OPTIMIZED AND VALIDATED RP-HPLC METHOD FOR QUANTIFICATION OF ABIRATERONE ACETATE (AN ANTI-PROSTATE CANCER DRUG) INPHARMACEUTICAL DOSAGE FORM

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ABSTRACT:

A simple, reliable, sensitive, precise, rapid, and reproducible RP -HPLC method was developed and validated for the determination of Abiraterone acetatein the pharmaceutical dosage form and the separation was carried out by using optimized chromatographic conditions on Agilent technologies -1260 infinity system, eclipse XDB C_{18} column, (250 mm × 4.6 mm i.d., particle size 5 µm, was maintained at ambient temperature). The mobile phase consisting of methanol: acetonitrile in the ratio 50:50 v/v. A gradient elution at a flow rate of 1 mL/ min using a 1260 DAD detector to monitor the elute at 255 nm. The retention time of Abiraterone acetate was found to be 7.453 and the calibration curve was a linear function of the drug in the concentration range of 2-10 µg/mL (r^2 = 0.9996).The limit of detection and the limit of quantitation was found to be 0.451 µg/mL and 1.369 µg/mL respectively. The recovery (accuracy) studies were performed and the percentage recovery was found to be 99.78. Analytical validation parameters such as selectivity, specificity, linearity, precision, and accuracy were studied and the % RSD value for all key parameters was less than 2 %. Thus the developed reversed-phase HPLC method was found to be feasible for the determination of Abiraterone acetate in bulk and pharmaceutical formulation.

KEYWORDS:RP -HPLC,Abiraterone acetate, Pharmaceutical formulation,Method Validation, ICH guidelines.

I.INTRODUCTION

Abiraterone acetate(ABTA)is an irreversible inhibitor of 17α-hydroxylase/C17,20-lyase (CYP17) which plays a part in the production of androgens in the testes and adrenal glands.ABTA was approved by the United States Food and Drug Administration (USFDA) in April 2011 for the treatment of castration-resistant prostate cancer[1-3].Thechemical nameof ABTAis[(3S,8R,9S,10R,13S,14S)-10,13-dimethyl-17-pyridin-3-yl-2,3,4,7,8,9,11,12,14,15-decahydro-[a]phenanthrene3yl]acetae with molecular formula $C_{26}H_{33}NO_2$.The chemical structure is shown in Fig. 1.Literature survey revealed that few analytical methods have been reported for estimation of ABTA individually or in combination with other drugs. The reported methods are Spectrophotometric[4-6],HPLC[7-14],Bioanalytical HPLC[15],LC-MS[16-20],UPLC-MS[21-

[23]methodswerereported.The present study was aimed to develop a simple, sensitive, rapid, precise and accurate and validated RP-HPLC method for the estimation of ABTA in Pharmaceutical dosage form.Developed method was validated according toICHguidelines by using high performance liquid chromatography[24-29].

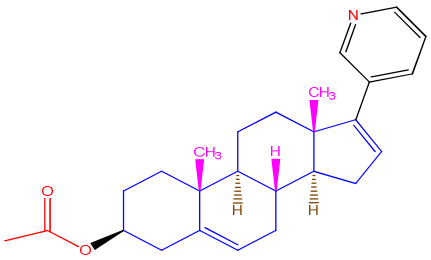

Fig.1. Chemical structure ofAbiraterone acetate

II. Materialsand Methods

2.1. Chemicals and Reagents:

A pure sample of ABTA was obtained as a gift sample from Hetero drugs Pvt.Ltd, Hyderabad, India. The marketed formulation of Zytiga, Yonsa tablets were procured from a local pharmacy store. HPLC grade of acetonitrile was procured from Thermo Fisher Scientific India Pvt. Ltd., Mumbai, India.HPLC grade water and methanol were purchased from Merck Specialties Pvt.Ltd, Mumbai, India.

2.2. Instrumentation:

The analysis was performed by using a chromatographic system, Agilent HPLC comprised of a 1260 Quaternary pump, a 20 µL injection loop, and a 1260 photodiode array detector and running on E-Z Chrome software with a reverse-phase Eclipse XDB C_{18} column having 250 x 4.6 mm internal diameter, 5 µm particle size. UV-Visible Spectrophotometer (Elico SL-210) Shimadzu electronic balance (AX-200) was used for weighing purposes. Ultra Sonicator (PCI Ltd., Mumbai) was used for the preparation and degassing of samples.

2.3. Method development and optimization of chromatographic conditions:

ABTA was analyzed with Eclipse XDB model C_{18} Column (4.6 mm i.d. X 250 mm, 5 µm particle size) for the chromatographic separation, and the column was maintained at ambient temperature.The mobile phase was composed of a mixture of methanol and acetonitrile in the ratio of 50:50 v/v and it was delivered at a flow rate of 1.0 mL/min and detection was monitored at 255 nm with a PDA detector. The mobile phase was used as a diluent. The injection volume was 20 µL.The run time was set at 10 min. The retention time ofABTA was found to be 7.453 min respectively.

2.4. Selection of detection wavelength:

The UV spectrum of diluted solutions of various concentrations of ABTA in methanol was recorded by using a UV spectrophotometer and scanned over a range of 200 - 400 nm. The maximum absorbance was found to be 255 nm.The spectrum of ABTAis shown in Fig.2.

Fig. 2.UV Spectrum of Aberaterone acetate

2.5. Preparation of stock and working standard solution:

The standard drug solution of ABTA was prepared by dissolving 100 mg of standard drug in a 20 mL mobile phase in a 100 ml volumetric flask. It was sonicated for 10 minutes for the complete solubility of the drug. After dissolving the drug, the final volume was brought up to 100 mL by adding a mobile phase to obtain an eventual concentration of 1000 µg/mL.The secondary stock solution is prepared by adding 1mL of primary stock solution in a 10 mL volumetric flask and filled up the volume with methanol having the concentration of 100 µg/mL. A series of five aliquotesolutions were prepared for calibration graph by adding defined volumes of the secondary stock solution and diluting with the mobile phase. The concentrations of ABTA are 2 µg/mL, 4 µg/mL, 6 µg/mL, 8 µg/mL and 10 µg/mL respectively.

2.6. Preparation of sample solution:

Accurately 20 tablets were weighed individually and the average weight was calculated and powdered. The tablet powder equivalent to 100 mg of ABTA and it was transferred into a 100 ml volumetric flask, to that 20 ml of mobile phase was added and sonicated for 10 minutes at a controlled temperature to dissolve the powder, further, the volume was made up with the same solvent. From the above solution pipette out 1.0 ml of the sample solution into a 10 mL volumetric flask and dilute with mobile phase up to the mark, mix well. Filtered through 0.45 µ membrane filter. & further dilutions were done with mobile phase as diluent to get eventual concentration (10 µg/ml) within the linearity range and Finally, the drug content in each tablet and also bulk drug was found by utilizing the standard graph.

2.7. Method development optimization:

Mixture of commanley used solvents like HPLC grade water, methanol, and acetonitrile with or without buffers in different combinations were tested as mobile phases in order to get an optimized HPLC conditions, Finally, the mobile phase consisting of a mixture of methanol and acetonitrile in the ratio of (50:50 v/v) at a flow rate of 1.0 mL/min was found to be satisfactory and proper system suitability parameters as per ICH guidelines.Optimized chromatographic conditions and SSP are tabulated in Table 1.

III. Method Validation

The method was validated for specificity, linearity, accuracy, precision, the limit of detection, the limit of quantification, and robustness by following procedures.

3.1. System suitability:

System suitability is an integral part of the chromatographic system. It is verification of resolution, capacity factor, tailing factor, theoretical plate count, relative retentions, etc. are calculated and compared with a standard specification of the system.

3.2. Precision:

The closeness of agreement (degree of scattering) between a series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions. Precision may be considered at three levels: System precision, method precision, intermediate precision and precision at different levels 50 %,100 %,150 %.

3.3. Linearity:

The linearity of the method was determined at five concentration levels ranging from 2-10 µg/ml for ABTA. Evaluation of the drug was performed with a PDA detector at 255 nm, peak area was recorded for all the peaks.The correlation coefficient value of ABTA was found to be 0.9996.

3.4. Accuracy (Recovery studies):

A known quantity of ABTAwas spiked with placebo at three different levels such as 50 %,100 %,150 %in triplicate preparations.the samples were analysed as per the proposed method.The mean % recovery at each level should be 98-102 %.

3.5. Robustness:

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. It should show the reliability of analysis concerning deliberate variations in method parameters. The robustness of the proposed method is estimated by changing the flow rate of the mobile phase and the composition of the mobile phase.

3.6. Stability of analytical solution:

Regarding the stability of both the standard and sample solutions were analyzed over a period of 48 hours at10°C. The results show that for both soultions, peak area and retention time almost

unchanged and no significant degradation with in the 48 hours.Which indicates that both soultions were stable for atleast 2 days which was sufficient to completholeanalyctical process.

3.7. LOD and LOQ:

Limit of detection (LOD) is defined as the lowest concentration of analyte that gives a detectable response. Limit of Quantification (LOQ) is defined as the lowest concentration of analyte that can be quantified with a specified level of accuracy and precision.The LODand LOQwere determined by injecting six replicates of the analyte at the progressively low concentrations of the standard solution using the developed HPLC method.

3.8. Analysis Abiraterone Acetate in tablet formulation :

Accurately 20 tablets were weighed individually and the average weight was calculated and powdered. The tablet powder equivalent to 100 mg of ABTA and it was transferred into a 100 ml volumetric flask, to that 20 ml of Mobile phase was added and sonicated for 5 minutes at a controlled temperature to dissolve the powder, further, the volume was made up with the same solvent. From the above solution pipette out 1.0 ml of the sample solution into a 10 mL volumetric flask and dilute with mobile phase up to the mark, mix well. Filtered through 0.45 µ membrane filter. & further dilutions were done with methanol to get eventual concentration (20 µg/ml) within the linearity range and Finally, the drug content in each tablet and also bulk drug was found by utilizing the standard graph.

IV. Results and Discussion

The HPLC procedure was optimized to develop an accurate assay method for the determination of ABTA in bulk and pharmaceutical dosage form by using Eclipse XDB C18 (4.6 x 250 mm internal diameter; 5 µm particle size) column with a mobile phase of methanol and acetonitrile in the ratio of 50:50 v/v. The flow rate of the mobile phase at 1.0 ml/min and the component was monitored and detected with a PDA detector at 255 nm. The eluted drug peaks in good shape and well resolved. The results of optimized chromatographic conditions were shown in Table 1. The retention time, the number of theoretical plates, and the tailing factor of ABTA were found to be 7.453 min, 8,960, and 1.150 respectively, which indicates the efficient performance of the column.

As per methodology system suitability solution and six replicate of standard preparation were injected into HPLC.The tailing factor, theoretical plates, % RSD were well within the acceptance criteria and study concludes the suitability of analytical system for the analysis.

The method was linear in the range of 2-10 µg/ml for ABTA with a correlation coefficient of 0. 9999. The regression equation of ABTA concentration over its peak area ratio was found to be Y = 6805.5x + 480.81, where X is the concentration of ABTA and Y is the respective peak area.

The results have shown that an excellent correlation exists between peak area and concentration of drug within the concentration range indicated.The linearity results were shown in Fig. 3.

Table1: Optimized chromatographic conditions and SSP for the proposed HPLC method

Parameter	**Chromatographic conditions**
SInstrument	Agilent HPLC quaternary-1260 Infinite-II series
Column	Eclipse XDB C18 250 mm×4.6 mm;5.0 µm
Detector	1260 Diode Array Detector (DAD).
Mobile phase	Methanol: Acetonitrile (50: 50 v/v)
Flow rate	1 mL/minute
Detection wavelength	255 nm
Run time	10 minutes
Temperature	Room temperature (25 ^{o}C).
Injection volume	20 µL
System suitability parameters (SSP)	
Retention time (R_t)	7.453 minutes
Number of Theoretical plates	8,960
Tailing factor (asymmetry)	1.150

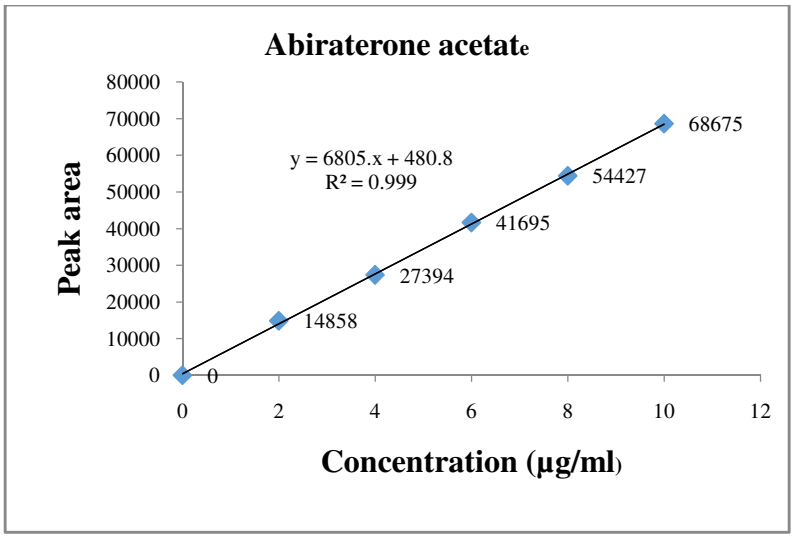

Fig. 3. Calibration graph of Abiraterone acetate

The mean % recoveries were found to be 99.6 - 99.8 % which indicates the method is accurate.The % RSD for intra-day precision and inter-day precision for ABTA was found to be 0.00028and 0.002208 respectively, the values were less than 2 % which indicates the method is precise.The limit of detection (LOD) and limit of quantification (LOQ) for ABTA were found to be 0.451µg/mL and 1.369 µg/mL respectively, which indicate the sensitivity of the method.

The robustness was performed for the flow rate variations like ±0.2 mL, wavelength variations like ± 5 nm, and mobile phase composition changes like ± 5 %. The results are summarized on evaluation of the above parameters variations in flow rate, wavelength, and mobile phase composition, results are not affected significantly. Hence it indicates that the method is robust. Overall % RSD was found to be less than 2% for all the variations which indicates that the proposed method is robust. The results of the study were shown in Table 2.

Table. 2: Robustness results of Abiraterone acetate

S. No	Parameter	Optimized	Used	Retention time (t_R), minutes	Plate count$	Peak asymmetry#	Remark
1.	Flow rate (± 0.2 mL/min)	1.0 mL/min.	0.8 mL/min	7.520	9980	1.190	*Robust
			1.0 mL/min	7.453	8603	1.150	*Robust
			1.2 mL/min	7.231	7700	1.110	*Robust
2.	Detection wavelength (±5 nm)	255 nm	250 nm	7,453	8605	1.150	Robust
			255 nm	7.453	8603	1.150	Robust
			260 nm	7.453	8610	1.150	Robust
3.	Mobile phase composition (± 5%) (MeOH : ACN)	50:50 v/v	55:45 v/v	7.552	8676	1.160	*Robust
			50:50 v/v	7.453	8600	1.150	*Robust
			45:55 v/v	7.357	8680	1.147	*Robust

*Acceptance criteria (Limits): #Peak Asymmetry < 1.5, $ Plate count > 2000, * Significant change in Retention time*

The validated method was applied for the determination of ABTA in commercial tablet formulation that was obtained by injected six replicates of the sample solutions. The amount of drug and Mean % recovery ± SDof the assay was found to be 499.3 mg/tablet and 99.86 ± 0.20%. 20 µL of each calibration standard solution (2,4,6,8,10 µg/mL) were injected into the HPLC system to get the chromatograms.The average peak area and retention time were recorded.A typical chromatogram of standard,blank with placebo and sample of ABTA were shown in Fig. 4 to 6. No interfering peaks were found in the chromatogram of the formulation within the run time indicating that excipients used in the formulation did not interfere with the estimation of the drug by the proposed method.Summary of method validation and assay results were shown in Table 3.

Table 3. Summary of validation parameters

Validation Parameters		Results
System suitability		Complies
Specificity		No interference observed, peak purity complies
Precision	System precision	% RSD: 0.022
	Method precision	% RSD: 0.028
	Intermediate precision	% RSD: 0.021; Cumulative % RSD:0.023
	Precision at different levels % RSD	50 % : 0.020; 100% : 0.015; 150% : 0.017
Linearity (r^2 should not less than 0.997)		0.9996
Accuracy (mean % should be 98-102 %)		50 % : 99.7 % ; 100 % : 99.8 % ; 150 % : 99.6 %
Limit of Detection (LOD) µg/ml		0.451 µg/mL
Limit of Quantification (LOQ) µg/ml		1.369 µg/mL
Assay reults of marketed formulation (ZYTIGA)		Label claim: 500 mg/tablet; *Amount found: 499.3 mg/tablet Mean % recovery± SD : 99.86 ± 0.20 ; % RSD*: 0.22.

** Average of six determinations, SD denotes standard deviation;RSD denotes % RSD: relative standard deviation.*

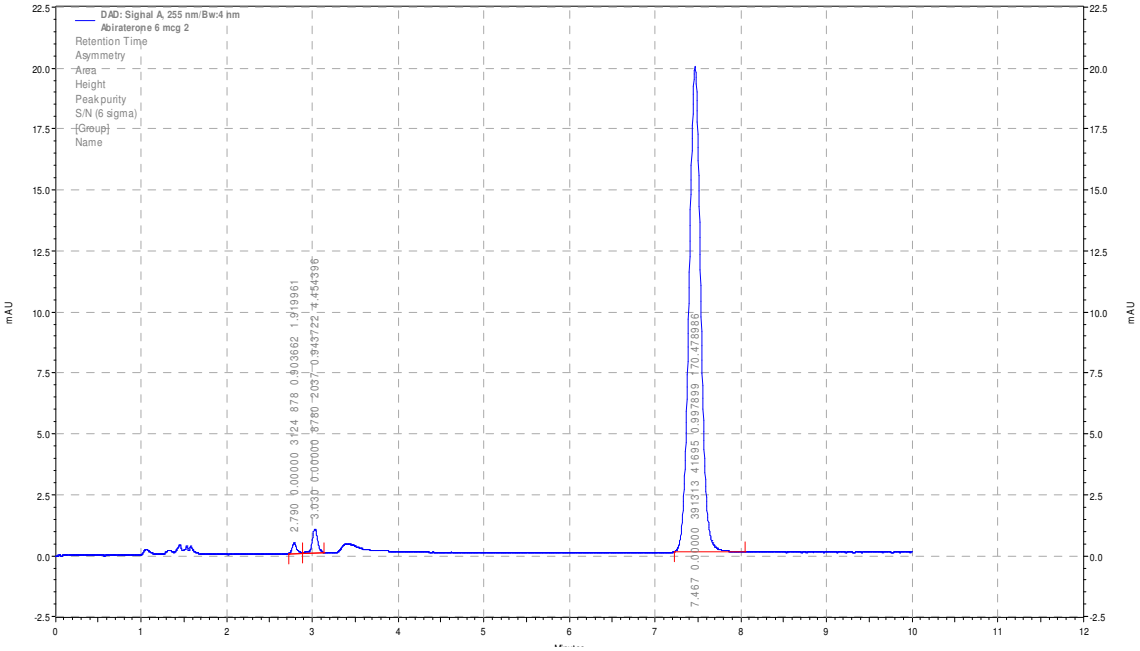

Fig. 4.Standard chromatogram ofAbiraterone acetate

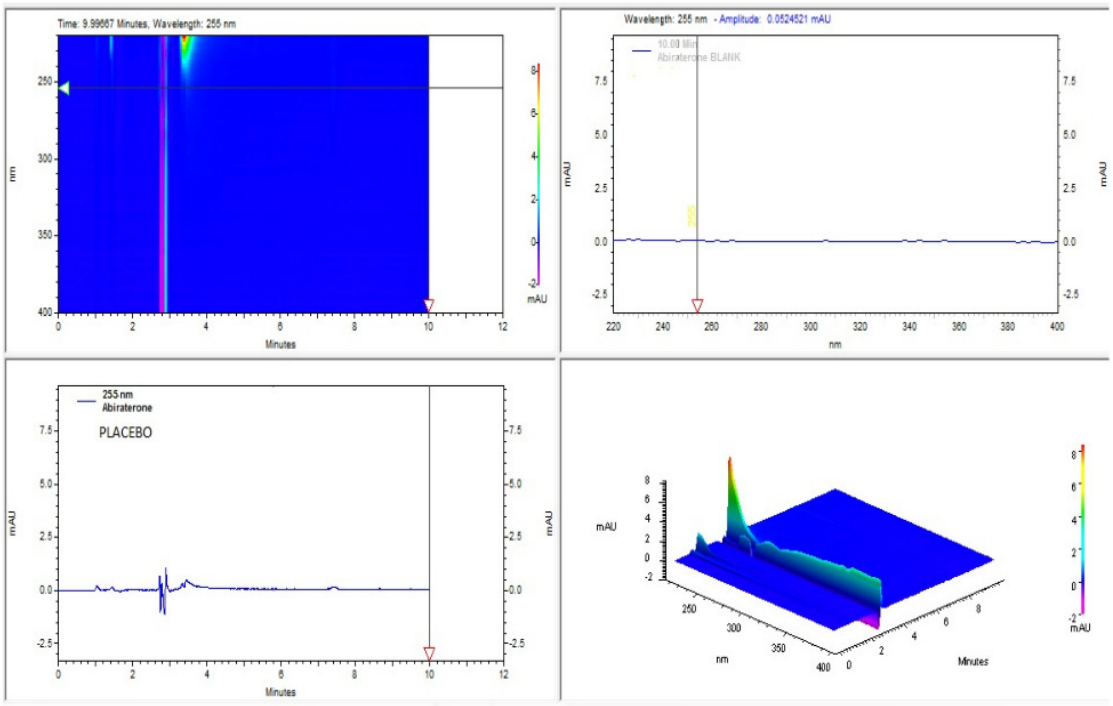

Fig. 5. Blank and Placebo chromatograms of ABTA

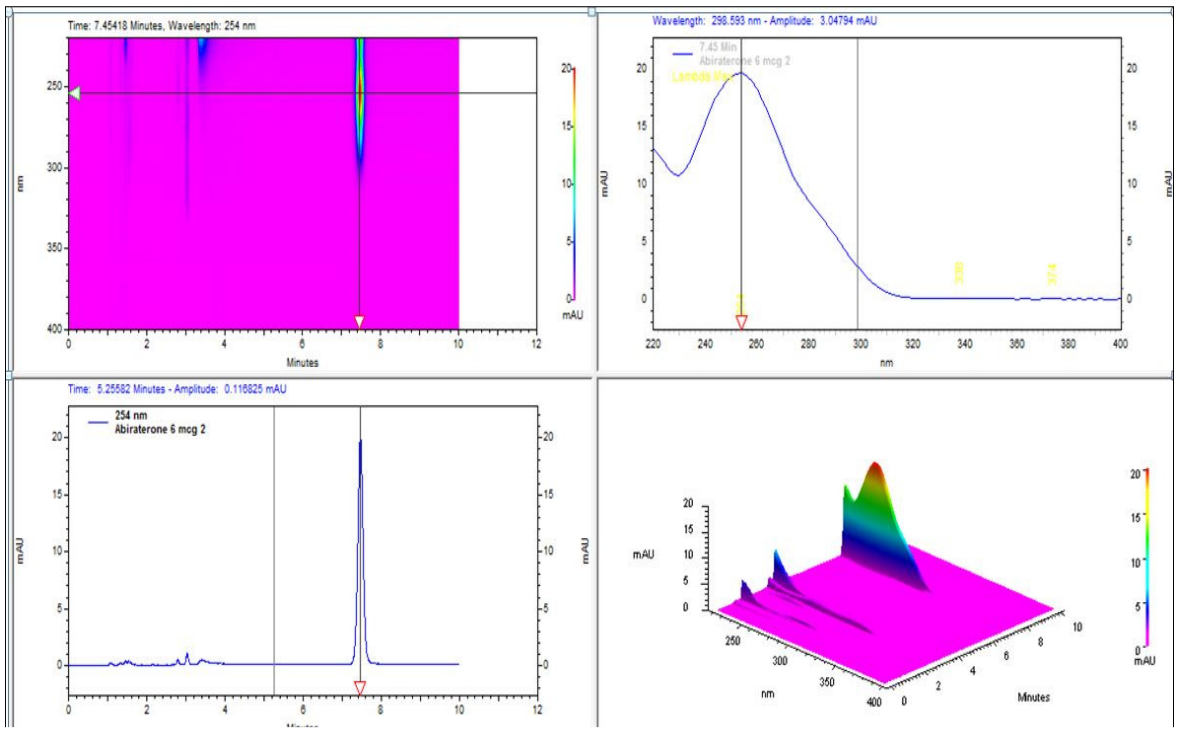

Fig. 6. Sample chromatogram of Abiraterone acetate

V. CONCLUSION

The proposed study describes a new HPLC method for the estimation of ABTA bulk and in its tablet formulation. From the above experimental data and results, the developed HPLC method is having the advantages such as The standard and sample preparation requires less time, No tedious extraction procedure was involved in the analysis of formulation, Run time required for recording chromatograms were less than 10 minutes. Hence, the chromatographic method developed for the ABTA said to be rapid, simple, specific, sensitive, precise, accurate and reliable that can be effectively applied for routine analysis in research institutions, quality control department in industries, approved testing laboratories.HPLC is just the leading technique for the trace analysis oforganic and inorganic substances[30].

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