International Journal of Chemistry and Pharmaceutical Sciences

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Method Development and Validation for Simultaneous Estimation of Bicalutamide and Atenolol by using RP-HP LC in Bulk and Pharmaceutical Dosage form

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A B S T R A C T

A reverse phase high performance liquid chromatographic method was developed for the determination of Bicalutamide and Atenolol in bulk and pharmaceutical dosage form. The separation was carried out on a [Column: Agilent C18 (4.6 x 250mm, 5µ m, Make: Waters)] using a mobile phase mixture of water, acetonitrile in an isocratic elution at a flow rate of 1ml/min. The detection was made at 254 nm. The retention time of Bicalutamide and Atenolol was found to be 2.325 and 4.322 min respectively, Calibration curve was linear over the concentration range of 1µg - 5 µg and 100µg-500µg of Bicalutamide and Atenolol. The propose method was validated as per the ICH guidelines. The meth od was accurate, precise, specific and rapid found to be suitable for t he quantitative estimation of related substances in drug an d pharmaceutical dosage form.

Keywords: Bicalutamide and Atenolol, HPL C, Validation studies

A R T I C L E I N F O

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Article History: Received 05 August 2015, **Accepted** 29 September 2015, **Available Online** 27 October 2015

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Manuscript ID: IJCPS2724

PAPER-QR CODE

Citation: Mulla Rafiyuddin, *et al.* Method Development and Validation for Simultaneous Estimation o f Bicalutamide and Atenolol by using RP-HPLC in Bulk and Pharmaceutical Dosage form. *Int. J. Chem, Pharm, Sci.*, 2015, 3(10): 2050-2054.

1. Introduction

Bicalutamide and Atenolol is an antihypertensive agent, adrenergic beta-1 Receptor antagonists. 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. Atenolol competes

with sympathomimetic neurotransmitters such as catecholamines for binding at beta (1)-adrenergic receptors in the heart and vascular smooth muscle, inhibiting sympathetic stimulation. This results in a reduction in resting heart rate, cardiac output, systolic and diastolic blood pressure, and reflex orthostatic hypotension. Higher doses of atenolol also competitively block beta (2)-adrenergic responses in the bronchial and vascular smooth muscles.

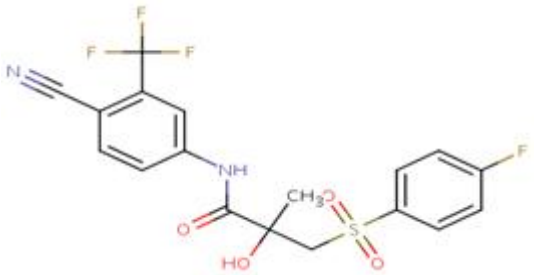


Figure 1: Bicalutamide

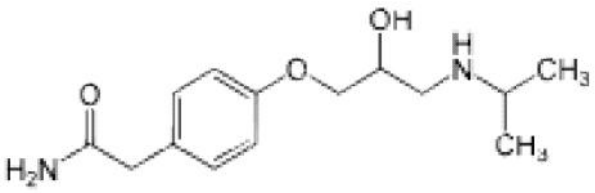


Figure 2: Atenolol

2. Materials and Methods

Preparation of mobile phase:

A mixture of Water 700ml (70%), 300 mL of ACN (30%) are taken and degassed in ultrasonic water bath for 5 minutes. Then this solution is filtered through 0.45 µ filter under vacuum filtration.

Preparation of standard solution (Atenolol and Bicalutamide):

Accurately weighed 10 mg of Atenolol and 10mg of Bicalutamide working standard were transferred into a 10mL and 100ml of clean dry volumetric flasks. About 7mL and 70ml of Diluents are added and sonicated to dissolve it completely and made volume up to the mark with the same solvent. (Stock solution) Further 0.3ml and 0.3ml of the above stock solution was pipetted into a 10ml volumetric flask and diluted upto the mark with diluents.

Method development & Optimization:

Using Mobile phase consisting of different buffers and methanol at different concentrations and different mobile phase's pH values are attempted .The peak was observed that the shape and retention time of voriconazole was found to be broad compared to the mixed phosphate buffer and acetonitrile composition of mobile phase. After selecting the best conditions based on peak performance , mixed water and acetonitrile in the ratio 70:30 and HPLC using column is Agilent C18 (150*4.6 *5µ) , the run times of the proposed method was 10 mins with isocratic solution. Column temperature is 25°C, flow rate is 1ml/min, PDA Detector is mainly used this purpose, after inject the standard solution volume was found to be 10mL.

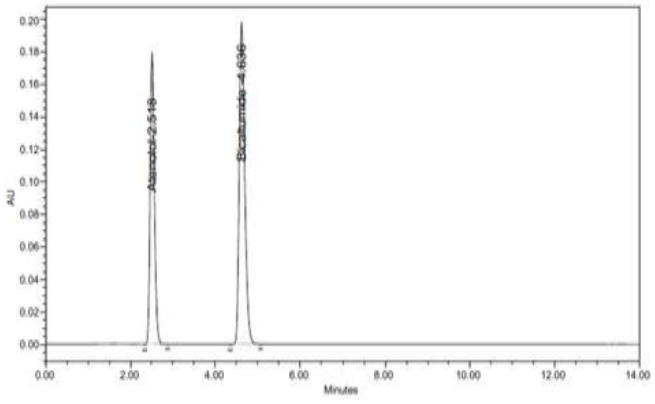
Method validation:

Bicalutamide and Atenolol standards taken to the10 mg was accurately weighed and transferred into a 25ml of volumetric flask containing HPLC grade Methanol s diluents. It was sonicated, dissolves completely and made volume up to the mark with the same solvent. The method was validated in accordance with ICH guidelines. The parameters assessed were precision, accuracy, linearity, specificity, robustness.

3. Results and Discussion

A new method was established for simultaneous estimation of Bicalutamide and Atenolol by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Bicalutamide and Atenolol by using Agilent C18 5µm (4.6*250mm) column, flow rate was 1ml/min, mobile phase ratio was Water: ACN (70:30%v/v), detection wave length was 254nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, PDA Detector 996, Empower-software version-2.The retention times were found to be 4.626 mins and 2.528 mins. The % purity of Bicalutamide and Atenolol was found to be 100.3% and 101.1% respectively.

The system suitability parameters for Bicalutamide and Atenolol such as theoretical plates and tailing factor were found to be 1.3, 5824.4 and 1.2, 2936.0 the resolution was found to be 9.4.The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study for Bicalutamide and Atenolol was found in concentration range of 20µg-100µg and 20µg-100µg and correlation coefficient (r2) was found to be 0.999 and 0.999, % mean recovery was found to be 102.5% and 101.0%, %RSD for repeatability was 0.6 and 0.5, % RSD for intermediate precision was 0.7 and 0.6 respectively. The precision study was precise, robust, and repeatable. LOD value was 3.1 and 3.02, and LOQ value was 10.1 and 10 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Bicalutamide and Atenolol in API and Pharmaceutical dosage form.



	Peak Name	RT	Area	Height	% Area	USP Resolution	USP Tailing	USP Plate Count
1	Atenolol	2.518	1371928	178985	41.266		1.18	3082.85
2	Bicaltumide	4.636	2042545	197563	58.744	9.36	1.23	6036.03

Figure 3

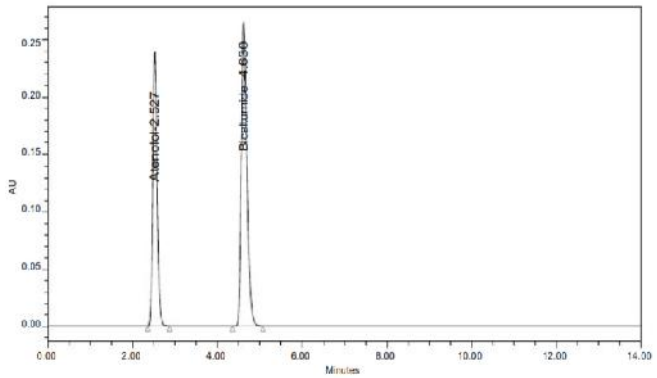


Figure 4

Table 1: Sample Chromatogram values for Repeatability

S.No	Peak name	RT	Area
1	Atenolol	2.527	1718661
2	Atenolol	2.528	1728892
3	Atenolol	2.528	1743398
4	Atenolol	2.529	1723892
5	Atenolol	2.527	1735820
6	Atenolol	2.528	1742589
Mean			1732209
Std.dev			9216.839
%RSD			0.5

Table 2

S.No	Peak name	RT	Area
1	Bicalutamide	4.630	2550539
2	Bicalutamide	4.628	2574105
3	Bicalutamide	4.626	2590892
4	Bicalutamide	4.632	2560539
5	Bicalutamide	4.626	2594102
6	Bicalutamide	4.628	2563982
Mean			2572360
Std.dev			15839.18
%RSD			0.61

Accuracy: The spiked level was found to be at 50,100,150 and the % recovery was found to be 102.5, 101.0% respectively.

Acceptance Criteria: The % Recovery for each level should be between 98.0 to 102.0%.

Linearity

Table 3: Linearity Results Atenolol

S.No	Linearity Level	Concentration	Area
1	I	20 ppm	471543
2	II	40 ppm	656277
3	III	60 ppm	794999
4	IV	80 ppm	946124
5	V	100 ppm	1002139
Correlation Coefficient			0.999

Table 4: Linearity Results of Bicalutamide

S.No	Linearity Level	Concentration	Area
1	I	20 ppm	471543
2	II	40 ppm	656277
3	III	60 ppm	794999
4	IV	80 ppm	946124
5	V	100 ppm	1002139
Correlation Coefficient			0.999

Acceptance Criteria: Correlation coefficient should be not less than 0.999

Plotting of calibration graphs:

The resultant areas of linearity peaks are plotted against Concentration

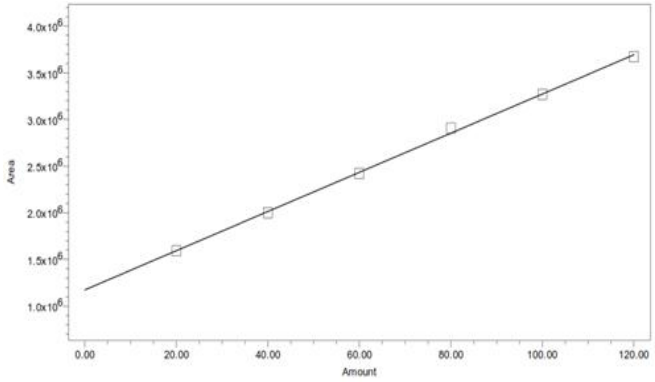


Figure 5: Calibration curve of Atenolol

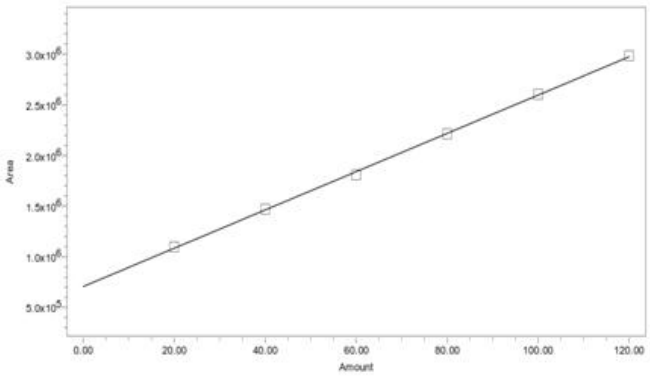


Figure 6: Calibration curve of Bicalutamide

Robustness:

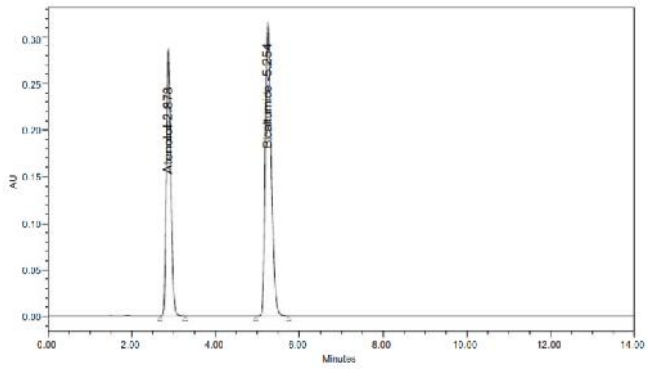


Figure 7

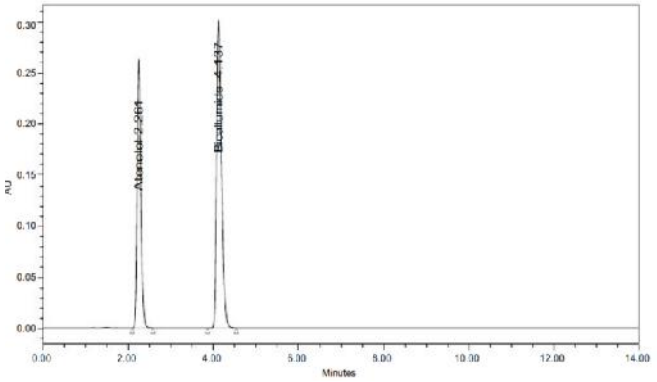


Figure 8: Chromatograms for Robustness Flow rate

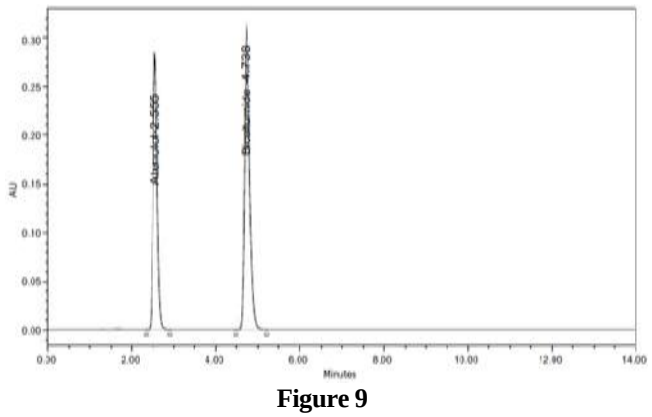


Figure 9

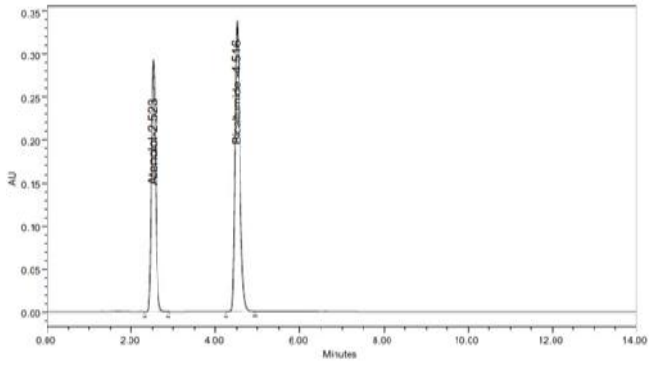


Figure 10: Chromatogram for Robustness organic composition

4. Conclusion

The method was found to be precise, accurate and linear over the linear concentration range. The method developed is unique in determining the impurities even at low levels than that of specifications. The analytical method validation of Atenolol and Bicalutamide in tablet dosage form by RP-HPLC was found to be satisfactory and could be used for the routine pharmaceutical analysis Method was validated as per ICH guidelines like system suitability, accuracy, precision, linearity, specificity, robustness and solution stability. Therefore, this HPLC method can be used as a routine analysis of these drugs in pharmaceutical formulations.

Table 5: Details of Instrument

S.No	Instrument	Model No.	Software	Manufacturer's name
1	HPLC Alliance	Waters 2695	Empower	Waters
2	PDA Detector	Waters 996	UV Win 5	Lab India
3	UV double beam	UV 3000	-	Satorius
4	spectrophotometer	BSA224SCW	-	Lab India
5	Balance	AD102U	-	-

Table 6: Accuracy results of Atenolol

% Concentration (at specification Level)	Area	Amount added (m)	Amount found (m)	% Recovey	Mean Recovery
50%	975578	5	5.0	101.3%	101.0%
100%	1718370	10	9.96	99.6%	
150%	1465857	15	14.9	99.3%	

Acceptance Criteria: The % Recovery for each level should be between 98.0 to 102.0%.

Table 7: Accuracy results of Bicalutamide

% Concentration (at specification Level)	Area	Amount added (m)	Amount found (m)	% Recovey	Mean Recovery
50%	1426646	5	4.9	101.8%	102.5%
100%	2551005	10	9.98	99.9%	
150%	2139845	15	15.0	100.0%	

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