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ORIGINAL ARTICLE

Development and validation of RP-HPLC Method for quantitative analysis of Abiraterone acetate in bulk drug

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ABSTRACT
This work was carried out with an aim to develop a simple, accurate, robust, precise and reproducible reverse phase High performance liquid chromatographic method for Abiraterone acetate in bulk drug. In the current developed method, SB C-18, 100x4.6 mm, 2.7 um, was used as stationary phase and eluent was 10 mM Ammonium formate in water: acetonitrile (10:90 v/v). Method validation and different studied parameters such as linearity range, system suitability, precision, accuracy, limit of detection and limit of quantitation were performed as per ICH guidelines. Forced degradation studies were conducted by drug exposure to various stress conditions like UV, water, acid, alkali, & oxidative. The method was found to be linear within the range 10-200 µg/ml with limit of detection 0.050321 ng/ml & limit of quantitation 0.150962 ng/ml whereas percent recovery was found to be 99.98% -100.77%. This method can be utilized for the routine quantitative analysis of Abiraterone acetate in bulk drugs.
Keywords: *Abiraterone acetate, prostate cancer, RP-HPLC, ICH, precision*

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INTRODUCTION

Abiraterone is steroid progesterone derivative which is FDA approved drug for prostate cancer treatment [1]. Abiraterone acetate is oral prodrug of Abiraterone. It is particularly indicated for treatment of metastatic castration and in metastatic high risk castration sensitive prostate cancer in combination with prednisone [1,2.] . It is reported as selective inhibitor of enzymes cytochrome P450 17x-hydrolyase and CYP17 which are involved in testosterone production. Drug is adrenal inhibitor therefore recommended as hormonal therapy in advanced breast and prostate cancer [2]. It is marketed under the brand name Zytiga. Taking into account high utility of drug, this research presents highly reproducible and sensitive RP-HPLC method for Abiraterone acetate.

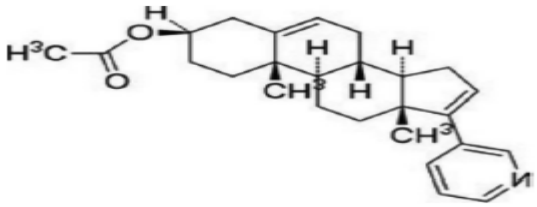

Fig.1. Abiraterone acetate structure

MATERIAL AND METHODS

Chemicals:

Abiraterone acetate received as gift sample from Glenmark Pharmaceuticals. All used chemicals were of AR grade. HPLC grade solvents were used and obtained from MERCK Ltd.

Instrumentation:
Agilent 1260 Series system was used which consist of quaternary pump, auto sampler unit, online degasser, column oven and PDA detector. Entire data was processed and monitored by using LC SOLUTION software.

Optimized chromatographic conditions:
SB C-18, 100x4.6 mm, 2.7 um, Agilent (Sr. USCEW04452) was adsorbent. The eluent was 10 mM Ammonium formate in water: acetonitrile (10:90 v/v). The optimized flow rate was 1ml/minute with injection volume of 20µl with a run time of 10 minutes. The chromatograms were recorded at wavelength 254 nm and temperature of column was set at 40°C during the study period. Different samples prepared as well as mobile phase was filtered using 0.22µm filter and degassed by ultrasonication prior to use.

Preparation of standard stock solution:
10mg of Abiraterone acetate was taken to a 10 ml volumetric flask containing methanol. Solutions of different concentration (10- 200µg/ml) were prepared by dilution of stock solution. Calibration curve was prepared from peak area and concentrations [3].

Method validation:
The method was validated as per prescribed ICH guidelines [3-5].

Linearity range:
Linearity range was determined by preparing standard solution of different concentrations. The linearity range was found to be 10-200µg/ml [6].

From stock solution of 1000µg/ml, different concentrations 10, 20, 30, 50, 100, 150 and 200 µg/ml were prepared. 20µl of each solution was injected into the column using auto sampler unit and the chromatograms were recorded. A graph of area under curve vs. concentration was then plotted to obtain calibration curve. From the calibration curve, equation, slope, intercept and regression coefficient (R^2) were determined. The general equation of the line is as per following:

$Y= mX+c$

In which,

Y- area under chromatogram

X- concentration of unknown

m- calibration curve slope

c – intercept[7].

Precision:
Precision indicates reproducibility of test results for developed analytical method. Precision of developed method was evaluated for inter and intraday variation[8]. The method precision was evaluated for three times on same replicates of 20 µg/ml concentration. Sets of three replicates of above concentration were analyzed on same day for intraday and on multiple days for inter day precision precision [7].

Accuracy:
In a preweighed solution of drug, a known quantity of drug was placed & chromatogram was recorded as per the optimized chromatographic conditions. The percentage recovery was then determined by fitting sample area in calibration curve equation [8].

Limit of detection (LOD) & limit of quantitation (LOQ):
These determined based on the response standard deviation and calibration curve slope as per ICH guidelines Q2 (R1) [9].

Robustness:
Robustness was evaluated by injecting prepared solutions as per method and by changing flow rate and wavelength [10].

Ruggedness:
The analysis of drug was performed by different analysts with same chromatographic conditions..

System suitability:
System suitability was determined by six replicate injections of 10 µg/ml concentration. Retention time, number of theoretical plates and tailing factor were determined [12].

Forced degradation studies:
The drug was exposed to various stress conditions which was analyzed with optimized chromatographic conditions to resolve the drug from potential degradation products [13].

The chromatograms were studied for peak area and appearance of secondary peaks. The decrease in the peak area and presence of secondary peaks shown degradation due to stress conditions. The percent degradation was calculated by using following equation [14].

$$\text{Percent degradation} = \frac{\text{Peak area of stressed drug sample}}{\text{Peak area of unstressed drug sample}} \times 100$$

Acid degradation studies:

Abiraterone acetate acid degradation was done by refluxing of 0.125 mg of Abiraterone acetate with 5 ml of 0.1N HCl in 100 ml flask. The flask was kept 80˚C for 2 hrs. It was subjected to optimized HPLC analysis [1, 15, 16].

Alkali degradation studies:

Abiraterone acetate alkali degradation was done by refluxing of 0.125 mg of Abiraterone acetate with 5 ml of 0.1N NaOH in 100 ml volumetric flasks. The flask was kept at 80˚C for 2 hrs. It was subjected to optimized HPLC analysis [1, 15, 16].

Oxidative degradation studies:

About 0.125 mg of Abiraterone acetate was dispersed in 5ml 10% H_2O_2. It was kept 80˚C for 2 hrs and analyzed by the optimized HPLC method [1, 15].

Water degradation studies:

0.125 mg of Abiraterone acetate was dispersed in 5 ml water. It was kept at 80˚C for 2 hrs and analyzed by the optimized HPLC method [15, 17].

UV degradation studies:

100 mg of pure drug was taken in petridish. It was then kept under ultraviolet chamber for 2 hours by maintaining 30 cm distance. Concentration of 12.5µg/ml was processed by serial dilution. The solution was filtered using 0.22 µm filter paper and analyzed by optimized chromatographic conditions [18].

RESULTS AND DISCUSSION

Screening of suitable chromatographic conditions:

Determination of appropriate column and mobile phase consist of mixture of 10 mM Ammonium formate in water: Acetonitrile. This mobile phase was screened with varying 10 to 90 % v/v organic phase composition and 0.8 to 1.2 ml/min flow rate on C8 and C18 columns. Flow rate selected for study was set at 1 ml/min as change in flow rate did not gave satisfactory results.

Column and mobile phase selection:

As per screening studies, C18 column and 10 mM Ammonium formate in water: acetonitrile (10:90 v/v) was selected for the analysis of Abiraterone acetate. The developed chromatographic conditions achieved good separation of Abiraterone acetate with 5.37 min retention time.

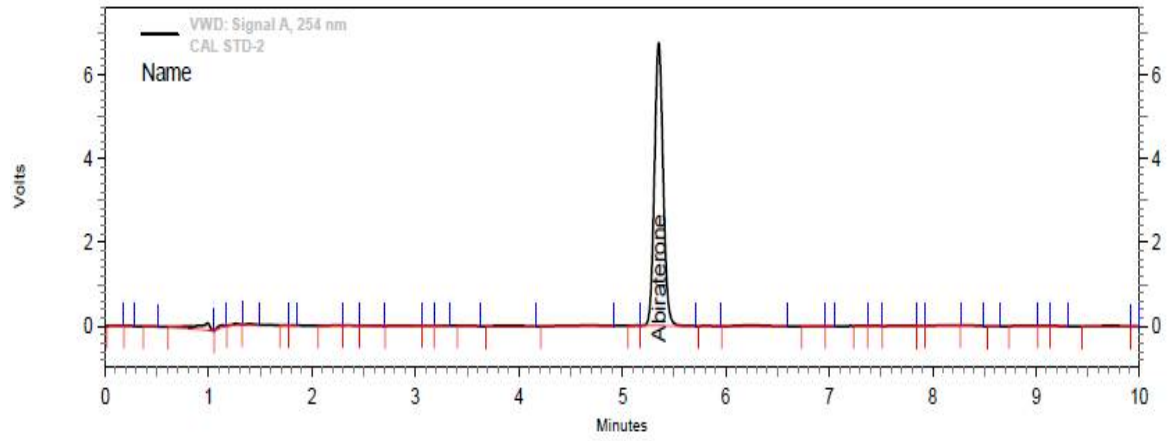

Fig 2. A typical chromatogram of Abiraterone acetate

Linearity & range:

The developed method was linear in 20µg/ml-200µg/ml range with a equation y=31847x+66834 and (R^2) of 0.996.

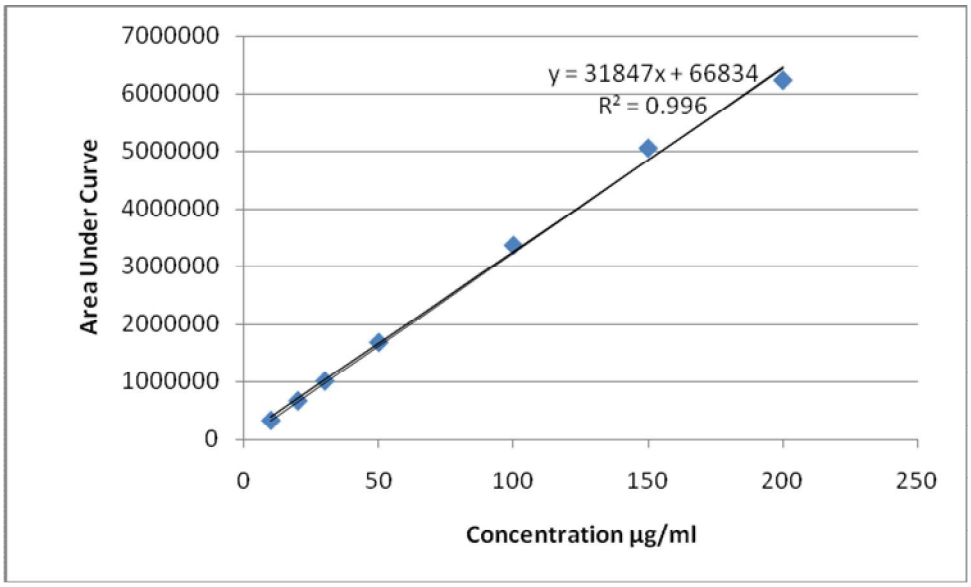

Fig 3. Calibration curve of Abiraterone aceatate

Precision:

The percent relative standard deviation for intra and inter day studies were less than 2 %, which confirms precision of method. Precision of developed method is given in Table1.

Table.1.Precision of proposed HPLC method

Con. (µg/ml)	Mean concentration		Standard Deviation		Standard Error		Co-efficient of Variation		% Co-efficient of Variation	
	Intra day	Inter day	Intra day	Inter day	Intra day	Inter day	Intra day	Inter day	Intra day	Inter day
15	14.7067	14.7068	0.063986	0.180084	0.036942	0.026878	0.004351	0.012245	0.4351	1.224492
95	94.1836	102.609	0.18742	10.98381	0.108207	1.639375	0.001149	0.107045	0.1149	10.70453
195	180.8894	178.5561	1.301416	2.975135	1.945695	0.751373	0.007195	0.016662	0.7195	1.666219

Accuracy:

Accuracy should be determined across the specified range of 98-102 %. The % accuracy was found within the specified limits.

Limit of detection and Limit of Quantitation:

The limit of detection and limit of quantitation values were 0.050321 and 0.150962 ng/ml respectively.

Robustness:

The results indicates no significant changes occur with changing the flow rate and wavelength. This indicates that, the developed method found to be robust.

Ruggedness

The results were not affected though performed by different analysts. This proves d ruggedness of developed method.

System suitability study:

For standard solutions, retention time, number of theoretical plates and peak area were determined. The values for retention time, number of theoretical plates and peak area were found to be 5.35 min, 332984 and 19085 respectively which were within acceptable limits.

Forced degradation studies:

The Abiraterone acetate was exposed to stress conditions to study drug degradation that may occur during storage or after drug administration into the body. This accelerated stability studies helps in determining real time or long term stability studies of drugs. The various degradation parameters studied were acid and basic hydrolysis, ultraviolet degradation, water and oxidative degradation. Abiraterone acetate was stable when exposed to UV light which is indicated by less degradation. The result of forced degradation studies are depicted in the following Table 2.

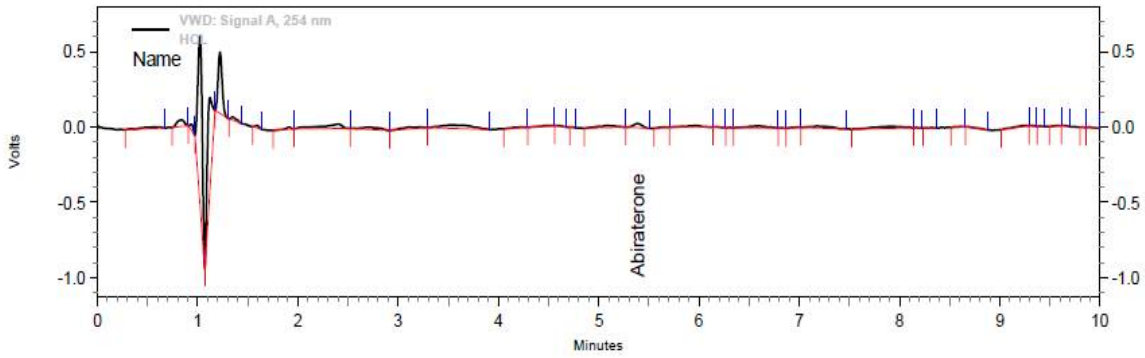

Fig 4. HCL mediated degradation

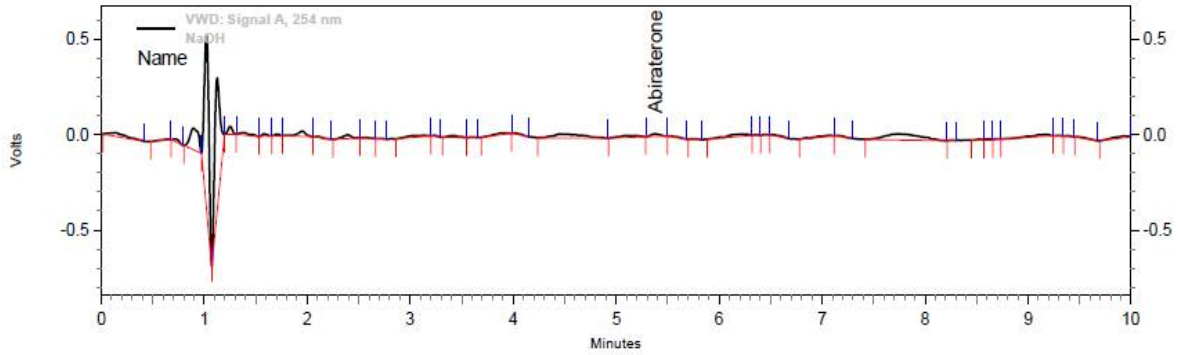

Fig.5. NaOH mediated degradation

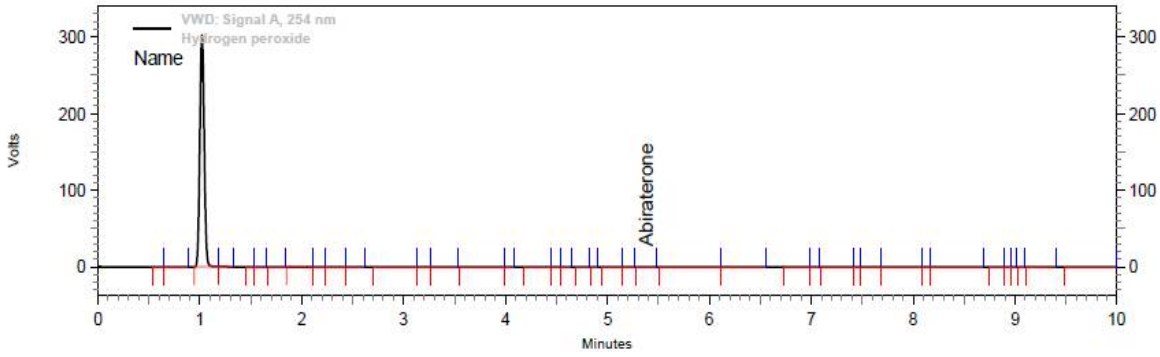

Fig.6. Hydrogen peroxide mediated degradation

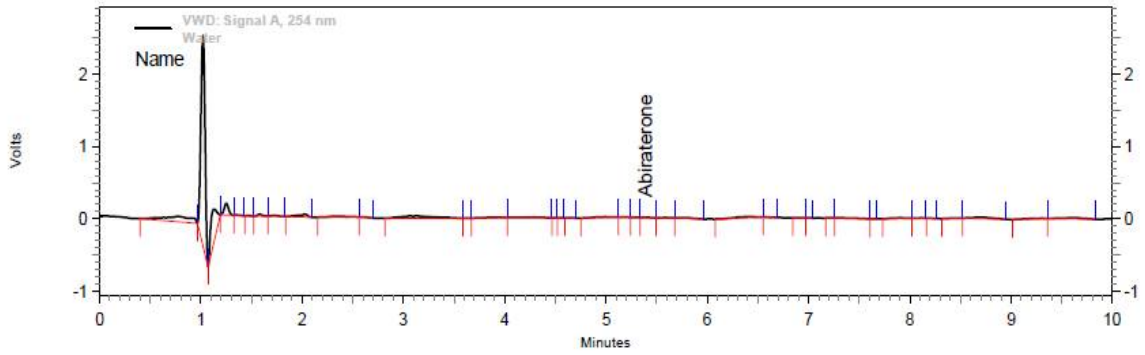

Fig.7.Water mediated degradation

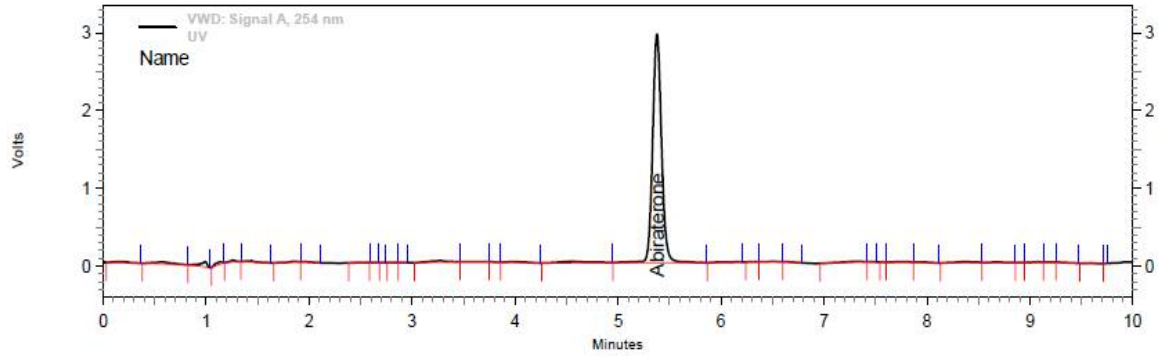

Fig.8. Degradation under UV light

Table.2.Forced degradation of Abiraterone acetate

Degradation parameter	% drug obtained	% degradation
HCL	3.08	96.92
NaOH	2.784	97.216
H_2O_2	2.784	97.216
Water	2.584	97.416
UV	67.024	32.976

CONCLUSION

In this study, a RP-HPLC method has been developed for quantitative evaluation of Abiraterone acetate in active pharmaceutical ingredient. This method has been validated for different parameters as per ICH guidelines. The drug was exposed to various stress conditions such as ultra violet, water, acid, alkali & oxidation to observe the rate and extent of drug degradation that may occur during storage or after drug administration into the body. The optimized method was found to comply as per ICH guidelines. This method can be used in future for the quantitative analysis of Abiraterone acetate in bulk drug. This method is economic due to less usage of organic content in the mobile phase and hence can be recommended for the routine quantitative analysis in the pharmaceutical industries.

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CONFLICT OF INTEREST

The authors do not have any conflict of interest.

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