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STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF APALUTAMIDE IN BULK AND ITS DOSAGE FORMS

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Apalutamide; RP-HPLC; Stability indicating; Validation

ABSTRACT

A Stability-indicating reverse phase-high performance liquid chromatographic (RP-HPLC) method was developed and validated for the determination of Apalutamide in tablet dosage form using C_{18} column phenomenix (250mm*4.6mm,5µm) with a mobilephase consisting of Methanol:2Mm Sodium Phosphate buffer pH 9.5(Adjusted with dil. NaOH)(25:75v/v).The mobilephase was sonicated for 10 minutes and filtered through a 0.45 µm membrane filter at a flow rate of 1ml/min. The detection was carried out at 272nm and retention time of apalutamide was found to be 3.273min.Method validation was Performed and Linearity was observed from50-150%. Apalutamide was subjected to stress conditions including acidic,alkaline, oxidative, photolysis,and thermal degradation and results showed that it was more sensitive towards Basic degradation.

INTRODUCTION

Apalutamide chemically known as4-[7-[6-cyano-5-(trifluoromethyl) pyridin-3-yl]-8-oxo-6-sulfanylidene-5,7-diazaspiro[3.4] octan-5-yl]-2-fluoro-N-methylbenzamide. Apalutamide, is a non steroidal antiandrogen (NSAA) medication which is used in the treatment of prostate cancer. It is specifically indicated for use in conjunction with castration in the treatment of non metastatic (NM-CRPC).Apalutamide is a potent androgen receptor (AR) antagonist that selectively binds to the ligand binding domain of AR and blocks AR nuclear translocation . It has been used for studying the treatment of Prostate Cancer, Hepatic impairment, Prostatic Neoplasms, Castration-Resistant Prostate Cancer, and Prostatic Neoplasms. Exerting an antitumour action, Apalutamide blocks the effect of androgens that promote tumour growth. It targets the AR ligand-bindingdomain and prevents AR nuclear translocation, DNA binding, and transcription of AR gene targets in prostate tumours. It is used for the treatment of patients with non-

metastatic prostate cancer that is resistant to treatment with hormone therapy(castration-resistant). It is available as oral tablets. Apalutamide is the first FDA-approved treatment for non-metastatic, castration-resistant prostate cancer. Extensive Literature reveals that there are no works reported on Apalutamide. So our Present work includes stability studies including Method Development and Validation of Apalutamide.

2. MATERIALS AND METHODS

2.1 Chemicals and reagents: The reagents used in this work were Methanol, HCL, NaOH (AR), and Hydrogen peroxide (3%) (AR), which were procured from, Merck India. Distilled water (HPLC) grade was obtained from Merck, Ltd; Mumbai.

2.2 Equipment: The instrument used in this study were electronic balance,sonicator,hot air oven,andDigital,p^Hmeter,HPLC(Shimadzu)was monitored and integrated using empower Software. Syringe system Additionally syringe[Hamiliton(Rheodye-20µL)]and syringe filter were used.

2.3 Chromatographic conditions: A number of HPLC systems were investigated to optimize the separation of apalutamide .HPLC system was composed of an prominence solvent delivery module,a manual rheodyne injector . With a 20µL fixed loop and an SPD-20A prominence UV- visible detector. Seperation was performed on a phenomenix C_{18} Column (250mm*4.6mm i.d; 5µm) phemomenex, Torrance at a ambient temperature.The mobile phase consisits of Methanol:2Mm Sodium Phosphate bufferpH 9.5(Adjusted with dil. NaOH)(25:75v/v). The Mobile phase was sonicated for 10 min and filtered through a 0.45µm membrane filter.The Mobilephase flow rate was maintained at 1ml/min and eluents were monitored .The samples were injected using a 20µL fixed loop.All determinations were performed at ambient temperature for a run time of 10 min.

2.4 Method development

2.4.1 Selection and preparation of mobilephase: Various mobilephase containing Methanol ,water in different ratios were tried with different ratios .Good symmetrical peak was found with the mobile phase comprising Methanol: Water in the ratio 25:75(v/v)(p^H was adjusted to 9.5 with dil.NaOH. Mobile phase was prepared by mixing 250 mL of HPLC grade methanol with 750 ml of water and the p^H was adjusted to 9.5 with dilute sodium hydroxide. Mobile phase was sonicated for 10 min and filtered through a 0.45µm membrane filter.

2.4.2 Preparation of standard stock solution

The standard stock solutions of 1000µg/ml of the drug were prepared by dissolving 50mg of the pure drug in the mobile phase in a 50ml volumetric flask and the volume was made up to the mark. Resulting solutions were further diluted with Mobile phase to obtain a final concentration of100µg/ml and stored under refrigeration.

2.4.3 Preparation of calibration curve

Aliquots of standard stock solutions were put in a 10ml volumetric flask and diluted up to the mark with mobilephase .In such a way ,the final concentration of the drug were in the range of 50-150 µg/ml.Triplicate injections of 20µL were made and analysed by chromatograph under the conditions as described above.Evaluation of the drugwas performed and peak areas were recorded .Calibration curves were constructed by plotting the peak area on y-axis against respective concentration of the drug on x-axis.The calibration curve was evaluated by its coefficient of determination(R^2).

2.5 Method validation: The developed method was validated by evaluating linearity, accuracy, precision, robustness, ruggedness, detection limit, quantification limit and stability. Coefficient of variation and relative errors of less than 2% were considered acceptable, except for the quantification limit, for which these values were established at 2%, as recommended in the literature

2.5.1 Linearity: A stock solution of Apalutamide of 1000 µg/mL was prepared with mobile phase.From it variousWorking standard solutions were prepared in the range of 50 to 150 µg/mL and injected into HPLC. It was shown that the selected drug had linearity in the range of 50-150 µg/mL.The calibration plot was generated by replicate analysis (n=9) at all concentration levels and the linear relationships was evaluated using the least square methpd within Microsoft program.

2.5.2. Accuracy

The Accuracy of the method was carried out using one set of different standard addition methods at different concentration levels, 50%, 100%, 150% and then comparing the difference between the spiked value (theatrical value) and actual found value.

2.5.3. Precision: The Precision of the method was ascertained from the peak area obtained by actual determination of six replicates of a fixed amount of the drug (50 µg/mL).The precision of the assay was also determined in terms of intra- and inter day variation in the peak areas of a set of drug solutions on three different days..The intra day and inter day variation in the peak area of the drug solution was calculated in terms of relative standard deviation.(RSD)

2-5.4 Robustness: Robustness of the proposed method for Apalutamide was carried out by the slight variation in flow rates ,p^H and mobilephase ratio. The percentage recovery and RSD were notes for Apalutamide.

2.5.5 Ruggedness: The test solutions were prepared as per test method and injected under variable conditions.Ruggedness of the method was studied by different analysts.

2.5.6 Detection limits and quantification limit

The limit of detection and limit of quantification were established based on the calibration curve parameters. According to the formula

LOD=3.3*S.D/slope

LOQ=10*S.D/slope

3. Forced degradation studies: The specificity of the method can be demonstrated through forced degradation studies conducted on the sample using Acid, Alkaline, Oxidative, Thermal studies, Photolytic and Ultraviolet Degradations. The sample was exposed to these conditions and the main peak was studied for the peak purity thus indicating that the method effectively separates the degradation products from pure active ingredients.

3.3.1- Degradation in acidic condition: Acid degradation of apalutamide was performed using 0.1N HCL.132.58 micrograms of the Apalutamide was weighed accurately and transferred into a 10 ml clean volumetric flask. The content of volumetric flask was dissolved in 5 ml of 0.1N HCL.then the volumetric flask was heated on a water bath at 65°c for 4 hours. Neutralize the sample with 0.1N NaOH and injected into HPLC system.

3.3.2. Degradation in Basic condition: Alkaline degradation was performed using 0.01M NaOH.132.58 micrograms of the Apalutamide was weighed accurately and transferred into a 10 ml clean volumetric flask. The content of volumetric flask was dissolved in 3 ml of 05N NaOH .then the volumetric flask was kept aside for 6 hours. Neutralize the sample with05N HCL and injected into HPLC system.

3.3.3 Oxidative degradation:

Alkaline degradation was performed using 0.01M NaOH132.58 micrograms of the Apalutamide was weighed accurately and transferred into a 10 ml clean volumetric flask. The content of volumetric flask was dissolved in 5ml of 3% H_2O_2.Then the volumetric flask was Kept aside for 1 day. and injected into HPLC system.

3.3.4 Photolytic degradation

132.58 micrograms of the Apalutamide was weighed accurately and transferred to a petridish then the closed petridish was placed

under direct sunlight for degradation.At different time intervals,10mg of sample was taken out .From it a stock solution of 1000µg/mL was prepared.Then it was sonicated for 5 min and diluted to obtain a working solution of 50 µg/mL in the mobilephase.it was then filtered through a 0.22µm filter and injected into the HPLC for analysis. The obtained chromatogram was observed for any degradation occurred during time

3.3.5.UV-degradation:

For UV degradation 100mg of the apalutamide was weighed accurately and transferred into a clean petridish .then the petridish was placed under a uv chamber 30cm at distance from the uv lamp After 3 hr the uv lamp was switched off and 10mg of sample was taken out .From it stock solution of 1000µg/mL was prepared.Then it was sonicated for 5 min and diluted to obtain a working solution of 50 µg/mLin the mobilephase.it was then filtered through a 0.22µm filter.

3.3.6 Thermal degradation:

Thermal degradation was performed by placing apalutamide in the incubator at 40°c.samples were drawn at different time intervals.132.58 weighed amount of sample was added to 5 ml of HPLC grade Methanol and heat at 80°C and sonicated for 5 min and injected into HPLC system.

4. DISCUSSION

In this RP-HPLC method the Linearity was within the range of 50-150µg/ml.The method was successfully validated in the optimized Conditions and the validation parameters were within the limits. In this chromatographic method , the LOD AND LOQ of Apalutmide was found to be 0.000023mg/ml,0.000076mg/ml. In the present study Apalutamide was subjected to stability studies under different conditions as per ICH guidelines.From acidic hydrolysis degradation peaks are observed at0.53 min and 3.023min along with the drug peak .The peak area showed that 9.18% of degradation occurred when the drug was kept in 0.1N HCL at 65°C for 4 hours.From alkaline degradation .Degradation peaks are observed at 3.023min along with the drug peak .The peak area showed that 11.60% of degradation occurred when the drug was kept in 0.5NaOH and kept aside for 4 hours. From Photolytic degradation Degradation peaks are observed at 2.52,2.62,4.32min along with the drug peak.The peak area showed that 7.73% of degradation occurred when the drug was exposed to sunlight and kept aside for 4 hours .From Oxidative degradation.Degradation peaks are observed at 2.52.4.1min along with the drug peak .The peak area showed that 10.80% of degradation occurred when the drug was treated with 3%H_2O_2 and kept aside for 1 day.From Thermal degradation Degradation peaks are observed at 2.52, 4.1min along with the drug peak .The peak area showed that 10.80% of degradation occurred when the drug was treated with 3%H_2O_2 and kept aside for 1 day.

5. CONCLUSION

The developed RP-HPLC method was found to be suitable for the analysis of Apalutamide in bulk form and was found to be simple,reliable,sensitive,economical and precise.The drug Apalutamide was found to be degraded when exposed to basic hydrolysis as it is degraded by 11.60%and least degraded by thermal degradation.Therefore ,this RP-HPLC method for estimation of Apalutamide can be used in various Laboratories for its quantitative determination in bulk and pharmaceutical dosage forms.

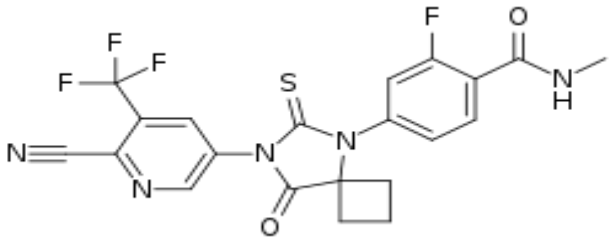


Figure 1:Chemical structure of Apalutamide

Table 1: Optimized chromatographic conditions of Apalutamide

Stationary Phase	Waters C_{18}, 250×4.6mm, 5µ, analytical column
Mobile Phase	2mM Sodium phosphate buffer pH 9.5(Adjusted with dil. NaOH): Methanol 75:25 v/v,
Flow rate	1.0 mL/min.
Detection Wavelength	272 nm.
Detector	Photo diode array
Injection Mode	Auto sampler
Injection volume	10 µl
Column Temperature	Ambient
Retention time	3.273 min.
Diluent	Mobile Phase

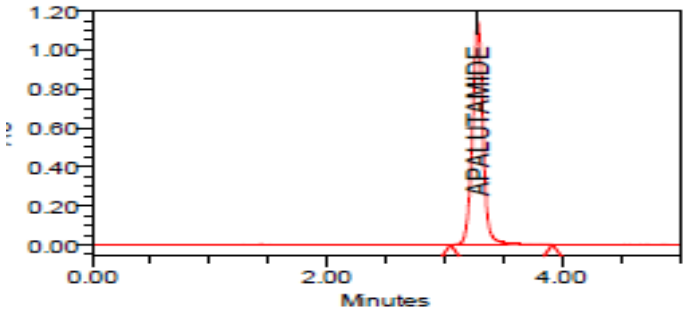


Figure 2: Optimised chromatogram of Apalutamide

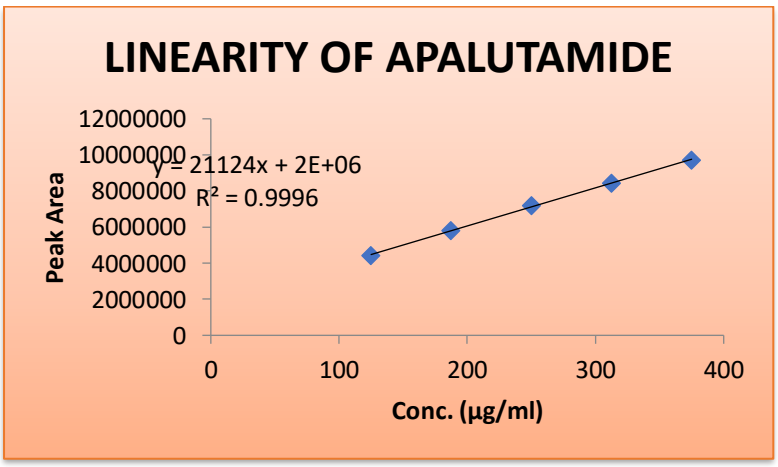


Figure 3: Linearity of Apalutamide

Table 2: Linearity studies of Apalutamide

Level	Conc (µg/ml)	Peak Area
LINEARITY50%	125	4442136
LINEARITY75%	187.5	5796681
LINEARITY100%	250	7189562
LINEARITY125%	312.5	8436630
LINEARITY150%	375	9723328

Table 3: Accuracy of apalutamide

Sample No.	Spiked Level	Sample Weight (mg)	Sample Area	µg/ml added	µg/ml found
1	50	66.29	3531749	124.97	123.58
2	50	66.29	3611606	124.97	126.37
3	50	66.29	3573683	124.97	125.04
4	50	66.29	3530428	124.97	123.53
5	50	66.29	3513458	124.97	122.94
6	50	66.29	3552716	124.97	124.31
7	100	132.58	7194479	249.94	251.74
8	100	132.58	7161061	249.94	250.57
9	100	132.58	7161150	249.94	250.57
10	150	198.87	10639927	374.91	372.29
11	150	198.87	10608563	374.91	371.20
12	150	198.87	10685958	374.91	373.90
13	150	198.87	10675024	374.91	373.52
14	150	198.87	10663028	374.91	373.10
15	150	198.87	10704679	374.91	374.56

Table 4: System suitability

S. No	STD Area	Theoritical Plates	Tailing Factor	RT
1	7289022	6839	1.00	3.282
2	7216189	6755	1.00	3.283
3	7154002	6616	0.99	3.276
4	7092252	6499	1.00	3.282
5	6972842	6576	0.99	3.278
Avg	7144861	6657	1.00	3.28
SD	120739	137.78	0.01	0.0030
%RSD	1.690	2.070	0.550	0.092

Table 5: System precision

S. No	Sample Weight	Sample Area	% Assay
1	132.58	7122699	99.69
2	132.58	7142606	99.97
3	132.58	7118974	99.64
4	132.58	7176367	100.44
5	132.58	7165881	100.30
6	132.58	7132006	99.82
Average Assay:			99.98
STD			0.33
% RSD			0.33

Table 6: Method precision

S. No	Sample Weight	Sample Area	% Assay
1	132.58	7165874	100.30
2	132.58	7186524	100.59
3	132.58	7198745	100.76
4	132.58	7175896	100.44
5	132.58	7174524	100.42
6	132.58	6974965	100.42
Avarage Assay:			100.49
STD			0.16
% RSD			0.16

Table 7: Results of LOD and LOQ

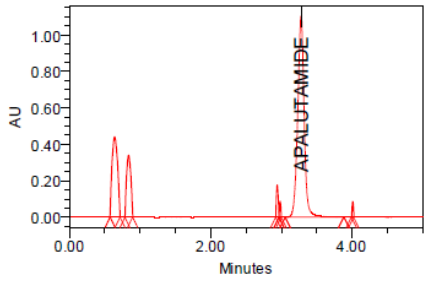
LOD	0.000023	mg/ml
LOQ	0.000076	mg/ml

The LOD AND LOQ of apalutamide were determined to be 0.000023mg/ml,0.000076mg/ml

Table 8: Results of robustness studies

S.No	**Condition**	**Peak area**	**% Assay**
1	0.8 ml/min	7108459	99.49
2	1 ml/min	7156845	100.17
3	1.2 ml/min	7157652	100.18
4	30 °C	7125339	99.73
5	35°C	7156845	100.17
6	40 °C	7142750	99.97

Acid studies



Base studies

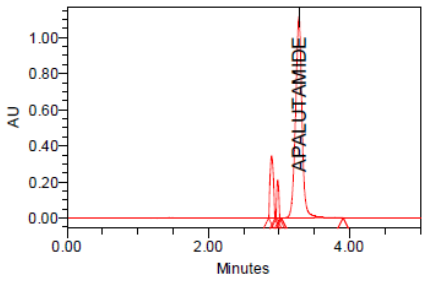
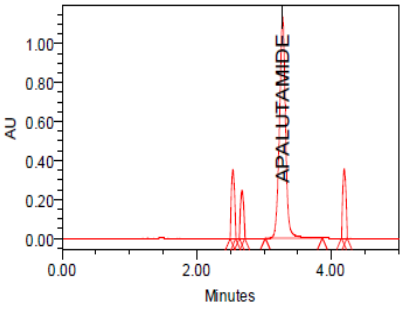


Figure 4: Acid degradation of Apalutamide

Figure 5: Base degradation of Apalutamide Photolytic studies

Thermal studies



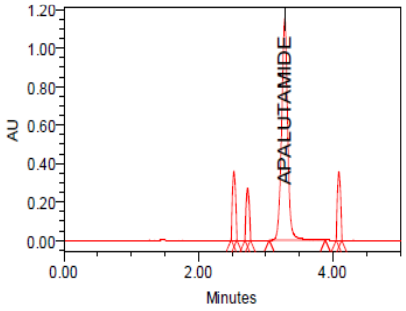


Fig 6: Photolytic degradation of Apalutamide

Fig 7: Thermal degradation of Apalutamide

Oxidative studies

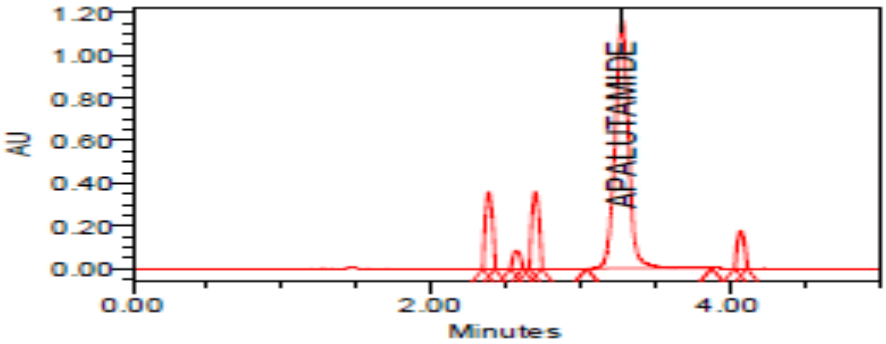


Fig 8: Oxidative degradation of Apalutamide

Table 9: Result of Forced degradation studies

Nature of the Sample	Sample Weight	Sample Area	% Assay	% of Degradation
Acid	132.58	6489022	90.82	9.18
Base	132.58	6316189	88.40	11.60
UV	132.58	6354002	88.93	11.07
Heat	132.58	6592252	92.27	7.73
H2O2	132.58	6372842	89.20	10.80

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