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Spectrofluorimetric Determination of Bicalutamide in Formulation and Biological Fluids

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A simple and reliable spectrofluorimetric method has been developed for the determination of bicalutamide in the pharma-ceutical preparation and biological fluids. The fluorescence of the product was found to have excitation at λ_{max} 272 nm and emission λ_{emi} at 328 nm. The method shows high sensitivity with linearity range from 0.1 to 0.6 µg/mL. The lower limit of detection (LOD) was found to be 5.264×10^{-3} µg/mL, 9.55×10^{-3} µg/mL and limit of quantification (LOQ) was found to be 1.595×10^{-2} µg/mL, 2.893×10^{-2} µg/mL in formulation and biologfical fluids, respectively. The different experimental parameters affecting the fluorescence intensity were carefully studied and optimized. The proposed method was applied successfully for determination of bicalutamide in the pharmaceutical preparation and biological fluids. The percentage recovery was found to be 98.279 ± 0.35; 99.98 ± 2.012 for formulation and biological fluids, respectively.

Key Words: Bicalutamide, Fluorimetry, Biological fluid.

INTRODUCTION

Bicalutamide (BCA) (+)-N-[4-cyano-3-(trifluoromethyl)phenyl]-3-[(4-fluorophenyl)sulfonyl]-2-hydroxyl-2-methyl propanamide is a bifunctional alkylating agent, non-steroidal which is used for the treatment of prostate cancer, competitively inhibits the action of androgens by binding to cystosi androgen receptors in the target tissue, prostatic carcinoma is known to be androgen and are removes the source of anderogen. When bicalutamide is combined with luteinzing hormone releasing hormone analogue therapy, the suppression of serum testosterone induced by the LHRH analogue is not affected. However in clinical trials with bicalutamide as a single agent for prostrate cancer[1,2] risosin serum testosterone and estradiol have been noted. It competes with testosterone and dihydrotestosterone for binding sites on the prostate and other sensitive tissues. It does not bind as tightly as to the receptors located outside the central nervous system and causes little increase in testosterone levels with little agonist activity[3-5].

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It is well absorbed in oral administration. Co-administration of bicalutamide with food has no clinically significant effect on rate or extent of adsorption, 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[6-8]. 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[9]. 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 *ca.* 99 % of total steady state plasma levels[10].

It is administered at dosages of 50 mg tablet once daily. The action has been started the same time as treatment with an LHRH analogue. The adverse effect of bicalutamide includes hot flashes, breast tenderness or pain and gynaecomastia. The studies have already been reported on this drug analyzed by UV-visible spectrophotometry[11,12] and HPLC method using the UV detector[13,14] in plasma[15-18]. The undifferentiated enantiomers in the human plasma were first collected on ODS column and then separated them by ES-OVM coloumn[18]. BCA was synthesized enantiometrically pure (S)-(+)-BCA and (R)-(-)-BCA and separated by chiralcel OJ column without reporting the chromatographic conditions[19]. The comparison of LC and SFC separations on cellulose derived chiralcel OD and amylase derived chiralpak AD chiral stationary phases using BCA and several other chiral compounds[20]. Recemic mixture of BCA by forming diastereomers on a Spherisorb-NH_2 column[21]. Evaluation of different CSPs for separation of enantiomers of BCA and its impurities[14]. This paper describes a simple, reliable method for assaying bicalutamide by spectrofluorimeter which has been used to analyze the formulation and plasma concentrations of bicalutamide in a patient.

EXPERIMENTAL

Bicalutamide sample was supplied by M/s Cipla Ltd., India as gift sample and used as such. Methanol used was spectro grade from S.D. Fine Chemicals Ltd., India. Ethyl acetate used was analytical reagent grade from Merck Ltd., India, Water used was HPLC grade generated from Milli-RO 10 plus Milli-Q purification system. (Milli Q Academic from Millipore (India) Pvt. Ltd. All other chemicals used were of analytical reagent grade supplied by M/s Fisher Inorganics and Aromatics Ltd., India.

Standard solutions

Stock solution: Bicalutamide (100 mg) was accurately weighed and dissolved in 100 mL of methanol and used as stock solution. Further dilutions were made with methanol to get required concentrations. For linearity study, serial dilutions were made for bicalutamide in the range of 0.1 to 0.6 mcg/mL concentrations were prepared by diluting the stock solution with methanol. The solutions were investigated in spectrofluorimeter by keeping the excitation[20,21] as 272 nm and emission as 328 nm.

For formulation: The average weight of the tablets were determined by weighing 20 tablets and powdered. Tablet powder equivalent to 25 mg of BCA was weighed and transferred to a 100 mL volumetric flask. About 60 mL of methanol was added and sonicated for 15 min complete dissolution of drugs, made up to the volume with methanol and filtered through filter paper. Dilutions were made with methanol to attain a concentration of 0.4 µg/mL and spectra was recorded. Six replicates of analysis were carried out with sample weighed individually. The average weight of the tablet was found to be 0.1874 g.

For biological fluids: About 100 mg of bicalutamide (BCA) powder was accurately weighed and taken in to a 100 mL volumetric flask, dissolved in methanol and made up to the volume with methanol. A series of dilution was made with water to get the concentrations of 5, 10, 15, 20, 25, 30 µg/mL. From each concentration 100 µL of drug solution was transferred in to a centrifuge tubes to which 900 µL of plasma was added, vortexed for 1 min for effective mixing of drug solution and plasma so that the final concentration will be 0.1 to 0.6 µg/mL. To the above mixture 5 mL of ethyl acetate was added and vortexed for 15 min on a rocking platform at moderate speed, followed by centrifugation at 1500 × g for 10 min. 4 mL of the supernatant fluid (organic solvent) was withdrawn and transferred to a 5 mL screw thread tapered disposable borosilicate centrifuge tube and evaporated to dryness under reduced pressure at room temperature in a vacuum centrifuge for 45 min. The residue was reconstituted in 2 mL of methanol, vortexed for 10 min and filtered through membrane filter (0.5 µ), filtrate was used for analysis. For each concentrtion six replicates were made by individual weighing. The spectrum was recorded by keeping 272 as λ_{max} for BCA and emission was scanned between 280 and 450 nm. Fluorescence maxima were observed at 328 as λ_{emi} for BCA and readings were noted. The calibration graphs were constructed taking mean fluorescence value at λ_{emi} in Y axis and concentration in X axis. The regression coefficient and intercept were calculated. The spectra of the solution were used for further linearity studies.

Calibration curves: Calibrtion standards of bicalutamide, covering the range 0.1-0.6 mcg/mL, were prepared by spiking drug free plasma with the suitably diluted bicalutamide, subjected to the extraction procedure and spectrum was taken as described above. The calibration curves were obtained by plotting the intensity of fluorescence against concentration of bicalutamide in spiked plasma. The slope and intercept of the calibration line were determined by linear regression using the least squares method.

Method validation: Method validation was performed in terms of specificity and selectivity, precision and accuracy, linearity and stabiilty.

Specificity and selectivity: The interference from endogenous compounds was investigated by the analysis of six different blank matrices.

Precision and accuracy: Method validation regarding reproducibility was achieved by replicate injections of extracted standard solutions at low, medium and high concentration levels, where intensity of fluorescence were measured in comparison to the intensity of fluorescence of the standard.

Intermediate precision study (day-to-day reproducibility) was conducted during routine operation of the system over a period of six consecutive days. Statistical evaluation revealed relative standard deviations at different values of six replicates. Within-day repeatability was studied by six replicate at three concentration levels.

Stability: Problems of stability are usually encountered with these compounds, mainly affecting plasma concentrations at room temperature. From blood sampling to analysis, storage in the freezer eliminates decomposition. The stability of bicalutamide was verified by storing sample solutions refrigerated for 6 months. Concentrations were measured once a week. For formulation the sample solutions were prepared and analyzed for 4 h in the interval of 0.5 h and found that the differences are within the limit.

RSULTS AND DISCUSSION

Calibration curves: Calibration standards for bicalutamide, covering the range 0.1-0.6 µg/mL, were prepared by serial dilutions with methanol for pure drug and in 1 mL of drug free plasma was subjected to the extraction procedure indicated above for biological fluids. The calibration curve was obtained by plotting the intensity of fluorescence of the bicalutamide *vs.* analyte concentration. The slope and intercept of the calibration line was determined by linear regression using the least squares method. In Figs. 1 and 2, regression analysis of the calibration curve showed a linear relationship between the intensity of fluorescence of bicalutamide and the concentration, with correlation coefficients higher than 0.9975 and 0.991 in all the curves assayed in pure form and in biological fluid, respectively. The precision and accuracy of the assay are presented in Tables 1 and 2.

TABLE-1
ANALYSIS OF TABLET FORMATION

S. No.	Formulation		Biological fluid	
	Drug content (mg/tab)	Label claim (%)	Drug content	Drug content (%)
1	51.4941	102.9884	50.2916	100.5834
2	51.6461	103.2922	51.6455	103.2910
3	51.8445	103.6892	49.9544	99.9088
4	50.5790	101.1582	49.1044	98.2089
5	50.5775	101.1550	50.1520	100.3041
6	51.1134	102.2669	48.7994	97.5989
Mean	51.2124	102.4250	49.9912	99.9825
SD	0.5436	1.0872	1.0060	2.0121

TABLE-2
ACCURACY OF BICALUTAMIDE

Level added (%)	Recovery (%)*	SD
50	97.98683	0.084478
75	98.39900	0.479297
100	98.45200	0.488849

*Mean of three determinations.

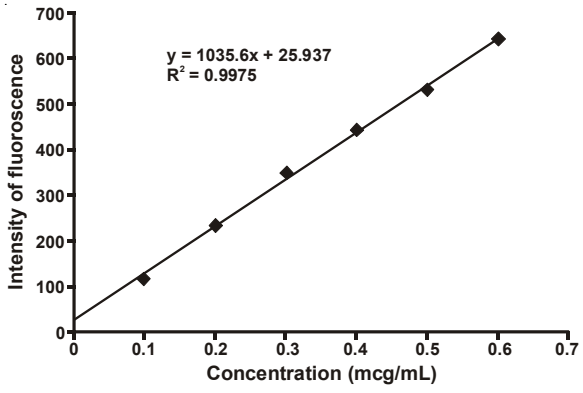

Fig. 1. Regression analysis of the calibration curve for bicalutamide showed a linear relationship between the intensity of fluorescence and the concentration, with correlation coefficients higher than 0.9975 in all the curves assayed

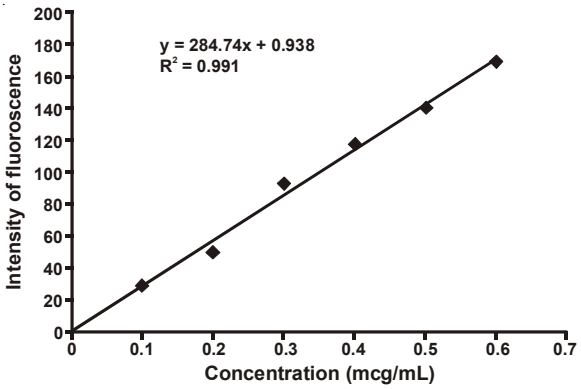

Fig. 2. Regression analysis of the calibration curve for bicalutamide in plasma showed a linear relationship between the intensity of fluorescence and the concentration, with correlation coefficients higher than 0.991 in all the curves assayed

Selectivity and specificity: The drug bicalutamide in the formulation and the plasma was well identified under this condition. There is no interferences was observed in six different blank plasma samples of bicalutamide. Fig. 1 shows the regression analysis of the calibration curve for bicalutamide in pure form showed a linear relationship between the intensity of fluoresence and the concentration, with correlation coefficients higher than 0.9975 in all the curves assayed. Fig. 2 shows the regression analysis of the calibration curve for bicalutamide in plasma showed a linear relationship between the intensity of fluorescence and the concentration, with correlation coefficients higher than 0.991 in all the curves assayed. Fig. 3 shows the absorption spectrum of bicalutamide and Fig. 4 shows fluoresence spectrum of bicalutamide at 272 nm as excitation.

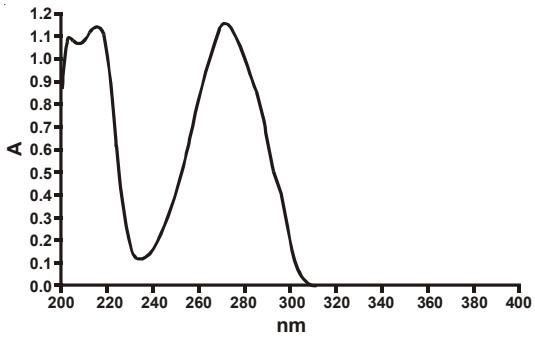

Fig. 3. Absorption spectrum of bicalutamide

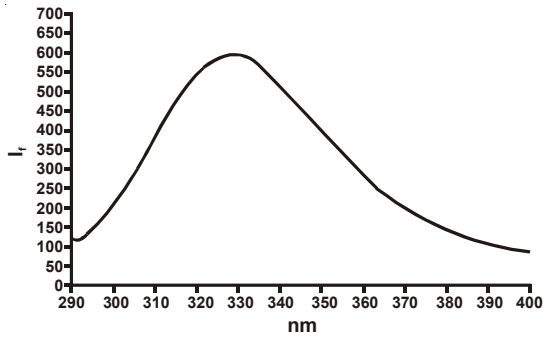

Fig. 4. Fluorescence spectrum of bicalutamide at 272 nm as excitation

Limit of detection (LOD) and limit of quantification (LOQ): The LOD determined as the amount of drug was found to be 5.264×10^{-3} µg/mL in formulation and 9.55×10^{-3} µg/mL in biological fluid. The LOQ was determined as the lowest concentration was found to be 1.595×10^{-2} µg/mL, 2.893×10^{-2} µg/mL in formation and in biological fluid, respectively.

Robustness: The method has been used by two different analysts with between person variability within the range of inter-assay variabilities observed for the same analyst. For the lack of resources, the method could not be repeated in a different laboratory or using different equipment.

Accuracy was estimated as the deviation to the observed mean concentration from actual concentrtion and found to be less than 2 % for all the concentrations.

Conclusion

A spectrofluorimetric method for quantifying bicalutamide in formulation and plasma samples has been developed and validated in human plasma. The assay is selective, precise, accurate and linear over the concentration range studied. Using 1 mL of plasma, concentrations of bicalutamide as low as 1.595×10^{-2} µg/mL in formulation and 2.893×10^{-2} µg/mL in plasma, respectively could be precisely quantified and LOD was *ca*. 5.264×10^{-3} µg/mL in formulation and 9.55×10^{-3} µg/mL mcg/mL in plasma, respectively. The method is simple and suitable for the determination of bicalutamide in formulation and plasma in pharmacokinetic studies.

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