QUANTITATIVE ANALYSIS OF FLUTAMIDE IN BULK DRUG AND ITS PHARMACEUTICAL DOSAGE FORM BY USING HPLC

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Abstract:

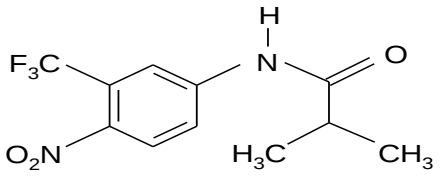
A simple, rapid and reproducible high performance reverse phase liquid chromatographic method has been developed for quantitative estimation of Flutamide in tablets using a C-8 column and UV detection at 294nm. The isocratic elution was used to quantify the analyte. The samples were chromatographed on C-8 column and the mobile phase was Acetonitrile: Water (50:50, v/v) was pumped at 1 mL/min. The method was linear between 10-125µg mL^{-1}, statistically validated for its linearity, precision and accuracy. The intra-and - inter day variation was found to be less than 1% showing high precision of the assay method. It was found that the excipients in the commercial tablets did not interfere with the method.

Key words:

Flutamide, High performance liquid chromatography (HPLC), Acetonitrile

Introduction

Flutamide[1-3] is an acetanilide, nonsteroidal compound with orally active antiandrogenic properties. It exerts its antiandrogenic action by inhibiting androgen uptake and/ or nuclear binding of androgen in target tissues. It is used, usually with gonadorelin analogues; in the palliative treatment of prostatic carcinoma. It is freely soluble in Acetone, Ethyl acetate, Methyl alcohol.



FLUTAMIDE

Fig: 1 Chemical structure of Flutamide (CAS No. 1311-84-7) molecular formula is $C_{11}H_{11}F_3N_2O_3$

Flutamide is chemically 2-Methyl-N-[4-nitro-3-(trifluoromethyl) phenyl] propanamide; [α′,α′,α′-Trifluoro-4′-nitroisobutyro-m- toluidide]. It is official only in B.P. Few analytical methods are reported Spectrophotometry[4-7], Spectroflurometry[8], HPLC[9] , and Polarography[10] for quantitative estimation of Flutamide and its metabolites.

The aim of this study is to develop a new HPLC method for the quantitative estimation of Flutamide in bulk drug and pharmaceutical dosage forms with high sensitivity, accuracy, precision and economical too. Specific HPLC assay is described that has been applied to quality control of drugs which require high sensitivity and selectivity. The results of the analysis were validated by statistical methods and recovery studies.

EXPERIMENTAL

Chemicals and reagents

Flutamide reference substance obtained from Cipla, Goa, India. Acetonitrile and Water (HPLC grade) were purchased from Merck Ltd. (Mumbai, India). The commercially available Flutamide tablets were obtained from local market. Each Flutamide tablet contains 250mg of active drug. The mobile phase and diluent composition were same which contains Acetonitrile and Water.

Equipment

Quantitative HPLC were performed on isocratic high performance liquid chromatograph (Waters 2965 separation module) with two Displacement pumps DNX2491, variable wave length programmable DAD/UV-Visible detector(Waters 2487) was used. A mobile phase contains Acetonitrile and water (50:50, v/v) pumped at a flow rate 1.0mL/min through a Agilent XDB C-8 column (4.6x150mm, 5µm). The injection volume 20µL and peaks were detected at 294nm. The total run time for assay was 8min. assay was done at

ambient temperature. The HPLC system was equipped with the software Empower-2.

Standard solution

The standard Flutamide solution was prepared by accurately weighing 25mg of Flutamide which was transferred to a 100ml volumetric flask, add about 70ml of diluents (Acetonitrile: Water, 50:50 v/v) and sonicate to dissolve it completely for 20min and make up to the mark with the diluents. From this stock take 1ml and make up to the mark with diluents to get final concentration of 25µg mL^{-1}.A typical chromatogram was shown in Fig 2. Prepare different concentrations of standard solutions into 10,25,50,75,100,125 µg mL^{-1} and injected into the column at a flow rate 1.0 mL/min. The peak areas of the different drug concentrations were calculated and were shown in Fig 3**.**

Sample preparation

For quantitative determination of Flutamide in tablets, twenty tablets were weighed to be obtaining the average tablet weight. The tablets were grounded up and powder sample equivalent to 25mg were transferred to a 100ml volumetric flask; add about 70mL of diluents and sonicate to dissolve it completely for 20min and make up to the mark with the diluent. Mix well and filter through 0.45µm filter paper. Further pipette out 1mL of the above solution in to a 10mL of volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45 µm filter.

The sample solution was injected five times at 20 µg mL^{-1} into the column at a flow rate 1.0 mL/min, the peak area of the sample drug were calculated.

Assay

20 tablets each containing 250mg were weighed and powdered. An accurately weighed portion to the powder equivalent to 100mg of Flutamide was transferred to 100ml volumetric flask containing 50ml of mobile phase. The contents of the flask were sonicated for 20min to dissolve Flutamide and made up to volume with mobile phase and resulting mixture filtered through 0.45µm filter paper. 2.5mL of the above solution was diluted to a 100mL with mobile phase. This solution (20 µL) was injected 5 times in to the column. The mean values of peak areas of five such determinations were calculated and the

content in the tablets was quantified using regression equation obtained above. The same procedure was followed for the estimation of Flutamide in other commercially available tablet dosage forms.

Results and Discussion

Flutamide can be analyzed by using proposed HPLC method both as pure drug and pharmaceutical dosage forms. The aim of the study was to develop a chromatographic procedure for the analysis of Flutamide in pure drug and pharmaceutical dosage forms. Water and Acetonitrile was chosen as the organic solvents because of Flutamide solubility and reversed phase chromatography was used to perform the assay. The column used in this research (150mm x 4.6mm) showed a high resolution separation. Moreover the mobile phase was modified. The influence of different organic solvents in the mobile phase was studied aiming to establish the best experimental conditions for the analysis of Flutamide.

The UV absorption spectrum of Flutamide was found at 294nm. The retention time for Flutamide was 4.54min for a run time of 8min. Each of the samples was injected 5 times and same retention times were observed in all cases. The system was validated for system suitability, precision, accuracy, linearity, ruggedness and robustness.

Linearity was obtained in the concentration range of 10-125µg mL^{-1}. A total 20µL volume of each sample was injected into the column. The peak areas were shown in Table 2. The standard calibration curve of Flutamide was constructed by plotting the peak area versus the respective drug concentration shown in Fig 4. The correlation coefficient was 1.000 indicating excellent linearity. The regression equation for Flutamide was calculated by the equation: $y = a+bx$, where **x** and **y** are concentration and area respectively.

The precision was determined for intra- and inter day on three different days. Precision was assessed by performing replicate analyses of quality control samples against calibration standard. The results obtained for Inter and Intraday variations were shown in Table 2.

Recovery studies were carried out at three different levels 50%, 100%, 150%.

The results were shown in Table 3. About 98% - 102% Flutamide could be recovered from the preanalysed sample indicating the high accuracy of the proposed HPLC method. No interfering peaks were found in the chromatograms due to tablet excipients. Flutamide was stable during all the procedure.

The robustness of the method was determined by carrying out the analysis under conditions during which the flow rate, mobile phase composition, temperature and different columns at intra-and inter day variation, there are insignificant variations were observed in those above analyses shown in Table 4.

The drug content in the tablets was quantified using the proposed method. The mean content of Flutamide in commercial tablet dosage form is shown in Table 5.

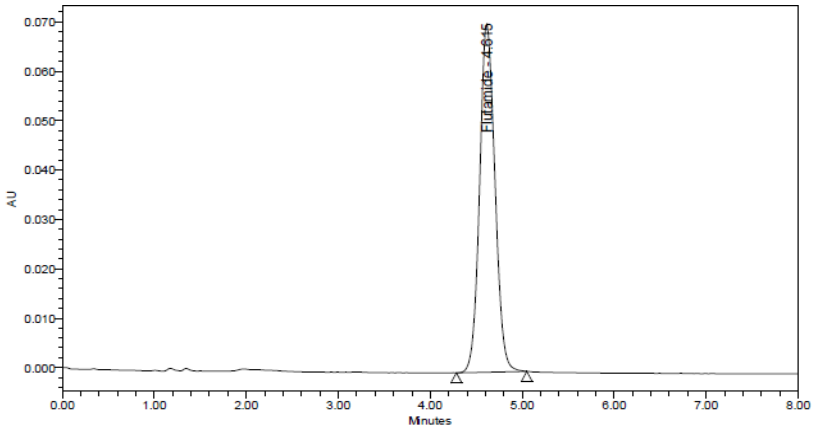


Fig 2: A typical chromatogram For Flutamide

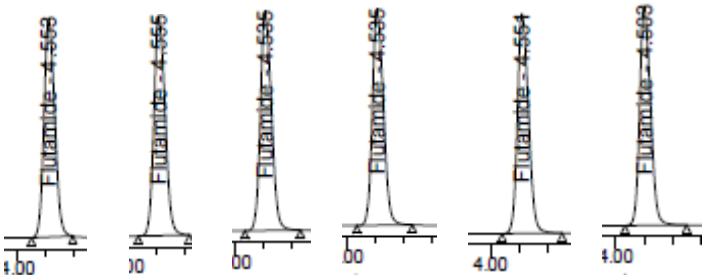


Fig 3. Typical chromatograms of Flutamide. Conditions XDB C-8 Column (150mm x 4.6mm), particle size 5µm; Acetonitrile: Water (50:50, v/v); flow rate: 1.0 mL min^{-1}; UV detector at 294nm; RT 4.5min; ambient temperature (25^0c).

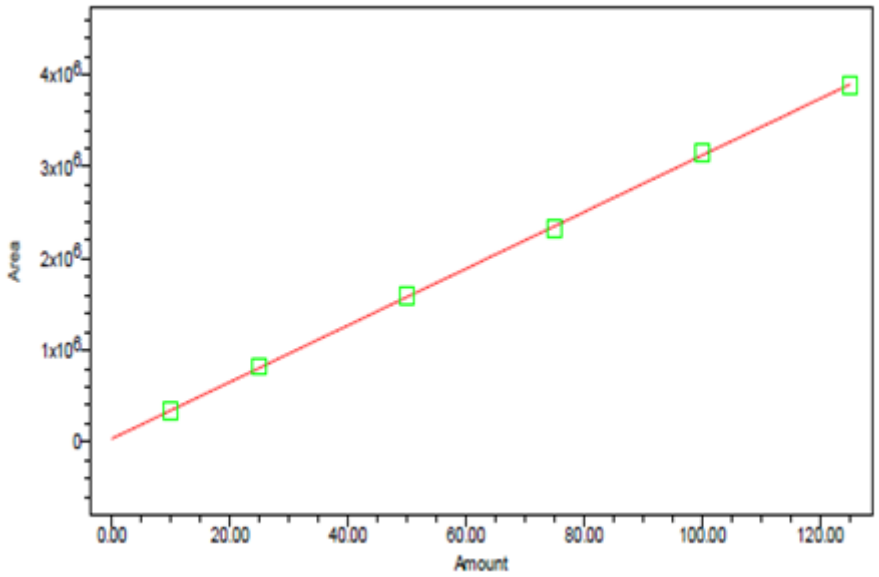


Fig 4. Calibration curve of Flutamide (10-125 µg mL^{-1}) at 294nm.

Table 1. Calibration of HPLC method for the estimation of Flutamide

Concentration of Flutamide (µg mL^{-1})	**Peak area**
10	331979
25	821870
50	1595079
75	2326370
100	3153750
125	3882846
Correlation coefficient	**1.000**

Table 2. Inter and Intra- day Precision for Flutamide assay in Pharmaceutical dosage forms by the proposed HPLC method

Concentration ($\mu g\ mL^{-1}$)	Peak area (Intra day)	Mean	SD	CV	Peak area (Inter day)	Mean	SD	CV
25	813148	815895.9	2457.0	0.3	864741	868000.7	3359.4	0.4
25	815460				866158			
25	815066				865997			
25	815945				870591			
25	819860				872517			

Table 3. Recovery of Flutamide using proposed HPLC method

%Concentration (at specification level)	Peak area	Amount of drug added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	518989	15.4	15.7	101.9%	99.9%
100%	817851	25.0	24.74	99.0%	
150%	175095	36.0	35.55	98.8%	

Table 4. Robustness table for Areas and Retention time

Parameters	Mobile phase		Flow rate		Temperature		%deviation
	Area	RT	Area	RT	Area	RT	
Initial	815632 (50:50)	4.61	815632 (1ml/min)	4.61	815632 (25^0C)	4.61	–
Changed	1089071 (40:60)	3.55	815632 (1ml/min)	4.61	815632 (25^0C)	4.61	4.615
	876305 (60:40)	5.52	815632 (1ml/min)	4.61	815632 (25^0C)	4.61	4.615
	815632 (50:50)	4.61	749908 (1.1ml/min)	3.99	815632 (25^0C)	4.61	8.08
	815632 (50:50)	4.61	911221 (0.9ml/min)	4.91	815632 (25^0C)	4.61	11.71962
	815632 (50:50)	4.61	815632 (1ml/min)	4.61	850048 (20^0C)	4.42	4.21995
	815632 (50:50)	4.61	815632 (1ml/min)	4.61	851751 (30^0C)	4.42	4.42835

Mobile composition (Acetonitrile: Water, 50:50 v/v)

Table 5. Mean amount of Flutamide in tablet dosage form by using proposed HPLC method

Tablet	Labeled claim(mg)	Amount found(mg)	Mean purity
T_1	250	249.75	99.9%

T_1 = Cytomid-250 (Cipla Ltd, Goa.)

CONCLUSION

The proposed reverse phase HPLC method was found to be simple, precise, highly accurate, specific and less time consuming for determination of Flutamide in pure drug and tablets. Method validation yields good results and presented good precision and accuracy. It was also found that the excipients in the commercial tablet dosage form did not interfere with the assay. Therefore, the proposed HPLC method is precise and accurate, and can be used for quantitative estimation of Flutamide in tablets.

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