STABILITY INDICATING ASSAY METHOD DEVELOPMENT AND VALIDATION OF ABIRATERONE BY RP-HPLC

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Abstract: *A specific, precise, and accurate RP-HPLC method has been developed and validated for the quantitative analysis of Abiraterone in tablet formulation. An Isocratic separation was achieved using Symmetry Waters C18 150 x 4.6 mm, 5µm. column with a flow rate of 0.8ml/min. The injection volume was 10µL and detection wavelength was at 235nm.The column and sample temperature was maintained at 30°C. Run time was 5 minutes. The retention time of Abiraterone peak was about 2.35 minutes. Isocratic elution was performed with mobile phase composition 0.01N $Na_2HPO_4.H_2O$(5 pH)Buffer : Acetonitrile, in the ratio of 70:30% v/v. The Mobile phase was used as Diluent. The method was validated for specificity, linearity, precision, accuracy and robustness. The specificity of the method was determined by assessing interference from the placebo and by stress testing the drug product (forced degradation). The method was linear over the concentration range from 25% of target concentration to 150% of target concentration (r^2 = 1.000). The method was found to be Robust and suitable for the quantitative analysis of Abiraterone in a tablet formulation. Degradation products resulting from the stress studies did not interfere with the detection of Abiraterone peak in chromatogram, demonstrating the stability-indicating method.*

IndexTerms **- Abiraterone, Stability-indicating, RP-HPLC. INTRODUCTION:**

Description:

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. FDA approved on April 28, 2011.

It has following structure:

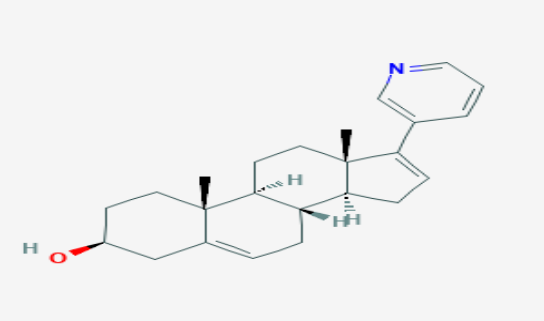

Figure 1: structure of Rivaroxaban

METHOD AND REQUIREMENTS:

Instruments:

Table 1: Instruments used

S.NO.	INSTRUMENTS
1	WATERS HPLC 2965 SYSTEM with Auto-injector and Empower 2 software and PDA Detector
2	UV-VIS spectrophotometer
3	Sonicator (Ultrasonic sonicator)
4	P^H meter (Thermo scientific)
5	Micro balance (Sartorius)

6	Vacuum filter pump

Chemicals:

Table 2: Chemicals and reagents used

S.NO.	Chemicals/Reagents
1	Methanol hplc grade (rankem)
2	Acetonitrile hplc grade (rankem)
3	HPLC grade water (rankem)
4	Glacial acetic acid

Chromatographic conditions:

The chromatographic separation was achieved by using isocratic elution mode with Symmetry Waters C18 150 mm x 4.6 mm, 5µm column. The mobile phase consisted of 0.01N $Na_2HPO_4.H_2O$: Acetonitrile (70:30). The flow rate was maintained as 0.8 mL/min. The column temperature was maintained at 30°C ± 2. The injection volume was 10µL. Run time was 5 min. The λ max was 235 nm.

Diluent preparation:
Based up on the solubility of the drugs, diluent was selected, Acetonitrile and 0.01N Na_2HPO_4 taken in the ratio of 50:50 v/v
Blank:
Diluent used as Blank.

Buffer preparation:

0.1%OPA Buffer: 1ml of ortho phosphoric acid was diluted to 1000ml with HPLC grade water.

0.01N $Na_2HPO_4.H_2O$ Buffer: Accurately weighed 1.37gm of disodium hyrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then PH adjusted to 5 with dil. Orthophosphoric acid solution.

Mobile phase preparation:
Mix 0.01N $Na_2HPO_4.H_2O$ Buffer : Acetonitrile in the ratio 70:30% v/v and sonicate to degas.

Preparation of Standard stock solutions: Accurately weighed 6.25mg of Abiraterone transferred 25ml 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 (250µg/ml of Abiraterone)

Preparation of Standard working solutions (100% solution): 1ml of Abiraterone from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (25µg/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 filters. (2500 µg/ml of Abiraterone)

Preparation of Sample working solutions (100% solution): 0.1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (25µg/ml of Abiraterone)

OPTIMIZED METHOD:

Table 3: Optimized chromatographic parameters and condition

PARAMETERS	CHROMATOGRAPHIC CONDITIONS
Column	Symmetry Waters C18 150 mm x 4.6 mm, 5µm
Elution	Isocratic
Mobile phase	0.01N $Na_2HPO_4.H_2O$ buffer : Acetonitrile (70:30 % v/v)
Flow rate	0.8 ml/min
Diluents	Acetonitrile and 0.01N Na_2HPO_4 taken in the ratio of 50:50 v/v
Detector	235 nm

wavelength	
Column temperature	30°C
Sample temperature	Ambient
Run time	5 min
Retention time	2.35 ±10%

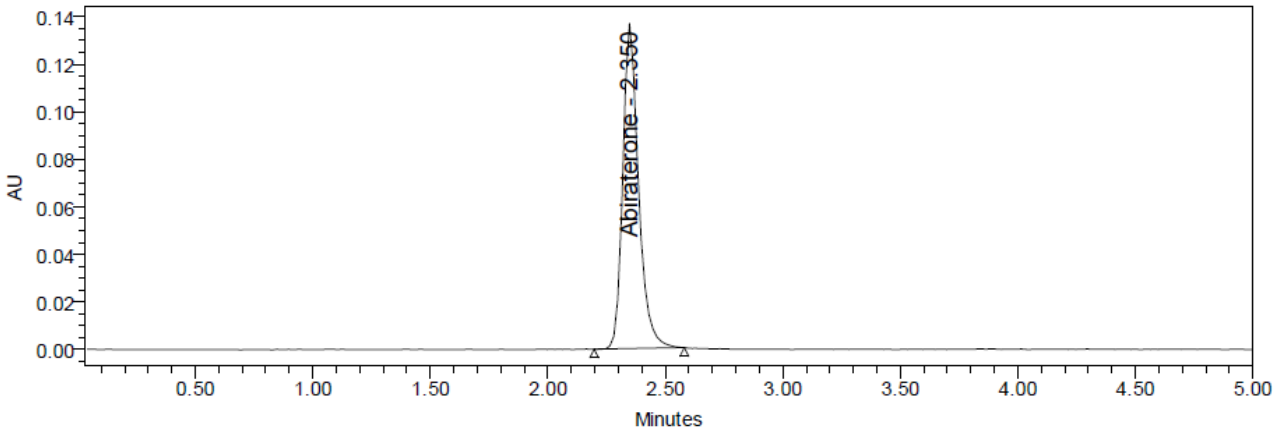

Figure 1: Optimized Chromatogram of Abiraterone

VALIDATION:

The method was validated for parameters like specificity, linearity, precision, accuracy and robustness as per ICH guidelines

System suitability:

The standard solution was prepared and injected into HPLC system. The system suitability parameters were evaluated as per the method and found to be within the limits.

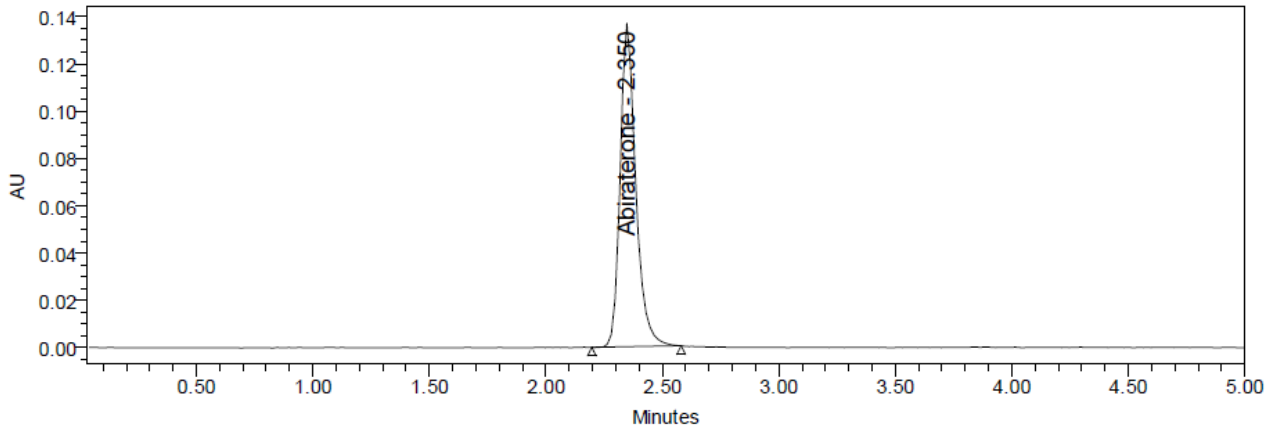

Figure 2: System suitability chromatogram of Abiraterone

System suitability data of Abiraterone:

Table 4: System suitability data of Abiraterone

Peak Name: Abiraterone

	Peak Name	RT	Area	USP Plate Count	USP Tailing
1	Abiraterone	2.349	669952	5977	1.26
2	Abiraterone	2.350	665666	6116	1.26
3	Abiraterone	2.351	673913	5982	1.26
4	Abiraterone	2.351	665705	6182	1.24
5	Abiraterone	2.351	667529	5939	1.26
6	Abiraterone	2.353	664199	6358	1.25
Mean			667827		
Std. Dev.			3577.1		
% RSD			0.5		

Specificity:

Blank, placebo and degradation product solution were injected and compared, there is no peak found in the blank, placebo and degradation solutions at retention time of drug so the method developed was specific and do not have any interference.

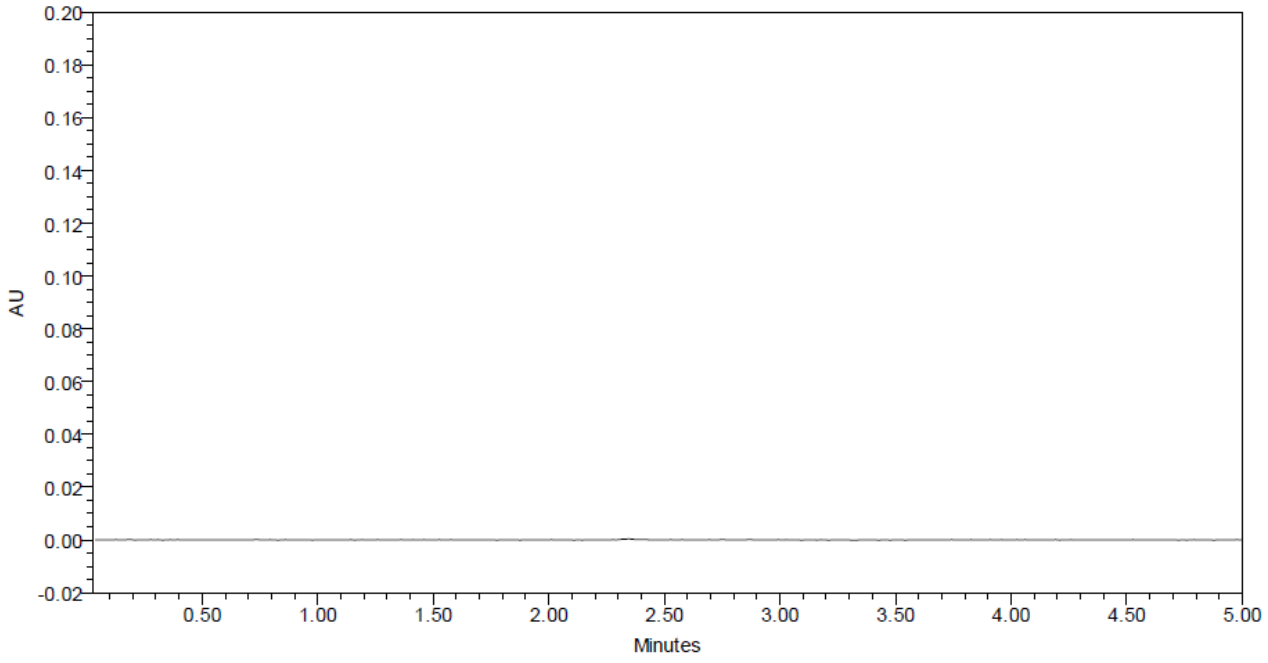

Figure 3: Typical chromatogram of Blank

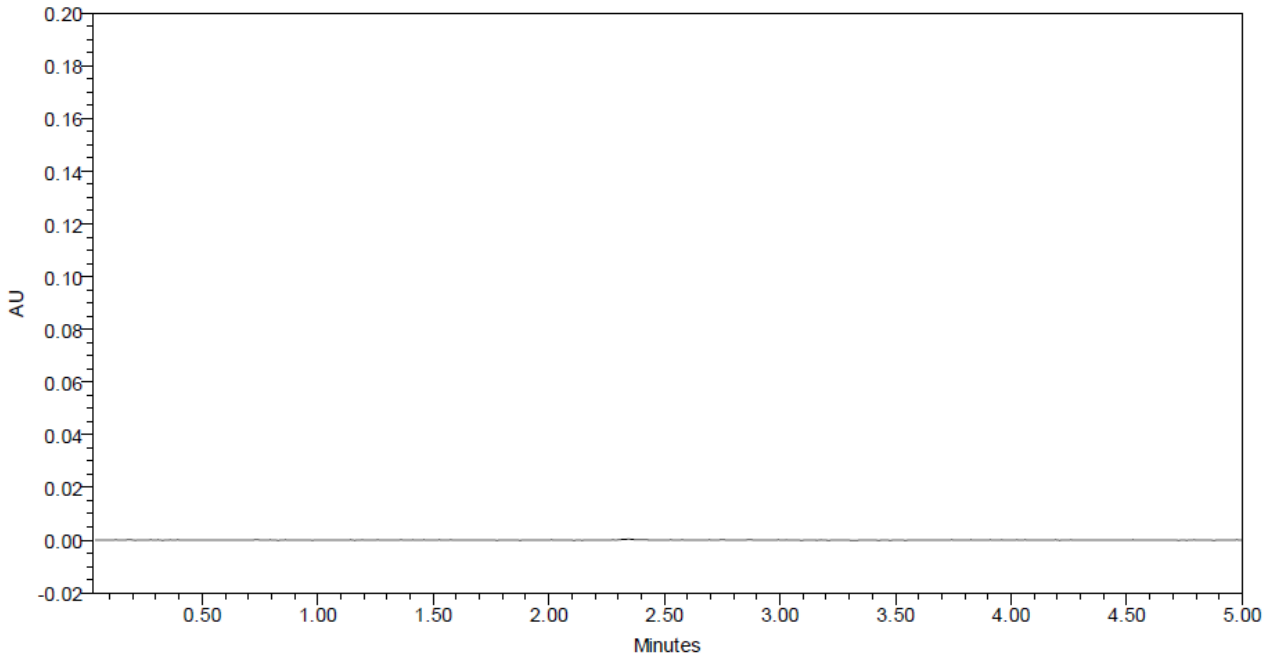

Figure 4: Typical chromatogram of Placebo

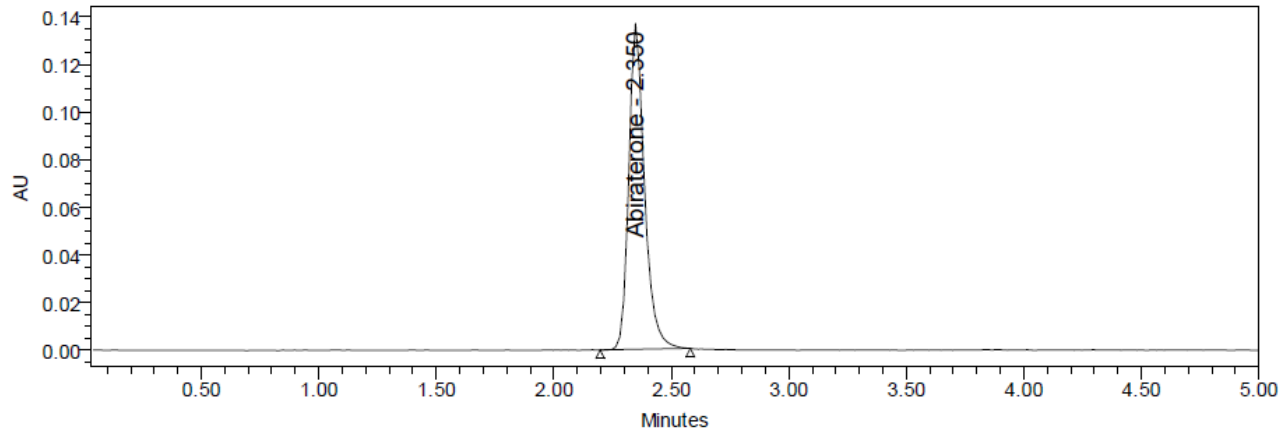

Figure 5: Typical chromatogram of Abiraterone

Interference from Degradation products (Forced degradation studies):
Degradation studies were performed with the formulation and degraded sample were injected. Assay of the injected sample was calculated and the entire samples passed the limits of degradation.

Table 5: Degradation data of Abiraterone

S.NO	Degradation Condition	% Drug UN Degraded	% Drug Degraded
1	Acid	93.18	6.82
2	Alkali	94.55	5.45
3	Oxidation	96.74	3.26
4	Thermal	97.22	2.78
5	UV	98.55	1.45

6	Water	98.55	1.45

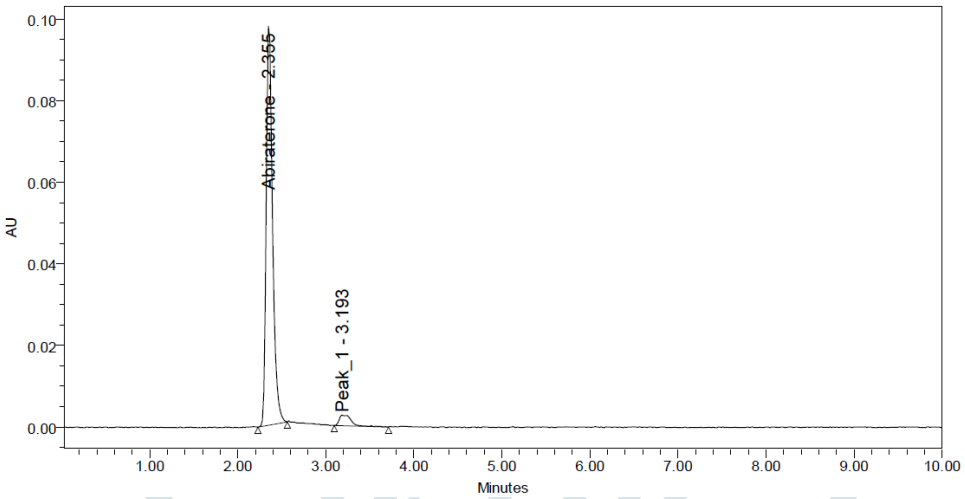

Figure 6: Typical chromatogram of Abiraterone in Acid degraded sample

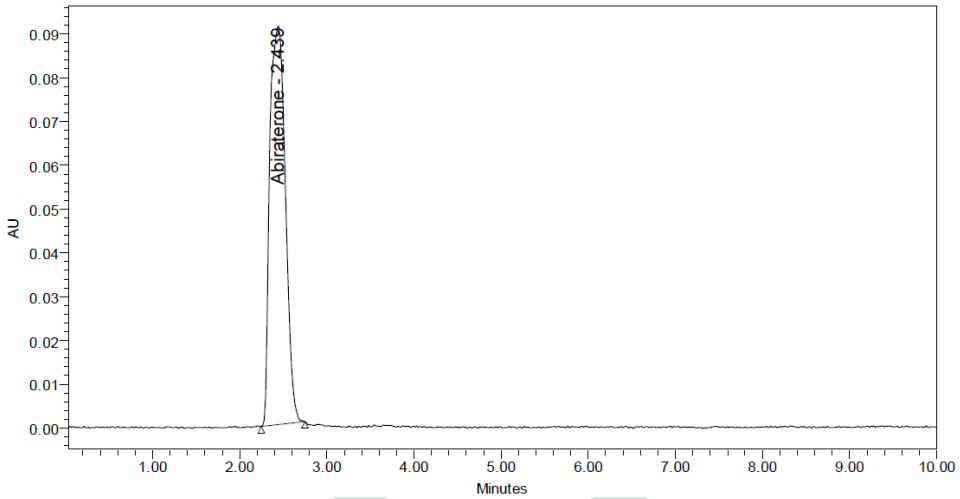

Figure 7: Typical chromatogram of Abiraterone in Base degraded sample

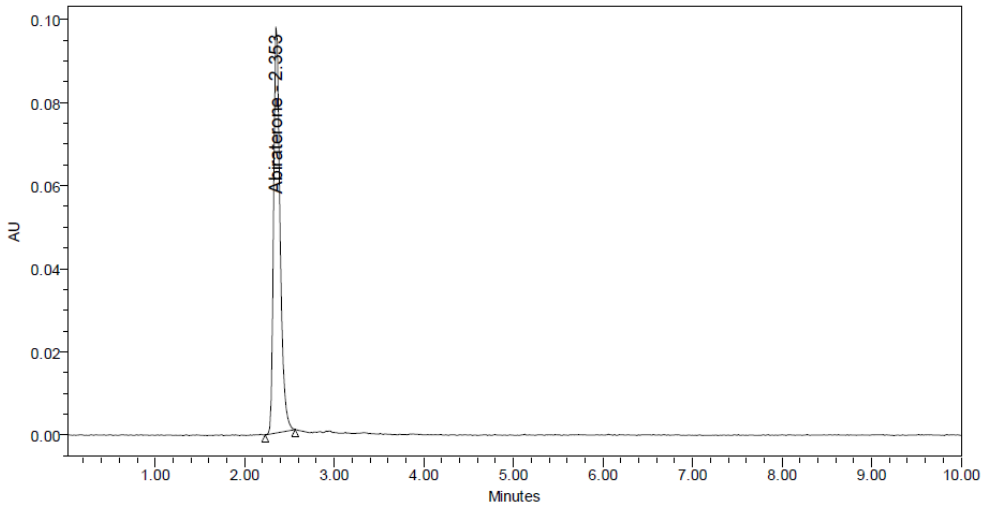

Figure 8:Typical chromatogram of Abiraterone in Peroxide degraded sample

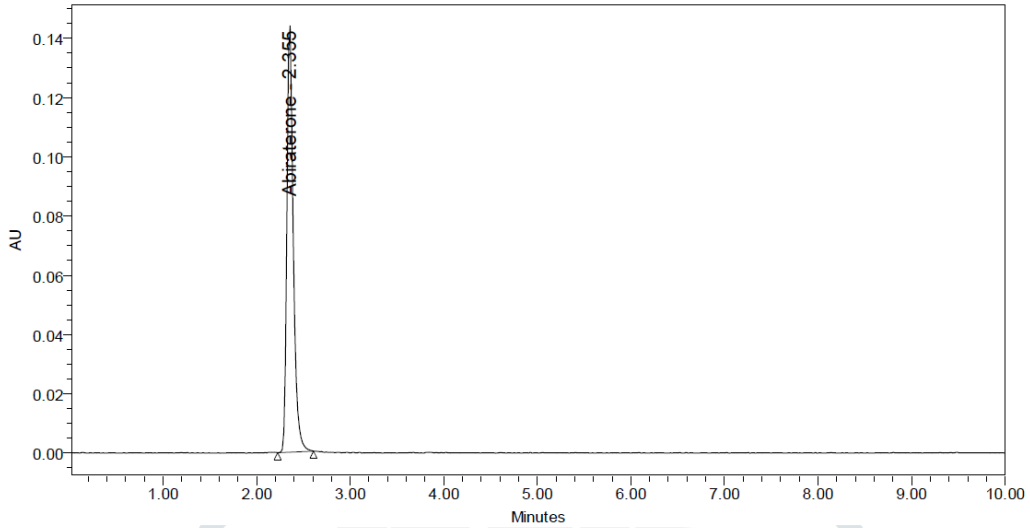

Figure 9:Typical chromatogram of Abiraterone in Water degraded sample

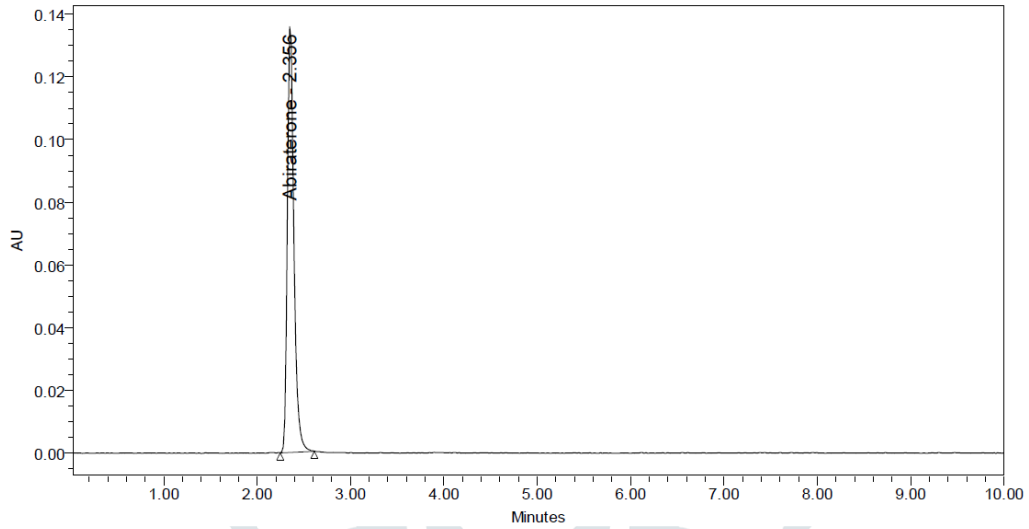

Figure 10:Typical chromatogram of Abiraterone in Thermal degraded Sample

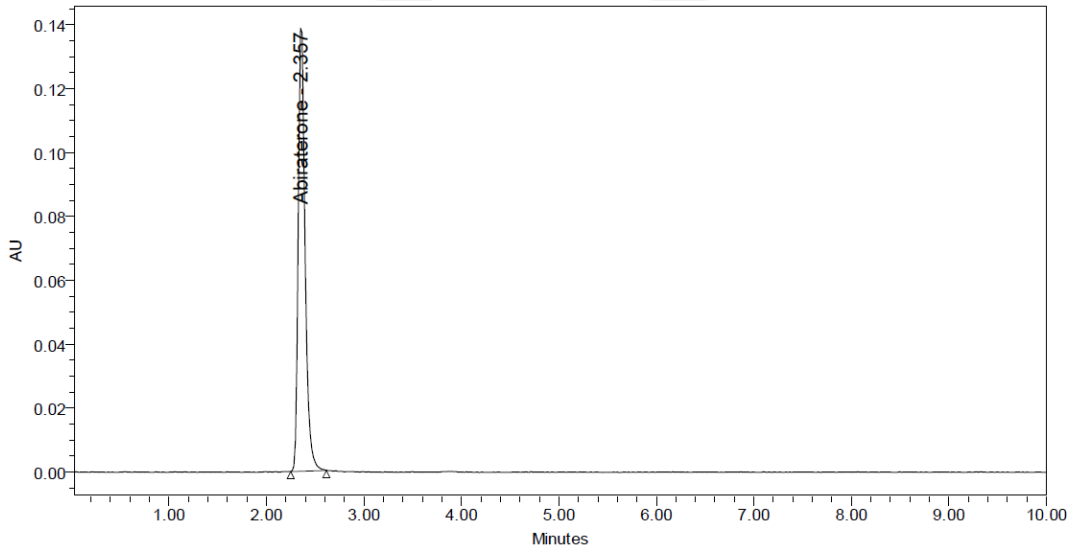

Figure 11:Typical chromatogram of Abiraterone in UV degraded sample

Accuracy:
Accuracy study was performed in triplicate (six replicate preparations for lower and higher concentrations) at different concentrations i.e. 50% to 150% of target sample concentration. Percent individual recovery, mean recovery, amount added and amount recovered were calculated.

Table 6: Accuracy results of Abiraterone

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	25	12.41	99.30	100.34%
	25	12.60	100.80	
	25	12.58	100.62	
100%	25	24.92	99.67	
	25	25.09	100.35	
	25	25.12	100.46	
150%	25	37.86	100.96	
	25	37.67	100.46	
	25	37.66	100.43	

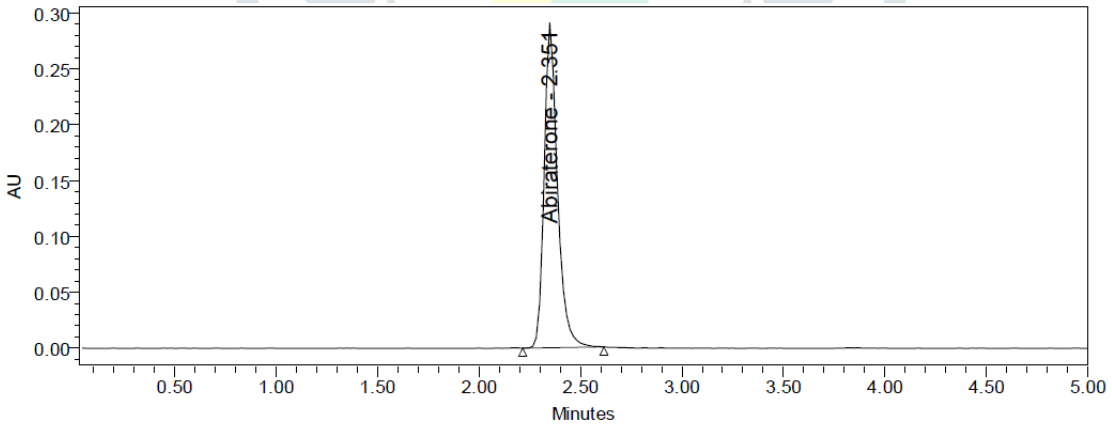

Figure 12:Accuracy 50% chromatogram of Abiraterone

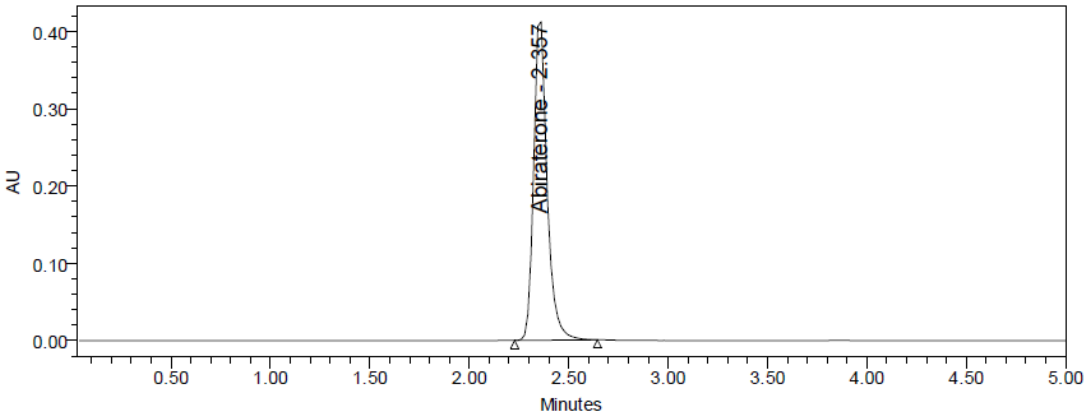

Figure 13: Accuracy 100% chromatogram of Abiraterone

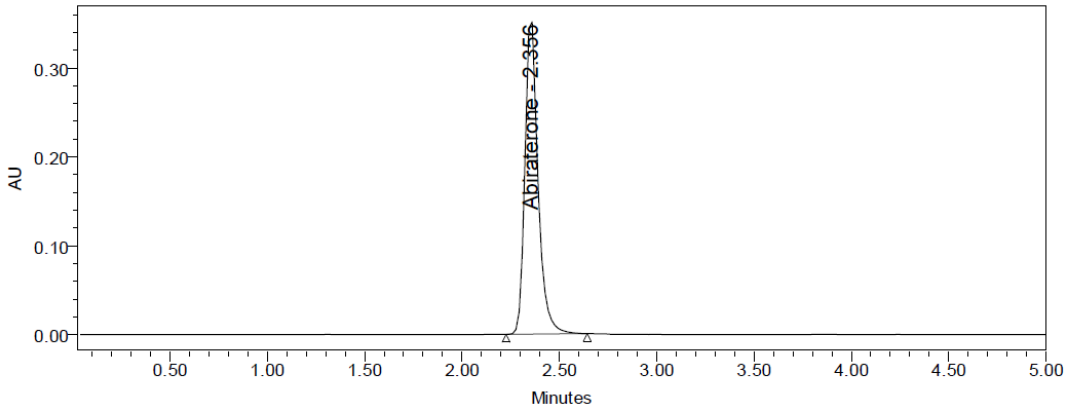

Figure 14: Accuracy 150% chromatogram of Abirateron

Precision:

Precision was evaluated by carrying out six different sample preparations and the results were found to be within the acceptance criteria.

Intraday precision:

Table 7: Intraday precision table of Abiraterone

S.No	Peak Area
1	669788
2	668456
3	662409
4	670000
5	669102
6	667538
AVG	667882
STDEV	2828.7
%RSD	0.4

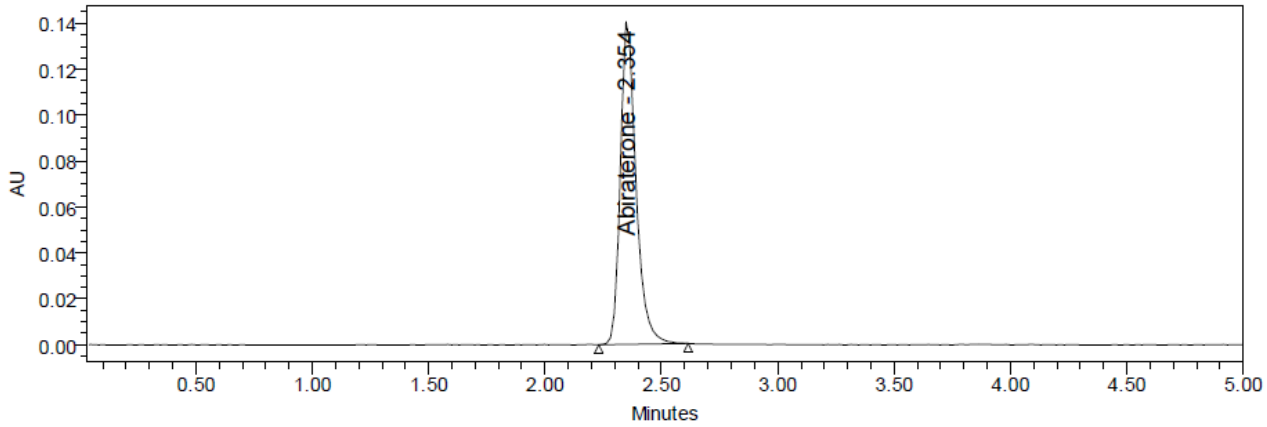

Figure 15: Intraday precision of Abiraterone

Intermediate precision:

The intermediate precision for Rivaroxaban compound was carried out on different day, by different analyst using different HPLC and different lot of column

Table 8: Intermediate precision table of Abiraterone

S.No	Peak Area
1	657084
2	658023
3	657911
4	655618
5	652393
6	653982
AVG	655835
STDEV	2279.7
%RSD	0.3

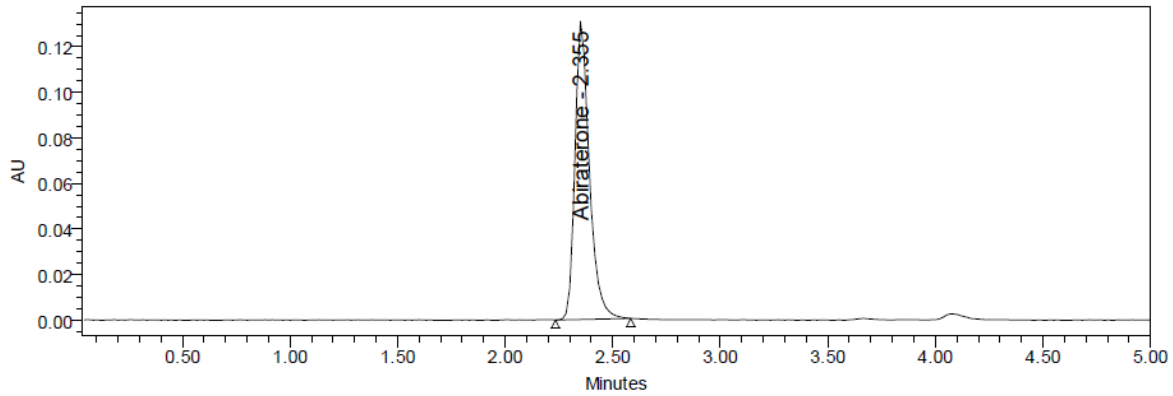

Figure 16: Intermediate precision graph of Abiraterone

Linearity:

To demonstrate the linearity of assay method, inject 5 standard solutions with concentrations of about 6.25ppm to 37.5ppm of Abiraterone . Plot a graph to concentration versus peak area. Slope obtained was y = 26265x + 3076 and Correlation Co-efficient was found to be 0.999.

Table 9: Linearity Concentration and Response

Linearity Level (%)	Concentration (ppm)	Area
0	0	0
25	6.25	170700
50	12.5	335701
75	18.75	481765
100	25	673973
125	31.25	820558
150	37.5	986138

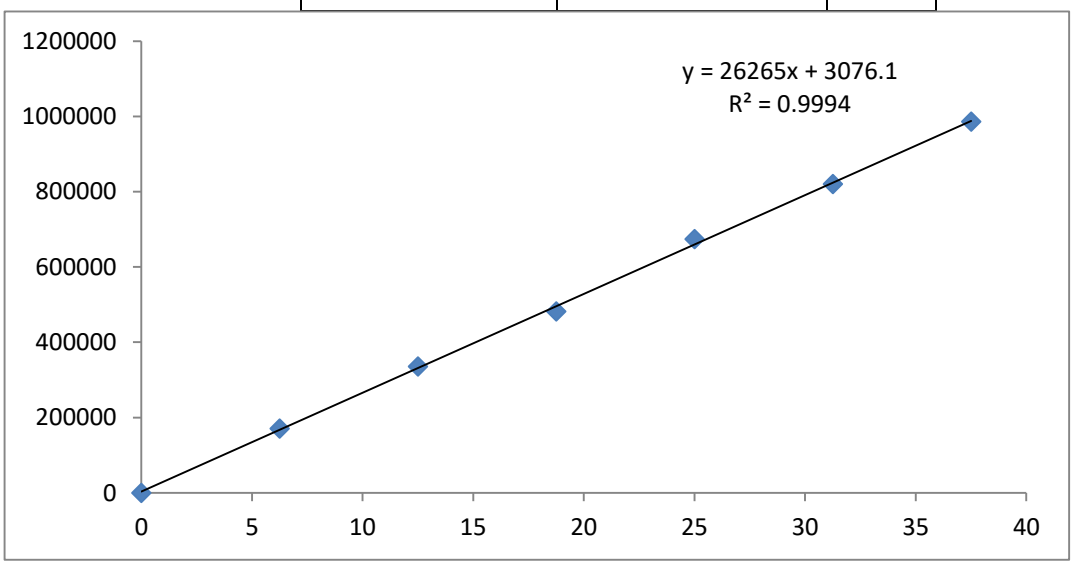

Figure 23: Linearity plot of Abiraterone

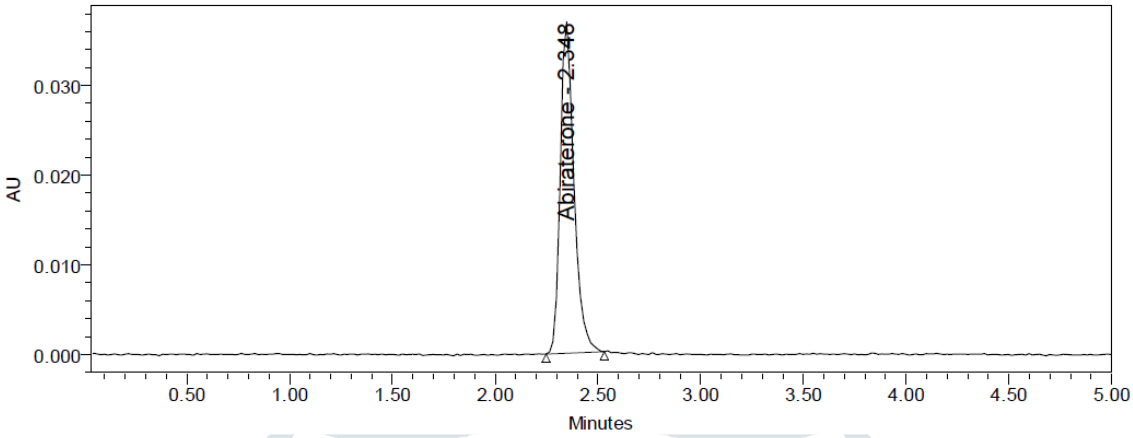

Figure 17: Linearity 25% chromatogram of Abiraterone

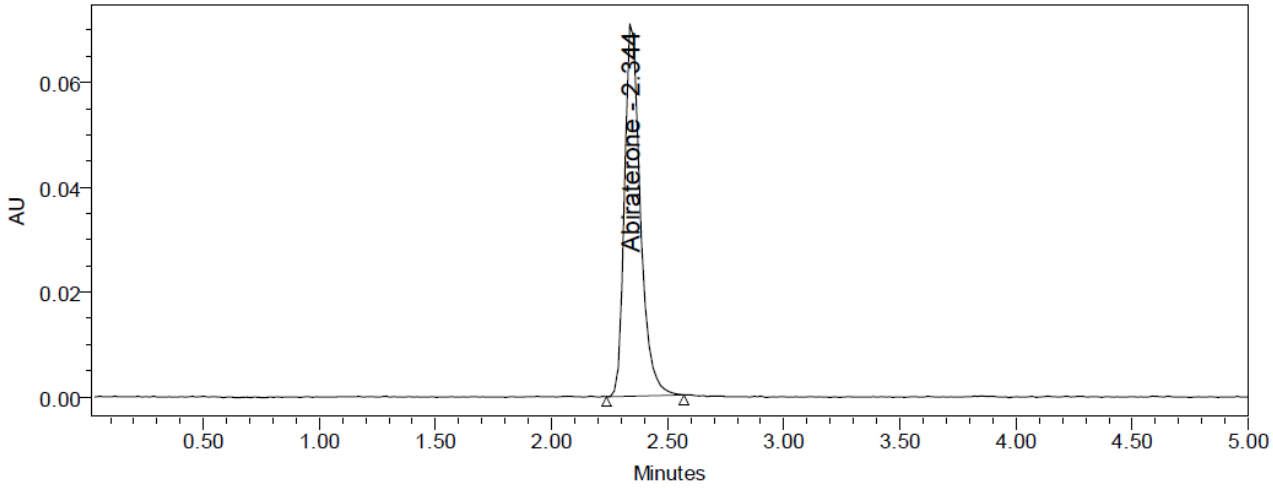

Figure 18: Linearity 50% chromatogram of Abiraterone

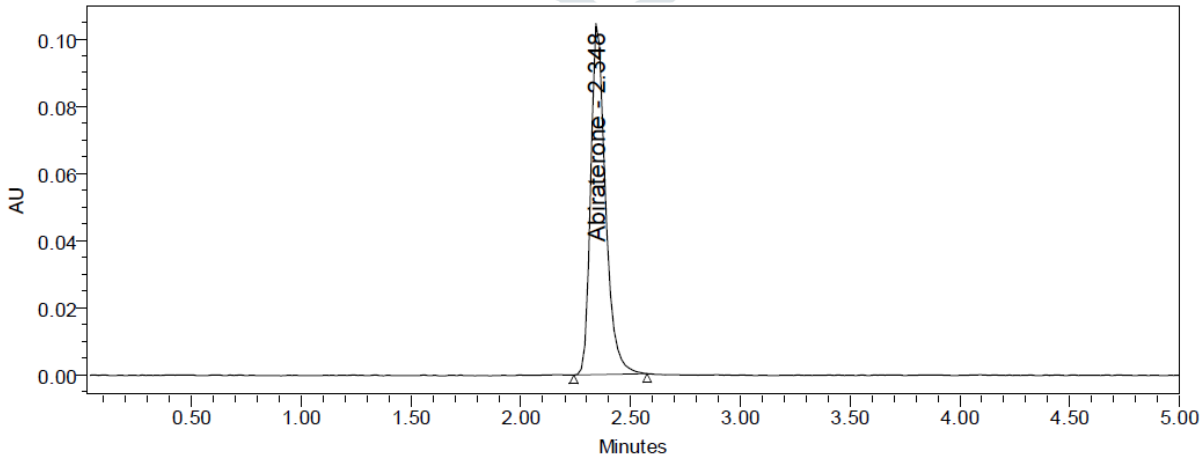

Figure19: Linearity 75% chromatogram of Abiraterone

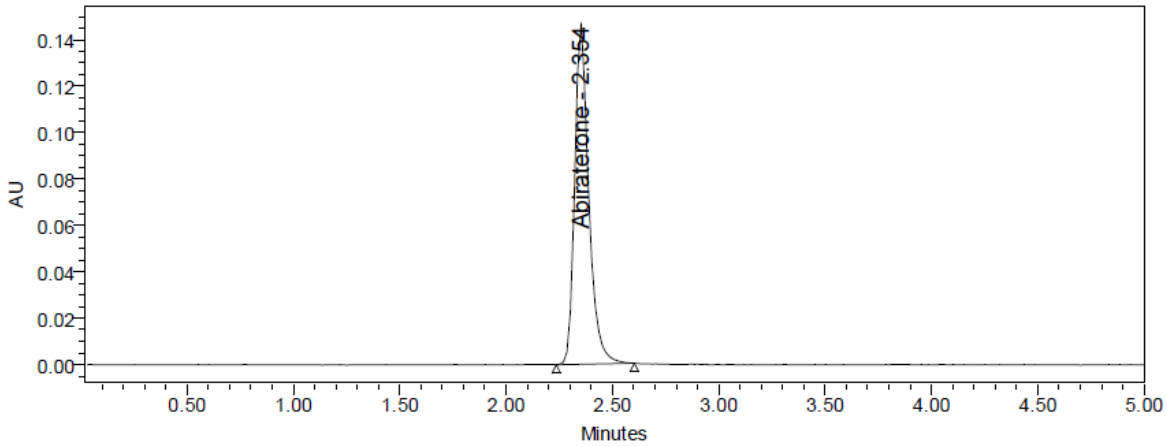

Figure 20: Linearity 100% chromatogram of Abiraterone

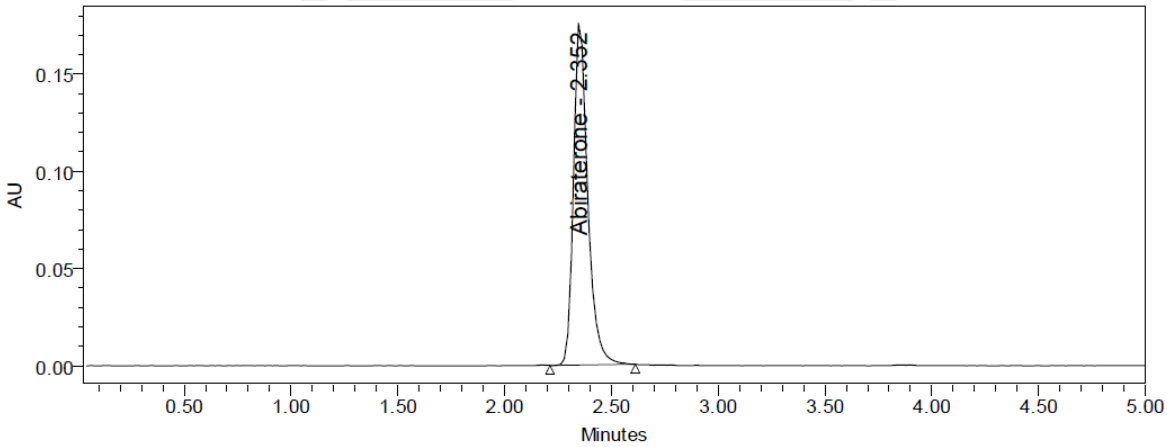

Figure 21: Linearity 125% chromatogram of Abiraterone

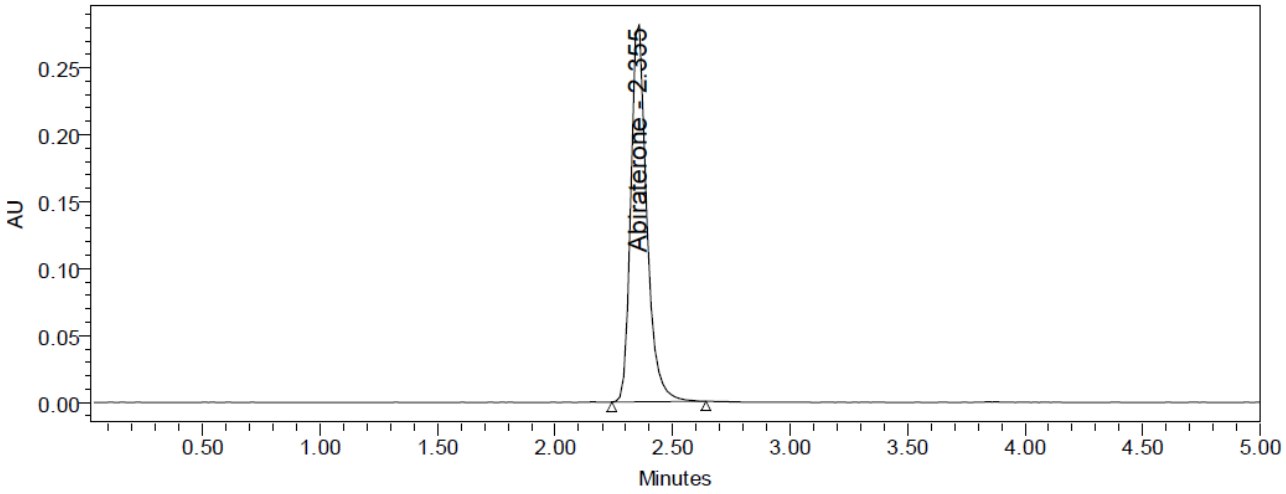

Figure 22: Linearity 150% chromatogram of Abiraterone

LOD: Ditection limit of the Abiraterone in this method was found to be 0.06µg/ml.

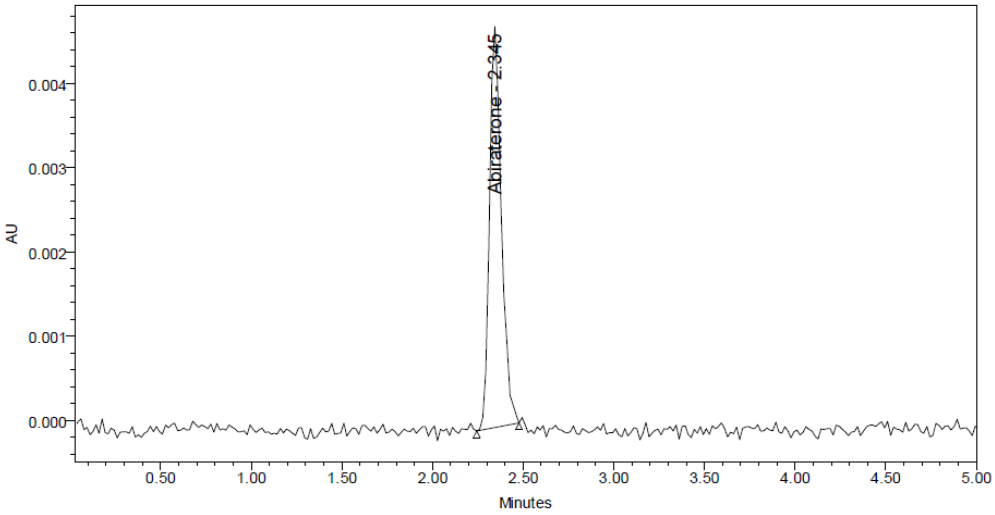

Fig 23:LOD Chromatogram of Abiraterone

LOQ: Quantification limit of the Abiraterone in this method was found to be 0.017µg/ml.

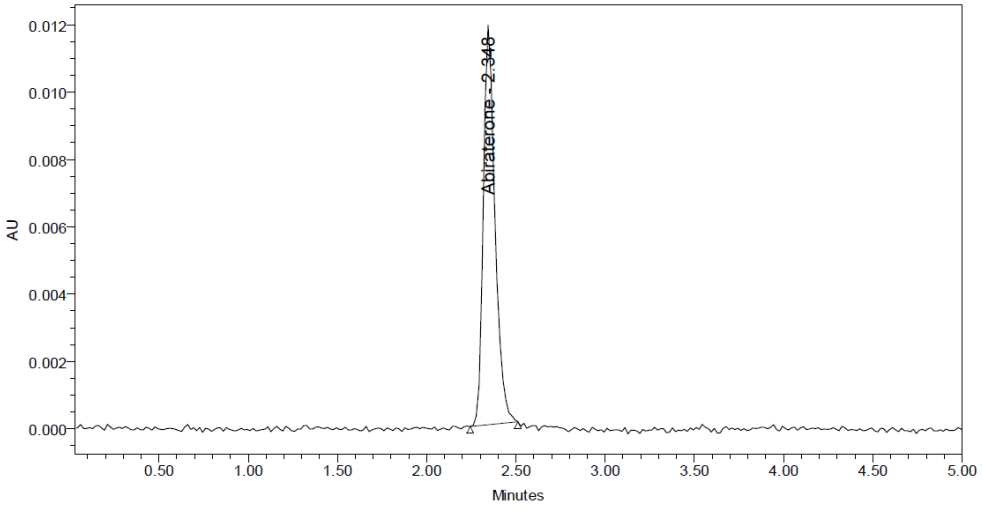

Fig 24: LOQ Chromatogram of Abiraterone

Robustness:

Small but deliberate changes in method like flow rate, column oven temperature, wavelength and filters were made but no recognized change in the results were observed and were within range as per ICH guidelines.

Table 10: Robustness Data

Parameter	%RSD
Flow Minus	0.5
Flow Plus	1.7
Mobile phase Minus	0.2

Mobile phase Plus	0.9
Temperature minus	1.0
Temperature plus	1.1

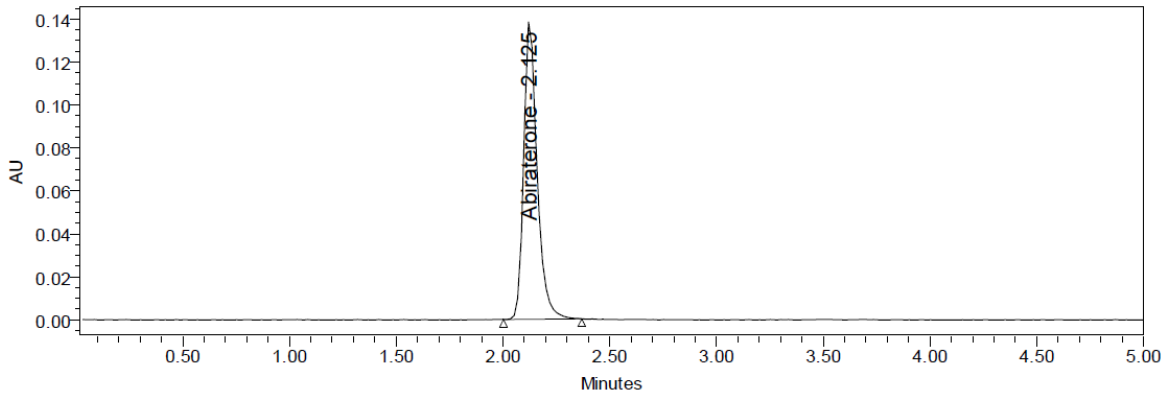

Figure 25: Flow plus (0.9 mL/min) chromatogram of Abiraterone

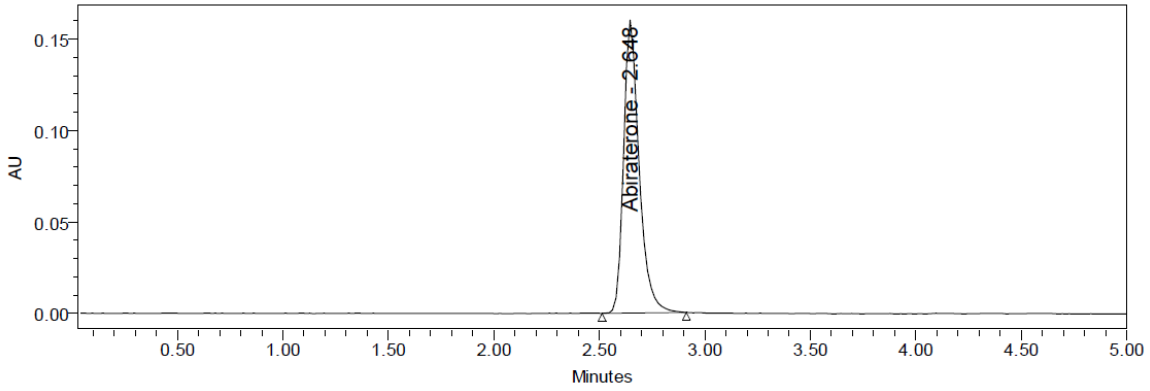

Figure 26: Flow minus (0.7 mL/min) chromatogram of Abiraterone

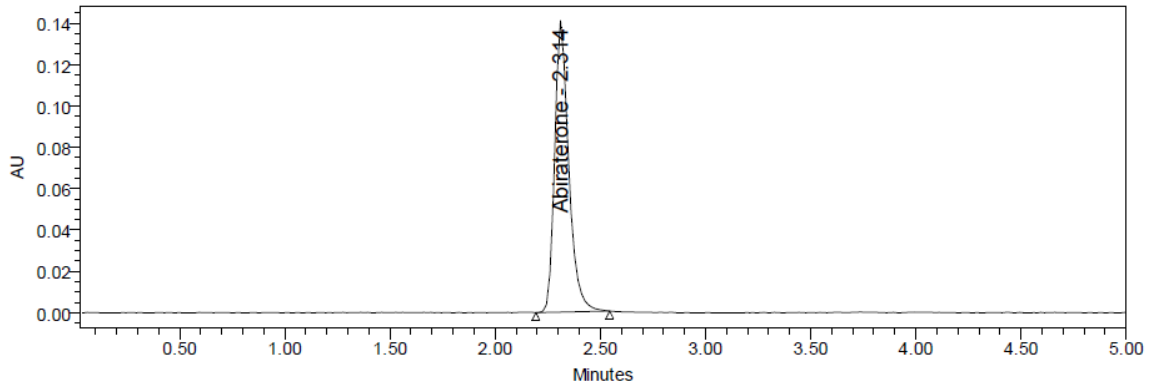

Figure 27: Column oven temperature plus (35°C) chromatogram of Abiraterone

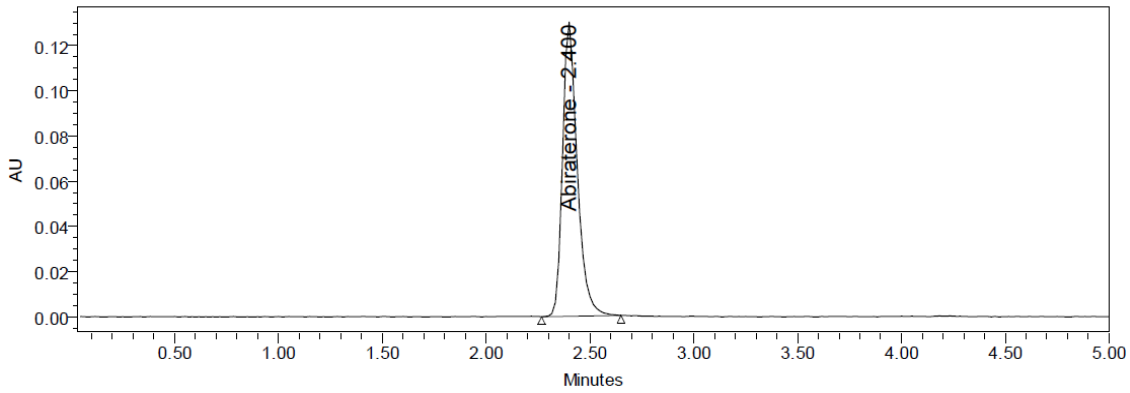

Figure 28: Column oven temperature minus (25° C) chromatogram of Abiraterone

Table 11: Validation summary data

Validation summary		
Parameters	**Results**	
Linearity Range (25% to 150%)	6.25 ppm to 37.5 ppm	
Regression line equation	26265x + 3076.1	
Slope	26265	
Intercept	3076.1	
Correlation coefficient (R^2)	0.9994	
%Accuracy	**%Recovery**	**%RSD**
50	100.24	0.8
100	100.16	0.4
150	100.61	0.3
Precision	**%RSD**	
Intraday precision n=6	0.4	
Interday precision n=6	0.3	
Robustness : No significant changes observed with deliberate changes in method parameters		

Table 12: Stress condition data

Stress condition	%Degradation
Initial (As such)	N/A
1ml 2N Sodium Hydroxide solution, 60°C 30 min.	5.45
1ml 2N Hydrochloric acid, heated at 60°C, 30 min.	6.82
1ml 20% Hydrogen peroxide, heated 60°C for 30 min.	3.26
1ml water, heated 60°C for 1 hr.	1.45
kept the standard and test solution in UV light(200 watt hr./m^2) for 1 day	1.45
Standard and samples were kept at 105°C for 1hr.	2.78

Conclusion

Chromatographic conditions used are stationary phase Symmetry Waters C18 (150mm*4.6mm 5µ.) Mobile phase 0.01N $Na_2HPO_4.H_2O$(5 pH): Acetonitrile in the ratio of 70:30v/v and flow rate was maintained at 0.8 ml/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 0.4 for repeatability and 0.3 for intermediate precision. LOD and LOQ are 0.06µg/ml and 0.17µg/ml respectively. By using above method assay of marketed formulation was carried out 99.61% 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.

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