetrical peaks for Dutasteride and impurities. i.e., Selection of the mobile phase and column.

3.4.4.3. Selection of Mobile phase:

Impurity-1, impurity-2 and impurity-3 were co-eluted using with different mobile phases. Dutasteride is steric derivative and the impurities of Dutasteride were having wide range of polarities and the separation of these impurities mainly depends on the column stationary phase. An isocratic method was mobile phase of buffer 0.01 di potassium dihydrogen phosphate in water pH adjusted to 4.5 and acetonitrile was suitable for the separation of Dutasteride and its related substances. Mobile phase was degassed and filtered through 0.22µm millipore filter paper.

3.4.4.4. Selection of stationary phase:

Separation was achieved with Zorbax CN, 250 x 4.6mm, I.D., 5.0µm column. Different stationary phases were studied for the separation of Dutasteride such as

![Figure- 3.5: UV Spectra of impurity-3.](image-url)
C8 and C18 using the mobile phase specified. The experimentation was started using Zorbax C18 250 X 4.6 mm, I.D., 5.0µm column.

**Trail-1:**
The complete experiment details are as follows.

<table>
<thead>
<tr>
<th>Column</th>
<th>Zorbax C18 250 X 4.6 mm, I.D., 5.0 µm column</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobile phase-A</td>
<td>Buffer and acetonitrile in the ratio of 50:50(v/v)</td>
</tr>
<tr>
<td>Sample preparation</td>
<td>5 mg in 10 mL of diluent</td>
</tr>
<tr>
<td>Wavelength</td>
<td>210 nm</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1.0 mL/min</td>
</tr>
<tr>
<td>Run time</td>
<td>26min</td>
</tr>
<tr>
<td>Injection volume</td>
<td>20µL</td>
</tr>
<tr>
<td>Oven temperature</td>
<td>40 °C</td>
</tr>
<tr>
<td>Diluent</td>
<td>Water and acetonitrile in the ratio of 8:2</td>
</tr>
<tr>
<td>Elution</td>
<td>Isocratic</td>
</tr>
</tbody>
</table>

**Figure- 3.6:** Typical HPLC Chromatogram of Dutasteride using Zorbax C18 250 X 4.6 mm I.D., 5.0µm column.

**Observation:** Dutasteride and impurity-3 are not separated while impurity-1 and impurity-2 are separated. Hence, Zorbax C18 250 X 4.6 mm I.D., 5.0µm column is not suitable for the separation of Dutasteride and impurity-3.

**Trail-2:**
The complete experiment details are as follows.
Column: Zorbax C8 250 X 4.6 mm I.D., 5.0µm column

Mobile phase: Buffer and acetonitrile in the ratio of 40:60 (v/v)

Sample preparation: 5 mg in 10 mL of diluent

Wavelength: 210 nm

Flow rate: 1.2 mL/min

Run time: 35 min

Injection volume: 20µL

Oven temperature: 45°C

Diluent: Water and acetonitrile in the ratio of 2:8

Elution: Isocratic

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**Figure- 3.7: Typical HPLC Chromatogram of Dutasteride using Zorbax C8 250 X 4.6 mm, I.D., 5.0µm column.**

**Observation:** Impurity-1 and impurity-2 are separated while Dutasteride and impurity-3 are coeluting each other. Hence, Zorbax C8 column is not suitable for the separation of Dutasteride and impurity-3.

**Trail-3:**

The complete experiment details are as follows.

Column: Zorbax CN 250 X 4.6 mm, I.D., 5.0µm column
Mobile Phase: Buffer and acetonitrile in the ratio of 50:50 v/v
Sample preparation: 5 mg in 10 mL of diluent
Wavelength: 210 nm
Flow rate: 1.0 mL/min
Oven temperature: 40°C
Diluent: Water and acetonitrile in the ratio of 2:8
Elution: Isocratic
Injection volume: 20µL
Runtime: 35 min

Figure-3.8: Typical HPLC Chromatogram of Dutasteride using Zorbax CN Column.

Observation: Dutasteride and impurity-3 are not resolved base line separation. Hence, CN column is suitable for the separation of Dutasteride and impurity-3. Need to change solvent and buffer ratio for better separation.

Trail-4:
The complete experiment details are as follows.

Column: Zorbax CN 250 x 4.6mm, I.D., 5.0µm column
Mobile phase: Buffer and acetonitrile in the ratio of 60:40 v/v
Sample preparation: 5 mg in 10 mL of diluent
Wavelength: 210 nm
Run time: 35 min
Injection volume : 20µL
Flow rate : 1.2 mL/min
Column temperature : 40°C

Figure- 3.9: Typical HPLC Chromatogram of Dutasteride using Zorbax CN column and trail-4 method conditions.

Observation: Impurity-1, impurity-2, Dutasteride and impurity-3 were separated very well. Hence, Zorbax CN 250 x 4.6mm,I.D., 5.0µm column is suitable for separation of all impurities and Dutasteride.

Conclusion:
Based on the above study on stationary phase, it was concluded that impurity-1, impurity-2, Dutasteride and impurity-3 were well separated from each other in column Zorbax CN 250 X 4.6mm ,I.D., 5.0µm column.

3.4.5. Optimized method:
Based on the above study, the below mentioned HPLC parameters was chosen for the separation and Quantification of impurity-1, impurity-2, Dutasteride and impurity-3.

Column : Zorbax CN 250 X 4.6 mm, I.D., 5.0µm column
Buffer preparation : 0.01M potassium dihydrogen ortho phoshate in water pH adjusted to 4.5 with dil. H₃PO₄
Mobile phase : Mix buffer and acetonitrile in the ratio of 40:60
Sample preparation : 5 mg in 10 mL of diluent
Wavelength : 210 nm  
Flow rate : 1.2 mL/min  
Oven temperature : 40°C  
Run time : 35 min  
Diluent : Water and acetonitrile in the ratio of 2:8  
Injection volume : 20 µL  

a) **Preparation of impurity-1 stock solution**: Transferred 5 mg of impurity-1 into a 10 mL volumetric flask, dissolved and diluted to volume with diluent.  
b) **Preparation of impurity-2 stock solution**: Weighed accurately 5 mg of impurity-2 into a 10 mL volumetric flask, dissolved and diluted to volume with diluent.  
c) **Preparation of impurity-3 stock solution**: 5 mg of impurity-3, transferred into a 10 mL volumetric flask, dissolved and diluted to volume with diluent.  
d) **Preparation of Sample solution**: Transferred 5 mg of sample into a 10 mL volumetric flask, dissolved and diluted to volume with diluent.  
e) **Preparation of system suitability solution**: Weighed accurately 5 mg of standard into a 10 mL volumetric flask, dissolved in 5 mL of diluent and added 75 µL of each impurity stock solution dissolved and diluted to volume with diluent.

**Procedure**: After equilibrated the column, separately injected 20 µL of diluent as a blank, system suitability solution, 20 µL of standard solution and test solution in the Liquid Chromatograph. Eliminate peaks due to the blank.

**System suitability criteria**: The resolution between Dutasteride and impurity-3 from system suitability solution should be not less than 2.0.  
The tailing factor for Dutasteride should be not more than 2.0.  
The number of theoretical plates for Dutasteride should be not less than 3000.

**Table -3.2: Specification:**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Name of the impurity</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Impurity-1</td>
<td>Not more than 0.15%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>-------------------------------</td>
<td>------------------------------</td>
</tr>
<tr>
<td>02</td>
<td>Impurity-2</td>
<td>Not more than 0.15%</td>
</tr>
<tr>
<td>03</td>
<td>Impurity-3</td>
<td>Not more than 0.15%</td>
</tr>
<tr>
<td>04</td>
<td>Any other impurity</td>
<td>Not more than 0.10%</td>
</tr>
<tr>
<td>05</td>
<td>Total impurities</td>
<td>Not more than 0.50%</td>
</tr>
</tbody>
</table>

Calculation: calculate the impurity using below formula

Known impurities: % area obtained for known impurity / RRF

Total impurities: % known impurities + % other unknown impurities calculated by area normalization.

RRF for Impurity-1: 0.92
RRF for Impurity-2: 1.37
RRF for Impurity-3: 0.74

### 3.5. RESULTS AND DISCUSSION

#### 3.5.1. Method validation:

Analytical method validation was performed as per ICH(8-10) and USFDA guidelines with specificity, precision, accuracy, linearity, limit of detection, limit of quantification, ruggedness and robustness.

#### 3.5.1.1. Related substances by HPLC:

#### 3.5.1.2. System suitability:

a) **Preparation of impurity-1 stock solution:** Transferred 5mg of impurity-1 into a 10 mL volumetric flask, dissolved and diluted to volume with diluent.

b) **Preparation of impurity-2 stock solution:** Transferred 5 mg of impurity-2 into a 10 mL volumetric flask, dissolved and diluted to volume with diluent.

c) **Preparation of Sample solution:** Weighed accurately 5mg of sample into a 10mL volumetric flask, dissolved and diluted to volume with diluent.

d) **Preparation of sample + all impurities spiked:** Transferred 5mg of sample into a 10 mL volumetric flask, dissolved in 5mL of diluent and added 10μL of each impurity stock solution dissolved and diluted to volume with diluent.
Injected all above solutions once and calculated the system suitability parameters i.e., the resolution between adjacent peaks, Tailing factor and tangent for each impurity.

**Conclusion:** Under optimized Chromatographic conditions, impurity-1, impurity-2, impurity-3 and Dutasteride were separated well, retention times being about 3.83, 12.18, 15.9 and 13.9 min respectively. The system suitability results are given in table - 3.3.

**Table- 3.3: System suitability results:**

<table>
<thead>
<tr>
<th>S. No</th>
<th>Name</th>
<th>Retention time (min)</th>
<th>Relative retention time (min)</th>
<th>Resolution, (Rs)</th>
<th>Theoretical plates (N)</th>
<th>Tailing factor (T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Impurity-1</td>
<td>3.83</td>
<td>0.29</td>
<td>---</td>
<td>3604</td>
<td>1.1</td>
</tr>
<tr>
<td>02</td>
<td>Impurity-2</td>
<td>12.18</td>
<td>0.24</td>
<td>10.2</td>
<td>1317</td>
<td>1.06</td>
</tr>
<tr>
<td>03</td>
<td>Dutasteride</td>
<td>13.9</td>
<td>1.00</td>
<td>3.1</td>
<td>14878</td>
<td>1.1</td>
</tr>
<tr>
<td>04</td>
<td>Impurity-3</td>
<td>15.9</td>
<td>1.14</td>
<td>3.5</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

3.5.1.3. **Specificity:**

a) **Acid hydrolysis:** Dissolved 50mg of sample in 50mL of 5N HCl solution and kept for 24 hours at 60°C with continuous stirring and analysed after 24 hours.

   **Observation:** Dutasteride sample was stable under acid hydrolysis.

b) **Base hydrolysis:** Dissolved 50mg of sample in 50mL of 0.1N NaOH solution and kept for 8 hours at 60°C with continuous stirring and analysed after 8 hours.

   **Observation:** Dutasteride was degraded under base hydrolysis.

c) **Oxidation degradation:** Dissolved 50mg of sample in 50mL of 0.01% peroxide solution and kept for 3 hours at 60°C with continuous stirring and analysed after 1 hour.

   **Observation:** Dutasteride was stable under peroxide solution.
d) **Thermal degradation:** About 1 gm of Dutasteride sample is taken and kept under thermal condition i.e., at 105°C for 7 days and sample collected after 48 hours and sample analyzed.

**Observation:** Dutasteride sample is stable under thermal condition.

e) **Photo degradation:** About 1 gm of sample is taken and kept in UV chamber i.e., at 254 nm for 48 hours and sample collected after 48 hours and sample analyzed.

**Observation:** Dutasteride sample is stable under photo condition.

f) **Water hydrolysis:** Dissolved 50 mg of sample in 100 mL of water and kept for 24 hours at 70°C with continuous stirring and injected after 24 hours.

**Observation:** Dutasteride was not degraded under water hydrolysis.

**Conclusion:**

Dutasteride samples are stable in thermal, photo degradation, acid hydrolysis, peroxide and water hydrolysis. Dutasteride was degraded under base hydrolysis. All samples are analyzed and found that degradation peaks are separated from known impurities and Dutasteride. Peak purity were established with PDA detector and proved that Dutasteride peak is pure in all above conditions. The studies are summarized in table 3.4.

**Table- 3.4: Dutasteride degradation data:**

<table>
<thead>
<tr>
<th>Stressed condition</th>
<th>Time (hrs)</th>
<th>% Purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>As such</td>
<td>Initial</td>
<td>99.95</td>
</tr>
<tr>
<td>Acid hydrolysis</td>
<td>24</td>
<td>99.4</td>
</tr>
<tr>
<td>Base hydrolysis</td>
<td>8</td>
<td>94.1</td>
</tr>
<tr>
<td>Oxidation degradation</td>
<td>3</td>
<td>99.86</td>
</tr>
<tr>
<td>Thermal degradation</td>
<td>7 x 24</td>
<td>99.92</td>
</tr>
<tr>
<td>Photo degradation</td>
<td>48</td>
<td>99.63</td>
</tr>
<tr>
<td>Water hydrolysis</td>
<td>24</td>
<td>99.86</td>
</tr>
</tbody>
</table>
Figure 3.10: A typical HPLC Chromatogram of acid degradation sample.

Figure 3.11: A typical HPLC Chromatogram of base degradation sample.

Figure 3.12: A typical HPLC Chromatogram of oxidation degradation sample.
Figure- 3.13: A typical HPLC Chromatogram of thermal degradation sample.

Figure- 3.14: A typical HPLC Chromatogram of photo degradation sample.

Figure- 3.15: A typical HPLC Chromatogram of water degradation sample.
3.5.1.4. Limit of Detection and Limit of Quantification:

a) **LOD/LOQ solution-1 preparation (0.05%)**: Transferred 5µL of each impurity stock solutions into 10 mL volumetric flask, dissolved and diluted to volume with diluent.

b) **LOQ solution-2 preparation**: Transferred 4.0µL of impurity-1, 7.0µL of impurity-2 and 7.5 µL of impurity-3 stock solutions into 10 mL volumetric flask, dissolved and diluted to volume with diluent.

c) **LOD solution-1 preparation**: Transferred 3.3mL of above LOQ solution-2 stock solutions into 10mL volumetric flask, dissolved and diluted to volume with diluent.

Injected all above solutions and calculated the Limit of Detection and Limit of Quantification for each impurity.

**Conclusion:**

The LOD for impurity-1, impurity-2 and impurity-3 were found to be 0.0110 %, 0.022% and 0.023% respectively. The LOQ for impurity-1, impurity-2 and impurity-3 were found to be 0.04 %, 0.07% and 0.075 % respectively. The results are summarized in table - 3.5.

**Table- 3.5: Limit of Detection and Limit of Quantification data:**

<table>
<thead>
<tr>
<th>Conc.</th>
<th>Impurity -1</th>
<th>Impurity -2</th>
<th>Impurity-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOD</td>
<td>0.0110</td>
<td>0.022</td>
<td>0.023</td>
</tr>
<tr>
<td>LOQ</td>
<td>0.040</td>
<td>0.070</td>
<td>0.075</td>
</tr>
</tbody>
</table>

3.5.1.5. Precision and accuracy at Limit of Quantification level:

a) **Solution preparation**: Transferred 40µL of impurity-1, 70µL of impurity-2 and 75µL of impurity-3 into 100mL volumetric flask, containing 50 mL of diluent dissolved and diluted to volume with diluent.

Prepared six times the solution as mentioned above and injected all the above solutions each preparation once, calculated the % RSD for six preparations for each impurity.
Accuracy:

b) **Sample + All impurities Solution preparation:** Transferred 50mg of sample into 100mL volumetric flask, dissolved in 50mL of diluent and added 40µL of impurity-1, 70µL of impurity-2 and 75µL of impurity-3 dissolved and diluted to volume with diluent.

c) **Sample solution preparation:** Transferred 50mg of sample into 100mL volumetric flask, dissolved and diluted to volume with diluent.

Prepared three times the solution as mentioned above and injected each preparation once to calculate the % recovery for each impurity at Limit of Quantification level.

**Conclusion:**

The repeatability and recovery the LOQ concentrations for impurity-1, impurity-2 and impurity-3 were 3.40%, 1.98% 1.62 % and 98.60%, 103.60%, 103.00% respectively. The results are summarized in the table - 3.6.

**Table- 3.6: Precision and accuracy at Limit of Quantification level data:**

<table>
<thead>
<tr>
<th>S. No</th>
<th>Impurity</th>
<th>% RSD (n=6)</th>
<th>% Recovery (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Impurity-1</td>
<td>3.40</td>
<td>98.60</td>
</tr>
<tr>
<td>2</td>
<td>Impurity-2</td>
<td>1.98</td>
<td>103.60</td>
</tr>
<tr>
<td>3</td>
<td>Impurity-3</td>
<td>1.62</td>
<td>103.00</td>
</tr>
</tbody>
</table>

**3.5.1.6. Linearity:**

a) **Linearity solution-1(0.075%):** 7.5µL of each impurity transferred into 10mL volumetric flask, containing 5 mL of diluent dissolved and diluted to volume with diluent.

b) **Linearity solution-2(0.1125%):** 11.25µL of each impurity transferred into 10mL volumetric flask, containing 5 mL of diluent dissolved and diluted to volume with diluent.

c) **Linearity solution-3(0.15%):** 15µL of each impurity transferred into 10mL volumetric flask, containing 5 mL of diluent dissolved and diluted to volume with diluent.
d) **Linearity solution-4(0.1875%)**: 18.75µL of each impurity transferred into 10mL volumetric flask, containing 5 mL of diluent dissolved and diluted to volume with diluent.

e) **Linearity solution-5(0.225%)**: 22.5µL of each impurity transferred into 10mL volumetric flask, containing 5 mL of diluent dissolved and diluted to volume with diluent.

Injected all above solutions each preparation once and calculated the Linearity parameters i.e. correlation coefficient, slope and intercept for each impurity.

**Conclusion:**
Linearity established for impurity-1, impurity-2 and impurity-3 at 0.075%, 0.1125%, 0.15%, 0.1875%, 0.225%. The correlation coefficient (r) are more than 0.99. The above result reveal that method is linear results are summarized in purity wise table -3.7.

**Table- 3.7: Dutasteride impurity-1 linearity data:**

<table>
<thead>
<tr>
<th>S. No</th>
<th>Level (%)</th>
<th>Concentration (%)</th>
<th>Area of impurity-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>0.075</td>
<td>8898</td>
</tr>
<tr>
<td>2</td>
<td>75</td>
<td>0.1125</td>
<td>12656</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>0.15</td>
<td>16959</td>
</tr>
<tr>
<td>4</td>
<td>125</td>
<td>0.1875</td>
<td>21542</td>
</tr>
<tr>
<td>5</td>
<td>150</td>
<td>0.225</td>
<td>25992</td>
</tr>
</tbody>
</table>

**Correlation coefficient(r)** 0.9993

**Slope** 114864

**Y-Intercept** -20.2

**(%Y-Intercept** -0.119
Figure- 3.16: Dutasteride impurity-1 linearity graph.

Table- 3.8: Dutasteride impurity-2 linearity data:

<table>
<thead>
<tr>
<th>S. No</th>
<th>Level (%)</th>
<th>Concentration (%)</th>
<th>Area of impurity-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>0.075</td>
<td>6400</td>
</tr>
<tr>
<td>2</td>
<td>75</td>
<td>0.1125</td>
<td>9284</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>0.15</td>
<td>12980</td>
</tr>
<tr>
<td>4</td>
<td>125</td>
<td>0.1875</td>
<td>16220</td>
</tr>
<tr>
<td>5</td>
<td>150</td>
<td>0.225</td>
<td>19540</td>
</tr>
</tbody>
</table>

Correlation coefficient(r) 0.9995
Slope 88576
Y-Intercept -401.6
(%)Y-Intercept -3.09
Figure- 3.17: Dutasteride impurity-2 linearity graph.

Table- 3.9: Dutasteride impurity-3 linearity data:

<table>
<thead>
<tr>
<th>S. No</th>
<th>Level (%)</th>
<th>Concentration (%)</th>
<th>Area of impurity-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>0.075</td>
<td>12200</td>
</tr>
<tr>
<td>2</td>
<td>75</td>
<td>0.1125</td>
<td>18587</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>0.15</td>
<td>24520</td>
</tr>
<tr>
<td>4</td>
<td>125</td>
<td>0.1875</td>
<td>30234</td>
</tr>
<tr>
<td>5</td>
<td>150</td>
<td>0.225</td>
<td>36567</td>
</tr>
</tbody>
</table>

Correlation coefficient (r) 0.9998
Slope 161016
Y-Intercept 269.2
(%)Y-Intercept 1.09
3.5.1.7. Accuracy:

a) **Accuracy solution-1 preparation (0.075%)**: Weighed accurately 5mg of sample into 10 mL volumetric flask, dissolved in 5mL of diluent and added 7.5µL of each impurity stock solution, dissolved and diluted to volume with diluent. Three solutions prepared as mentioned above.

b) **Accuracy solution-2 preparation- (0.15%)**: Weighed accurately 5 mg of sample into 10 mL volumetric flask, dissolved in 5mL of diluent and added 15µL of each impurity stock solution, dissolved and diluted to volume with diluent. Three solutions prepared as mentioned above.

c) **Accuracy solution-3 preparation (0.225%)**: Weighed about 10mg of sample into 10 mL volumetric flask, dissolved in 5mL of diluent and added 22.5µL of each impurity stock solution, dissolved and diluted to volume with diluent. Three solutions prepared as mentioned above.

Injected each above preparation once and calculated the recovery for each impurity at each level.

**Conclusion:**
The percentage recovery of all three impurities in Dutasteride samples is shown in the table-3.10.
Table 3.10: % Recovery in accuracy:

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Impurity-1(%)</th>
<th>Impurity-2(%)</th>
<th>Impurity-3(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50%</td>
<td>101.45</td>
<td>97.32</td>
<td>102.11</td>
</tr>
<tr>
<td>100%</td>
<td>96.61</td>
<td>102.19</td>
<td>99.10</td>
</tr>
<tr>
<td>150%</td>
<td>97.79</td>
<td>95.59</td>
<td>96.10</td>
</tr>
</tbody>
</table>

3.5.1.8. Precision:

a) **Sample preparation:** About 10mg of sample into 10mL volumetric flask, dissolved and diluted to volume with diluent.

b) **Sample + 0.10% spiked preparation:** Transferred 10mg of sample into 10mL volumetric flask, dissolved in 5mL of diluent added 10µL of each impurity stock solution dissolved and diluted to volume with diluent. Prepared the solution six times as mentioned above.

Injected all above sample preparations and calculated the % RSD for each impurity.

**Results and discussion:**

The precision of the related substance method was checked by injecting six individual preparations of Dutasteride spiked with 0.10% of impurity-1, impurity-2 and impurity-3. The % RSD of the area for each of impurity-1, impurity-2 and impurity-3 were calculated. The results were summarized in the table 3.11.

Table 3.11: Precision data:

<table>
<thead>
<tr>
<th>Name</th>
<th>Impurity-1</th>
<th>Impurity-2</th>
<th>Impurity-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>% RSD</td>
<td>3.40</td>
<td>1.98</td>
<td>1.62</td>
</tr>
</tbody>
</table>

3.5.1.9. Robustness:

Flow variation:

a) **Sample solution preparation:** About 5mg of sample into 10mL volumetric flask, dissolved and diluted to volume with diluent.
b) **Sample + 0.10% spiked preparation:** About 5mg of sample into 10mL volumetric flask, dissolved in 5mL of diluent added 10µL of each impurity stock solution dissolved and diluted to volume with diluent. Injected the above sample solution at flow rates 1.0mL/min and at 1.4mL/min and observed the system suitability parameters and impurities relative retention times and compared with 1.2mL/min results.

**Temperature variation:**

a) **Sample solution preparation:** Transferred 5mg of sample into 10mL volumetric flask, dissolved and diluted to volume with diluent.

b) **Sample + 0.10% spiked preparation:** Transferred 5mg of sample into 10mL volumetric flask, dissolved in 5mL of diluent added 10µL of each impurity stock solution dissolved and diluted to volume with diluent. Injected the above sample solution at temperature 35°C and at 45°C and observed the system suitability parameters and impurities relative retention times and compared with 40°C results.

**Conclusion:**

The results are summarized in the table- 3.12.

**Table- 3.12: Robustness data:**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>35°C</th>
<th>45°C</th>
<th>1.2 mL/min</th>
<th>1.4 mL/min</th>
<th>As such</th>
</tr>
</thead>
<tbody>
<tr>
<td>Impurity -1 RRT</td>
<td>0.28</td>
<td>0.31</td>
<td>0.29</td>
<td>0.29</td>
<td>0.29</td>
</tr>
<tr>
<td>Impurity -2 RRT</td>
<td>0.23</td>
<td>0.25</td>
<td>0.24</td>
<td>0.24</td>
<td>0.24</td>
</tr>
<tr>
<td>Impurity -3 RRT</td>
<td>0.23</td>
<td>0.25</td>
<td>0.24</td>
<td>0.24</td>
<td>0.24</td>
</tr>
<tr>
<td>Theoretical plates for Dutasteride</td>
<td>12363</td>
<td>11614</td>
<td>13282</td>
<td>12084</td>
<td>14878</td>
</tr>
<tr>
<td>Tailing factor for Dutasteride</td>
<td>1.3</td>
<td>1.3</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Resolution between Impurity-2,3 and Dutasteride</td>
<td>2.4</td>
<td>2.2</td>
<td>2.5</td>
<td>2.2</td>
<td>2.4</td>
</tr>
</tbody>
</table>
3.5.1.10. Solution stability:

**Sample solution preparation:** Weighed accurately 5 mg of sample into 10 mL volumetric flask, dissolved and diluted to volume with diluent.

Injected the solution for 0 hrs(Sample solution initial), 12hrs, 24 hrs and 48 hrs and performed the impurity content.

**Conclusion:**

Impurity-1, and impurity-2 is not increased and other impurities are also not observed during the solution stability and mobile phase stability experiments when performed using the related substance method. The solution stability and mobile phase stability experiment data confirms that the sample solutions and mobile phases used during the related substance determination were stable for at least 48 hours. The results are summarized in the table- 3.13.

**Table- 3.13: Solution stability data:**

<table>
<thead>
<tr>
<th>Duration</th>
<th>Impurity-1 (%)</th>
<th>Impurity-2 (%)</th>
<th>Impurity-3 (%)</th>
<th>Any other impurity (%)</th>
<th>Total impurities (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample solution initial</td>
<td>0.01</td>
<td>Not detected</td>
<td>Not detected</td>
<td>0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>After 12 hrs</td>
<td>0.01</td>
<td>Not detected</td>
<td>Not detected</td>
<td>0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>After 24 hrs</td>
<td>0.01</td>
<td>Not detected</td>
<td>Not detected</td>
<td>0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>After 48 hrs</td>
<td>0.01</td>
<td>Not detected</td>
<td>Not detected</td>
<td>0.01</td>
<td>0.02</td>
</tr>
</tbody>
</table>

**Table- 3.14: Mobile phase stability data:**

<table>
<thead>
<tr>
<th>Duration</th>
<th>Impurity-1 (%)</th>
<th>Impurity-2 (%)</th>
<th>Impurity-3 (%)</th>
<th>Any other impurity (%)</th>
<th>Total impurities (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample solution initial</td>
<td>0.01</td>
<td>Not detected</td>
<td>Not detected</td>
<td>0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>After 12 hrs</td>
<td>0.01</td>
<td>Not detected</td>
<td>Not detected</td>
<td>0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>After 24 hrs</td>
<td>0.01</td>
<td>Not detected</td>
<td>Not detected</td>
<td>0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>After 48 hrs</td>
<td>0.01</td>
<td>Not detected</td>
<td>Not detected</td>
<td>0.01</td>
<td>0.02</td>
</tr>
</tbody>
</table>
3.5.1.11. Batch analysis:

Using the above validated method, Dutasteride sample was analyzed and the data is furnished in table 3.15.

Table- 3.15: Batch analysis data:

<table>
<thead>
<tr>
<th>Lot Number</th>
<th>Impurity-1</th>
<th>Impurity-2</th>
<th>Impurity-3</th>
<th>Any other impurity</th>
<th>Total impurities</th>
</tr>
</thead>
<tbody>
<tr>
<td>001</td>
<td>0.01</td>
<td>0.01</td>
<td>Not detected</td>
<td>0.01</td>
<td>0.03</td>
</tr>
</tbody>
</table>

3.6. SUMMARY AND CONCLUSION

The present study describes the development of reversed phase liquid chromatographic (RPLC) method for Dutasteride in the presence of its impurities. Successful separation of Dutasteride from the synthetic impurities achieved on a Inertsil CN, 250X4.6mm ID., 5.0µm column. The developed HPLC method was validated with respect to linearity, accuracy, precision, specificity and ruggedness. To ensure the quality of Dutasteride. To the best of our knowledge, validated stability indicating Liquid Chromatography method which separates all the impurities disclosed in this investigation was not studied elsewhere. This method can be used for routine analysis of production samples in bulk drugs.
3.7. REFERENCES


