NOVEL RP-HPLC METHOD FOR THE QUANTIFICATION OF ABIRATERONE ACETATE

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Abstract

Simple, rapid, accurate and specific RP-HPLC method has been developed for quantitative estimation of Abiraterone acetate in bulk and tablet. Separation was attained by using Acclaim C18 column (50mm x 4.6 mm, 5µ) at 25°C and 80:20 v/v acetonitrile:ammonium acetate buffer(10mM) of pH 6.0 with flow rate of 1ml/min as mobile phase and the drug was detected at 254 nm. Analytical method was said to be linear over a range of 0.125-60 µg/ml with correlation constant of 0.999. Method was appeared to be precise, robust and rugged. LOD and LOQ were found to be 10 ng/ml and 50 ng/ml respectively and the mean recovery value as 100.52%. Stability indicating capability of this method has been demonstrated by analyzing stressed samples of drug and found labile to acidic, alkaline, oxidative, thermal and photolytic conditions. Above method was applied for the estimation in API, formulation and dissolution testing of the dosage form. Percentage assay of the formulation was found to be 101.66% w/w. Drug released to an extent of 85.29% at 30 minutes as per FDA published method. Thus, the analytical procedure is reliable and offers advantage in terms of speed; so could be effectively used for the routine quality control purpose.

Keywords: Abiraterone acetate, stress testing, RP-HPLC, Dissolution.

INTRODUCTION

Abiraterone acetate \{(3S,8R,9S,10R,13S,14S)-10,13-dimethyl-17-pyridin-3-yl-2,3,4,7,8,9,11,12,14,15-decahydro-1H-cyclopenta[a]phenanthren-3-yl acetate\} - is an acetyl ester of Abiraterone [1-5]. It is a prodrug of its active metabolite Abiraterone. It is potent, orally bioavailable androgen receptor blocker and is proved to be far more potent than ketoconazole and liarozole in CYP17A1 inhibition; a rate-limiting enzyme in androgen biosynthesis resulting in inhibition of testosterone production in the adrenals, testes and the prostatic tumor tissues. Mainly it is indicated for use in combination with prednisone for the treatment of men with metastatic castration-resistant prostate cancer who have already received prior chemotherapy containing docetaxel. It improves the overall survival rate by nearly 4 months. A literature review revealed that bioanalytical methods [6-8] as well as a stability indicating LC-MS [9] method and determination by spectrofluorimetry [10] have been developed for the estimation of Abiraterone acetate and Abiraterone. As there was no reported analytical method and not official in any pharmacopoeia a simple, sensitive, precise, accurate, specific, robust and stability indicating HPLC method was developed. For the estimation of bulk drug and formulation.

MATERIALS AND METHODS

Materials

Pure standard: - The drug Abiraterone acetate was obtained from Sun Pharma,
Figure 1: Chemical structure of Abiraterone acetate

Vadodara, India as a gift sample. Batch number: AHNABRFL013, purity: >99%

Abstet tablets: - The Abstet tablet (Abiraterone acetate, 250 mg) was obtained from Vision marketing, New Delhi.

Chemicals and Reagents
Chemicals and reagents including ammonium acetate, sodium hydroxide, glacial acetic acid, hydrochloric acid, hydrogen peroxide are of AR grade and acetonitrile (HPLC grade) were obtained from Merck Laboratories Pvt Ltd., Mumbai. Methanol HPLC grade from Finar reagents, Ahmedabad.

HPLC Instrumentation and Conditions
Alliance WATERS 2695 separation module coupled with Waters 2487 Dual wavelength absorbance detector controlled by Empower software.

Chromatographic Conditions
Column: Acclaim C18 (50mm x 4.6 mm, 5µm)
Mobile phase: Acetonitrile: ammonium acetate (10mM), pH adjusted to 6.0 with glacial acetic acid.
Composition: 80:20
Sample temperature: 25°C
Column temperature: 25°C
Flow rate: 1 ml/min
Injection volume: 10 µl
Detection: 254 nm
Runtime: 10 minutes
Retention time: 6.00 ±0.5 minutes

Preparation of Reagents and Solutions
10 mM of ammonium acetate buffer (pH 6.0 ± 0.02):- 0.77 gram of ammonium acetate was dissolved in 1000mL of ultra-clear water and pH was adjusted to 6.0 ±0.02 with glacial acetic acid. The resulting solution was passed through 0.45µ filter paper and filtered using vacuum filtration and sonicated for about 15 minutes.

Mobile phase: 800 ml acetonitrile and 200 ml ammonium acetate buffer.

Diluent: 50:50 (water: methanol) v/v was used as diluent for all trials.

Primary stock solution: 10 mg Abiraterone acetate was dissolved in methanol in a 10 ml volumetric flask and the volume was made with methanol up to the mark to get a concentration of 1000 µg/ml.

Working stock solution: 1 ml from primary stock solution was pipette out in to a 10 ml volumetric flask and the volume was made with methanol up to the mark to get a concentration of 100 µg/ml.

Tablet solution: Powder equivalent to 250 mg was accurately weighed and transferred to a volumetric flask of 250 ml. The powder was dissolved in 100ml of the methanol. The flask was then sonicated and shaken for 30 minutes. It was then cooled to room temperature and the volume was made with diluent (50:50 water: methanol) up to the mark. 1ml of supernatant was pipette out into a 10 ml volumetric flask and the volume was made up with diluents to the mark. 1ml from the above 10 ml solution was pipette out into a 10 ml volumetric flask whose volume was made up with mobile phase to the mark and injected into the HPLC system.

Stress Testing
Acid Degradation
2.5 mg of Abiraterone acetate was weighed and 1mL methanol was added to dissolve the drug and the volume was made to 25 ml with 0.1 M hydrochloric acid, it was then transferred to a round bottom flask(RBF) and refluxed at 80 °C. The resulted concentration of the sample solution was 100 µg/ml. Aliquots were withdrawn initially (0 hr) and at different time points (30mins, 1,2,4 and 8 hr); neutralized to pH 7.0 with counter base 0.1 M sodium hydroxide and then diluted to 10 µg/ml with mobile phase and injected into the HPLC system [11-12].
The standard solution of 10 μg/ml was considered as 100 % and the percentage degradation of drug was calculated by area normalization method.

**Base Degradation**

2.5 mg of Abiraterone acetate was exactly weighed and 1mL methanol was added to dissolve the drug and the volume was made to 25 ml with 0.05 M sodium hydroxide, it was then kept aside at room temperature. The resulted concentration of the sample solution was 100 μg/ml. Aliquots were withdrawn initially (0 hr) and at different time points (30mins, 1,2,4 and 8 hr); neutralized to pH 7.0 with counter acid 0.05 M hydrochloric acid and then diluted with mobile phase to 10 μg/ml and injected into the HPLC system.

The standard solution of 10 μg/ml was considered as 100 % and the percentage degradation of drug was calculated by area normalization method.

**Oxidative Degradation**

2.5 mg of Abiraterone acetate was exactly weighed and 1 ml methanol was added to dissolve the drug and the volume was made to 25 ml with 10% v/v hydrogen peroxide, it was then kept aside at room temperature. The resulted concentration of the sample solution was 100 μg/ml. Aliquots were withdrawn initially (0 hr) and at different time points (30mins, 1, 2, 4 and 8 hr); diluted to 10 μg/ml with mobile phase and injected into the HPLC system.

The standard solution of 10 μg/ml was considered as 100 % and the percentage degradation of drug was calculated by area normalization method.

**Thermal Degradation**

Sufficient amount of Abiraterone acetate was placed in a Petri dish and kept in the hot air oven at 80 °C for 24 hours. Aliquots were withdrawn initially (0 hr) and at different time points (30mins, 1, 2, 4 and 8 hr); diluted to 10 μg/ml with mobile phase and injected into the HPLC system.

The standard solution of 10 μg/ml was considered as 100 % and the percentage degradation of drug was calculated by area normalization method.

**Photolytic Degradation**

**Preparation of Sample**

A standard solution of 10 μg/ml was prepared and kept inside the UV chamber. Aliquots were collected initially (0 hr) and at different time intervals like 1, 2, 3, 4 hrs and injected into the HPLC system.

The standard solution of 10 μg/ml was considered as 100 % and the percentage degradation of drug was calculated by area normalization method.

**Validation [13]**

**Specificity**

Interference from blank: It includes the interference from the blank i.e. mobile phase. Blank should not show any response (peak) at the retention time of the Abiraterone acetate peak.

Interference from impurities: It includes interference from the degradation product generated during stress testing. The drug peak should be homogenous and there should be no co-eluting peaks. Peak purity for drug peak should pass.Interference from excipients: The excipients from the tablet should not show any response (peak) at the retention time of the drug.

**Linearity**

A series of solutions were prepared at concentration levels as 125 ng/ml 250 ng/ml, 500 ng/ml, 1 mcg/ml, 5 mcg/ml, 10 mcg/ml, 20 mcg/ml, 40 mcg/ml and 60 mcg/ml. A 10μl volume from each concentration of solution were injected twice into the HPLC system. Chromatograms were recorded under optimized chromatographic conditions. A graph was plotted considering peak areas on Y-axis and concentration on X-axis. The linear equation, y-intercept, slope of regression line and regression constant ($r^2$) were calculated.

**Accuracy**

A series of solutions were prepared in triplicate by spiking the known standard concentrations of Abiraterone acetate in the range of 80-120% on the tablet solution and analyzed. The accuracy of method was provided at three different concentration levels at 8, 10 and 12 μg/ml of Abiraterone.
acetate standard. The percentage recoveries of three different concentrations were found to be within the range of 98 to 102 % as per ICH Q2R1 guidelines.

**Precision**

Repeatability or intra-day precision: The peak areas of 10 mcg/ml were analyzed on the same day by injecting it six times into the system. The chromatogram was recorded and RSD was calculated.

Intermediate or inter-day precision: The peak areas of 10 mcg/ml were analysed on two consecutive days by injecting it six times into the system. The chromatogram was recorded and RSD was calculated.

Different systems: Abiraterone acetate standard solution (10μg/mL) was analyzed six times on different equipment (WATERS and SHIMADZU HPLC system) under optimized chromatographic conditions and the RSD was calculated for the resultant peak area.

**Limit of Detection and Limit of Quantitation**

LOD and LOQ can be calculated based on the signal to noise ratio approach, visual evaluation and standard deviation of the response and slope of the calibration curve. The slope (S) is calculated from the equation of straight line in calibration curve of the analyte. The standard deviation (σ) is calculated based on its blank response or the y-intercepts of regression line.

**Robustness**

The robustness of a method is its ability to remain unaffected under changes in parameters. Robustness was carried out by altering the pH of mobile phase (±0.2 units), change in mobile phase composition (±5.0 units), change in detection wavelength (± 2nm), change in column oven temperature (±5°C) and change in flow rate (±0.1mL/min). The standard solution comprising of Abiraterone acetate (10μg/mL) was injected six times and the %RSD was calculated for the resultant area of the peak.

**System Suitability Parameter**

The chromatograms of 10 μg/ml were analyzed by injecting it six times into the system and the system suitability parameters like plate count, tailing factor, capacity factor and reproducibility were determined.

**Dissolution Testing [14]**

Formulation: Tablet  
Type: Immediate Release  
Strength: 250 mg  
Dissolution Apparatus: Type II (Paddle)  
Medium: 0.25% SLS in 56.5 mM phosphate buffer, pH 4.5  
Volume: 900 ml  
RPM: 50  
Temperature: 37.5 ºC

**Assay [15]**

Five tablets of abstet were selected randomly and their average weight was determined and the tablets were crushed in mortar and pestle until a fine powder was obtained. From this powder an amount equivalent to 250mg was weighed and dissolved in 100ml of methanol in 250ml of volumetric flask. The flask was then sonicated and shaken for 30 minutes. It was then cooled to room temperature and the volume was made up to the mark with diluent. (1000μg/ml). The solution was then diluted to 10μg/ml with mobile phase and injected into the HPLC system. The sample is prepared in triplicates.

\[
\text{% assay} = \frac{\text{Area of unknown} \times \text{Concentration of standard}}{\text{Area of standard} \times \text{Concentration of unknown}} \times 100
\]

**RESULTS AND DISCUSSION**

**Optimization of Chromatographic Conditions**

Type of Chromatography: Abiraterone acetate is having affinity towards non polar stationary phase (log P 5.12). Hence, reverse phase HPLC was selected for initial separation because of its simplicity, suitability and wide usage.

Selection of detection wavelength: From the UV scan 254nm was selected as wavelength for the detection and quantification of Abiraterone acetate.
Selection of mobile phase: Acetonitrile was used as an organic mobile phase because of lower UV cutoff, lower viscosity than methanol which resulted in decreased system back pressure. Various buffers like phosphate buffer and ammonium acetate buffer etc., were tried along with acetonitrile as mobile phase but finally ammonium acetate buffer found to produce clear peaks without any interferences and the tailing factor also reduced. To optimize the pH and buffer strength, trials were made and it was found that pH and buffer strength does not have much effect on the retention time of the drug so pH 6.0 and strength as 10 mM were selected. Trials were also made for the composition of mobile phase and 80:20 was optimized composition.

Selection of stationary phase and column oven temperature: Trials were made on different columns like genesis phenyl, BDS Hypersil, C8 BDS, Gracesmart RP 18, Grace Vydac and Acclaim C18; out of which Acclaim C18 was the optimized stationary phase. The optimized column temperature was 25°C.

Stability Indicating Assay Method

Acid degradation: Initially 0.1M hydrochloric acid at room temperature was used for the degradation of Abiraterone acetate but it was found that the drug was stable as there was no degradation. So, 0.1M hydrochloric acid under reflux condition at 80 °C was used for the degradation of Abiraterone acetate. The area of drug peak decreased and a corresponding degradation peak was observed at 2.051 minutes. The %degradation of drug after 30 minutes was found to be 54.80%. This indicated that the drug is hydrolyzed under acidic condition. The peak purity index (1.00) was more than single point threshold (0.983) which indicates that there was no overlapping of impurity peak and peak purity was passed.

Alkali degradation: Initially 0.05M sodium hydroxide at room temperature was used for the degradation of Abiraterone acetate. The area of drug peak decreased and a degradation peak was observed at 2.058 minutes. The %degradation of drug after 2 hours at room temperature was found to be 47.62%. This indicated that the drug is hydrolyzed under basic condition. The peak purity index (1.00) was more than single point threshold (0.980) which indicates that there was no overlapping of impurity peak and peak purity was passed.

Oxidative hydrolysis: Hydrogen peroxide 10% v/v at room temperature for 24hr was used for the degradation of Abiraterone acetate. The area of drug peak decreased, without corresponding rise in new peak. The %degradation of drug after 30 minutes was found to be 7.5%. This indicated that the drug is hydrolyzed oxidative condition. The peak purity index (0.999) was more than single point threshold (0.993) which indicates that there was no overlapping of impurity peak and peak purity was passed.

Thermal degradation: The drug degraded to an extent of 25% when kept at 80 °C inside the hot air oven. The peak purity index (1.00) was more than single point threshold (0.995) which indicates that there was no overlapping of impurity peak with the analyte peak and peak purity was passed.

Photolytic degradation

The drug degraded to an extent of 1.4% when kept inside the UV chamber for 3 hours. The peak purity index (1.00) was more than single point threshold (0.993) which indicates that there was no overlapping of impurity peak with the analyte peak and peak purity was passed.

The results are summarized in Table-1

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Type of degradation</th>
<th>Stress Conditions</th>
<th>Percentage degradation observed</th>
<th>Peak purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Acid hydrolysis</td>
<td>0.1 M Hydrochloric acid at 80°C reflux for 30 minutes</td>
<td>55</td>
<td>Passed</td>
</tr>
<tr>
<td>2</td>
<td>Alkali hydrolysis</td>
<td>0.05 M Sodium hydroxide at room temperature for 2 hours</td>
<td>48</td>
<td>Passed</td>
</tr>
<tr>
<td>3</td>
<td>Oxidative degradation</td>
<td>10% v/v Hydrogenperoxide at room temperature for 30</td>
<td>8</td>
<td>Passed</td>
</tr>
</tbody>
</table>
4 minutes
Thermal degradation
Inside hot air oven at 80°C for 24 hours
Passed

5 minutes
Photolytic degradation
Inside UV chamber for 3 hours
1.4 Passed

Validation

Specificity

No interference was observed in the blank and tablet solution at the retention time of the Abiraterone acetate. The peak purity of the Abiraterone acetate peak was found to be passing in all degradation conditions. This is shown in figure-2

![Chromatogram showing specificity](image)

Figure 2: Chromatogram showing specificity

Linearity

The calibration curve was made by plotting the concentration on X-axis against peak area on Y-axis. A series of Abiraterone acetate standard solution were prepared in the range of 0.125 to 60μg/mL. The correlation coefficient of the curve was found to be 0.999 with a regression equation of y=14771x + 2249. This is shown in figure-3

![Calibration curve of Abiraterone acetate](image)

Figure 3: Calibration curve of Abiraterone acetate
Accuracy

The mean recovery was found to be 100.52%. The results are shown in Table-2.

Table 2: Table for recovery studies

<table>
<thead>
<tr>
<th>Spiked level</th>
<th>Amount added (µg/ml)</th>
<th>Amount recovered (µg/ml)</th>
<th>Percentage recovery</th>
<th>Mean percentage recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>80%</td>
<td>18</td>
<td>18.03</td>
<td>100.21</td>
<td>100.04</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>17.83</td>
<td>99.09</td>
<td></td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>18.15</td>
<td>100.84</td>
<td></td>
</tr>
<tr>
<td>100%</td>
<td>20</td>
<td>20.18</td>
<td>100.99</td>
<td>100.93</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>20.23</td>
<td>101.18</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>20.12</td>
<td>100.64</td>
<td></td>
</tr>
<tr>
<td>120%</td>
<td>22</td>
<td>22.13</td>
<td>100.61</td>
<td>100.59</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>21.94</td>
<td>99.77</td>
<td></td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>22.30</td>
<td>101.39</td>
<td></td>
</tr>
</tbody>
</table>

Mean percentage recovery across all levels (n= 9) 100.52

Precision

The RSD of the below results was found to less than 1% and are shown in Table-3.

Table 3: Results of precision

<table>
<thead>
<tr>
<th>Type</th>
<th>Percentage RSD (Relative Standard Deviation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intra-day precision</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>0.27</td>
</tr>
<tr>
<td>Inter-day precision</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>0.28</td>
</tr>
<tr>
<td>Different system</td>
<td>0.47</td>
</tr>
</tbody>
</table>

LOD and LOD

The limit of Detection and limit of Quantification were found out to be 10 ng/ml and 50 ng/ml respectively.

Robustness

The RSD of the below results was found to be less than 1% and are shown in Table-4

Table 4: Results of robustness testing

<table>
<thead>
<tr>
<th>Type</th>
<th>Condition</th>
<th>Percentage RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change in pH</td>
<td>5.8</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td>6.2</td>
<td>0.12</td>
</tr>
<tr>
<td>Change in wavelength</td>
<td>252 nm</td>
<td>0.62</td>
</tr>
<tr>
<td></td>
<td>256 nm</td>
<td>0.34</td>
</tr>
<tr>
<td>Change in flow rate</td>
<td>0.9 ml/min</td>
<td>0.83</td>
</tr>
<tr>
<td></td>
<td>1.1 ml/min</td>
<td>0.25</td>
</tr>
<tr>
<td>Change in mobile phase</td>
<td>75:25</td>
<td>0.39</td>
</tr>
<tr>
<td>composition</td>
<td>85:15</td>
<td>0.65</td>
</tr>
<tr>
<td>Change in column oven</td>
<td>20 °C</td>
<td>0.39</td>
</tr>
<tr>
<td>temperature</td>
<td>30°C</td>
<td>0.28</td>
</tr>
</tbody>
</table>

System Suitability Parameter

The system suitability parameter passes for Abiraterone acetate and the results are shown in Table-5.

Table 5: Summary of system suitability testing

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Observation</th>
<th>Acceptance criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>% RSD of retention time</td>
<td>0.15</td>
<td>Not more than 1.0</td>
</tr>
<tr>
<td>of 6 injections</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% RSD of area of 6</td>
<td>0.40</td>
<td>Not more than 1.0</td>
</tr>
<tr>
<td>injections</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plate count</td>
<td>4738</td>
<td>More than 2000</td>
</tr>
</tbody>
</table>
Tailing Factor | 1.12 | Not more than 2.0
---|---|---
Capacity Factor | 62.14 | More than 2.0

**Dissolution Testing**

The percent release at 30 minutes was found out to be 85.29 and are shown in Figure-4

![Figure 4: Release profile of Abstet tablet](image)

**Assay**

Average assay value was found to be 101.66 and 101.70% from the calibration curve and formula respectively and the results are shown in Table-6.

**Table 6: Results of assay of Abstet tablets**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Sample</th>
<th>Peak area</th>
<th>% assay from calibration curve</th>
<th>% assay from formula</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Standard</td>
<td>Tablets</td>
<td>Tablets</td>
</tr>
<tr>
<td>1</td>
<td>10 µg/ml solution</td>
<td>149872</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>A</td>
<td>152089</td>
<td>101.4</td>
<td>101.4</td>
</tr>
<tr>
<td>2</td>
<td>B</td>
<td>152899</td>
<td>101.9</td>
<td>102.01</td>
</tr>
<tr>
<td>3</td>
<td>C</td>
<td>152563</td>
<td>101.7</td>
<td>101.7</td>
</tr>
<tr>
<td>Mean</td>
<td>-</td>
<td>-</td>
<td>101.66</td>
<td>101.70</td>
</tr>
</tbody>
</table>

**CONCLUSION**

An easy, rapid and efficient reverse-phase HPLC method was developed for quantitative estimation of Abiraterone acetate in pharmaceutical dosage forms. The method was validated as per ICH Q2(R1) guideline. A precise, accurate, linear, robust and rugged method was found during validation. The above method for Abiraterone acetate was found to be selective and stability indicating under different stress conditions. It was applied for the dissolution testing of the marketed formulation, as the results were successful it could be efficiently used for the routine quality control purpose.

**ACKNOWLEDGEMENTS**

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