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Original Article

Stability Indicating Method Development and Validation for Determination of Abiraterone in Bulk and Pharmaceutical Dosage Form by RP-HPLC

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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 Discovery C18 150mm x 4.6 mm, 5µ ,Mobile phase 0.01N KH2PO4:Acetonitrile in the ratio of 60:40 and flow rate was maintained at 1.1 ml/min, detection wave length was 235 nm, column temperature was set to 30^{o}C 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 0.2 and for repeatability 0.3. LOD and LOQ are 0.953µg/ml and 2.888µg/ml respectively. By using above method assay of marketed formulation was carried out 99.84% was present. Degradation studies of Abiraterone were done, in all conditions purity threshold was more than purity angle and within the acceptable range.

Key words: HPLC Abiraterone, Method development. ICH Guidelines

INTRODUCTION

Abiraterone is a derivative of steroidal progesterone and is an innovative drug that offers clinical benefit to patients with hormone refractory prostate cancer. Abiraterone is administered as an acetate salt prodrug because it has a higher bioavailability and less susceptible to hydrolysis than abiraterone itself. Literature survey revealed that there were few analytical methods reported for Abiraterone in RP-HPLC. However, an extensive literature search didn't reveal any estimation method for Abiraterone in API and Pharmaceutical dosage form. Therefore an attempt has been made to develop and validate simple, precise, accurate economical RP-HPLC method as per ICH guidelines for the estimation of Abiraterone in API and Pharmaceutical dosage form.

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MATERIALS AND METHODS

Chemicals and Reagents: Acetonitrile (HPLC grade), orthophosphoric acid (HPLC grade), water (HPLC grade) were purchased from Mark (India) Ltd, Worli, Mumbai, India. All active pharmaceutical ingredients (APIs) of Abirateroneas reference standards were procured from Spectrum Pharma labs, Hyderabad, India.

Instruments and Chromatographic Conditions
Electronics Balance-Denver, P^H meter -BVK enterprises, India, Ultrasonicator-BVK enterprises, WATERS HPLC Acuitysystem equipped with quaternary pumps, UV detector and Auto sampler integrated with Empower 2 Software was used for LC peak integration and Data processing. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz cells integrated with UV-win 6 Software was used for measuring absorbance of Abiraterone solution. The mobile phase used was 0.01N Potassium di hydrogen phosphate: Acetonitrile (60:40A) at a flow rate of 1.1ml/min, samples were analyzed at 235 nm detector wavelength and at an injection volume of 10 µL using discovery C_{18}150 x 4.6 mm, 5 with run time of 10 min.

Methods

Diluent: Based up on the solubility of the drugs, diluent was selected, Acetonitrile and Water taken in the ratio of 50:50.

Buffer: 0.01N KH2PO4 Buffer (1ml of Ortho phosphoric acid was diluted to1000ml with HPLC grade water.)

Standard Preparation: 1 ml of Abiraterone from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent.

Sample Preparation: 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 filters.

Method Validation
As per ICH guidelines the method was validated and the parameters like Linearity, Specificity, Accuracy, Precision, Limit of Detection (LOD) and Limit of Quantitation (LOQ) were assessed.

Specificity: Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

Linearity: Stock solutions of Abiraterone is taken into 6 different volumetric flasks and diluted to 10ml with diluents to get 62.5ppm, 125ppm, 187.5ppm, 250ppm, 312.5ppm, 375ppmLinearity solutions are prepared such that 0.25, 0.5, 0.75, 1, 1.25, 1.5ml from the

Accuracy: Preparation of Standard stock solutions: Accurately weighed 125mg of Abiraterone transferred to two separately 50ml and volumetric flasks, 3/4 th of diluents was added and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution.
Preparation of 50% Spiked Solution: 0.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.
Preparation of 100% Spiked Solution: 1.0ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.
Preparation of 150% Spiked Solution: 1.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Robustness: Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines.
Robustness conditions like Flow minus (1ml/min), Flow plus (1.2ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much effected and all the parameters were passed. %RSD was within the limit.

LOD sample Preparation: 0.25ml of Standard stock solution was pipetted out and transferred to 10ml volumetric flasks and made up with diluents. From the above solution 0.1ml Abiraterone, were transferred to 10ml volumetric flasks and made up with the same diluents

LOQ sample Preparation: 0.25ml of Standard stock solution was pipetted out and transferred to 10ml volumetric flasks and made up with diluents. From the above solution 0.3ml Abiraterone, were transferred to 10ml volumetric flasks and made up with the same diluents

System suitability parameters: The system suitability parameters were determined by preparing standard solutions of Abiraterone (250ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.
The % RSD for the area of six standard injections results should not be more than 2%.

Assay of Abiraterone: Assay of the marketed formulation was carried out by injecting sample corresponding to equivalent weight into HPLC system

RESULTS & DISCUSSIONS

Optimization of Chromatographic Conditions: To develop and establish a suitable RP-HPLC method for estimation of Abiraterone in bulk and tablet dosage forms, different preliminary tests were performed and different chromatographic conditions were tested and optimized chromatographic conditions were developed which were given in Table-1.The final analysis was performed by using 60% Ortho phosphoric acid:40% Acetonitrile at a flow rate of 1ml/min, samples were analyzed at 235 nm detector wave length and at an injection volume of 10µL using Discovery C18 4.6 x 150mm, 5µmwith run time of 5min. The proposed method was optimized to give sharp peak with good resolution and minimum tailing effect for Abiraterone, the optimized chromatogram was obtained as shown in (Figure-2).

Validation: Linearity was established (62.5-375µg/ml) at six different concentrations each were injected in a duplicates and average areas were determined and linearity equations were obtained asy = 11056x + 7871., correlation coefficient (R^2) was determined as 0.999. The Linearity calibration curves were plotted as shown in (Figure-3). Retention time of Abirateronewas2.986min where no interfering peaks in blank and placebo were found in this method. So this method holds its specificity. Three levels of Accuracy samples 50%, 100%, 150% were prepared and triplicates of injections were given for each level of accuracy and mean% Recovery was obtained as 99.39% was shown in (Table-2).% RSD was calculated from the corresponding peaks obtained by injecting six times a known concentration of Abiraterone was obtained as 0.20% and the % RSD for Repeatability was obtained as 0.30%, Low % RSD values indicates that the method developed was precise as shown in (Table-3). The LOD and LOQ values were evaluated based on Relative standard deviation of response and slope of the calibration curve Abiraterone. The detection limit value was obtained as 0.953 and Quantitation limit was found to be 2.888as given in (Table-4).Robustness conditions like Flow minus (1.0ml/min), Flow plus (1.2ml/min), mobile phase minus (55:45), mobile phase plus (65:35), temperature minus (25°C) and temperature plus (35°C) were maintained and samples were injected in duplicate manner(Table -5). System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit (Table -6).Abiraterone pure drug (API) was obtained from Spectrum Pharma research solutions (Zytiga), bearing the label claim 250mg.Assay was performed with the above formulation. Average % Assay obtained was 99.84%the result was shown in (Table-7) and the chromatogram of standard drugs and pharmaceutical dosage forms were shown in (Figure-4, 5) respectively.

Degradation Studies: Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation (Table 6.8).

CONCLUSION

Chromatographic conditions used are stationary phase Discovery C18 (150mm*4.6mm 5□.) Mobile phase 0.01N KH2PO4: Acetonitrile in the ratio of 60:40 and flow rate was maintained at 1.1ml/min, detection wave length was 235 nm, column temperature was set to 30ºC 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 0.2, for repeatability 0.3. LOD and LOQ are 0.953µg/ml and 2.888µg/ml respectively. By using above method assay of marketed formulation was carried out 99.84% 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. Retention times are decreased and that run time was decreased so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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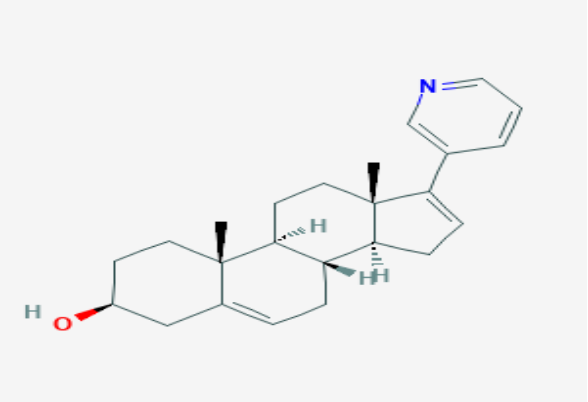

Figure-1: Chemical Structure of Abiraterone

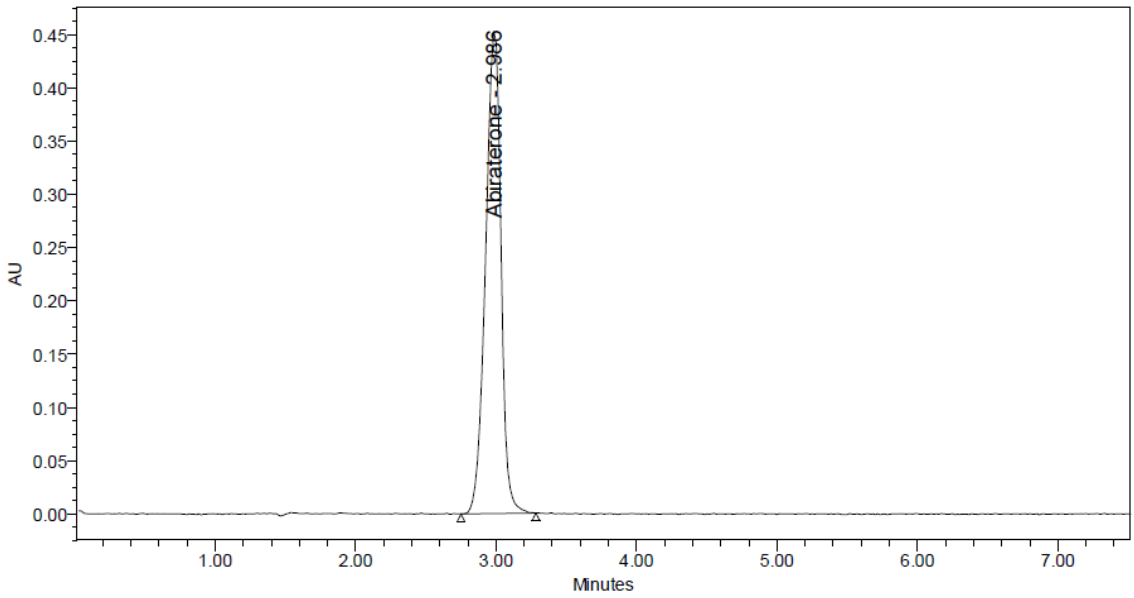

Figure-2: Optimized Chromatogram of Abiraterone

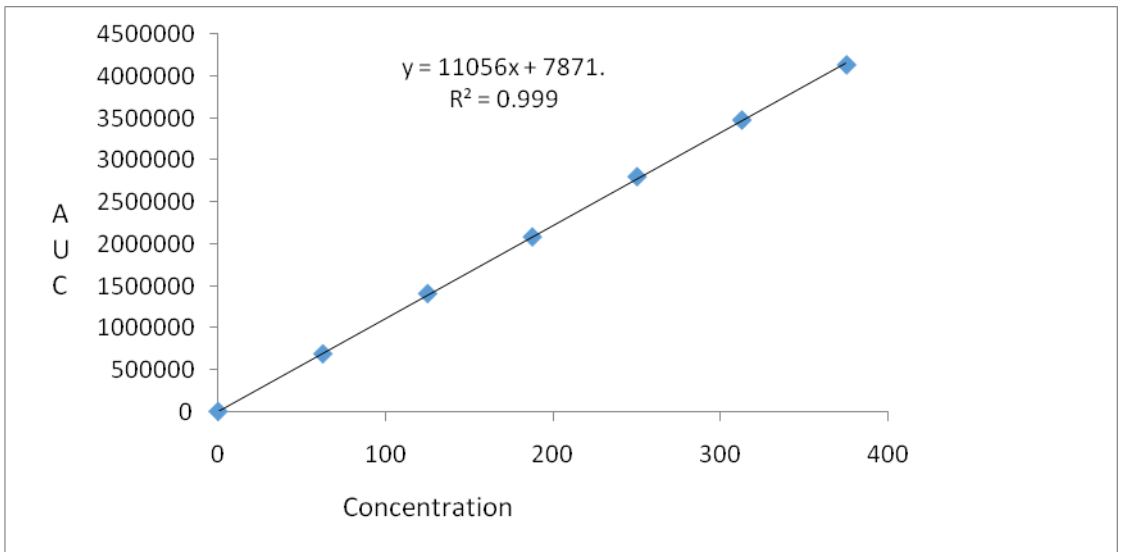

Figure-3: Linearity Curve of Abiraterone

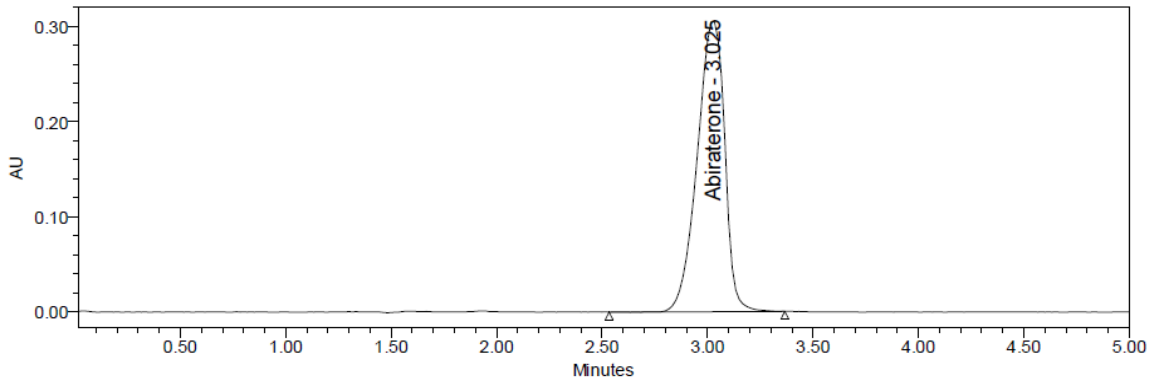

Figure-4: Standard Chromatogram of Abiraterone

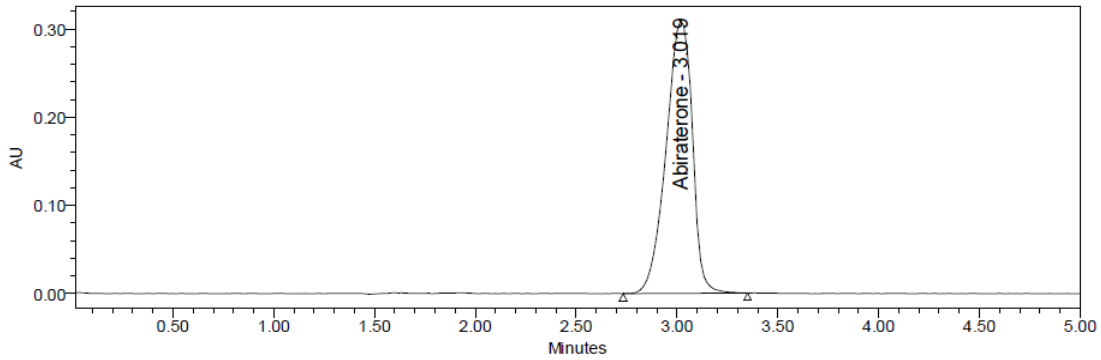

Figure-5: A Sample Chromatogram of Abiraterone Pharmaceutical Dosage Form

Table-1: Optimized Chromatographic Conditions

Parameter	**Condition**
RP-HPLC	WATERS HPLC SYSTEM equipped with quaternary pumps with PDA detector
Mobile phase	60%KH2PO4 (0.1%) : 40% Acetonitrile
Flow rate	1ml/min
Column	Discovery 150x4.6mm, 5µ
Detector wave length	235nm
Column temperature	30°C
Injection volume	10µL
Run time	10 min
Diluent	Water and Acetonitrile in the ratio 50:50
Retention Time	Abiraterone2.986min
Theoretical Plates	Abiraterone2820

Table-2: Accuracy results of Abiraterone

% Level	**Amount Spiked (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**	Mean %Recovery
50%	125	124.89	99.91	99.39%
	125	123.45	98.76	
	125	124.88	99.91	
100%	250	249.94	99.97	
	250	249.04	99.61	
	250	248.65	99.46	
150%	375	368.56	98.28	
	375	371.69	99.12	
	375	372.89	99.44	

Table-3: Precision Result of Abiraterone

S.no	Repeatability	Intermediateprecision
1.	2803133	2801836
2.	2792633	2796128
3.	2801715	2814167
4.	2794249	2796333
5.	2803918	2813837
6.	2801579	2808212
Mean	2799538	2805086
S.D	4830.4	8200.5
%RSD	0.2	0.3

Table-4: LOD and LOQ values of Abiraterone

Molecule	LOD	LOQ
Abiraterone	0.085	0.258

Table-5 Robustness Data of Abiraterone

S.no.	Condition	%RSD of Abiraterone
1	Flow rate (-) 1ml/min	0.7
2	Flow rate (+) 1 ml/min	0.4
3	Mobile phase (-) 40 OPA : 60A	0.7
4	Mobile phase (+) 50 OPA:50A	0.5
5	Temperature (-) 30°C	0.6
6	Temperature (+) 30°C	0.2

Table-6: System Suitability Parameters Result of Abiraterone

S.no.	Abiraterone		
Inj	**RT(min)**	**USP Plate Count**	**Tailing**
1	3.015	2736	0.85
2	3.022	2773	0.86
3	3.023	2677	0.86
4	3.025	2669	0.85
5	3.037	2821	0.86
6	3.040	2729	0.86

Table -7: Assay Results of Abiraterone

S. No.	% Assay Abiraterone
1	99.97
2	99.60
3	99.92
4	99.65
5	100.00
6	99.91
AVG	99.84
STDEV	0.1723
%RSD	0.17

Table - 8. Degradation Data of Abiraterone

S.NO	Degradation Condition	%Drug Degraded	Purity Angle	Purity Threshold
1	Acid	4.61	0.278	0.464
2	Alkali	4.22	0.221	0.415
3	Oxidation	3.50	0.098	0.324
4	Thermal	2.66	0.219	0.417
5	UV	1.57	0.220	0.420
6	Water	0.24	0.223	0.418

REFERENCES

1. R. S. Satoskar, S. D. Bhandarkar and S. S. Ainapure. "Pharmacology and Pharmacotherapeutics", 17th edition, Popular Prakashan, Mumbai, India, 2001.
2. "Burger's Medicinal Chemistry and drug discovery", 6 th edition, Wiley Interscience, New Jersey, 2007.
3. Foye's "Principles of Medicinal Chemistry", 6th edition, Lippincott Williams & Wilkins, New york, 2008.
4. Drugs & Cosmetics Act, 1940 & Rules, 1945, 2nd edition, Susmit publishers, Mumbai, India, 2000.
5. B. Jala chandra reddy et al, development and validation of a novel rp-hplc method for stability-indicating assay of abiraterone acetate journal journal of liquid chromatography & related technologies volume 39, 2016 - issue 7
6. Bedi smriti, gude sushmitha, krishna muddu, vasantharaju sg et al,.(2016); novel rp-hplc method for the quantification of abiraterone acetate journal of global pharma technology © 2009-2016, jgpt. all rights reserved.
7. Gadhave r. v., tamnar a. b., bansode a. s., choudhari v. p. et al stability indicating rp-hplc-pda method for determination of abiraterone acetate and characterization of its base catalyzed degradation product by lc-ms vol 8, issue 2, 2016 > v.
8. Divyashree s, veena mk, channabasavaraj kp et almethod validation for simultaneous estimation of prednisolone and abiraterone acetate by rp-hplc issue 1| vol 7| 2016|jchrdd
9. M. ramesh babu et al stability indicating method development and validation for the estimation of residue of abiraterone by hplc int j pharm 2016; 6(2): 267-276
10. Arun kumar kuna, s. ganapaty and g.v. radha et al analytical method development and validation for theestimation of abiraterone acetate in pharmaceutical formulation by uv and rp-hplc int j pharm bio sci 2016 april; 7(2): (p) 57 – 61.