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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF BICALUTAMIDE IN PHARMACEUTICAL FORMULATION USING RP-HPLC

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ABSTRACT

The estimation of Bicalutamide in tablets was done by Reverse Phase HPLC. The mobile phase used consists of Buffer containing sodium dihydrogen phosphate and mobile phase ratio of Sodium dihydrogen phosphate (pH 3.5) : Acetonitrile(50:50 v/v). A C18 column containing Octadecyl silane (ODS) chemically bonded to porous silica particles (250 × 4.6mm, 5µ particle size) was used as the stationary phase. The detection was carried out using UV detector set at 271nm. The solutions are chromatographed at a constant flow rate of 1.3 ml/ min. The retention time for Bicalutamide was around 9.5 min. The quantitative estimation was carried out on the tablets using RP HPLC. The quantitative results obtained are subjected to the statistical validation. The values of %RSD are less than 2.0%, indicating the accuracy and precision of the method. The percentage recoveries were found to be 99.7% to 101.6% for Bicalutamide.

Key words: Bicalutamide, RP-HPLC, Validation.

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INTRODUCTION

Bicalutamide (*N*-[4-cyano-3-(trifluoromethyl)phenyl]-3-[(4-fluorophenyl)sulfonyl]- 2-hydroxy-2-ethylpropanamide) is an oral non-steroidal antiandrogen (Fig-1).

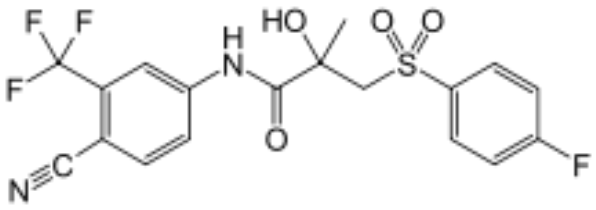

Fig-1 Structure of Bicalutamide

Bicalutamide acts as a pure anti-androgen by binding to the androgen receptor(AR) and preventing the activation of the AR and subsequent upregulation of androgen responsive genes by androgenic hormones. In addition, bicalutamide accelerates the degradation of the androgen receptor. Bicalutamide has been used as a molecular template for the design of selective androgen receptor modulators (SARMs) such as Andarine and Ostarine Literature survey reveals the availability of some methods for the estimation of Bicalutamide includes UV spectrometry and RP-HPLC. Only very few HPLC estimations have been reported in the literature for the determinations of Bicalutamide present in bulk and formulations (1-8). The objective of this experiment was to optimize the assay method for estimation of Bicalutamide in tablets based on the literature survey made. So here planned to develop accurate, precise, specific method for estimation of Bicalutamide in tablets.

MATERIALS AND METHODS

Optimized method

Buffer preparation: 0.5g of sodium dihydrogen phosphate was dissolved in 10ml of HPLC grade water , sonicate and make up to 100ml with water and adjust pH to 3.0 with ortho phosphoric acid.

Mobile phase: Prepare a filtered and degassed mixture of buffer and acetonitrile in the ratio of 50:50 v/v.

Chromatographic conditions

Flow rate-1.3ml/min, Detector wave length-271nm, Column temperature-25^{o}C, Injection volume-20µl, Run time-12 mins.

Standard preparation

10 mg of Bicalutamide was accurately weighed into 10ml volumetric flask and volume is made up to the mark with mobile phase. Solution was cooled to room temperature. 0.5 ml of above solution transferred into 10ml volumetric flask and diluted to volume with methanol.

Sample preparation

Portion of the powder, equivalent to 10mg of Bicalutamide was weighed and transferred into 100ml volumetric flask. 50ml of diluents was added and sonicated for 30mins with occasional shakings. Solution was cooled to room temperature and diluted to volume with dilluent solution is filtered through 0.45µm water filter. 1ml of above solution was transferred into 10ml volumetric flask and volume diluted with mobile phase.

VALIDATION PARAMETERS

Linearity

The linearity study was performed for the concentration of 50µg/ml to 250 µg/ml level. Each level was injected into chromatographic system .The area of each level was used for calculation of correlation coefficient.

Accuracy

The accuracy study was performed for 50 µg/ml, 100 µg/ml and 150 µg/ml for Bicalutamide. Each level was injected in triplicate into chromatographic system. The area of each level was used for calculation of % recovery.

Precision

The method and system precision study was performed for six injections of Bicalutamide. Each standard injection was injected into chromatographic system. The area of each standard injection was used for calculation of %RSD.

Ruggedness

It is the degree of reproducibility of test results obtained by the analysis of same sample under variety of test conditions i.e different analysts , laboratories, equipments, days.(from laboratory to laboratory analyst to analyst.

System Suitability

The system suitability studies were done with the 60 mg of standard drug. The % of RSD values are below 2%, theoretical plate count is above 2000 and tailing factor is less than 2, indicating that the method is suitable.

LOD and LOQ

The detection limit is characteristic of limit test only. It is the lowest amount of analyte in a sample that can be detected but not necessarily quantified under stated

experimental conditions. The limit of quantification is the lowest amount of analyte in the sample that can be quantitatively determined with definite precision with stated experimental conditions (9).

RESULTS AND DISSCUSION

Good reproducibility was produced in mobile phase ratio of Sodium dihydrogen phosphate (pH 3.5) : Acetonitrile(50:50 v/v) at a flow rate of 1.3 ml/ min. The system suitability studies were done with accurately weighing equivalent to 10mg of Bicalutamide dosage form. The % of RSD values are below 2%, theoretical plate count is above 2000 and tailing factor is less than 2, indicating that the method is suitable. The chromatogram is recorded and are shown in fig-2

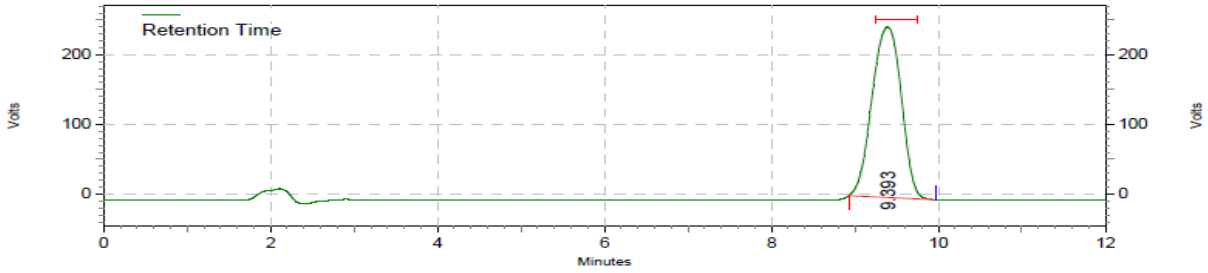

Fig-2 Chromatogram showing system suitability

The linearity study was performed the correlation coefficient of Bicalutamide was found to be 0.998 respectively (Fig-3)

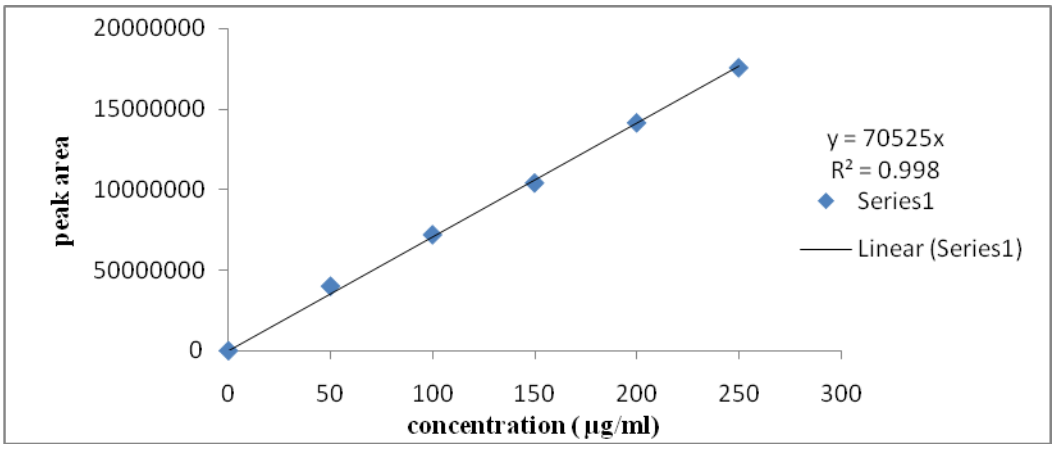

Fig-3 Calibration curve of Bicalutamide

The specificity test was performed for Bicalutamide. It was found that there was no interference of impurities in retention time of analytical peak. The method show excellent specificity with Bicalutamide eluting at retention of 9.513 minutes. No interference was observed with mobile phase. The accuracy study was performed for % recovery. The % recovery was found to be 101.6 to 99.70% respectively (Table-1)

Table-1 Showing result from accuracy study

Level of recovery	Amount of drug spiked(µg/ml)	Drug recovered	%Recovery	Mean	SD	%RSD
50	49.6	49.62	100.2	100.4	0.346	0.34
		49.62	100.2			
		49.68	100.8			
100	99	99.23	101.9	101.6	0.974	0.95
		99.08	100.6			
		99.31	102.5			
150	149.4	149.26	99.02	99.70	0.6451	0.64
		149.21	99.8			
		149.25	99.6			

The precision of method and system was determined by replicate injection of sample solution (Table-2 and 3). The %RSD of area of intraday precision is 0.3%, 0.10% and 0.06%. %RSD of interday precision was found to be 0.3%, 0.09% and 0.07%. Precision results are within the limits (Table-4 and 5).

Table -2 Showing result from method precision study

S.No	Peak Name	Peak area
1	Bicalutamide	71970422
2	Bicalutamide	71972303
3	Bicalutamide	71972167
4	Bicalutamide	71971955
5	Bicalutamide	71972016
6	Bicalutamide	71970975
Mean		71971639
SD		1502.76
%RSD		0.10

Table -3 Showing result from system precision study

S.No	Peak Name	Peak area
1	Bicalutamide	71972014
2	Bicalutamide	71972290
3	Bicalutamide	71971988
4	Bicalutamide	71972209
5	Bicalutamide	71972017
6	Bicalutamide	71972065
Mean		71972097

SD		1648.33
%RSD		0.12

Table-4 Showing results from precision study- Intraday

Conc (µg/ml)	Peak area	Statistical parameters
50	42728675	Mean:42725146 S.D:3123.5 %R.S.D:0.34
	42729089	
	42717674	
100	71972209	Mean:71972018 S.D:1407.15 %R.S.D:0.10
	71972017	
	71972069	
150	100161405	Mean:100161530 S.D:1227.72 %R.S.D:0.06
	100161609	
	100161576	

Table-5 Showing results from precision study- Interday

S.No	Peak Name	Peak Area (Day 1)	Peak Area (Day 2)	Peak Area (Day 3)
1	Bicalutamide	71972014	71970422	71971955
2	Bicalutamide	71972290	71972303	71972016
3	Bicalutamide	71971988	71972167	71970975
4	Bicalutamide	71972209	71971955	71971639
5	Bicalutamide	71972017	71972016	71971988
6	Bicalutamide	71972065	71970975	71972209
Mean		71972097	71971639	71972017
SD		1648.33	1502.76	1799.67
%RSD		0.12	0.10	0.14

Table-6 Summary of HPLC results

Validation Parameters	Acceptance Criteria	HPLC Results
Specificity	The peaks of diluent and impurities should not interfere with the main Peak	The peaks of diluent and impurities are not interfering with the main peaks of Bicalutamide.
Linearity	The Correlation coefficient shall be NLT 0.99	0.998
Accuracy	The % recovery at each spike level should be between 98%-102%	99.7%-101.6%
System Precision	The %RSD of peaks obtained	0.12%

	from the 6 replicate injections should be NMT 1.0%	
Method Precision	The % RSD for the six determinations shall be NMT 2.0.	0.10%
Ruggedness	The %RSD of the peaks is NMT 2.0%	0.09%
LOD	0.14 µg/ml	-
LOQ	0.44µg/ml	-

CONCLUSION

An isocratic reverse phase liquid chromatography (RP-HPLC) method has been developed and subsequently validated for the determination of Bicalutamide. The developed method (Table-6) is simple, fast, accurate and precise hence can be applied for routine quality control analysis of Bicalutamide.

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