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DETERMINATION AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF BICALUTAMIDE TABLET

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ABSTRACT: A sensitive and direct spectrophotometric method is developed which is free from extraction, derivatization, evaporation and complexation for the determination of Bicalutamide in pharmaceutical formulation. The optimum conditions for the analysis of the drug are established. The method permits the determination of Bicalutamide over a concentration range of 5µg/ml to 15µg/ml. Detection and quantification limit was found to be that 0.0117µg/ml and 0.0355µg/ml respectively. The attained results shows that good recoveries of 99.72%, with relative standard deviation of 0.21. All the calibration curve shows a linear relation between the absorbance and concentration with correlation coefficient higher than 0.999. Precision and Accuracy of the method was established with recovery studies. The proposed method is applicable for the assay of Bicalutamide investigation in dosage form and the results are in good agreement.

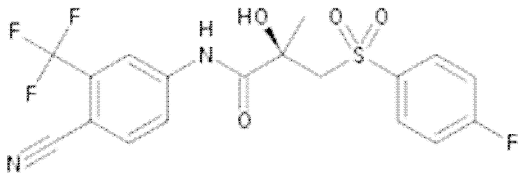
Keywords: Bicalutamide, Spectroscopy, Determination

1. INTRODUCTION

Bicalutamide (BCA) *N*-[4-cyano-3-(trifluoromethyl)phenyl]-3-[(4-fluorophenyl)sulfonyl]-2-hydroxy-2-methylpropanamide

Bicalutamide is an non-steroidal anti-androgen. It competitively inhibits the action of androgen by binding to cytosl androgen receptors in the target tissue, prostatic carcinoma is known to be androgen and or removes the source of androgen [1-3]

The structural formula of BCA by AM 1 method is illustrated below:



Bicalutamide drug

It is well absorbed in oral administration. Co-administration of Bicalutamide with food has no clinically significant effect on rate or extent of absorption, highly protein bound (96%), undergoes stereo specific metabolism. Medicinal chemistry is concerned with the understanding of chemical and biological mechanism by which the action of drug molecule can be explained [4 - 6]. It also tries to establish relation between chemical structure and biological activity and to link the later to the physical properties of the drug molecules. The discovery of a new and biologically important active compound usually gives rise to an extended search for closely related compounds of similar more effective, more specific or even opposite activity [7] The S-isomer (inactive) is metabolized primarily by glucuronidation. The R-isomer (active) also undergoes glucuronidation but is predominantly oxidized to an inactive metabolite followed by glucuronidation. Both the

parent metabolite glucuronides are eliminated in the urine and feces. The S-enantiomer accounting for about 99 % of total steady state plasma levels.

It is administered at dosages of 50 mg tablet daily. The adverse effect of Bicalutamide includes hot flashes, breast tenderness or pain and gynaecomastia. The literature reveals that various methods for the determination of BIC in biological fluids and pharmaceutical formulations. Among these methods are UV – visible Spectrophotometry [13], HPLC method using the uv detector [16], Rp-HPLC method [18-20], LC method [15,22] in plasma were reported. The objective of the present study is to develop a simple, precise, accurate and economic analytical method with a better detection range for the estimation of BIC in bulk drugs and in pharmaceutical formulations. No extraction, derivatization, or evaporation step, no complexation agent, and no baleful chemicals are involved in the proposed method, thereby decreasing the time and the error in the quantization. This paper describes a simple, reliable method for assaying bicalutamide by Spectrophotometer which has been used to analyze the formulation of BCA.

2. EXPERIMENTAL

2.1 Apparatus

A double-beam spectrophotometer Shimadzu UV 1601 PC model was used.

2.2 Chemicals and reagents

Determination and Validation of Bicalutamide was carried at M/s Cipla Ltd, India. Methanol used was spectro grade from S.D fine chemicals Ltd, India.

2.3 Standard solutions

Stock Solution

A methanolic primary stock solution of Bicalutamide (50 mg) was prepared in methanol. All the measurements were performed at room temperature. The standard solutions were prepared by the proper dilution of the primary stock solution with methanol to obtain working standard. For linearity study, serial dilutions were made for Bicalutamide in the range of 5 to 15 µg/ml concentrations were prepared by diluting the stock solution with methanol. The absorbances of these solutions were fitted in the calibration curve to calculate the accuracy and precision of the method.

2.4 For Formulation

The average weight of the tablets were determined by weigh 20 tablets and powdered. Tablet powder equivalent to 50mg of BCA was weighed and transferred to a 100 ml volumetric flask. About 60 ml of methanol was added and sonicated for 15 minutes complete dissolution of drugs, made up to the volume with methanol and filtered through whatman no 41 filter paper. Dilutions were made with methanol to attain a concentration of 10 µg/ml and spectra was recorded. Six replicates of analysis were carried out with sample weighed individually.

3. METHOD VALIDATION

3.1 Linearity

The method was validated according to ICH Q2B guidelines [8] for validation of analytical procedures in order to determine the Linearity, sensitivity, precision, and accuracy of the analyte [8 - 10]. For BIC five point calibration curves were generated with the appropriate volumes of the working standard solutions for UV methods. The linearity was evaluated by the least-squares regression method using unweighted data.

3.2 Precision and Accuracy

Precision is the degree of repeatability of an analytical method under normal operational conditions. The precision and accuracy were determined with standard quality control samples (in addition to calibration standards) prepared in triplicate at different concentration levels covering the entire linearity range[12]. The precision of the assay was determined by repeatability (intraday) and intermediate precision (inter-day) and reported as RSD % for a statistically significant number of replicate measurements [11]. The intermediate precision was studied by comparing the assays on three different days and the results are documented as the standard deviation and RSD %. Accuracy is the percent of analyte recovered by assay from a known added amount. Data from nine determinations over three concentration levels covering the specified range were obtained.

3.3 LOD and LOQ

The limit of detection (LOD) is defied as the lowest concentration of an analyte that an analytical process can reliably differentiate from back-ground levels. In this study, LOD and LOQ were based on the standard deviation of the response and the slope of the corresponding curve using the following equations

$LOD = 3s/m$; $LOQ = 10s/m$

Where s, the noise of estimate, is the standard deviation of the absorbance of the sample and m is the slope of the related calibrations graphs [14].

The limit of quantification (LOQ) is defined as the lowest concentration of the standard curve that can be measured with an acceptable accuracy, precision, and variability. The values of LOD and LOQ were given in Table 1.

3.4 Recovery study

Recovery of the analyte of interest from a given matrix can be used as a measure of the accuracy or the bias of the method. The same range of concentrations as employed in the linearity studies was used. To study the accuracy, precision, and reproducibility of the proposed method and dosage forms, recovery experiments were carried out using the standard addition method [17]. These studies were performed by the addition of known amounts of pure BIC to the pre-analyzed tablet formulation and the mixtures were analyzed using the proposed techniques. After parallel analyses, the recovery results were calculated using the related calibration equations.

4. RESULTS AND DISCUSSION

The development of a simple, rapid, sensitive, and accurate analytical method for the routine quantitative determination of samples will reduce unnecessary tedious sample preparations and the cost of materials and labor. BIC is a UV-absorbing molecule with specific chromophores in the structure that absorb at a particular wavelength and this fact was successfully employed for their quantitative determinations using the UV spectrophotometric method. The absorption spectrum of BCA in methanolic solution is shown in Fig. 1.

4.1 Calibration curves

Calibration curve data was constructed in the range of the expected concentrations of 5 to 15 µg/ml. Beer's law was obeyed over this concentration range. The regression equation was found to be y = 56.319x +0.0007. The correlation coefficient (r) of the standard curve was found to be greater than 0.9999. The stock solutions and working standards were made in methanol. The λ_{max} of the drug for analysis was determined by taking scans of the drug sample solutions in the entire UV region.

The characteristic of the calibration plot is presented in Table 1 and the analytical characteristics and necessary validation parameters for the UV techniques for BCA is presented.

Performing replicate analyses of the standard solutions was used to assess the accuracy, precision, and reproducibility of the proposed methods. The selected concentration within the calibration range was prepared in methanol and analyzed with the relevant calibration curves to determine the intra- and inter-day variability. The intra- and inter-day precision were determined as the RSD %. The precision, accuracy, and reproducibility of the results given in table 1and 2 demonstrate a good precision, accuracy, and reproducibility.

The proposed methods can be successfully applied for assay in tablet dosage forms without any interference. The assay showed the drug content of this product to be in accordance with the labeled claim (Table 2). The recovery of the analyte of interest from a given matrix can be used as a measure of the accuracy of the method (Table 2). In order to check the accuracy and precision of the developed method and to prove the absence of interference by excipients, recovery studies were carried out after the addition of known amounts of the pure drug to various pre-analyzed formulations of all drugs. The application of this procedure is explained in the experimental section. The obtained results demonstrate the validity and accuracy of the proposed method for the determination of all drugs in tablets (Table 2). These results reveal that the developed method have an adequate precision and accuracy, and consequently, can be applied to the determination of BCA tablet in pharmaceuticals without any interference from the excipients.

5. CONCLUSIONS

A spectrophotometric method for quantifying Bicalutamide in formulation samples has been developed and validated. The assay is selective, precise, accurate and linear over the concentration range studied. LOD was approximately 0.0117µg/ml in formulation. In summary, the proposed method can be used for the drug analysis in routine quality control.

Table 1: Regression data of the calibration lines for quantitative determination of BCA by UV method.

Parameters	Bicalutamide
Measured wavelength (λ_{max})	270
Linearity range, µg/ml	5-15
Slope	56.319
Intercept	0.0007
Correlation coefficient (r)	1.0000
SE of slope	23.07
SE of intercept	1.387
LOD, µg/ml	0.0117
LOQ, µg/ml	0.0355
Repeatability of absorbance, RSD %	0.17
Repeatability of wavelength, RSD %	0.12
Reproducibility of absorbance, RSD %	0.39
Reproducibility of wavelength, RSD %	0.02

Table 2: Assay results from BCA tablets and mean recoveries in spiked tablets

Parameters	Bicalutamide
Labelled claim, mg	50
Amount found, mg*	49.1
RSD %	0.44
Added, %	150, 170, 190, 200 220, 250
Found, %**	99.31, 99.64, 99.80, 99.82, 99.85, 99.9
Recovery, %	99.72
RSD, % of recovery	0.21

*Mean of six determinations, ** three determinations

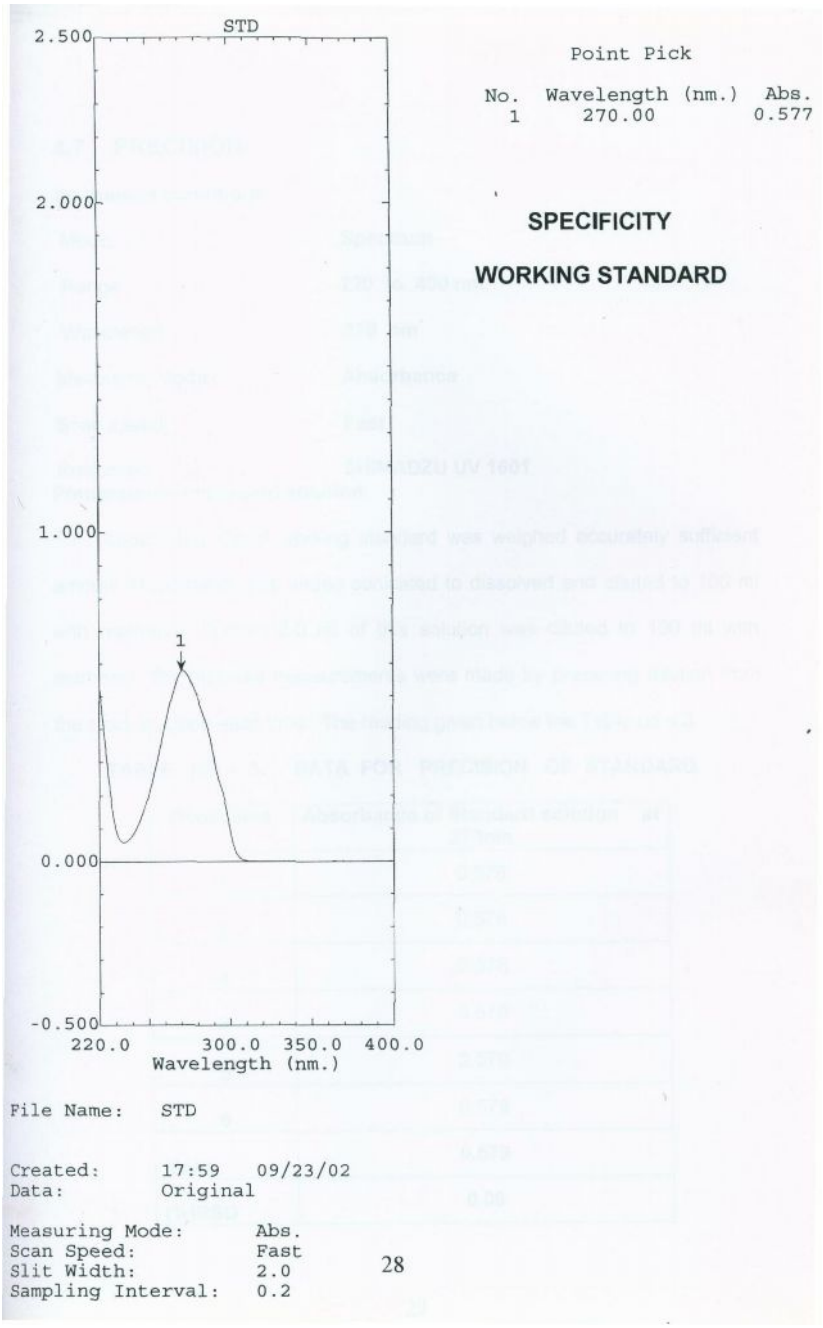


Figure 1: Absorbance

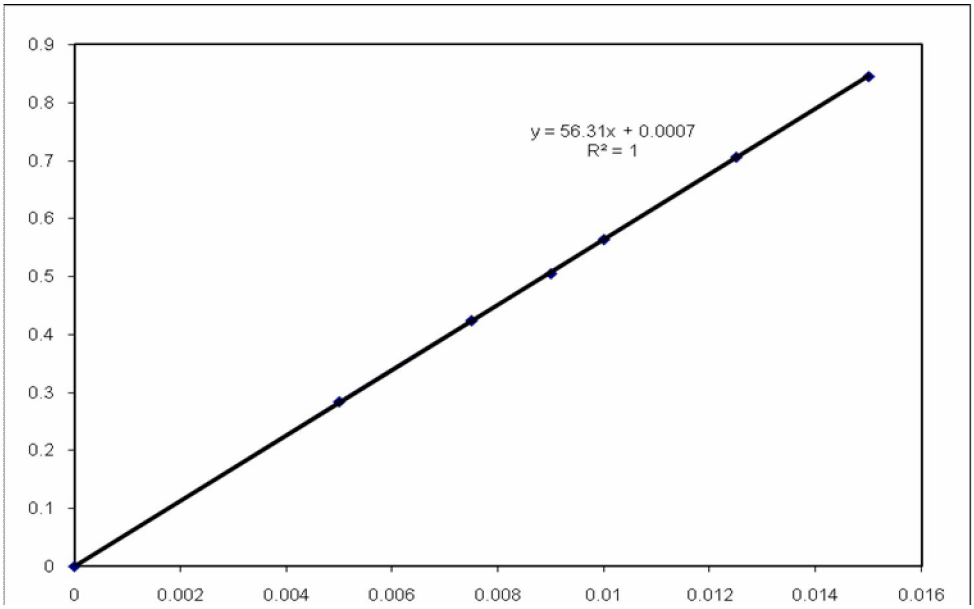


Figure 2: Linearity of Concentration Vs Absorbance

Figure 2: Regression analysis of the calibration curve for Bicalutamide showed a linear relationship between the intensity of absorbance and the concentration, with correlation coefficients higher than 0.9999 in all the curves assayed.

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