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Development and Validation of RP-HPLC Method For Quantitative Analysis Of Abiraterone In Pure and Pharmaceutical Dosage Form

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ABSTRACT

A simple, Precised, Accurate method was developed for the estimation of Abiraterone by RP-HPLC technique. Chromatographic conditions used are stationary phase Azilent C18 (150mm x 4.6 mm, 5µ)Mobile phase 0.01%KH_2PO_4:Acetonitrile in the ratio of 60:40 and flow rate was maintained at 1.0 ml/min, detection wave length was 235 nm, column temperature was set to 30^oC and diluent was mobile phase Conditions were finalized as optimized method. System suitability parameters were studied by injecting the standard six times and results were well under the acceptance criteria. Linearity study was carried out between 25% to150 % levels, R^2 value was found to be as 0.999. Precision was found to be 0.7 for repeatability and 0.2for intermediate precision. LOD and LOQ are 1.629µg/ml and 4.937µg/ml respectively. By using above method assay of marketed formulation was carried out 100.81% was present. Degradation studies of Abiraterone were done, in all conditions purity threshold was more than purity angle and within the acceptable range .Full length method was not performed ; if it is done this method can be used for routine analysis of Abiraterone

Keywords: HPLC , Abiraterone , Method development , ICH Guidelines

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INTRODUCTION

Abiraterone is a derivative of steroid progesterone approved by the FDA for the treatment of prostate cancer. [1]The approval was accelerated based on progression-free survival, therefore confirmatory trials by the sponsor to demonstrate clinical efficacy in prostate cancer treatment are in progress of being conducted.[2-4] Abiraterone is marketed by under the brand name Zytiga. After an expedited six-month review, Abiraterone was approved by the U.S .Food and Drug Administration(FDA) in April 2011 .It has received FDA (28 APRIL 2011), EMA (23 September 2011), MHRA (5 September 2011) and TGA 1(1 March 2012) approval for this indication . In the present study, a new RP-HPLC method was developed which shown high reproducibility and sensitivity. The developed method was validated as per ICH guidelines. [5-11]

MATERIALS AND METHOD

Standards and Chemical Used

Abiraterone was gift sample for Noverties company, Hyderabad. All the chemicals Acetonitrile HPLC Grade, HPLC grade Water.

Instrumentation

HPLC instrument used was of WATERS HPLC 2965 SYSTEM with Auto Injector and PDA Detector. Software used is Empower .UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz was be used for measuring absorbance for Abiraterone solutions.

Preparation of Mobile phase:

Into a 1000ml cleaned volumetric flask, HPLC grade, acetonitrile 500ml and Potassium dihydrogen phosphate 500ml (0.01N) which are filtered through 0.25mm membrane filters by vacuum filtration were slowly added, mixed well and sonicated upto 20min. Cool the above solution. This solution is again sonicated to 10min. Cool the solution to room temperature and use for chromatography method

Preparation of Standard stock solutions:

Accurately weighed 50mg of Abiraterone transferred 50ml of volumetric flask, and 3/4 th of diluents was added and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution (1000µg/ml of Abiraterone)

Preparation of Standard working solutions (100% solution):

1ml of Abiraterone each stock solution was pipette out and taken into a 10ml volumetric flask and made up with diluents. (100µg/ml of Abiraterone)

Preparation of Sample stock solutions:

5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100 ml volumetric flask, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filter.(2500 µg/ml of Abiraterone)

Preparation of Sample working solutions (100% solution):

0.4ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. 100µg/ml of Abiraterone)

RP-HPLC METHOD DEVELOPMENT

Based on nature and solubility characteristics of Abiraterone, reverse phase mode of HPLC was selected for chromatography. Among different RP-HPLC stationary phases tried, C_{18} column was found to be optimum.

In order to get sharp peak with base line separation from interfering peaks carried out a number of experiments by varying the composition of solvents and mobile phase flow rate. To have an ideal separation of the drug under isocratic conditions, mixtures of solvents like methanol, water and acetonitrile with or without different buffers in different combinations were tested as mobile phase. A mixture of 0.01N KH_2PO4 : Acetonitrile (50:50) (v/v) was proved to be the most suitable of all the combinations, since the chromatographic peak obtained was better defined and resolved and almost free from tailing . This chromatographic conditions for the estimation of Abiraterone was discussed in table.

Table 1. Optimized chromatographic conditions for estimation of Abiraterone

Parameter	Condition
Mobile phase	0.01N KH_2PO_4: Acetonitrile (50:50) (V/V)
Pump mode	Isocratic
Diluents	Mobile phase
s	ODC C18 Column (150 x 4.6 mm, 5µ)
Column Temp	30^0C
Wavelength	230nm
Injection Volume	10µL
Flow rate	1.0 ml/min
Run time	10min

RESULTS AND DISCUSSION

Analysis of Formulation

The sample solution was injected and a chromatogram was recorded. The injections were repeated five times and the peak areas were recorded. The amount of drug present in the

pharmaceutical formulation was calculated using standard calibration curve (concentration in µg/ml was taken on X –axis and average peak area on Y –axis). A representative chromatogram has been given in Figure. 1

Validation of The Proposed Method

As an integral part of analytical method development is validation. The proposed method was validated as per ICH guidelines.

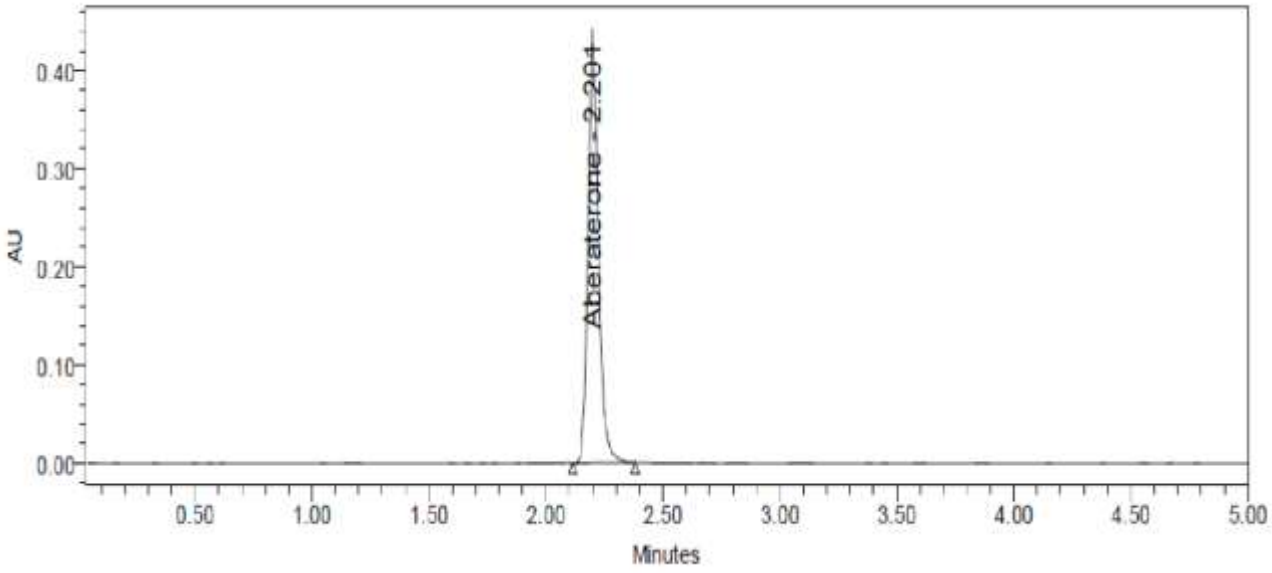

Figure 1: Chromatogram of Abiraterone

Linearity

To demonstrate the linearity of assay method, inject 5 standard solutions with concentrations of about 25 ppm to 150ppm of Abiraterone. Plot a graph to concentration versus peak area. Slope obtained was y = 14912x + 5759 and Correlation Co-efficient was found to be 0.999 and Linearity plot was shown in table

Table 2 Linearity Results of Abiraterone

Linearity Level(%)	Concentration (ppm)	Peak Area
0	0	0
25	25	375311
50	50	741258
75	75	1152591
100	100	1498279
125	125	1872841
150	150	2228723

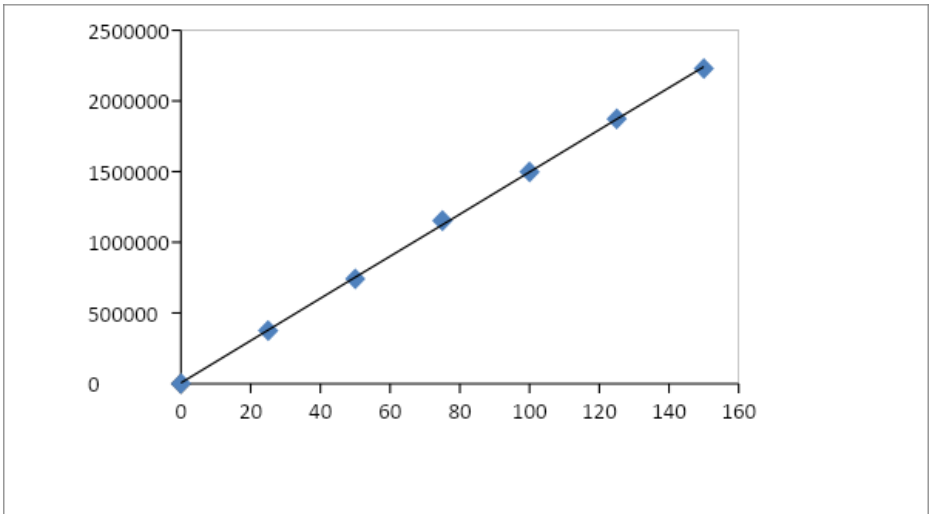

Figure 2 Linearity Plot

Precision:

Repeatability:

Six working sample solutions of 20ppm are injected and the % Amount found was calculated and %RSD was found to be 0.2

Intermediate precision:

Five working sample solutions of 20ppm are injected on the next day of the preparation of samples and the % Amount found was calculated and %RSD was found to be 0.3

Ruggedness

The ruggedness of the method was determined by carrying out the experiment on different instruments like Shimadzu HPLC 2965 SYSTEM, Azlient HPLC .By different operators using different columns of similar type like Hypersil C_{18} Hichron C_{18}. It was observed that there were no marked changes in the chromatograms, which demon started that the RP-HPLC method developed, is ruggedness.

Limit of Detection and Limit of Quantification

A Calibration curve was prepared using concentrations in the range of 5-30 µg/ml (expected detection limit range). The standard deviation of Y-intercepts of regression line was determined and kept in following equation for the determination of Detection limit and Quantitation limit. The results were reported in table 3.

$$\text{Limit of detection} = \frac{\sigma \times 3.3}{S}$$

$$\text{Limit of quantification} = \frac{\sigma \times 10}{S}$$

Where,

σ = the standard deviation of the response.

S = the slope of the calibration curve

Table 3. Limit of Detection and Limit of Quantification for Abiraterone

Parameter	Values
Limit of Quantification	0.151µg/ml
Limit of Detection	0.050 µg/ml

Accuracy: The accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed sample solution Three Concentrations of 50%, 100%, 150% are Injected in a triplicate manner and %Recovery was calculated as 100.15%. And chromatograms were shown in fig 3 Recovery ranging from 99.12 to 101.04% were obtained by the proposed method.

Table 3 Accuracy data

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	10	10.104	101.04	100.15%
	10	10.023	100.23	
	10	10.040	100.40	
100%	20	20.181	100.90	
	20	20.057	100.28	
	20	20.005	100.02	
150%	30	30.039	100.13	
	30	29.771	99.24	
	30	29.737	99.12	

Robustness: Small Deliberate change in the method is made like Flow minus, flow plus, Mobile phase minus, Mobile phase plus, Temperature minus, Temperature Plus. %RSD of the above conditions are calculated

Table 4 Robustness Data

Parameter	%RSD
Flow Minus	1.6
Flow Plus	0.6
Mobile phase Minus	0.9
Mobile phase Plus	1.0
Temperature minus	0.8
Temperature plus	0.5

System Suitability

A Standard solution of Abiraterone working standard was prepared as per procedure and was injected five times into the HPLC system. The system suitability parameters were evaluated from standard Chromatograms obtained by calculating the % RSD of retention time, tailing factor, theoretical plates and peak areas from five replicate injections are within range and Results were shown in table 5

CONCLUSION

A convenient, rapid, accurate, precise RP-HPLC method has been developed for estimation of Abiraterone. The proposed method followed the ICH guidelines .The proposed method can be used for the routine analysis of Abiraterones in bulk preparations of the drug and in pharmaceutical dosage forms without interference of excipients.

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