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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 XTERRA RP-$C_{18}$ column(4.6x150mm i.d., particle size 5 µm) with mobile phase consisting of Potassium dihydrogen phosphate buffer pH 2.8 and acetonitrile in the ratio of 50:50v/v was used. The detection wavelength is 270nm and the flow rate 1.0ml/min.The linearity was found in the range of 5-25 µg/ml and shows a correlation coefficient of 0.999. 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[1-4]

Bicalutamide is a Non steroidal antiandrogen receptor. 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 and HPLC methods 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.

## EXPERIMENTAL[1-4]

### Instrumentation

The separation was carried out on HPLC system 2695(Waters) with Waters Quaternary pump, PDA detector(2998), Waters Empower software and XTERRA RP-$C_{18}$ column (100mmx4.6mm I.D; particle size 5µm).

### Chemicals and Reagents

Bicalutamide was a gift sample by Celon pvt Ltd., Hyderabad. Acetonitrile of HPLC grade were purchased.

Potassium dihydrogen phosphate and ortho phosphoric acid of AR grade were obtained

## 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 50:50v/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 8min. 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

10 mg of Bicalutamide standard was accurately weighed and transferred to a 50 ml volumetric flask, dissolved in 30ml of diluent and diluted to 50ml with diluent then sonicated for 10 min. By using the working standard, aliquots of 5, 10, 15, 20 and 25 µg/ml were prepared with diluent. 10 µl of each dilution was injected into the column with a flow rate of 1.0 ml/min and the chromatogram was recorded in Fig.1. The retention time of bicalutamide was found to be 5.281. 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..

## Assay of Bicalutamide in Tablets

Twenty tablets were weighed and finely powdered. An accurately weighed quantity of the powder equivalent to 10 mg of Bicalutamide was transferred to 50 ml volumetric flask containing 40 ml of diluent and the contents of the flask were sonicated for 15 min, to ensure the complete solubility of the drug, then the mixture was made up to 50 ml with diluent. The resulting solution was thoroughly mixed and filtered through a 0.45 µm membrane filter. From this a working standard solution of 100 µg/ml of strength was prepared. Aliquots of 5, 10, 15, 20 and 25 µg/ml were prepared in 10 ml volumetric flasks & diluted with diluent. This each concentration (10 µl) was injected six times into the column. The mean values of peak areas were calculated and the drug content in the tablets was quantified. The amount of bicalutamide present in tablet formulation was determined by comparing the peak area from the standard. The results are shown 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, 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 5-25µg/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. The results are furnished in Table 1 and Fig 2.

## 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.

**Fig.1**: **Typical chromatogram of bicalutamide**

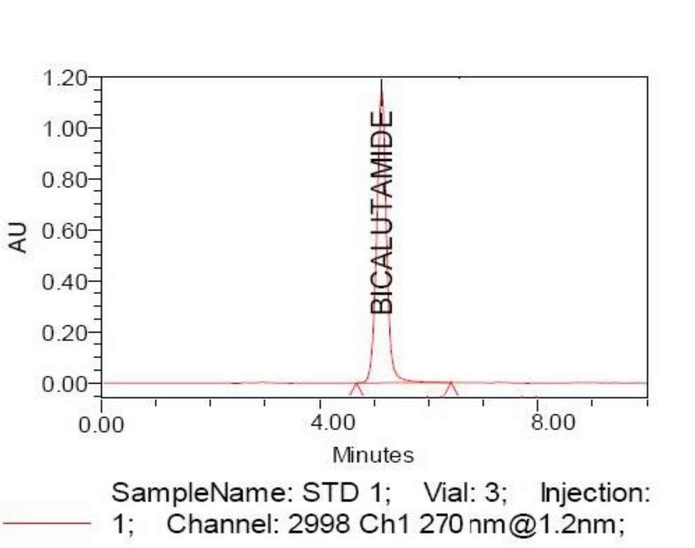

**Table-1**: **Calibration of the proposed HPLC method for the estimation of bicalutamide**

| S.No. | Concentration(µg/ml) | Peak area ratio |
|---|---|---|
| 1 | 5 | 167234 |
| 2 | 10 | 344272 |
| 3 | 15 | 511354.3 |
| 4 | 20 | 667407 |
| 5 | 25 | 835508 |

Slope-33401, intercept-3452, regression equation-33401x+3452, correlation coefficient-0.999

**Fig 2: Linearity curve for Bicalutamide**

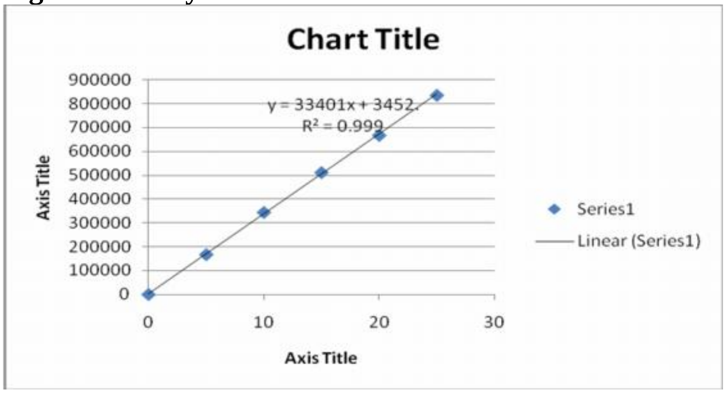

**Table-2**: **Assay and recovery studies:**

| S.No. | Label claim(mg) | Amount found (mg) | %Amount found |
|---|---|---|---|
| 1 | 50 | 50.02 | 100.04 |
| 2 | 50 | 50.03 | 100.06 |
| 3 | 50 | 50.08 | 100.16 |
| 4 | 50 | 50.02 | 100.04 |
| 5 | 50 | 49.99 | 99.98 |
| 6 | 50 | 50.02 | 100.04 |

**Table-3: System suitability parameters**

| S. No | Parameter | Result |
|---|---|---|
| 1 | Linearity(µg/ml) | 5-25(µg/ml) |
| 2 | Correlation coefficient | 0.999 |
| 3 | Theoretical plates (N) | 7632 |
| 4 | Tailing Factor | 1.13 |
| 5 | LOD (µg/ml) | 0.02 |
| 6 | LOQ (µg/ml) | 0.08 |
| 7 | Percentage recovery | 99.6-101 |

**Table-4**: **Precision of the proposed HPLC method**

| Concentration of Bicalutamide (µg/ml) | Conc. Of Bicalutamide Found on | | | |
|---|---|---|---|---|
| | Intra-Day | | Inter-Day | |
| | Mean (n=6) | C.V. (%) | Mean (n=3) | C.V. (%) |
| 1 | 666368.2 | 0.004 | 666201.3 | 0.0657915 |

**Table-5: Accuracy Studies**

| Sample | %Recovery | Mean | Std.Dev | %RSD |
|---|---|---|---|---|
| **50%** | 100.37 | 100.1067 | 4677 | 0.186 |
| | 100.15 | | | |
| | 99.94 | | | |
| | 100.2 | | | |
| | 99.85 | | | |
| | 100.13 | | | |
| **100%** | 100.45 | 100.19 | 7475 | 0.22 |
| | 100.09 | | | |
| | 100.04 | | | |
| **150%** | 100.11 | 100.09 | 10272 | 0.24 |
| | 99.8 | | | |
| | 100.5 | | | |
| | 100.2 | | | |
| | 99.9 | | | |
| | 100.05 | | | |

## RESULTS AND DISCUSSION

By applying the proposed method, the retention time of bicalutamide in a typical chromatogram was found to be 5.281min, which indicates a good base line. Linearity range was observed in concentration 5-25µg/ml. The regression equation of bicalutamide concentration over its peak area ratio was found to be Y=33194X+7250(r=0.999) 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 number of theoretical plates was found to be 7632, which indicates efficient performance of the column. The limit of detection and limit of quantitation was found to be 0.02 and 0.08µg/ml.

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 50:50(v/v) resulted in peak with good shape and resolution. The high percentage of recovery of bicalutamide ranging from 99.6 to 101indicates 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.

## REFERENCES:

1. Anton Smith A, Manavalan R and Kannan K., Simple and sensitive spectrophotometric method for determination of Bicalutamide in bulk and formulations, Rasayan J. Chem., 2009, 2, 204.
2. Nageswara Rao R, Narasa Raju A and Narsimha R, Isolation and characterization of process related impurities and degradation products of bicalutamide and development of RP-HPLC method for impurity profile study, J. Pharm. Biomed. Anal., 2008, 46, 505.
3. Palleshwar Rao G , JVLNS Rao, Lanka A, Rama Prasad, Srinivasu Pamidi, Development and Validation of a new stability indicating HPLC method for Quantification of process related and Degradation impurities of Bicalutamide in tablet dosage forms, Int J Pharm.,2012, 2, 218.
4. Lakshmana Rao A , Taraka Ramesh G , JVLNS Rao, Development and Validation of RP-HPLC Method For the Estimation of Bicalutamide in Pure and Pharmaceutical Dosage Forms. Rasayan J.chem., 2009, 2, 512.

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