International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS | Volume 7 | Issue 1 | JAN-MAR | 2017 | 179-187
Original Research Article | Pharmaceutical Sciences | Peer Reviewed | Referred Journal
UGC Approved Journal

DEVELOPMENT AND METHOD VALIDATION OF BICALUTAMIDE RP-HPLC IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT s

A simple, rapid, specific and accurate reverse phase high performance liquid chromatographic method has been developed for the validated of Bicalutamide in bulk as well as in marketed pharmaceutical dosage form. This separation was performed on a Symmetry ODS C18 (4.6×250mm, 5µm) column with Methanol: Phosphate Buffer (35:65) V/V as mobile phase at a flow rate of 1.0 mL min−1 with UV detection at 235 nm; the constant column temperature was Ambient. The runtime under these chromatographic conditions was less than 8 min. The retention time of Bicalutamide was found to be 2.252. The calibration plot was linear over the concentration range of 6–14µg mL−1 with limits of detection and quantification values of 1.2 and 3.6ng mL−1 respectively. The mean % assay of marketed formulation was found to be 99.86%, and % recovery was observed in the range of 98-102%. Relative standard deviation for the precision study was found <2%. The developed method is simple, precise, specific, accurate and rapid, making it suitable for estimation of Bicalutamide in bulk and marketed pharmaceutical dosage formdosage form.

KEY WORDS

Bicalutamide, RP-HPLC, Validation, ICH Guidelines.

Received on: 11.11.2016
Accepted on: 17.12.2016
Published on: 01.01.2017

1. INTRODUCTION

High Performance Liquid Chromatography (HPLC) is a technique that has arisen from the application to liquid chromatography the use of an instrumentation that was originally developed for gas chromatography. High Pressure Liquid Chromatography was developed in the mid-1970 and was improved with the development of column packing material and the additional convenience of on-line detectors. The various components of HPLC are pumps (solvent delivery system), mixing unit, gradient controller and solvent degasser, injector (manual or automatic), guard column, analytical columns, detectors, recorders and/or integrators. Recent models are equipped with computers and software for data acquisition and processing. The mobile phase in HPLC refers to the solvent being continuously applied to the column or stationary phase at a flow rate of 1-5 cm3/min. The mobile phase acts as a carrier for the sample solution. The chemical interactions of the mobile phase and sample with the column determine the degree of migration and separation of components contained in the sample. The mobile phase can be altered in order to manipulate the interactions of the sample and the stationary phase.

Types of Chromatography

1. Normal-phase chromatography

Mechanism: Retention by interaction with the polar surface of the stationary phase with polar parts of the sample molecules.

Stationary phase: SiO2, Al2O3, -NH2, -CN, -Diol, -NO2, etc.

Mobile phase: Heptane, hexane, cyclohexane, CHCl3, CH2Cl2, dioxane, methanol, etc.

Application: Separation of non-ionic, non-polar to medium polar substances. Disadvantage: Lack of reproducibility of retention times as water or protic organic solvents change the hydration state of the silica or alumina chromatographic media.

2. Reversed-phase chromatography

Mechanism: Retention by interaction of the stationary phase's non-polar hydrocarbon chain with non-polar parts of the sample molecules.

Stationary phase: n-octadecyl (RP-18), n-octyl (RP-8), ethyl (RP-2), phenyl, (CH2)n-CN, (CH2)n-diol, etc.

Mobile phase: Methanol, Acetonitrile, water, buffer (sometimes with additives of THF orDioxane), etc.

Application: Separation of non-ionic and ion forming non-polar to medium polar substances (carboxylic acids, hydrocarbons). If ion forming substances (as carboxylic acids) are to be separated, a pH control by buffers is necessary.

3. Reversed-phase ion-pair chromatography

Mechanism: Ionic sample molecules are ionically bound to an ion-pair reagent. The ion- pair reagent contains an unpolar part suitable for interaction with the unpolar hydrocarbon chain of the stationary phase.

Stationary phase: Reversed phase materials (RP-18, RP-8, CN), etc.

Mobile phase: Methanol, Acetonitrile, buffer with added ion-pair reagent in the concentration range of 0.001 to 0.01 M, etc.

Application: Ionic substances often show very poor retention in reversed phase chromatography. To overcome this difficulty an ion-pair reagent is added to the eluent.

4. Ion-exchange chromatography

	Strong	Weak
Cation exchanger	SO_3^-	COO^-
Anion exchanger	NR_3^+	NHR_2^+

Mechanism: Retention of reversible ionic bonds on charged groups of the stationary phase

Stationary phase:

Mobile phase: Aqueous buffer systems.

Application: Separation of substances which can form ions such as inorganic ions, organic acids, organic bases, proteins, nucleic acids.

Advantages of HPLC

1) It provides specific, sensitive and precise method for analysis of the different complicated sample.

2) There is ease of sample preparation and sample introduction.

3) There is speed of analysis.

4) The analysis by HPLC is specific, accurate and precise.

5) It offers advantage over gas chromatography in analysis of many polar, ionic substances, high molecular weight substances, metabolic products and thermo labile as well as nonvolatile substances.

Applications of HPLC

a) Natural Products: HPLC is an ideal method for the estimation of various components in plant extracts which resemble in structure and thus demand a specific and very sensitive method e.g., analysis of digitalis, cinchona, liquorice, and ergot extracts.

b) Stability studies: HPLC is now used for ascertaining the stability of various pharmaceuticals. With HPLC the analysis of the various degradation products can be done and thus stability indicating HPLC systems have been developed.

c) Bioassays and its complementation: Complex molecules as antibiotics and peptide hormones are mainly analyzed by bioassay which suffers from high cost, necessity replicates, poor precision and length of time required. Also, bioassay gives an overall estimate of potency and gives no guidance about the composition. Thus, HPLC can be used to complement bioassays and give an activity profile. It has been used for analysis of chloramphenicol, penicillins and clotrimoxazole, sulfas and peptides hormones.

d) HPLC has also been used in the cosmetic industry for quality control of various cosmetics.

MATERIALS AND METHODS

Bicalutamide (Pure)-Sura labs, Water and Methanol for HPLC-LICHROSOLV (MERCK), Acetonitrile for HPLC

Merck, HPLC WATERS Alliance 2695 separation module, **Software:** Empower 2, 996 PDA detector, pH meter-Lab India, Weighing machine-Sartorius, Volumetric flasks-Borosil, Pipettes and Burettes-Borosil, Beakers-Borosil, Digital ultra sonicator- Labman.

HPLC METHOD DEVELOPMENT:

TRAILS

Preparation of standard solution:

Accurately weigh and transfer 10 mg of Bicalutamide working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.1ml of the above Bicalutamide stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure:

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization:

Initially the mobile phase tried was Methanol and Methanol: Water with varying proportions. Finally, the mobile phase was optimized to Methanol: Phosphate Buffer in proportion 35:65% v/v.

Optimization of Column:

The method was performed with various C18 columns like, X- bridge column, Xterra, and C18 column. Symmetry ODS C18 (4.6 x 250mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS:

Instrument used	: Waters HPLC with auto sampler and PDA detector 996 model.
Temperature	: Ambient
Column	: Symmetry ODS C18 (4.6×250mm, 5µm)
Mobile phase	: Methanol: Phosphate BufferpH-3.6 (35:65)
Flow rate	: 1ml/min
Wavelength	: 235nm
Injection volume	: 10µl
Run time	: 8minutes

VALIDATION

PREPARATION OF BUFFER AND MOBILE PHASE:

Preparation of Potassium dihydrogen Phosphate (KH2PO4) buffer (pH-3.6):

Dissolve 6.8043 of potassium dihydrogen phosphate in 1000 ml HPLC water and adjust the pH 3.6 with diluted orthophosphoric acid. Filter and sonicate the solution by vacuum filtration and ultrasonication.

Preparation of mobile phase:

Accurately measured 350 ml (35%) of Methanol, 650 ml of Phosphate buffer (65%) were mixed and degassed in digital ultra sonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)

Mobile phase ratio	: Methanol: Phosphate Buffer (35:65) V/V
Column	: Symmetry ODS C18 (4.6×250mm, 5µm)
Column temperature	: Ambient
Wavelength	: 270nm
Flow rate	: 1ml/min
Injection volume	: 10µl
Run time	: 8min

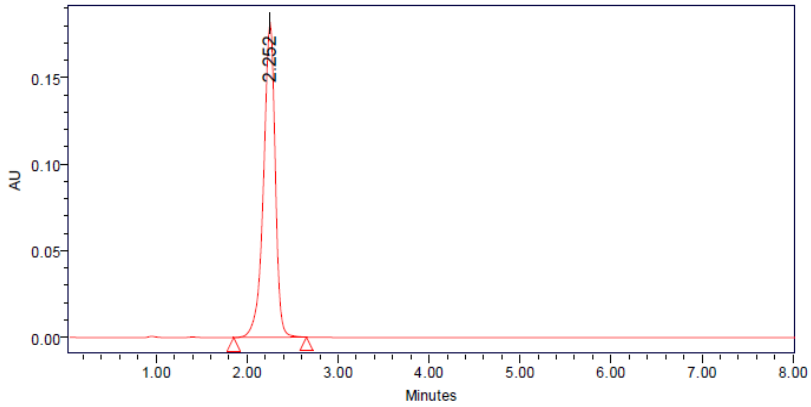

Figure: Optimized Chromatogram (Standard)

Table: Optimized Chromatogram (Standard)

S. No.	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Bicalutamide	2.252	1658242	185421	1.24	6569

Observation: In this trial it shows proper separation of peak and more plate count in the chromatogram and the tailing factor is within the limit. So, it is an optimized chromatogram.

Optimized Chromatogram (Sample)

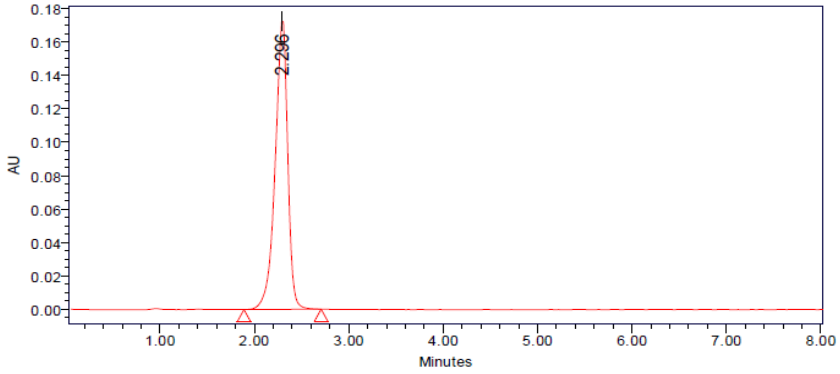

Figure-10: Optimized Chromatogram (Sample)

Table-8: Optimized Chromatogram (Sample)

S.N	Name	RT	Area	Height	USPTailing	USPPlate Count
1	Bicalutamide	2.296	1689654	185231	1.28	6659

Acceptance criteria:

- Theoretical plates must be not less than 2000.
- Tailing factor must be not less than 2.

METHOD VALIDATION

Blank:

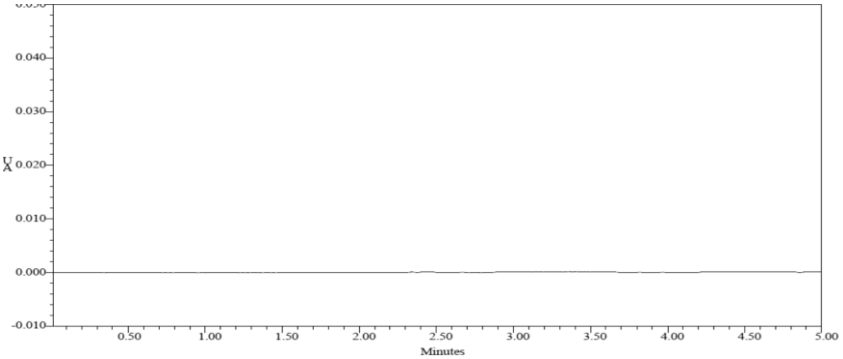

Fig: Chromatogram showing blank (mobile phase preparation)

Table: Results of system suitability for Bicalutamide

S. No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Bicalutamide	2.277	1652847	185647	6589	1.24
2	Bicalutamide	2.277	1653658	186254	6587	1.26
3	Bicalutamide	2.267	1654521	185475	6584	1.28
4	Bicalutamide	2.265	1653564	186594	6582	1.29
5	Bicalutamide	2.277	1658745	185684	6895	1.24
Mean			1654667			
Std.Dev.			2705.764			
%RSD			0.142371			

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is suitable.

SPECIFICITY: Assay (Standard):

Table: Peak results for assay standard

S. No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Bicalutamide	2.265	1658254	185468	1.24	6391	1
2	Bicalutamide	2.267	1658475	184524	1.23	6549	2
3	Bicalutamide	2.267	1658471	186598	1.25	6682	3

Table: Peak results for Assay sample

S. No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Bicalutamide	2.246	1645879	184574	0.85	6458	1
2	Bicalutamide	2.246	1645875	183598	0.86	6584	2
3	Bicalutamide	2.246	1658423	185472	0.85	6457	3

%ASSAY =

$$\frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \text{Weight of tablet} \times \text{Label claim}$$

The % purity of Bicalutamidein pharmaceutical dosage form was found to be 99.86%.

LINEARITY: CHROMATOGRAPHIC DATA FOR LINEARITY STUDY:

Table: Data for Linearity of Bicalutamide

Concentration µg/ml	Average Peak Area
6	1078475
8	1461129
10	1808358
12	2211573
14	2593778

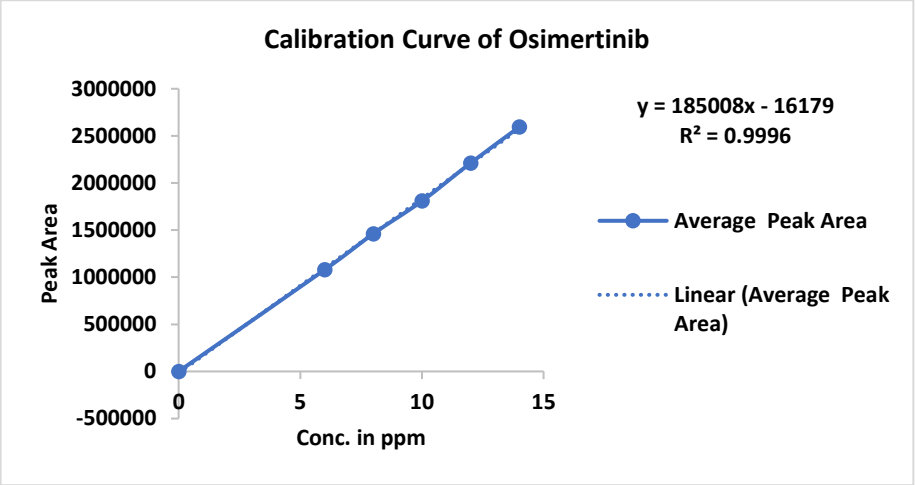

Fig: Linearity Curve of Bicalutamide

REPEATABILITY

Obtained Five (5) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

Table: Results of repeatability for Bicalutamide:

S. No	Peak name	Retention Time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Bicalutamide	2.293	1658954	186958	1.26	6785
2	Bicalutamide	2.276	1658745	187548	1.27	6854
3	Bicalutamide	2.286	1659865	189854	1.26	6852
4	Bicalutamide	2.277	1653254	186985	1.25	6784
5	Bicalutamide	2.280	1654781	189542	1.24	6895
Mean			1657120			
Std. dev			2913.592			
%RSD			0.175823			

Acceptance criteria:

- %RSD for sample should be NMT 2.
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Intermediate precision:

Table: Results of Intermediate precision for Bicalutamide

S. No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Bicalutamide	2.274	1678541	186589	6587	1.26
2	Bicalutamide	2.258	1685985	186598	6321	1.26
3	Bicalutamide	2.267	1685745	186985	6385	1.25
4	Bicalutamide	2.270	1685987	187854	6580	1.26
5	Bicalutamide	2.264	1698526	187549	6721	1.27
6	Bicalutamide	2.265	1685943	186598	6637	1.26
Mean			1686788			
Std. Dev.			6463.466			
%RSD			0.383182			

Acceptance criteria:

- %RSD of Six different sample solutions should not more than 2.

Table: Results of Intermediate precision Analyst 2 for Bicalutamide

S. No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Bicalutamide	2.277	1665847	167481	6854	1.25
2	Bicalutamide	2.255	1658989	167854	6785	1.26
3	Bicalutamide	2.265	1659845	167895	6854	1.24
4	Bicalutamide	2.255	1665964	167854	6895	1.26
5	Bicalutamide	2.253	1659863	168585	6459	1.25
6	Bicalutamide	2.252	1665986	167859	6456	1.26
Mean			1662749			
Std.Dev.			3501.766			
%RSD			0.210601			

Acceptance criteria: %RSD of Six different sample solutions should not more than 2.

ACCURACY:

Table: The accuracy results for Bicalutamide

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	109068.3	5	5.021	100.420%	
100%	202187	10	10.054	100.540%	100.72%
150%	297032.3	15	15.181	101.206%	

Acceptance Criteria:

- The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

LIMIT OF DETECTION FOR BICALUTAMIDE

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

LOD= 3.3 × σ / s

Where,

σ = Standard deviation of the response

S = Slope of the calibration curve

Result:

= 1.2µg/ml

Quantitation limit

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

LOQ=10×σ/S

Where,

σ = Standard deviation of the response

S = Slope of the calibration curve

Result:

= 3.6 µg/ml

ROBUSTNESS

The robustness was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Bicalutamide. The method is robust only in less flow condition. The standard of Bicalutamide was injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

Table: Results for Robustness

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	1658242	2.312	6569	1.24
Less Flow rate of 0.9 mL/min	1854215	2.458	6865	1.35
More Flow rate of 1.1 mL/min	1758468	2.032	6254	1.32

Acceptance criteria:

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

SUMMARY

The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to be at 270 nm and the peak purity was excellent.

Injection volume was selected to be 10µl which gave a good peak area.

The column used for study was Symmetry ODS C18 (4.6×250mm, 5µm) because it was giving good peak.

Ambient temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area and satisfactory retention time.

Mobile phase is Methanol: Phosphate Buffer pH-3.6 in the ratio of 35:65% v/v was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study.

Methanol was selected because of maximum extraction sonication time was fixed to be 10min at which all the drug particles were completely soluble and showed good recovery.

Run time was selected to be 8min because analyze gave peak around 2.252minand also to reduce the total run time.

The percent recovery was found to be 98.0-102 was linear and precise over the same range. Both system and method precision were found to be accurate and well within range.

The analytical method was found linearity over the range of 6-14ppm of the Bicalutamide target concentration.

The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

CONCLUSION

- In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Bicalutamide in bulk drug and pharmaceutical dosage forms.
- This method was simple, since diluted samples are directly used without any preliminary chemical derivatization or purification steps.
- Bicalutamide was found to be soluble in organic solvents such as ethanol, DMSO, and dimethyl formamide.
- Methanol: Phosphate Buffer (35:65) V/V was chosen as the mobile phase. The solvent system used in this method was economical.
- The %RSD values were within 2 and the method was found to be precise.
- The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods.
- This method can be used for the routine determination of Bicalutamide in bulk drug and in pharmaceutical dosage forms.

ACKNOWLEDGEMENT

Firstly, Authors acknowledge the Management of Venkateswara Institute of pharmaceutical sciences, Nalgonda for providing the laboratory facilities. The authors gratefully like to acknowledge Ravuri Ramya Krishna for her contribution in major funding and guiding during experimental work. Special thanks to Hechhu Ramana for revising and editing the research paper. The authors acknowledge the Nampelly Karnakar for assistance in statistical analysis. Finally, the authors thank for Poteti Karunakar for assisting throughout the entire research work.

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