INTERNATIONAL JOURNAL OF PHARMACY AND ANALYTICAL RESEARCH

ISSN: 2320-2831

IJPAR |Vol.10 | Issue 3 | July - Sep -2021
Journal Home page: www.ijpar.com

Research Study Open Access

A novel analytical development and validation of an rp-hplc assay method for the quantification of apalutamide in bulk and its marketed formulation

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ABSTRACT

A simple, rapid, specific and accurate reverse phase high performance liquid chromatographic method has been developed for the validated of Apalutamide in bulk as well as in marketed pharmaceutical dosage form. This separation was performed on a Symmetry ODS C18 (4.6×250mm, 5µm) column with Methanol: Phosphate Buffer (35:65) V/V as mobile phase at a flow rate of 1.0 mL min−1 with UV detection at 235 nm; the constant column temperature was Ambient. The run time under these chromatographic conditions was less than 8 min. The retention time of Apalutamide was found to be 2.252. The calibration plot was linear over the concentration range of 6–14 µg mL−1 with limits of detection and quantification values of 1.2 and 3.6 ng mL−1 respectively. The mean % assay of marketed formulation was found to be 99.86%, and % recovery was observed in the range of 98-102%. Relative standard deviation for the precision study was found <2%. The developed method is simple, precise, specific, accurate and rapid, making it suitable for estimation of Apalutamide in bulk and marketed pharmaceutical dosage form dosage form.

Keywords: Apalutamide, RP-HPLC, Validation, ICH Guidelines.

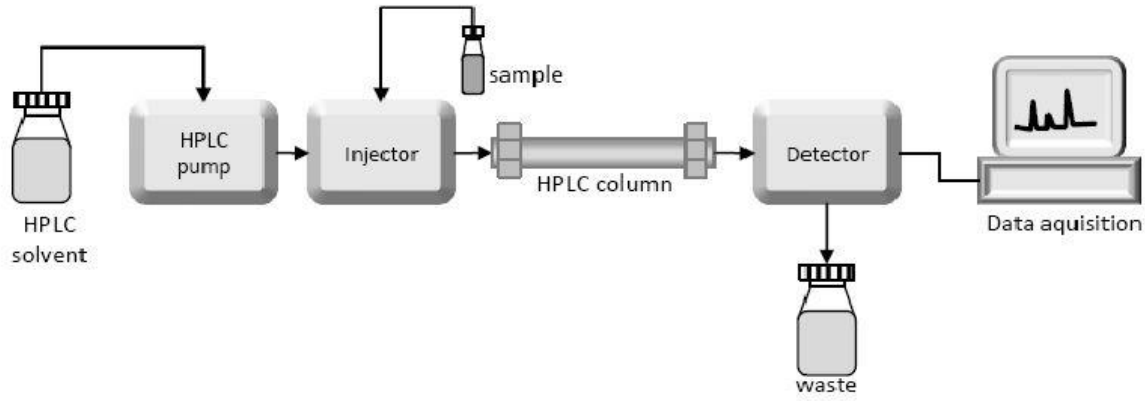
INTRODUCTION

High Pressure Liquid Chromatography (HPLC)

High Performance Liquid Chromatography (HPLC) is a form of column chromatography that pumps a sample mixture or analyte in a solvent (known as the mobile phase) at high pressure through a column with chromatographic packing material (stationary phase). The sample is carried by a moving carrier gas stream of helium or nitrogen. HPLC has the ability to separate, and identify compounds that are present in any sample that can be dissolved in a liquid in trace concentrations as low as parts per trillion. Because of this versatility, HPLC is used in a variety of industrial and scientific applications, such as pharmaceutical, environmental, forensics, and chemicals.

High-performance liquid chromatography (HPLC; formerly referred to as high-pressure liquid chromatography) is a technique in analytical chemistry used to separate, identify, and quantify each component in a mixture. It relies on pumps to pass a pressurized liquid solvent containing the sample mixture through a column filled with a solid adsorbent material. Each component in the sample interacts slightly differently with the adsorbent material, causing different flow rates for the different components and leading to the separation of the components as they flow out of the column. HPLC has been used for manufacturing (e.g., during the production process of pharmaceutical and biological products), legal (e.g., detecting performance enhancement drugs in urine), research (e.g., separating the components of a complex biological sample, or of similar synthetic chemicals from each other), and medical (e.g., detecting vitamin D levels in blood serum) purposes.[1]

Instrumentation of HPLC



Advantages

- HPLC separations can be accomplished in a minutes, in some cases even in seconds.
- High resolution of complex sample mixture into individual components.
- Rapid growth of HPLC is also because of its ability to analyze substances that are unsuitable for gas liquid chromatographic (GLC) analysis due to non-volatility or thermal-instability.
- Quantitative analysis is easily and accurately performed and errors of less than 1 % are common to most HPLC methods.
- Depending on sample type and detector used, it is frequently possible to measure 10^{-9} g or 1 ng of sample. With special detectors, analysis down to 10-12 pg has been reported.

The primary objective of proposed work is

- ✓ To develop new simple, sensitive, accurate and economical analytical method for the estimation of Apalutamide in bulk and marketed pharmaceutical dosage form.
- ✓ To validate the proposed method in accordance with USP and ICH guidelines for the intended analytical application i.e., to apply the proposed method for analysis of the Apalutamide in bulk and marketed pharmaceutical dosage form.

MATERIALS

Table-1: Instruments used

S. No	Instruments and Glass wares	Model
1	HPLC	WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA detectors.
2	pH meter	Lab India
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital ultra sonicator	Labman

Table-2: Chemicals used

S. No	Chemical	Brand names
1	Apalutamide (Pure)	Sura labs
2	Water and Methanol for HPLC	LICHROSOLV (MERCK)
3	Acetonitrile for HPLC	Merck

METHODOLOGY

HPLC METHOD DEVELOPMENT

Preparation of standard solution

Accurately weigh and transfer 10 mg of Apalutamide working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with Methanol.
Further pipette 0.1ml of the above Apalutamide stock solution into a 10ml volumetric flask and dilute up to the mark with Methanol.

VALIDATION

PREPARATION OF BUFFER AND MOBILE PHASE

Preparation of Potassium dihydrogen Phosphate (KH2PO4) buffer (pH-3.6)

Dissolve 6.8043 of potassium dihydrogen phosphate in 1000 ml HPLC water and adjust the pH 3.6 with diluted orthophosphoric acid. Filter and sonicate the solution by vacuum filtration and ultra sonication.

Preparation of mobile phase

Accurately measured 350 ml (35%) of Methanol, 650 ml of Phosphate buffer (65%) were mixed and degassed in digital ultra sonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluents Preparation

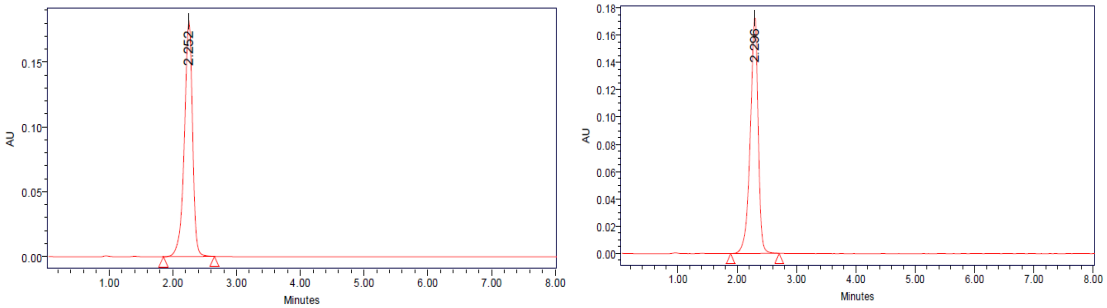
The Mobile phase was used as the diluents

OPTIMIZED CHROMATOGRAPHIC CONDITIONS

Instrument used	:	Waters HPLC with auto sampler and PDA detector 996 model.
Temperature	:	Ambient
Column	:	Symmetry ODS C18 (4.6×250mm, 5µm)
Mobile phase	:	Methanol: Phosphate Buffer pH-3.6 (35:65)
Flow rate	:	1ml/min
Wavelength	:	235nm
Injection volume	:	10µl
Run time	:	8minutes

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard) Optimized Chromatogram (Sample)



Observation: In this trial it shows proper separation of peak and more plate count in the chromatogram and the tailing factor is within the limit. So it is an optimized chromatogram.

Acceptance criteria

- Theoretical plates must be not less than 2000.
- Tailing factor must be not less than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.

METHOD VALIDATION

System Suitability

Table-3: Results of system suitability for Apalutamide

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Apalutamide	2.277	1652847	185647	6589	1.24
2	Apalutamide	2.277	1653658	186254	6587	1.26
3	Apalutamide	2.267	1654521	185475	6584	1.28
4	Apalutamide	2.265	1653564	186594	6582	1.29
5	Apalutamide	2.277	1658745	185684	6895	1.24
Mean			1654667			
Std.Dev.			2355.764			
%RSD			0.142371			

Specificity

Table-4: Peak results for assay standard

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Apalutamide	2.265	1658254185468		1.24	6391	1
2	Apalutamide	2.267	1658475184524		1.23	6549	2
3	Apalutamide	2.267	1658471186598		1.25	6682	3

Table-5: Peak results for Assay sample

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Apalutamide	2.246	1645879184574		0.85	6458	1
2	Apalutamide	2.246	1645875183598		0.86	6584	2
3	Apalutamide	2.246	1658423185472		0.85	6457	3

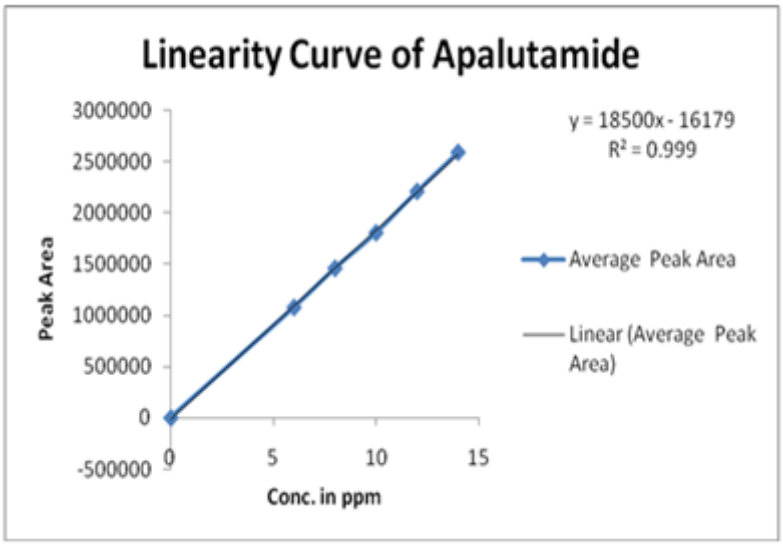
$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Apalutamide in pharmaceutical dosage form was found to be 99.86%.

Linearity

Table-6: Data for Linearity of Apalutamide

Concentration µg/ml	Average Peak Area
6	1078475
8	1461129
10	1808358
12	2211573
14	2593778



CONCLUSION

Correlation Coefficient (r) is 0.99, and the intercept is 0.16179. These values meet the validation criteria.

Precision

Repeatability

Table-7: Results of repeatability for Apalutamide

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Apalutamide	2.293	1658954	186958	1.26	6785
2	Apalutamide	2.276	1658745	187548	1.27	6854
3	Apalutamide	2.286	1659865	189854	1.26	6852
4	Apalutamide	2.277	1653254	186985	1.25	6784
5	Apalutamide	2.280	1654781	189542	1.24	6895
Mean			1657120			
Std.dev			2913.592			
%RSD			0.175823			

Intermediate precision:

Table-8: Results of Intermediate precision for Apalutamide by Analyst 1

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Apalutamide	2.274	1678541	186589	6587	1.26
2	Apalutamide	2.258	1685985	186598	6321	1.26
3	Apalutamide	2.267	1685745	186985	6385	1.25
4	Apalutamide	2.270	1685987	187854	6580	1.26

5	Apalutamide	2.264	1698526	187549	6721	1.27
6	Apalutamide	2.265	1685943	186598	6637	1.26
Mean			1686788			
Std.Dev.			6463.466			
%RSD			0.383182			

Table-9: Results of Intermediate precision Analyst 2 for Apalutamide

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Apalutamide	2.277	1665847	167481	6854	1.25
2	Apalutamide	2.255	1658989	167854	6785	1.26
3	Apalutamide	2.265	1659845	167895	6854	1.24
4	Apalutamide	2.255	1665964	167854	6895	1.26
5	Apalutamide	2.253	1659863	168585	6459	1.25
6	Apalutamide	2.252	1665986	167859	6456	1.26
Mean			1662749			
Std.Dev.			3501.766			
%RSD			0.210601			

Acceptance criteria

- %RSD of Six different sample solutions should not more than 2.

Accuracy

Table-10: The accuracy results for Apalutamide

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	109068.3	5	5.021	100.420%	100.72%
100%	202187	10	10.054	100.540%	
150%	297032.3	15	15.181	101.206%	

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Limit Of Detection For Apalutamide

LOD= 3.3 × σ / s

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Result

= 1.2µg/ml

Limit Of Quantitation For Apalutamide

LOQ=10×σ/S

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Result

= 3.6µg/ml

Robustness

Variation in flow

Table-11: Results for Robustness

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	1658242	2.312	6569	1.24
Less Flow rate of 0.9 mL/min	1854215	2.458	6865	1.35
More Flow rate of 1.1 mL/min	1758468	2.032	6254	1.32

Acceptance criteria

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Apalutamide in bulk drug and pharmaceutical dosage forms.

This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps.

Apalutamide was freely soluble in methanol, ethanol, and chloroform, soluble in ether, sparingly soluble in acetonitrile and octanol, and practically insoluble in water.

Methanol: Phosphate Buffer (35:65) V/V was chosen as the mobile phase. The solvent system used in this method was economical.

The %RSD values were within 2 and the method was found to be precise.

The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods.

This method can be used for the routine determination of Apalutamide in bulk drug and in Pharmaceutical dosage forms.

ACKNOWLEDGEMENT

The Authors are thankful to Sura Labs, Dilshukhnagar, and Hyderabad for providing the necessary facilities for the research work.

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