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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-C_{18} column (100mm x 4.6mm I.D., 5µm particle size) with mobile phase consisting of phosphate buffer pH 2.8 and acetonitrile in the ratio of 60:40v/v was used. The detection wavelength is 270nm and the flow rate 1.0ml/min. The linearity was found in the range of 25-150µg/ml and shows a correlation coefficient of 0.9998. The proposed method was validated by determining sensitivity, accuracy, precision and linearity. The proposed method is simple, fast, accurate, precise and reproducible hence can be applied for routine quality control analysis of bicalutamide in pure and pharmaceutical dosage forms.

Keywords: Bicalutamide, HPLC, Validation.

INTRODUCTION

Bicalutamide is a non-steroidal peripheral androgen receptor inhibitor[1]. It competitively inhibits the action of androgens by binding to cytosol androgen receptors in the target tissue. It is chemically, N-[4-cyano-3(trifluoromethyl) phenyl]-3-[(4-fluorophenyl) sulfonyl)]-2-hydroxy-2-methyl propanamide. Literature survey reveals that various spectrophotometric[2] and HPLC methods[3-4] 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.

EXPERIMENTAL

Instrumentation

The separation was carried out on HPLC system (Waters) with Waters 1525 binary HPLC pump, Waters 2487 dual absorbance detector, Waters Empower software and RP-C_{18} column (100mmx4.6mm I.D; particle size 5µm).

Chemicals and Reagents

Bicalutamide was a gift sample by Dr. Reddy's Laboratories Ltd., Hyderabad. Acetonitrile of HPLC grade were purchased from E.Merck (India) Ltd., Mumbai. Potassium dihydrogen phosphate and orthophosphoric acid of AR grade were obtained from S.D. Fine Chemicals Ltd., Mumbai.

HPLC conditions

The mobile phase consisting of phosphate buffer (pH 2.8 adjusted with orthophosphoric acid) and acetonitrile (HPLC grade) were filtered through 0.45µ membrane filter before use, degassed and were pumped from the solvent reservoir in the ratio of 60:40v/v was pumped into the column at a flow rate of 1.0ml/min. The detection was monitored at 270nm and the run time was 15min. The volume of injection loop was 10µl prior to injection of the drug solution the column was equilibrated for at least 30 min. with the mobile phase flowing through the system.

Procedure

Stock solution of bicalutamide was prepared by dissolving 50mg of bicalutamide in 50ml standard volumetric flask containing 25ml of acetonitrile and the solution was sonicated for 20 min. and then made upto the mark with acetonitrile to get a concentration of 1mg/ml. 0.5ml of the above stock solution was transferred to 25ml volumetric flask and the volume was made up to the mark with mobile phase. Subsequent dilutions of this solution were made with mobile phase to get concentration of 25-150µg/ml. The solutions were injected into the 10µl loop and the chromatogram was recorded in Fig. 1. The retention time of bicalutamide was found to be 6.116min. The calibration curve was constructed by plotting concentration vs peak area ratio. The amount of bicalutamide present in sample was calculated through the standard calibration curve. The linearity experiment was carried out in triplicate to ascertain accuracy and precision of the method. The peak area ratios of the drug vs concentration were found to be linear and the results are furnished in Table-1.

Assay

Two commercial brands of tablets were chosen for testing suitability of the proposed method to estimate bicalutamide in pharmaceutical dosage forms. Twenty tablets were weighed accurately and powdered. A quantity equivalent to 50mg of bicalutamide was weighed accurately and transferred to 50ml volumetric flask. About 30ml of acetonitrile was added and kept in ultrasonic bath for 20min. This solution is filtered through a membrane filter and the volume was made up to the mark with mobile phase to get 1mg/ml concentration. The solution obtained was diluted with the mobile phase so as to obtain a concentration in the range of linearity previously for the pure drug determined. Sample solution was injected under the chromatographic conditions and the chromatogram was recorded. The amount of bicalutamide present in tablet formulation was determined by comparing the peak area from the standard. The results are furnished in Table-2.

Validation of proposed method

Selectivity of the method was assessed on the basis of elution of bicalutamide using the above mentioned chromatographic conditions. To study the specificity, linearity, precision, accuracy, limit of detection, limit of quantitation, robustness and system suitability parameters has been validated for the determination of bicalutamide. The results are furnished in Table-3.

Specificity

The specificity was established by preparing a bicalutamide standard at 0.5% level of test concentration and injected 6 times into HPLC system as per the test procedure.

Linearity

The standard curve was obtained in the concentration range of 25-150µg/ml. The linearity was evaluated by linear regression analysis using the least square method. It was found that correlation coefficient and regression analysis are within the limits.

Precision

The precision of the assay was determined in terms of intra-day and inter-day precision. The intra-day and inter-day variation in the peak area of drug solution was calculated in terms of coefficient of variation (C.V.) obtained by multiplying the ratio of standard deviation to mean with 100. The results are furnished in Table-4.

Limit of detection (LOD) and limit of quantitation (LOD)

The LOD and LOQ for bicalutamide were predicted basing on the parameters of standard error of estimate and slope, calculated from linearity of the response data of bicalutamide.

Accuracy

The accuracy of the HPLC method was assessed by adding known amounts of sample solutions of bicalutamide at 50%, 100% and 150% of the specification were prepared in triplicate to the test solutions and injected into the HPLC system as per the proposed method. The results are furnished in Table-5.

RESULTS AND DISCUSSION

By applying the proposed method, the retention time of bicalutamide in a typical chromatogram was found to be 6.116min, which indicates a good base line. Linearity range was observed in concentration

range of 25-150µg/ml. The regression equation of bicalutamide concentration over its peak area ratio was found to be Y=147872X+66052 (r=0.9998) where Y is the peak area ratio and X is the concentration of bicalutamide(µg/ml). The proposed HPLC method was also validated for intra-day and inter-day variation. When the solution containing 50µg/ml of bicalutamide were repeatedly injected on the same day, the coefficient of variation in the peak area of drug for three replicate injections was found to be less than 1%. Also, the inter-day variation on three different days was found to be less than 1%. The asymmetry factor was found to be 1.09, which indicated asymmetric nature of peak. The number of theoretical plates was found to be 5877, which indicates efficient performance of the column. The limit of detection and limit of quantitation was found to be 0.045µg/ml and 0.656µg/ml, indicates the sensitivity of the method. To optimize the chromatographic conditions, various combinations of phosphate buffer with acetonitrile were tested. The use of phosphate buffer and acetonitrile in the ratio of 60:40v/v resulted in peak with good shape and resolution. The high percentage of recovery of bicalutamide ranging from 98.58 to 100.24 indicates that the proposed method is highly accurate. No interfering peaks were found in the chromatogram indicating that excipients used in tablet formulations did not interfere with the estimation of the drug by proposed HPLC method.

CONCLUSION

The proposed HPLC method was found to be simple, precise, accurate and sensitive for the determination of bicalutamide in pharmaceutical dosage forms. Hence, this method can easily and conveniently adopt for routine quality control analysis of bicalutamide in pure and its pharmaceutical dosage forms.

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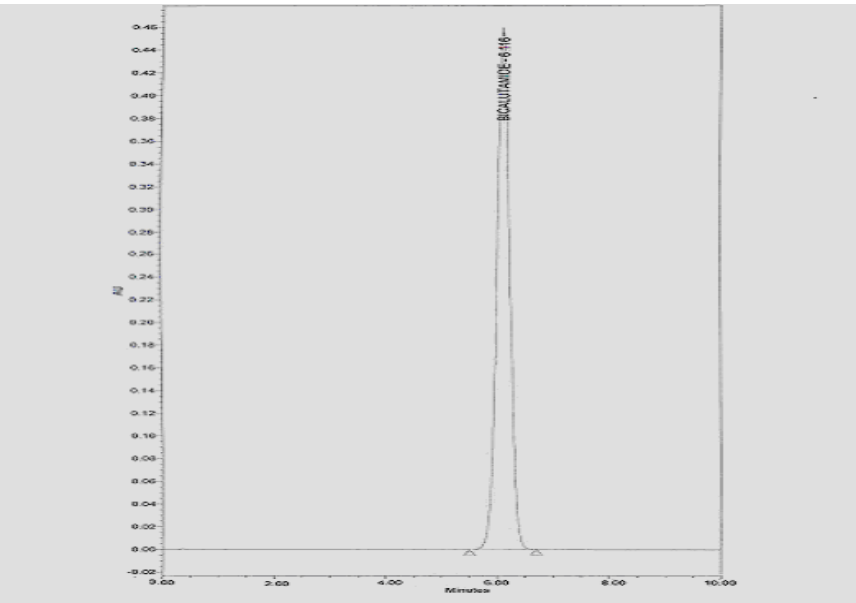

Fig-1. Typical Chromatogram of Bicalutamide

Table-1: Calibration of the proposed HPLC method for the estimation of bicalutamide

Concentration (μg/ml)	Peak area ratio
25	3728753
50	7447485
75	11286862
100	15012604
125	18455729
150	22163815

Slope : 147872 ; Intercept : 66052 ; Regression equation : Y=147872X+66052 ; Correlation coefficient : 0.9998

Table-2: Assay and recovery studies

Formulation	Label claim (mg)	Amount found (mg)	% Amount found	% Recovery
Brand-1	50	49.85	99.70	100.10
Brand-2	50	49.94	99.88	99.64

Table-3: System suitability parameters

S. No.	Parameter	Result
1	Linearity(μg/ml)	25-150
2	Correlation coefficient	0.9998
3	Theoretical plates (N)	5877
4	Tailing Factor	0.98
5	Asymmetry factor	1.09
6	LOD (μg/ml)	0.045
7	LOQ (μg/ml)	0.656
8	Percentage recovery	98.58-100.24

Table- 4: Precision of the proposed HPLC method

Concentration of bicalutamide(μg/ml)	Measured concentration of bicalutamide(μg/ml)			
	Intra-day		Inter-day	
	Mean (n=3)	% C.V.	Mean (n=3)	%C.V.
25	25.14	0.38	25.12	0.87
50	49.09	0.27	50.4	0.64
100	100.15	0.64	100.25	0.93

Table -5: Accuracy studies

Concentration	Amount added (μg)	Amount found (μg)	% Recovery	Statistical Analysis	
50% Sample 1	25	24.76	99.52	Mean	99.80
50% Sample 2	25	25.12	100.24	%RSD	0.38
50% Sample 3	25	24.82	99.64		
100 %Sample 1	50	50.2	100.2	Mean	99.96
100% Sample 2	50	48.5	98.58	%RSD	1.2
100% Sample 3	50	51.1	101.1		
150% Sample 1	75	75.24	100.16	Mean	99.92
150% Sample 2	75	74.31	99.54	%RSD	0.3
150% Sample 3	75	75.11	100.07		

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