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Developmentand Validation for the Estimation of Bicalutamide in Bulk and Pharmaceutical Dosage Form by RP-HPLC method

Amrit Kumar Rath*, Rishabh Jain

Faculty of Pharmacy, Department of Pharmaceutical Analysis, Danteswari College of Pharmacy, Borpadar, Raipur Road, Jagdalpur, CG – 494221, India.

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ABSTRACT: Background: Bicalutamide is an anti-androgen medication that is primarily used to treat prostate cancer. Bicalutamide used together with a gonadotropin-releasing hormone (GnRH) analogue for the treatment of advanced prostate cancer. **Aim:** The present study was aimed to develop a specific, accurate and precise Reverse Phase High Performance liquid chromatographic (RP-HPLC) method for the estimation of Bicalutamide in bulk and tablet dosage forms. **Methods:** A Symmetry C18 (250 × 4.6 mm, 5µ) a column with a mobile phase of acetonitrile: water (90:10) was used for the estimation of Bicalutamide. A flow rate of 0.5 ml/min was maintained. UV detection was performed at 270 nm. The method was validated as per ICH Q2 (R1) guidelines, for specificity, linearity, accuracy, precision, and robustness. **Results:** The retention time of Bicalutamide was 6.15 min, and therefore the total run time was 20 min. The recovery of Bicalutamide in tablets was found to be within the range of 99 to 100.83 %. **Conclusion:** The studied RP-HPLC method was found to be a suitable technique for the determination of Bicalutamide in pharmaceutical dosage form without any interference.

Corresponding author*
Mr. Amrit Kumar Rath
Asst. Professor
Danteswari College of Pharmacy
Borpadar, Raipur Road,
Jagdalpur, CG, India.
Tel: +91-9439397301
Mail ID: amritkumar052@gmail.com

Keywords: Bicalutamide, HPLC, Validation, Antiandrogen, Linearity, ICH.

INTRODUCTION:

Bicalutamide is an oral non-steroidal anti-androgen drug, utilized within the treatment of prostate cancer and hirsutism[1,2]. It competitively inhibits the action of androgens by binding to cytosol androgen receptors within the target tissue. It is chemically, N-[4-cyano-3(trifluoromethyl) phenyl]-3-[(4-fluorophenyl) sulfonyl)]-2-hydroxy-2-methyl propanamide (Fig 1). Literature survey reveals that various spectrophotometric and HPLC methods are reported for the determination of Bicalutamide in bulk and pharmaceutical dosage forms [3-5]. In this study an easy, rapid, accurate, sensitive and

precise HPLC method was developed for the estimation of Bicalutamide in pharmaceutical dosage forms.

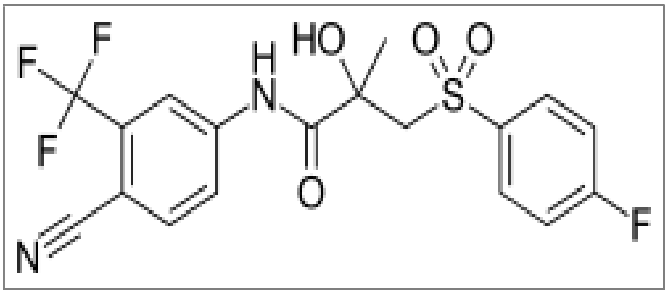

Fig 1. The chemical structure of Bicalutamide.

MATERIALS AND METHODS:

Bicalutamide was procured as a gift sample by Hetero Labs Pvt. Ltd, Hyderabad. Acetonitrile of HPLC grade was 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.

Instrument:

The separation was carried out on HPLC system (Waters) with Waters 1525 binary HPLC pump, UV absorbance detector; LC Solutions software and Enable C18H 250 × 4.6 mm. all glassware used in this study were of analytical grade procured from Borosil, India.

HPLC Methodology:

The mobile phase was consisting of Potassium dihydrogen orthophosphate and acetonitrile (HPLC grade). The mobile phase was filtered through 0.2 µm membrane filters before use. The solvent was degassed and the mixture of acetonitrile and HPLC water was prepared with a ratio of 90: 10 v/v. This solvent mixture was pumped into the column at a flow of 1.0 ml/min. The detection was monitored at a wavelength of 270 nm and therefore the run time was 15 min. The volume of the injection loop was 20 µL. Before injection of the drug solution, the column was equilibrated for a minimum of 30 min with the mobile phase flowing through the system [6,7].

Preparation of working standard solution:

About 10 mg o of Bicalutamide was weighed and transferred into a 10 ml clean, dry volumetric flask. The volume of the flask was made up to volume with mobile phase to get concentration 1000 µg/ml (Stock solution). From the stock solution, 1 ml was diluted with 10 ml using mobile phase to get a concentration of 100 µg/ml. From this solution, 1 ml of solution was transferred into a 10 ml volumetric flask and volume was adjusted with mobiles phase to urge 10 µg/ml [5,8].

Preparation of sample drug solution:

About 10 Bicalutamide tablets were weighed. All tablets were crushed into uniform fine powder in a suitable Powdering device. The powder equivalent to about 10 mg Bicalutamide was accurately weighed and transferred to a 10 ml clean, dry graduated tube. About 10 ml of acetonitrile was added and Sonicated (Bandelin SONOREX Digital 10P, Sigma Aldrich, India) for about 20 min at room temperature with intermittent shaking. Allow the mixture to chill temperature and dilute the volume with the mobile phase, which was blended and filtered through a 0.2 µ filter [5,8].

Assay:

Two commercial brands of tablets were chosen for testing the suitability of the proposed method to estimate Bicalutamide in pharmaceutical dosage forms. Twenty tablets were weighed accurately and powdered [8]. A quantity like 50mg of Bicalutamide was weighed accurately and transferred to a 50 ml volumetric flask. About 30ml of acetonitrile was added and kept in an ultrasonic bath for 20min. This solution was filtered through a membrane filter and therefore the volume was made up to the mark with mobile phase to urge 1000 µg/ml concentration. The solution obtained was diluted with the mobile phase so on obtain a degree within the range of linearity previously for the pure drug determined. The sample solution was injected under the chromatographic conditions and therefore the chromatogram was recorded. The amount of Bicalutamide present in tablet formulation was decided by comparing the height area from the quality.

Validation of proposed method:

The selectivity of the tactic was assessed on the idea of elution of Bicalutamide using the above mentioned chromatographic conditions. To study the specificity, linearity, precision, accuracy, the limit of detection (LOD), Limit of Quantitation (LOQ), robustness, and system suitability, parameters have been validated for the determination of Bicalutamide.

***Specificity*:**

System suitability for specificity was administered to work out whether there's any interference of any impurities at the retention time of analytical peak.

The study was performed by injecting blank. The chromatogram was observed and recorded.

***Linearity*:**

The linearity study was performed for the concentration ranges of 100 to 3000 ng/ml. Each dilution was injected into the HPLC system. The area of each dilution was used to calculate the correlation coefficient. The chromatogram was observed and recorded.

***Precision*:**

The standard solution was prepared as per the proposed assay method in six determinations and was injected into the HPLC system. The retention time and peak area of six determinations were measured and % RSD was calculated. The LOD and LOQ were determined based on the visualization of the chromatogram and recorded.

***Accuracy*:**

The accuracy study was performed for 80, 100, and 120 % of Bicalutamide. Each dilution was injected in triplicate into the HPLC system. The area of each level was used for the calculation of % RSD. The chromatogram was observed and recorded.

RESULTS AND DISCUSSION:

The UV absorption was maximum at a wavelength of 270 nm. The purity of the sample was also confirmed by RP-HPLC analysis using mobile phase acetonitrile: Water (90: 10). The results indicated that one prominent peak appeared with a retention time of 6.122 min and there were no minor impurities were observed, thus confirming the sample is pure.

The retention time of Bicalutamide was found to be 6.12 min and the system suitability studies were done with an 800 ng/ml concentration of the standard drug. The % RSD values are below 2 %. The percentage purity of Bicalutamide in pharmaceutical dosage form was found to be 109.312 %. The chromatograms for sample, standard, and blank injection.

The % RSD of the area of system precision was found to be 1.75. Precision results are within the limits. The % RSD for the area of all replicate injections found to be within the limits. Method precision should be performed to intraday and inter-day. The linearity study was performed and therefore the coefficient of correlation of Bicalutamide was found to be 0.999. Accuracy values were found to be within the bounds (should not be quite 2).

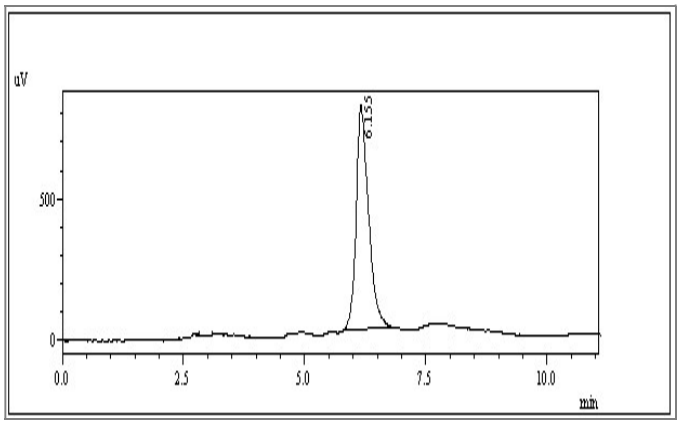

Fig 2. Chromatogram standard preparation.

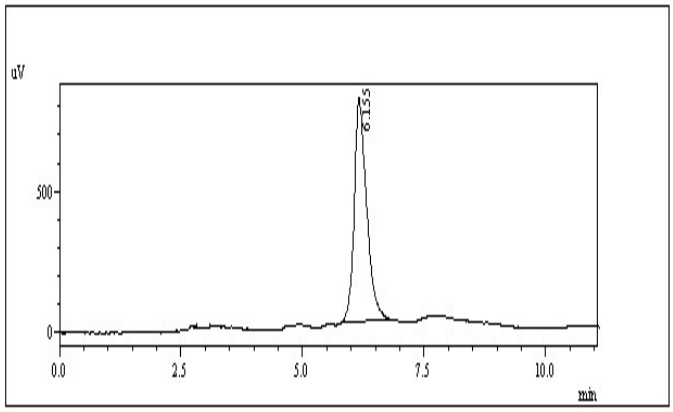

Fig 3. The chromatogram sample preparation.

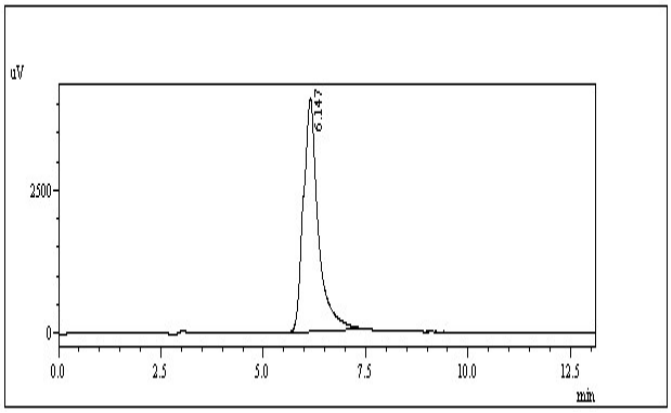

Fig 4. Chromatogram specificity of standard.

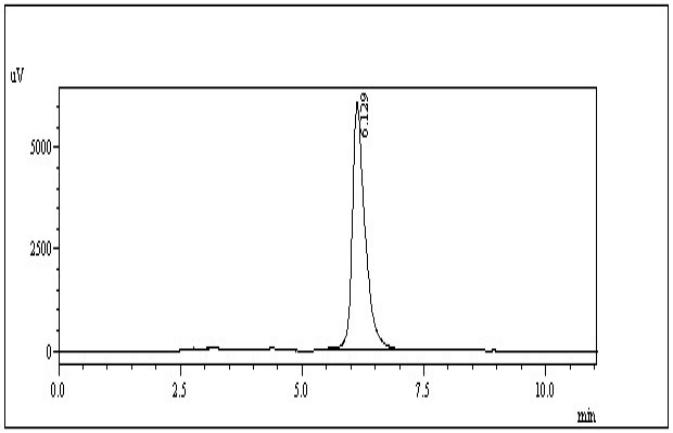

Fig 5. Chromatogram specificity of sample.

The assay method was established for estimation of Bicalutamide in tablets and therefore the % purity was found to be 101.3 %.

Table 1. The System precision.

Sl. No.	Retention time	Area
1	6.120	20848
2	6.122	20811
3	6.119	20873
4	6.123	20805
5	6.123	21647
6	6.223	20578
Avg.		21460.33
SD		368.157
% RSD		1.75

Table 2. The Intra-day precision data.

Sl. No.	Conc. (ng/ml)	MM	AM	EM	SD	% RSD
1	600	10559	10563	10571	42	0.39
2	1000	12765	12769	12762	54	0.42
3	1200	14521	14536	14541	48	0.33

MM - Morning mean, AM – Afternoon mean, EM – Evening mean.

Table 3. Inter-day precision data.

Sl. No.	Conc. (ng/ml)	MM	AM	EM	SD	% RSD
1	600	10559	10563	10531	46.5	0.64
2	1000	12760	12775	12762	39	0.30
3	1200	14702	14735	14741	44	0.29

MM - Morning mean, AM – Afternoon mean, EM – Evening mean.

Table 4. The Percentage purity of Bicalutamide.

Conc. (µg/ml)	Peak area	Mean	% RSD	SD
80	10637	10643	38	0.35
	10643			
	10650			
100	12801	12806	32	0.30
	12803			
	12814			
120	14770	14776	36	0.24
	14779			
	14881			

CONCLUSION:

The assay method was established for estimation of Bicalutamide in tablets. And the % purity was found to be 101.1. The proposed RP-HPLC method is a suitable technique for the determination of Bicalutamide in pharmaceutical dosage form without any interference. The developed method can be used for routine and quality control analysis of the investigated drug to provide simple, accurate and reproducible quantitative analysis for determination of Bicalutamide.

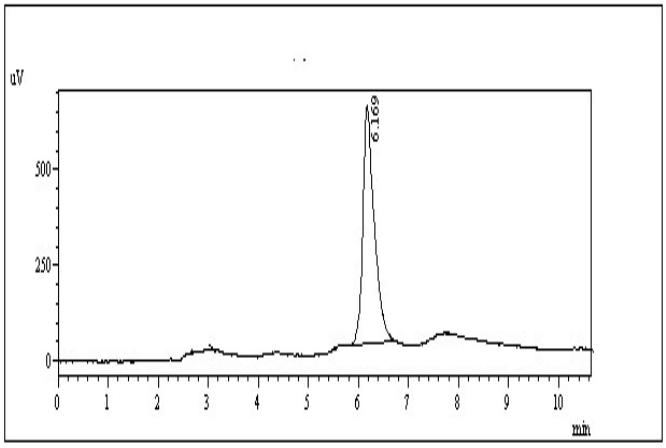

Fig 6. The Chromatogram system precision.

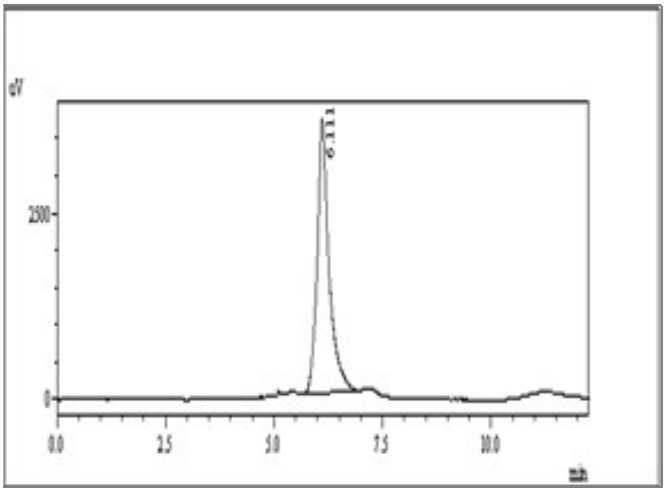

Fig 7. The Chromatogram method precision 100 %.

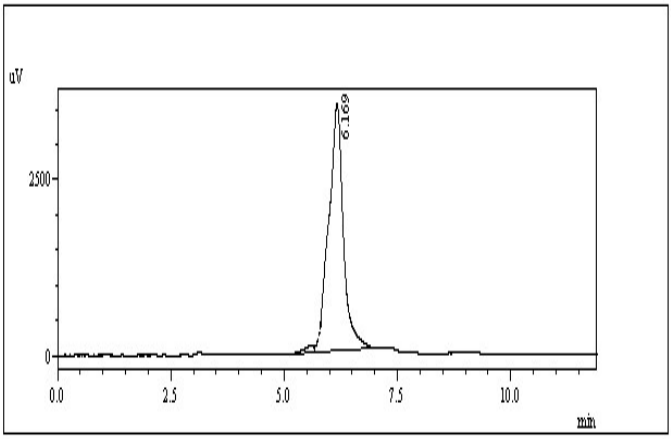

Fig 8. The Chromatogram showing assay of Bicalutamide tablets.

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REFERENCES:

1. Tripathi KD. Essential of Medical Pharmacology, 5th ed. New Delhi: Jaypee Brothers Medical publisher; 2012.
2. Wellington K, Keam SJ. Bicalutamide 150mg: a review of its use in the treatment of locally advanced prostate cancer. Drugs, 2006; 66(6): 837-850.
3. Finkel R, Clark MA, Cubeddu LX. Pharmacology. Philadelphia: Lippincott Williams & Wilkins; 2009. Pp. 48-489.
4. Dole EJ. Holdsworth MT. Nilutamide: an antiandrogen for the treatment of prostate cancer. Annals Pharmacother, 1997; 3(1): 65-75.
5. Schellhammer PF. An evaluation of bicalutamide in the treatment of prostate cancer. Expert Opin Pharmacother, 2002; 3(9): 1313-1328.
6. Cockshott ID. Bicalutamide: clinical pharmacokinetics and metabolism. Clinical Pharmacokinet, 2004; 43(13): 855-878.
7. Jain D, Jain S, Jain D, Maulik A. Simultaneous Estimation of bicalutamide by RP-HPLC in Tablet Formulation. J Chromatogr Sci, 2008; 46: 501-504.
8. Sravanthi C, Priya DKS, Pragada VR, Haque MA, Bakshi V, Narender B. Development and Validation of Spectrophotometric method for estimation of Levomilnacipran in Bulk and Pharmaceutical dosage form by Area Under Curve. J Pharm Adv Res, 2020; 3(10): 1024-1031.

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