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Bicalutamide quantification in human plasma by high-performance liquid chromatography: Application to bioequivalence study

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ABSTRACT

A liquid chromatographic procedure was developed for the determination of a new anticancer agent bicalutamide in plasma using fluorescence detection. Chromatography was performed using a reversed phase trimethylsilyl bonded silica column with a mobile phase of water: acetonitrile (30:70) at a flow rate of 1 ml/min. Detection of the eluted peaks was observed using excitation and emission wavelengths of 272 and 328 nm, respectively. Chromatographic run time did not exceed 10 minutes with no interference of endogenous material. The calibration curve was linear over the concentration range of 0.05 to 0.8 ng/µl and inter – and intra – assay imprecision was less than 10%. The lower limit of quantitation was assessed at 0.03 ng/µl.

Key words: Bicalutamide, HPLC, Fluorescent detector, Bioequivalance studies

INTRODUCTION

Bicalutamide (+) – 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[4,5] risosin serum testosterone and estradiol have been noted.

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[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 faces. The S – enantiomer accounting for about 99 % of total steady state plasma levels[10]. It is administered at dosages of 50 mg tablet once daily. Action will be started the same time as treatment with an LHRH analogue. The adverse effect of Bicalutamide includes hot flashes, breast tenderness or pain and gynaecomastia. There were studies already have reported on this drug analyzed by UV – Visible and HPLC method using the UV detector from 10 – 250 mg/ml concentrations[11]. This paper describes a simple,

reliable method for assaying bicalutamide by HPLC using fluorescence detection which has been used to analyze plasma concentrations of bicalutamide in a patient.

EXPERIMENTAL

Chemicals and reagents

Bicalutamide was obtained from Cipla Limited. Spectro grade solvents were purchased from E – Merck Chemical Company. The purity of drug was checked by similar fluorescence spectra when excited with different wavelengths. Double distilled water was used for the preparation of aqueous phases. Serum samples were kindly provided from the blood donation unity of Rajah Muthiah Medical College and Hospital, Annamalai University, Annamalai Nagar.

Instruments

Absorption spectral measurements were carried out with a Shimadzu UV 1601 PC model UV – Visible spectrophotometer and the fluorescence measurements were made using a Perkin Elmer LS 55 specftrofluorimeter. Chromatograms were obtained with a Perkin Elmer HPLC with series 200 pump.

Instrumentation and conditions

A Perkin-Elmer Model Series – 200 pump was used to deliver a mixture of mobile phase acetonitrile, water (70:30, v/v) at 1 ml/min and sample was injected through Rheodyne injector 7725i separation was carried out by Spheri – 5 RP – 18 with a particle size of 5 µm and its dimension is 250 × 4.6 mm column. Detection was performed with a Waters Model 420 – AC fluorescence detector equipped with filters of 272 nm (excitation) and 328 nm (emission). Peaks were recorded with a networking channel Perkin – Elmer Model series interface NCI 900.

Bicalutamide is a highly fluorescent compound, with maximum excitation and emission wavelengths of 272 and 328 nm, respectively. Therefore, an excitation filter of 280 nm and an emission filter cut-off of 320 nm were selected for the detection in the chromatographic system. Chromatograms of blank plasma and plasma with bicalutamide are shown in Fig. 2a & 2c, where it can be observed that there are no interfering peaks of endogenous plasma components and that peaks of bicalutamide.

Stock solutions

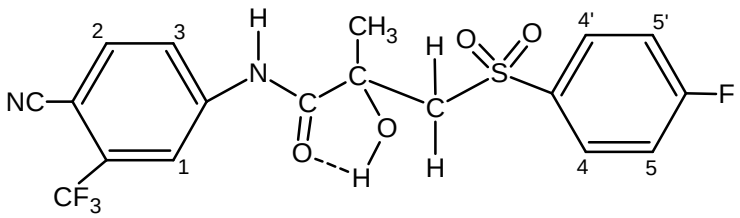
Stock standard solutions were prepared by dissolving an accurate weight of 100 mg of drug in 100 ml of Acetonitrile. Serial dilutions were made in the range of 0.05 – 0.8 ng/µl with Acetonitrile: water (7:3). A 20 ml aliquot was injected into the column for quantitative analysis was based their peak area measurement as ratio towards the peak area of internal standard. Pooled human drug free plasma obtained from healthy volunteers was spiked from the stock solutions to attain 0.4 – 0.8 ng/µl.

Internal Standard

A stock solution was prepared by dissolving 100 mg of 3, 3' - diamino diphenyl sulphone in a 100 ml volumetric flask. The solution was stored at 4° C. the working solution was prepared by dilution with mobile phase to get the final concentration of 0.5ng/µl.

Pharmaceutical sample preparation

A number of commercial tablets were finely powdered in a porcelain mortar and an accurately weighed portion of the sample equivalent to the



Scheme - 1: Structure of Bicalutamide

bicalutamide content of 25mg, was quantitatively transferred to a volumetric flask and dissolved and diluted with Acetonitrile. Further dilutions were made to get a concentration of 0.4 ng/µl with Mobile phase.

Extraction from spiked plasma

Aliquots of 900 µl of human blood plasma were spiked with 100 µl (10 µg) of standard solution at different concentration levels. The mixture was vortexed for 1 minute and then 5ml ethyl acetate was added. The contents were mixed for 15 min on a rocking platform at 'moderate' speed, followed by centrifugation at 1500 × g for 10 min. The supernatant (organic) phase was transferred to a 5ml screw thread tapered disposable borosilicate centrifuge tube and evaporated to dryness under reduced pressure at room temperature in a vacuum centrifuge for 45 min. To the residue, 100µl of mobile phase was added, vortexed and injected. A nine point calibration curve was constructed in drug free plasma in the range of 0.05 – 0.8 ng/µl and processed similarly with each batch of samples.

Method Validation

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

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 peak areas were measured in comparison to the peak area of the internal standard.

Intermediate precision study (day – to – day reproducibility) was conducted during routine operation of the system over a period of nine consecutive days. Statistical evaluation revealed relative standard deviations at different values for six injections. Within – day repeatability was studied by eight 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.

RESULTS AND DISCUSSION

Calibration curves

Calibration standards for bicalutamide, covering the range 0.05 – 0.8 ng/µl, were prepared in 1 ml of drug free plasma and subjected to the

Table - 1: Precision and accuracy of bicalutamide assay in plasma

Nominal conc. (ng/µl)	Conc.found (ng/µl)	Accuracy (fund /nomial, %)	Precision (RSD)	Relative Error (%)
		Intra – assay (n=6)		
0.05	50.3±0.5	102	4.9	1.1
0.4	406.4±0.8	102	0.8	- 0.1
0.8	813.7±0.3	103	3.1	0.1
		Inter – assay (n=6)		
0.05	50.9±1.0	104	0.6	- 8.5
0.4	401.1±1.0	100	0.8	1.8
0.8	809.3±1.4	101	1.5	0.0

extraction procedure indicated above. The calibration curve was obtained by plotting the peak – height ratio of bicalutamide/internal standard versus analyte concentration. The slope and intercept of the calibration line was determined by linear regression using the least squares method. In Fig.- 1, regression analysis of the calibration curve showed a linear relationship between the peak-height ratio of bicalutamide and the bicalutamide concentration, with correlation coefficients higher than 0.992 in all the curves assayed. The accuracy and precision of the assay are presented in Table -1.

Selectivity and Specificity

The drug bicalutamide and the internal standard were well separated under the HPLC condition applied. Retention times were 5.5 minutes for bicalutamide and 6.9 minutes for internal standard. After addition of acetonitrile to the mobile phase, it was possible to separate small interfering peaks from the analytes, so that no interferences was observed in six different blank plasma samples around the retention times of bicalutamide and internal standard. Fig.2 shows the chromatograms of (a) a blank plasma sample, (b) a quality control standard with a bicalutamide concentration of 0.4 ng/ml and chromatogram of drug and (c) internal standard in plasma.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD determined as the amount of drug corresponding to the signal to noise ratio of 3:1 was 0.01 ng/µl. The LOQ was determined as the lowest concentration of the analyte in plasma that could be quantified with an inter assay was found to be 0.01 ng/µl.

Intra – Assay and Inter – Assay Variation and Accuracy

The intra – assay RSD for the analyte ranged from 0.8 to 4.9 and inter – assay RSD for the analyte ranged from 0.6 to 2.4. The accuracy of the measurements was determined using the six quality control samples for each compound in every run and the results are reported in Table -1.

Robustness

The method has been used by three 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 entration and found to be less than 5% for all

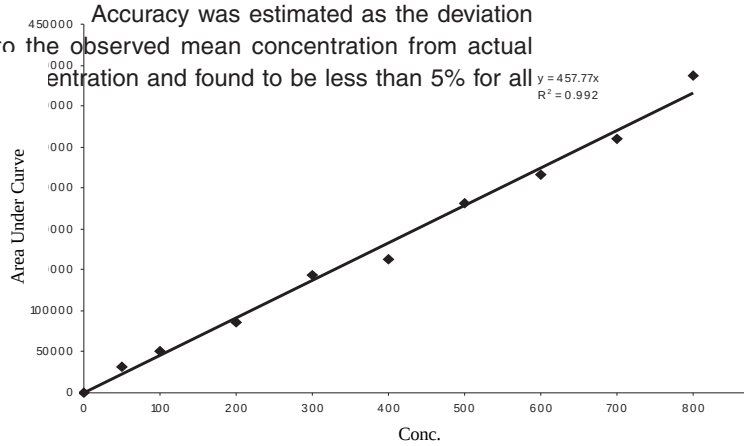


Fig. - 1: Regression analysis of the calibration curve showed a linear relationship between the peak-height ratio of bicalutamide and the bicalutamide concentration, with correlation coefficients higher than 0.992 in all the curves assayed.

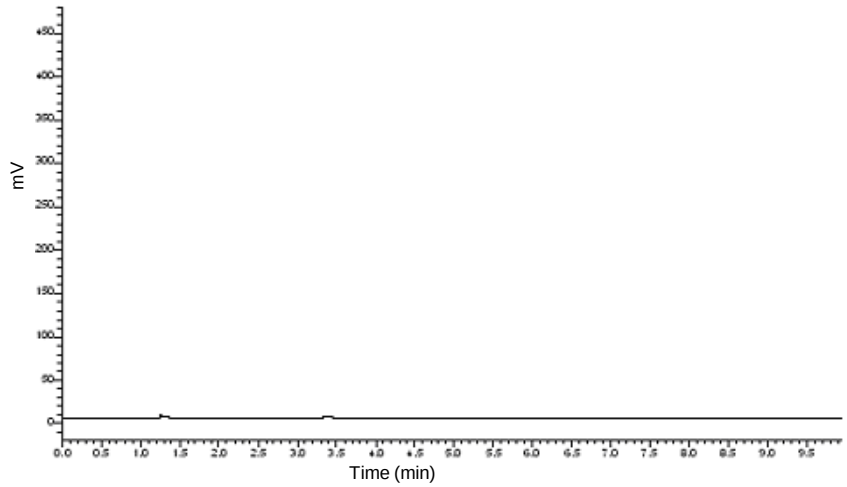


Fig. - 2(a): Blank plasma sample

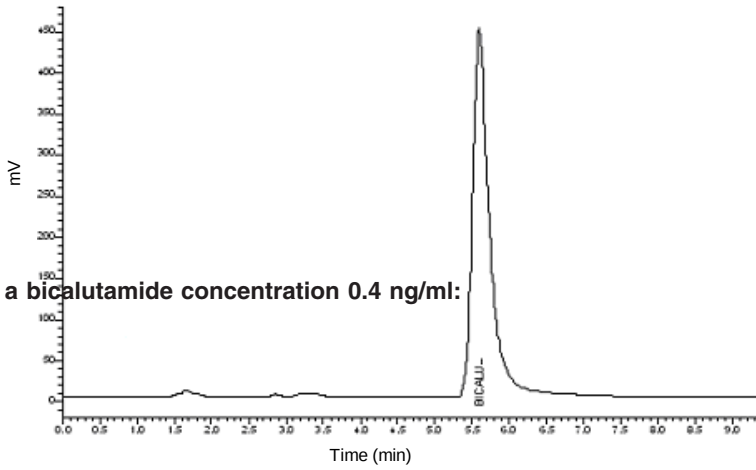


Fig. - 2(b): A quality control standard with a bicalutamide concentration 0.4 ng/ml:

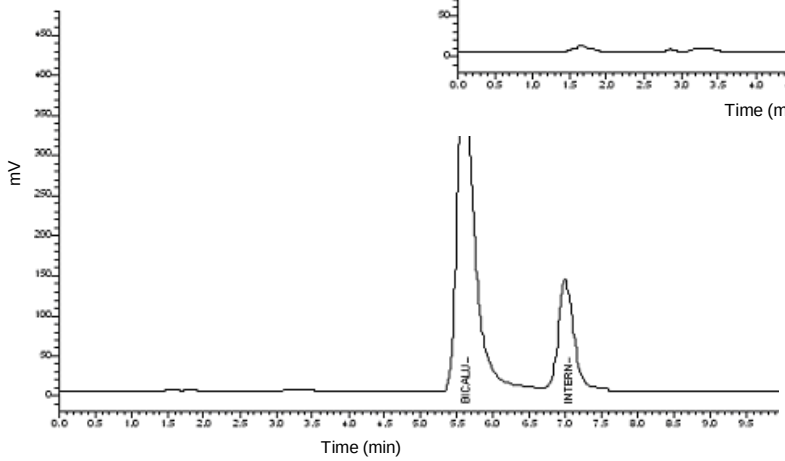


Fig. - 2(c): Chromatogram of drug and internal standard in plasma:

the concentrations. The intra- and inter-day RSDs were lower than 10% for all concentrations assayed. With this method, concentrations of bicalutamide in plasma were as low as 0.03ng/µl could be precisely quantified (LOQ) with a LOD of approximately 0.01ng/µl, which based on a signal to-noise ratio of 3. The recovery at 0.05 – 0.8ng/ml range was found to be 70±5%. The chromatograms obtained with this method do not have interfering peaks of plasma components and the LOD is found to be low (0.03ng/µl).

Conclusions

A liquid chromatographic method for quantifying Bicalutamide in 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 0.03ng/µl could be precisely quantified and LOD was approximately 0.01 ng/µl. The method is simple and suitable for the determination of plasma bicalutamide in pharmacokinetic studies.

REFERENCES

1. Clarke, J. S. *et al., J. Pharm, Biomed. Anal.,* **12:** 643 (1994).
2. Hsu. H, *et al., C. S. J. Food and Drug Anal.,* **2:** 161 (1994).
3. Cockshott. I. D, *et al., Biopharm. Drug Dispos.* **18:** 499-507 (1997).
4. D. A. William and T. C. Lenke: Foye's Principle of Medicinal Chemistry, 5th Ed. B. I. Pbs., 711 (2005).
5. C. I. Carswell and P. Figgitt, *Drugs:* **2471,** 62 (2002).
6. Steffen Bauer *et al., J. Pharm. And Biomed Anal.* **31:** 551-555 (2003).
7. Samanidou *et al., J. Chrom. B.* **788:** 147-158 (2003).
8. Luis Renato Pires de Abreu *et al. AAPS Pharm Sci*. **5**(2). Available at website http://www.pharmasci.org accessed on December (2006).
9. J.F.Li, Y.X.Wei, L.H.Ding, and C.Dong: *Spectrochim. Acta.* **59A,** 2759 (2003).
10. D.M. Brahmankar and S.B. Jaiswal: Biopharmaceutics and Pharmacokinetics, A Treiatise, Vallabh prakasham Pub., P – 302 (2006).
11. Nageswara Rao, R., Narasa Raju, A., Nagaraju, D., *Journal of Pharmaceutical and Biomedical Analysis,* **42**(3): 347-353 (2006).