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Research Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE ESTIMATION OF BICALUTAMIDE IN PURE AND PHARMACEUTICAL DOSAGE FORMS

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Abstract: A simple, accurate, rapid, sensitive and precise reverse phase high performance liquid chromatographic method has been developed for the estimation of bicalutamide in pure and pharmaceutical dosage forms. In this method RP-C18 Innersil ODS column with mobile phase consisting of Sodium dihydrogen phosphate buffer(NaH_2PO_4) adjusted pH to 3.5 with Ortho phosphoric acid and acetonitrile in the ratio of 65:35v/v was used. The detection wavelength is 272nm and the flow rate 1.5ml/min. The linearity was found in the range of 50-150µg/ml and shows a correlation coefficient of 0.9997. The proposed method was validated by determining sensitivity, accuracy, precision and linearity. The proposed method is simple, fast, accurate and precise hence can be applied for routine quality control analysis of bicalutamide in pure and pharmaceutical dosage forms.

Key words: Bicalutamide, HPLC, Validation

INTRODUCTION

Bicalutamide is a non-steroidal peripheral androgen receptor inhibitor. Bicalutamide competes with androgen for the binding of androgen receptors, consequently blocking the action of androgens of adrenal and testicular origin which stimulate the growth of normal and malignant prostatic tissue.[1-2] It is chemically, N-[4-cyano-3(trifluoromethyl) phenyl]-3-[(4-fluorophenyl) sulfonyl)]-2-hydroxy-2-methyl propanamide and chemical formula of Bicalutamide is $C_{18}H_{14}F_4N_2O_4S$.3

Literature survey reveals that UV spectrophotometric [4] and HPLC [5-6] method have been reported for the determination of bicalutamide in pure and pharmaceutical dosage forms. In this study a simple, rapid, accurate, sensitive and precise HPLC method was developed for the estimation of bicalutamide in pharmaceutical dosage forms.

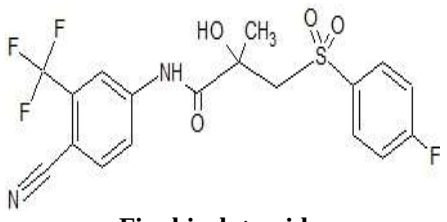


Fig: bicalutamide

MATERIALS AND METHODS:

Bicalutamide were obtained as a memento sample from Dr. Reddy's Hyderabad. Acetonitrile HPLC grade rankem New Delhi, mille-Q water it was purified by miili Pore Corporation's system mfg Barnstead. Ortho phosphoric acid AR grade, Fisher scientific pvt ltd. NaH_2PO_4 Fisher Scientific. The analysis was carried out on HPLC Waters 2695 connected with PDA detector 2998 and Empower2 soft ware.

Chromatographic Conditions

Column: Innersil BDS C_{18}
Flow rate: 1.5ml/min
Injection volume: 10µl
Column Temperature: 40°C
Mobile phase A: NaH_2PO_4 Buffer: Acetonitrile (65:35)
Detection: 272nm.
Run time: 5.0mins

Preparation of Mobile Phase:

To optimize the HPLC parameters several mobile phase compositions were tried. Satisfactory peak symmetry (Tailing factor and theoretical plates as shown in Table 1) were obtained with mobile phase as mentioned above.

Preparation of Diluent:

Prepare 50:50 ratios of Methanol and Water.

Preparation of standard solution:

Accurately weighed quantities, 50.2 mg of Bicalutamide was transferred into 50ml of volumetric flask and adds 20ml of diluent and sonicate for 20 mins make up the volume with Diluent.Transfer above solution 5ml into 10ml

volumetric flask and make up the volume with diluents.

Preparation of sample preparation:

An accurately weigh 10 tablets and calculate average weight of those tablets and crushed. Transfer the tablet powder weight of 130mg of standard drug. Into 50ml of volumetric flask add 25ml of diluents and sonicate for 30mins and filter through the 0.45µm filter paper and make up the volume with diluent. Transfer the 5 ml of above solution into 10 ml volumetric flask and make up the volume with diluents.

METHOD VALIDATION

Specificity:

To determine the specificity of the drug carried out by inject the blank, excipients standard one by one and same manner blank ,excipients and sample at this time blank and excipients peaks are not interference with standard and sample peaks. It proves method is highly selective.

Linearity:

Method linearity is performed by prepare 5 replicate samples in different concentration levels (50%, 75%, 100%, 125%, 150%) inject into HPLC system. Plot the graph concentration versus area and calculate correlation co efficient.

Accuracy:

The method accuracy was determined by recovery studies using method of standard addition to pre analyzed formulation of Bicalutamide. Known amount of three spike levels (50%,100%,150%) six replicate samples are inject into HPLC then accuracy was calculate as per test method assay results.

Precision:

Method precision was carried out by prepare six replicate samples from single formulation and samples run by on the same day and on three different days over a period of one week.

Robustness:

Method robustness was evaluated by carrying deliberate changes in flow rate ±0.2, temperature ±5 run the samples as per test method. Those results are compared to other trails they were not observed significant changes.

Limit of detection (LOD) and Limit of quantification(LOQ):

LOD and LOQ were calculated for the sensitivity of the method they were qualified based on the signal to noise ratio. LOD is lowest detectable concentration of analyte by the method while LOQ is the minimum quantifiable concentration. LOD and LOQ were calculated according to ICH guidelines.

LOD= 3.3× SD/slope

LOQ= 10×SD/slope

RESULTS AND DISCUSION

System suitability results were given by table1 and table 2 six replicate standards system suitability parameters are retention time, tailing and plate count were shown uniformity and %RSD was less than 1 so we can say system is suitable for analysis method specificity was concluded by fig:1 and fig:2 those figures are Bicalutamide standard chromatogram and other one is formulation they were not observed placebo and excipients peaks interference with standard and analytic peak so it proves method is selective. The method accuracy was evaluated by recovery studies those values are given by table3. Bicalutamide recovery was founded 99-101% as per ICH 97%- 103% and also percentage RSD was very low so method is accurate. Linearity calibration curve was given below fig: 3 and plot the graph three different concentrations versus areas to construct the linear regression equation and to calculate the value of correlation co efficient. Linear correlation was found to be 0.9997. Precision results were shown by table 4. The intraday and inter day variations was calculated in terms of %RSD and results was found to be intraday and inter day respectively. Method robustness results was given by table5 they were not observed marked changes of those trails compared to other trails so it proves method was robust. Limit of detection and Limit of quantification values are given in table6.

CONCLUSION

The %RSD was very low and also standard deviation as required by ICH guidelines it indicates high degree of precision. The accuracy results was found within the limit hence it proves method is highly validated so it use full quality control for estimation of Bicalutamide in bulk and tablet dosage forms.

Table 1: System Suitability Test Parameters for the proposed method

Parameters	Bicalutamide
Retention Time (min)	3.2
Theoretical plates	6891
Tailing factor	1.01

Table2: Standard 2 result of Bicalutamide

	Sample name	inj	Name	RT	Area	Tailing	Plate count
1	STD2	1	Bicalutamide	3.282	7289022	1.000	6839
2	STD2	2	Bicalutamide	3.283	7216189	1.000	6755
3	STD2	3	Bicalutamide	3.276	7154002	0.990	6616
4	STD2	4	Bicalutamide	3.282	7092252	1.000	6499
5	STD2	5	Bicalutamide	3.278	6972842	0.990	6576
Mean					7144862		
Std.dev					120739		
%RSD					1.7		

Table3: Accuracy results of Bicalutamide

Spiked level	Sample weight	Sample area	µg/ml added	µg/ml found	%recovery	mean
50%	80.10	4731749	333.75	329.7851	98.81	99
50%	80.00	4711606	333.3333	328.3812	98.51	
50%	80.00	4673683	333.3333	325.7381	97.72	
50%	80.00	4730428	333.3333	329.693	98.91	
50%	80.00	4713458	333.3333	328.5103	98.55	
50%	80.00	4752716	333.3333	331.2464	99.37	
100%	120.20	7194479	500.8333	501.4281	100.12	101
100%	108.30	6461061	451.25	450.3116	99.79	
100%	110.30	6761150	459.5833	471.2267	102.53	
150%	144.60	8839927	602.5	616.1097	102.26	101
150%	142.40	8408563	593.3333	586.0452	98.77	
150%	143.50	8385958	597.9167	584.4697	97.75	
150%	120.40	7375024	501.6667	514.0114	102.46	
150%	125.20	7663028	521.6667	534.0842	102.38	
150%	120.00	7304679	509.1086	509.1086	101.82	

Table4: precision results of Bicalutamide

S.NO	Sample weight	Area	%Assay
1	130.2	7922699	102
2	130.1	7642606	98
3	135.0	8018974	99
4	130.1	7776367	100
5	130.0	7765881	100
6	112.0	6632006	99
Mean		7626422	100
Std.dev		----------	1.19
%RSD		----------	1.19

Table5: Robustness of Bicalutamide

	Sample name	change	Name	RT	Area	Tailing	Plate count
1	Flow1	1.7ml/min	Bicalutamide	4.070	8057652	1.07	8154
2	Flow2	1.3ml/min	Bicalutamide	2.900	5508459	0.92	3735
3	Temp1	50° C	Bicalutamide	3.290	6325339	0.94	4770
4	Temp2	40°C	Bicalutamide	3.260	6242750	0.92	4607

Table6: LOD and LOQ

	Sample name	inj	name	RT	Area
1	LOD	1	Bicalutamide	3.279	894878
2	LOQ	1	Bicalutamide	3.281	447805

Fig:1 A typical standard chromatogram of Bicalutamide

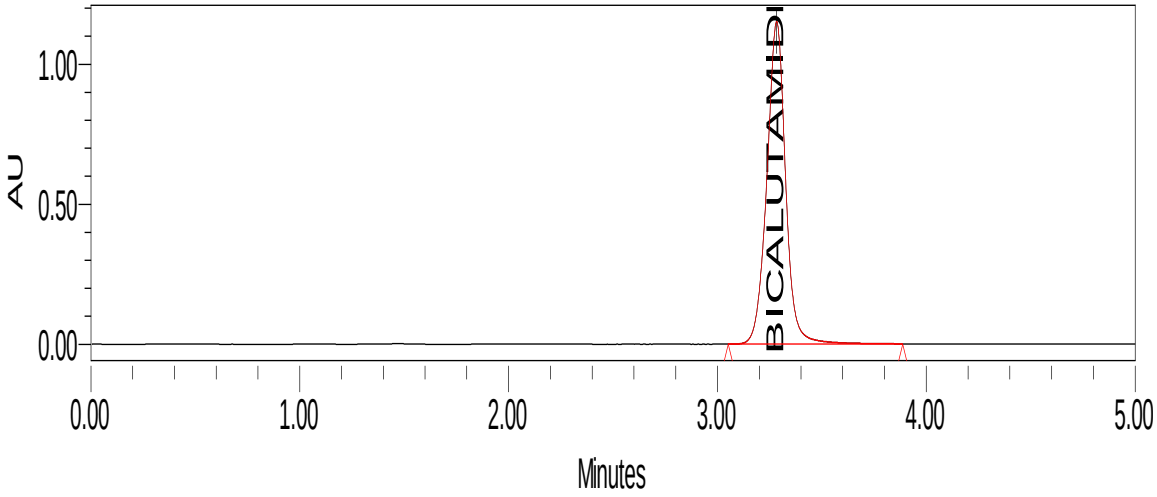


Fig:2 A typical formulation chromatogram of Bicalutamide

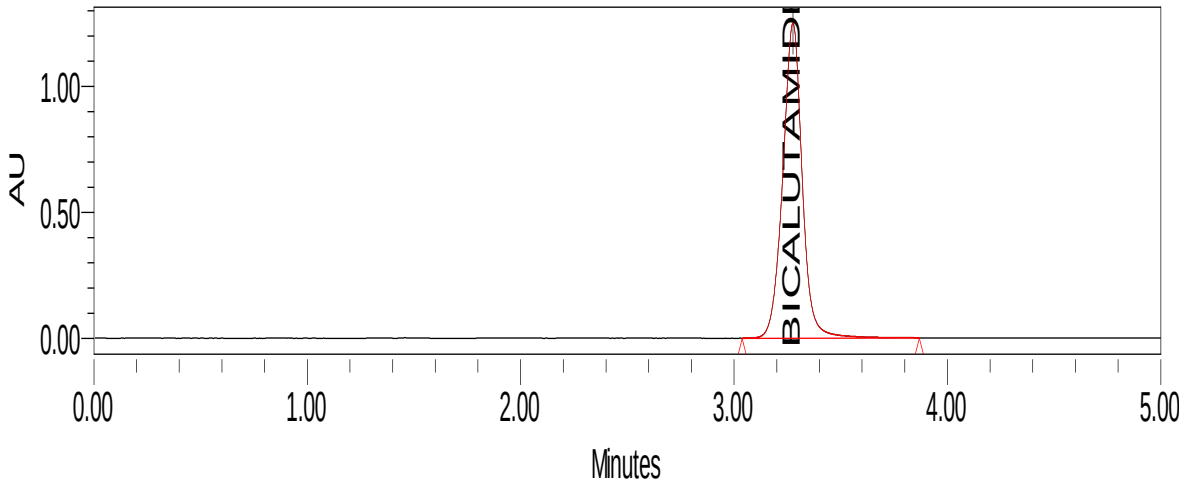
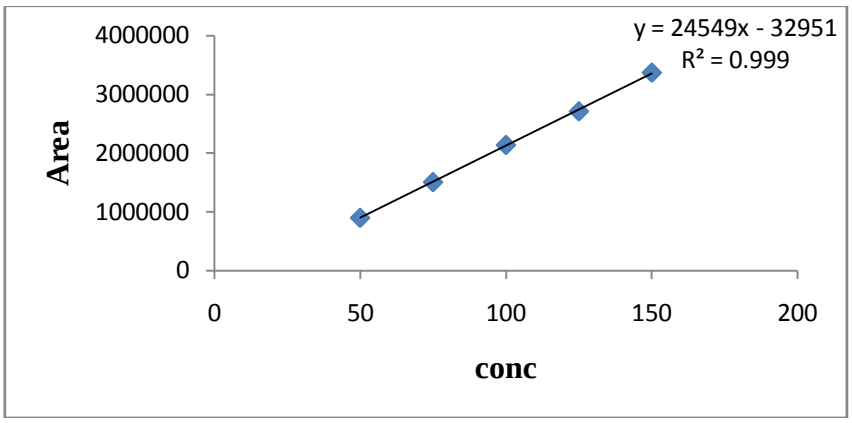


Fig:3 Calibration curve of Bicalutamide



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