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Analytical Method Development and Validation for the Estimation of Abiraterone and its Impurity in Pharmaceutical Formulation By RP-HPLC

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ABSTRACT

A simple, specific and accurate reverse phase high performance liquid chromatographic method was developed for the determination of Abiraterone and its impurity in pharmaceutical dosage form. The column used was Discovery 250 mm × 4.6 mm, 5m., with mobile phase containing buffer and acetonitrile in the ratio of 50: 50 v/v, the flow rate was 1.0 ml/ min and eluent was monitored at 235nm. The retention time for Abiraterone was 4.372 and for impurity it is 3.182. The proposed method was validated and successfully applied to the estimation of Abiraterone and impurity in formulations.

Keywords: RP-HPLC, Validation, Abiraterone, Impurity.

INTRODUCTION

Abiraterone is a derivative of steroidal progesterone with hormone refractory prostate cancer. Abiraterone is administered as a salt prodrug because of higher bioavailability and less susceptible to hydrolysis than Abiraterone itself. Abiraterone is marketed under the trade 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 March 2012) approval for this indication. Abiraterone, the active metabolite of abiraterone, inhibits

CYP17A1, which manifests as two enzymes, 17α-hydroxylase (IC50 = 2.5 nM) and 17,20-lyase (IC50 = 15 nm) (six-fold more selective for inhibition of 17α-hydroxylase over 17,20-lyase), that are expressed in testicular, adrenal, and prostatic tumor tissues. Abiraterone also acts as an antagonist of the androgen receptor (AR) (to some extent) and as an inhibitor of the enzymes 3β-hydroxysteroid dehydrogenase, CYP11B1 (steroid 11β-hydroxylase), and other CYP450s (e.g., CYP1A2, CYP2C9, and CYP3A4). Abiraterone is able to reduce serum testosterone levels to less than 1 ng/dL (i.e., undetectable), and decreases the weights of the prostate gland, seminal vesicles, and testes, in accordance with its anti-androgen action.

Literature survey revealed that only few analytical methods -high performance liquid chromatography (HPLC) method have been reported. Hence, a new sensitive and efficient HPLC method was developed and validated for the assay of the drug in injection. The structure of Abiraterone is shown in Figure 1.

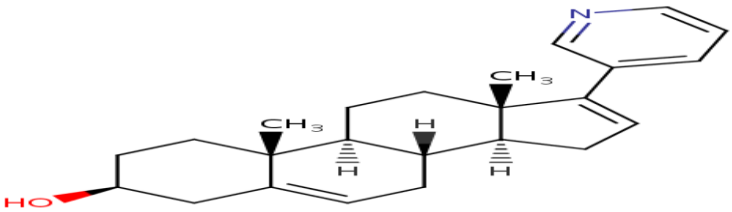


Figure 1: Structure of Abiraterone.

Abiraterone impurity is chemically known as [(3S,8R,9S,10R,13S,14S)-10,13-dimethyl-17-pyridine-3-yl-2,3,4,7,8,9,11,12,14,15-decahydro-1H-cyclopenta[a]phenanthren-3-yl]. Structure of impurity in Figure 2.

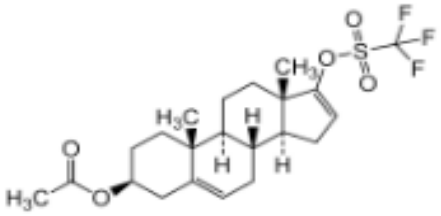


Figure 2: Structure of impurity.

Regulatory requirements for the identification, qualification, and control of Impurities in drug substances and their formulated products are defined, through the International Conference on Harmonization (ICH) [1,2]. Few analytical methods have been

reported in the literature for estimation of Abiraterone [3-7]. The aim of current study was to develop and validate HPLC method for the simultaneous determination of Abiraterone and its impurity.

MATERIALS AND METHODS

Chemicals and Reagents

Abiraterone pure analytical sample was obtained as a gift sample from Nishka Labs, Hyderabad. All chemicals and reagents used were of AR grade and all the solvents used were of HPLC grade. Tablets were procured from the local pharmacy labelled to contain Abiraterone equivalent to 100 mg.

Instrumentation

HPLC system used was Waters 2695 system. UV Visible Spectrophotometer of Labindia TG1800. Ultra sonicator of fast clean and weighing balance of Denver. A chromatographic column Discovery (250 × 4.6 mm, 5µm) was used for separation.

Chromatographic conditions

Chromatographic separation carried using buffer and acetonitrile in the ratio of 50:50 v/v as the mobile phase which gave good resolution and acceptable peak parameters. Separation was carried out at flow rate of 1 ml/min and detection at 235 nm. The peak purity was checked with the UV-Visible spectrophotometer detector. The sample injection volume was 10µl.

Preparation of mobile phase

0.1% OPA was prepared by 1 ml of ortho phosphoric acid in a 1000 ml of volumetric flask and about 900 ml of milli-Q water added and degas to sonicate and finally make up the volume with water. Mobile phase was prepared by mixing buffer and acetonitrile in the ratio of 50: 50 v/v.

Preparation of solution

Standard solution

1 mg of Abiraterone drug was weighed accurately and transferred to 10 ml volumetric flask and dissolved in a small amount of diluent. The solution is sonicated for 5min and the solution was made up with diluent to 10 ml to achive 100 ppm solution. 0.1 ml of the above solution was transferred to another 10 ml volumetric flask and was made up with the diluent to give 1 ppm solution.

Linearity

Preparation of stock solution

1 mg of Abiraterone and 1 mg of Impurity are weighed accurately and transferred into two 10 ml volumetric flasks which are labled as 'Abiraterone' and 'Impurity' respectively and are made up with the diluent. This gives the 400 ppm of Abiraterone and Impurity each stock solution.

Preparation of 25% level solution

0.25 ml of sample from the stock solution 'Abiraterone' and 0.25 ml of sample from the stock solution 'Impurity' are transferred to a 25 ml volumetric flask and was makeup with the diluent. This gives the solution containing both Abiraterone and Impurity with 0.25 ppm concentration

Preparation of 50% level solution

0.5 ml of sample from the stock solution 'Abiraterone' and 0.5 ml of sample from the stock solution 'Impurity' are transferred to a 25 ml volumetric flask and was makeup with the diluent. This gives the solution containing both Abiraterone and Impurity with 0.5 ppm concentration

Preparation of 75% level solution

0.75 ml of sample from the stock solution 'Abiraterone' and 0.75 ml of sample from the stock solution 'Impurity' are transferred to a 25 ml volumetric flask and was makeup with the diluent. This gives the solution containing both Abiraterone and Impurity with 0.75 ppm concentration.

Preparation of 100% level solution

1 ml of sample from the stock solution 'Abiraterone' and 1 ml of sample from the stock solution 'Impurity' are transferred to a 25 ml volumetric flask and was makeup with the diluent. This gives the solution containing both Abiraterone and Impurity with 1 ppm concentration.

Preparation of 125% level solution

1.25 ml of sample from the stock solution 'Abiraterone' and 1.25 ml of sample from the stock solution 'Impurity' are transferred to a 25 ml volumetric flask and was makeup with the diluent. This gives the solution containing both Abiraterone and Impurity with 1.25 ppm concentration.

Preparation of 150% level solution

1.50 ml of sample from the stock solution 'Abiraterone' and 1.50 ml of sample from the stock solution 'Impurity' are transferred to a 25 ml volumetric flask and was makeup with the diluent. This gives the solution containing both Abiraterone and Impurity with 1.5 ppm concentration.

Accuracy

Preparation of impurity stock solution

1 mg of Impurity is accurately weighed and transferred to a 10 ml volumetric flask and was makeup with the diluent gives 100 ppm solution of impurity.

50% spiking

Tablet powder equivalent to 10 mg of Abiraterone is weighed and transferred to a 10 ml volumetric flask labelled 50% spiked. 0.05 ml of solution from impurity stock is transferred to the volumetric flask labelled 50% spiked and makeup to 10 ml with diluent.

100% spiking

Tablet powder equivalent to 10 mg of Abiraterone is weighed and transferred to a 1 ml volumetric flask labelled 100% spiked. 0.1 ml of solution from impurity stock is transferred to the volumetric flask labelled 100% spiked and makeup to 10 ml with diluent.

150% spiking

Tablet powder equivalent to 10 mg of Abiraterone is weighed and transferred to a 10 ml volumetric flask labelled 150% spiked. 0.15 ml of solution from impurity stock is transferred to the volumetric flask labelled 150% spiked and makeup to 10 ml with diluent.

$$Amount\ added\ \left(\%\ w/w\right) \frac{=\ Imp\ wt\ Dil-2\ Dil-4\ spl.dil\ imp\ potency\ Avg.wt}{Dil-1\ Dil-3\ Dil-5\ spl\ wt\times 100L}\times 100$$

$$Amount\ found\ \left(\%\ w/w\right) = \frac{Imp\ area\ std.wt\ Dil-2\ Dil-4\ spl.\ Dil\ std\ potency\ Avg.wt}{Avgstd\ area\ Dil-1\ Dil-3\ Dil-5\ spl.wt\times 100\ L\ RRF}\times 100$$

$$Amount\ recoverd\left(\%w/w\right)\ =\ Amount\ found-Amount\ present\ in\ controlled\ sample$$

$$\%\ Recovery\ =\ \frac{Amount\ recovered}{Amount\ added}\times 100$$

Method precision

Preparation of impurity stock solution

1 mg of impurity is accurately weighed and transferred to a 10 ml volumetric flask and was makeup with the diluent gives 100 ppm solution of impurity.

Spiking impurity

Tablet powder equivalent to 10 mg of Abiraterone is weighed and transferred to a 10 ml volumetric flask labelled precision spiked. 0.1 ml of solution from impurity stock is transferred to the volumetric flask labelled precision spiked and makeup to 10 ml with diluent.

$$\%\ w/w\ = \frac{Imp\ area\ Stdwt\ 5\times 1\ spl.dil\ Potency\times 100\ Avg.wt}{Avg\ .std\ \ area\ 50\times 50\times 100\ splwt\times 100\ RRF\ label\ claim}$$

Intermediate precision

Preparation of impurity stock solution

1 mg of Impurity is accurately weighed and transferred to a 10 ml volumetric flask and was makeup with the diluent gives 100 ppm solution of impurity.

Spiking impurity

Tablet powder equivalent to 10 mg of Abiraterone is weighed and transferred to a 10 ml volumetric flask labelled precision spiked. 0.1 ml of solution from impurity stock is transferred to the volumetric flask labelled precision spiked and makeup to 10 ml with diluent.

$$\%\ w/w\ = \frac{Imp\ area\ Stdwt\ 5\times 1\ spl.dil\ Potency\times 100\ Avg.wt}{Avg.std\ area\ 50\times 50\times 100\ splwt\times 100\ RRF\ label\ claim}$$

RESULTS AND DISCUSSION

Development and Optimization

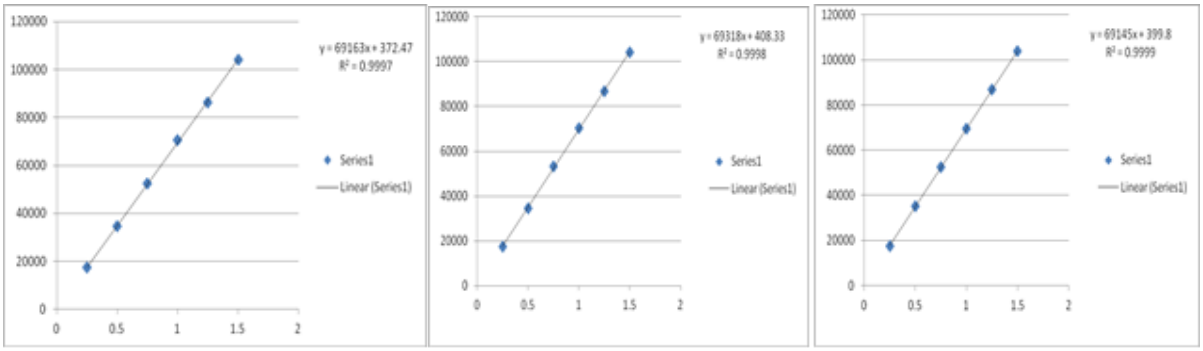
The HPLC procedure was optimized with a target to achieve separation of Impurity and main component Abiraterone. Based on literature survey and review of physic -chemical properties of analytes, preliminary chromatographic conditions were selected. Initially, trials were carried out using methanol, water in various proportions along with buffer to obtain the desired system suitability parameters. After few trials, mixing buffer and acetonitrile in the ratio of 50: 50 v/v was chosen as the mobile phase which gave good resolution and acceptable peak parameters. Separation was carried out at flow rate of 1 ml/min and detection at 235 nm. The injection volume was 10µl.

Linearity

The method was found to be linear over the range. The data generated was analysed by linear regression analysis shows the satisfactory result with correlation coefficient greater than 0.998. Linearity Curves of Abiraterone shown in Figure 3 and impurity in Figure 4. Linearity results are given in Tables 1 and 2.

Table 1: Linearity results of abiraterone.

Linearity details of Abiraterone				
%Level	**Conc (ppm)**	**Resp 1**	**Resp 2**	**Resp 3**
25	0.25	17455	17629	17377
50	0.5	34664	34478	35144
75	0.75	52537	53146	52487
100	1	70488	70181	69536
125	1.25	86246	86803	87025
150	1.5	103951	104134	103843



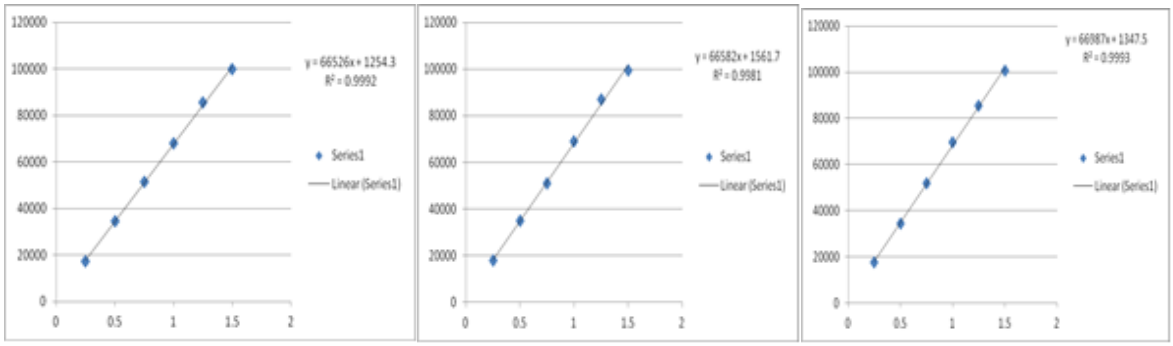
Linearity Curve of Resp 1 Linearity Curve of Resp 2 Linearity Curve of Resp 3

Figure 3: Linearity curve of impurity.

Table 2: Linearity results of impurity.

Linearity details of Impurity				
%Level	**Conc (ppm)**	**Resp A**	**Resp B**	**Resp C**
25	0.25	17323	17794	17679
50	0.50	34596	34800	34490
75	0.75	51332	51044	51767
100	1.00	68138	68931	69631
125	1.25	85642	86856	85428

150	1.50	99754	99502	100770



Linearity Curve of Resp A Linearity Curve of Resp B Linearity Curve of Resp C

Figure 4: Linearity curve of impurity.

Accuracy

The % mean recoveries for Abiraterone impurity was in the range of 99.30-99.67. The overall % RSD was observed as less than 2%. Accuracy details are given in Tables 3 and 4.

Table 3: Accuracy results of abiraterone.

Accuracy details of Abiraterone					
% Spike level	**Amount added (%w/w)**	**Avg amount added (%w/w)**	**% Recovery**	**Mean % Recovery**	**% RSD**
50	0.0490	0.049	99.06	99.30	0.3
	0.0490		99.60		
	0.0490		99.23		
100	0.0980	0.098	99.90	99.42	0.7
	0.0980		99.76		
	0.0980		98.60		
150	0.1470	0.147	99.85	99.67	0.2
	0.1470		99.46		
	0.1470		99.68		

Table 4: Accuracy results of impurity.

Accuracy details of Impurity					
% Spike level	**sample wt (mg)/ No. of units**	**Area**	**Amount found (%w/w)**	**Amount recovered (%w/w)**	**Avg amount recovered (%w/w)**
50	10	34098	0.049	0.049	0.049
	10	34285	0.049	0.049	
	10	34158	0.049	0.049	
100	10	68775	0.098	0.098	0.097
	10	68680	0.098	0.098	
	10	67880	0.097	0.097	
150	10	103115	0.147	0.147	0.147
	10	102710	0.146	0.146	
	10	102940	0.147	0.147	

Precision

The % RSD in precision studies was found to be 0.50. This indicates that the method is precise. Precision results are given in Table 5.

Table 5a: Precision results.

Standard response number	**Standard response**	**Precision data**		
		RRF = 1		
		Sample	**Area**	**%w/w**
1	70431	Sample-1	68948	0.098
2	69031	Sample-2	69403	0.099
3	70763	Sample-3	69356	0.099
4	69354	Sample-4	69814	0.099
5	70552	Sample-5	68935	0.098
6	70941	Sample-6	69559	0.099
Mean	70179	--	--	--
% RSD	1.1	--	--	--

Table 5b: Method precision details of abiraterone.

S. No	RT	Area	USP Plate Count	USP Tailing	USP Resolution
1	4.366	11985577	4000	0.83	0.82
2	4.366	11931883	4067	0.82	0.82
3	4.370	11945682	4097	0.82	0.82
4	4.371	12007944	4154	5.3	5.4
5	4.373	11979015	4034	5.4	5.5
6	4.379	11915411	4245	5.4	5.5
Mean	--	11960919	--	--	--
Std. Dev	--	35476.9	--	--	--
%RSD	--	0.3	--	--	--

Table 5c: Method precision details of impurity.

S. No	RT	Area	USP Plate Count	USP Tailing
1	3.173	68948	7029	1.19
2	3.175	69403	6697	1.19
3	3.175	69356	5982	1.17
4	3.178	69814	6279	1.16
5	3.182	68935	5812	1.22
6	3.186	69559	6284	1.18
Mean	--	69336	--	--
Std. Dev	--	344.80	--	--
%RSD	--	0.5	--	--

Solution Stability details

Solution stability details of Abiraterone and its impurity for zero hours and 24 hours as represented below. Table 6 contains details of solution stability.

Table 6a: Accuracy results of abiraterone.

Solution Stability at Zero hours					
Peak Name	**RT**	**Area**	**% Area**	**Height**	**USP resolution**
Impurity	3.182	53937	0.45	8353	--
Abiraterone	4.372	11923634	99.55	1172384	5.3

Table 6b: Solution stability at zero hours.

Peak Name	RT	Area	%Area	Height	USP resolution
Impurity	3.182	53937	0.45	8353	--
Abiraterone	4.372	11923634	99.55	1172384	5.3

Table 6c: Solution Stability at 24 hours for Abiraterone.

S. No	RT	Area	USP Plate Count	USP Tailing	USP resolution
1	4.374	12143617	3525	0.86	4.9
2	4.379	12132053	3466	0.86	4.9
3	4.395	12171410	3537	0.86	4.9
4	4.396	12140187	3494	0.86	4.9
5	4.396	12149897	3553	0.86	4.9
6	4.397	12218416	3550	0.86	4.9
Mean	--	12159263	--	--	--
Std. Dev	--	31885.5	--	--	--
% RSD	--	0.3	--	--	--

Table 6d: Solution stability at 24 hours for impurity.

S. No	**RT**	**Area**	**USP Plate Count**	**USP Tailing**
1	3.185	56204	5225	1.19
2	3.191	59973	4329	1.14
3	3.194	60455	5069	1.08
4	3.197	57146	5220	1.07
5	3.199	68242	3974	0.96
6	3.200	61940	4545	1.08
Mean	--	60660	--	--
Std. Dev	--	4285.7	--	--
% RSD	--	7.1	--	--

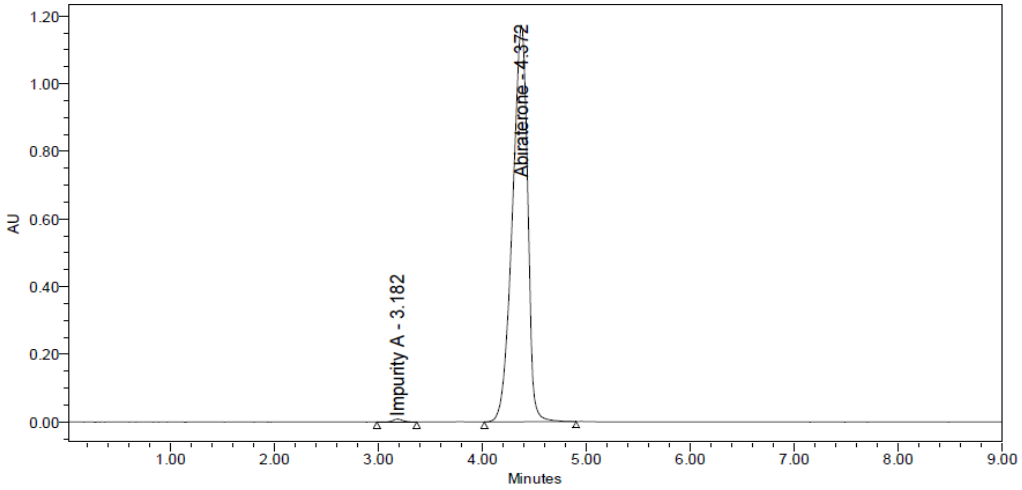


Figure 5: Chromatogram of abiraterone and its impurity.

CONCLUSION

The proposed HPLC method for simultaneous estimation of Abiraterone and its impurity was developed and validated. The method was found to be linear in stated range. Statistical analysis proved that the method is specific and sensitive. The method is suitable for simultaneous quantitative analysis of Abiraterone and its Impurity in formulations without any interference from the excipients. Thus, the proposed method can be successfully employed for routine quality control.

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