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Improved Liquid Chromatographic method for the determination of Flutamide in Pharmaceutical Formulation

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Abstract : A simple and reliable liquid chromatographic method has been developed for the determination of flutamide in the pharmaceutical preparation. The fluorescence of the product was found to have excitation λ_{max} at 255 nm and emission λ_{max} at 375 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 8.697 x 10^{-3} µg / ml and limit of quantification (LOQ) was found to be 26.355 x 10^{-3} µg/ml in formulation. The different experimental parameters affecting the fluorescence intensity were carefully studied and optimized. Common excipients used as additives in pharmaceutical preparations which do not interfere in the proposed method. The proposed method was applied successfully for determination of flutamide in the pharmaceutical preparation. The percentage recovery was found to be 99.53 ± 0.5283.

Keywords: Formulation, Fluorimetry, Reduction, Evaluation, HPLC

1. Introduction

Flutamide chemically 2–methyl–N–[4–nitro–3–(trifluoromethyl)phenyl]propanamide is used as antineoplastic and antiandrogen drug[1]. Flutamide is a powerful nonsteroidal androgen antagonist which is used to treat prostate cancer, is believed to block androgen receptor sites. This drug and its primary hydroxy metabolite decreases metabolism of C – 19 steroids by the cytochrome P – 450 system at the target cells in the secondary sex organ[2] .The objective of the work is to develop a HPLC method for the determination of flutamide in pharmaceutical formulation. A survey of literature reveals that there are not many methods for the assay of the drug. The reported methods include polarography[3], gas chromatography[4], and high performance liquid chromatography[5,6]. The polarographic, UV spectrophotometric and HPLC determination of flutamide in tablets has been reported[7]. The spectrophotometric methods available for the determination of flutamide include the formation of yellow color with hydrochloric acid with $_{max}$ of 380 nm suffers from various drawbacks[8], using promethazine hydrochloride or resorcinol, or N – (1 – naphthyl) ethylenediamine dihydrochloride (NEDA), two more spectrophotometric methods which do not give much information about the optical characteristics makes use *p* – dimethylamino cinnamaldehyde or NEDA or resorcinol[9,10], using chromotropic acid alone as coupling agent[11] and flow injection analysis method[12] .The authors have made some attempts in this direction, and succeeded to develop a new method by reducing the nitro group to amino group. This method offers the advantages of sensitivity, selectivity, rapidity and more stability when compared to other existing method without any need for extraction.

2. Experimental

2.1 Chemicals and reagents

Flutamide 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. Hydrochloric acid 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.

2.2 Instruments

Absorption spectral measurements were carried out with a Perkin Elmer Lambda 25 model UV – Visible spectrophotometer and the fluorescence measurements were made using a Perkin Elmer LS 55 spectrofluorimeter. Chromatograms were obtained with a Perkin Elmer HPLC with series 200 pump.

2.3 Chromatographic 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 Perkin-Elmer Model Series - 200 fluorescence detector equipped with filters of 255 nm (excitation) and 375 nm (emission). Peaks were recorded with a networking channel Perkin – Elmer Model series interface NCI 900. Chromatograms of blank and flutamide with internal standard were shown in Fig. 2a & 2b, where it can be observed that there is no interfering.

2.4 Stock Solution

Flutamide (50 mg) was accurately weighed transferred and dissolved in 100 ml of standard flask containing 4 ml of methanol, 0.5 g of Zinc dust and 1 ml of concentrated hydrochloric acid for reduction[11]. The mixture was kept aside for 30 min, and at the end of 30 min sodium bicarbonate was added to neutralize the above solution, till the effervescence subside and made up to the volume with water. Then the solution was filtered. Further dilutions were made with water to get required concentration. For linearity study, serial dilutions were made in the range of 0.1 to 0.6 mcg/ml concentrations were prepared by diluting the stock solution with mobile phase. For each concentration six replicates were made by individual weighing. The solutions were investigated by liquid chromatographic method using the excitation as 255 nm and emission as 375 nm.

2.5 Internal Standard

A stock solution was prepared by dissolving 100 mg of 3,3′-diaminodiphenylsulphone in a 100 ml volumetric flask. The solution was stored at 4^{o} C. The working solution was prepared by further dilution with mobile phase to getting the final concentration of 0.5ng/µl.

2.6 For Formulation

The average weight of the tablets were determined by weigh 20 tablets and powdered. Tablet powder equivalent to 50 mg of flutamide was weighed and transferred to a 100 ml volumetric flask containing 4 ml of methanol, sonicated for 15 minutes for the complete dissolution of drugs, 0.5 g of Zinc dust and 1 ml of concentrated hydrochloric acid were added. The mixture was kept aside for 30 min and at the end of 30 min sodium bicarbonate was added to neutralize the above solution, till the effervescence subside and made up to the volume with water. Then the solution was filtered through 0.2µ membrane filter, further dilutions were made with mobile phase to get required concentration of 0.4 µg/ml. Six replicates of analysis were carried out with sample weighed individually. The average weight of the tablet was found to be 0.75185 g.

3. Method Validation

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

3.1 Specificity and Selectivity

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

3.2 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.

3.3 Stability

Sample solution was kept at room temperature and the fluorescence intensity was measured at the interval of one hour up to for 7 hours and found stable within these periods.

4. Results and Discussion

4.1 Calibration curves

Calibration standards for flutamide, covering the range 0.1 – 0.6 g/ml, were prepared by the method mentioned above and serial dilutions were made with mobile phase. The calibration curve was obtained by plotting the peak – height ratio of flutamide/internal standard versus analyte concentration. The slope and intercept of the calibration line was determined by linear regression using the least squares method. In Figure 1, regression analysis of the calibration curve showed a linear relationship between the peak-height ratio of flutamide and the IS concentration, with correlation coefficients higher than 0.9955 for pharmaceutical preparation in all the curves assayed. The precision and accuracy of the assay are presented in table 1 & 2.

Table 1: Analysis of tablet formulation

	Formulation	
Sl no.	Drug ontent (mg/tab)	% Label claim
1	249.06939	99.62776
2	248.36174	99.3447
3	252.20301	100.8812
4	253.25593	101.3024
5	256.23638	102.4946
6	254.89895	101.9596
Mean	252.33757	100.935
SD	3.1340417	1.253617

4.2 Selectivity and Specificity

The drug flutamide and the internal standard were well separated under the HPLC condition applied. Retention times were 3.95 minutes for flutamide and 6.9 minutes for internal standard. After addition of acetonitrile to the

Table 2: Accuracy of Flutamide

Levels	Flutamide content in the sample (mg)	Flutamide added (mg)	Total Flutamide (mg)	Amount found (mg)	% Recovery
25%	100.92	25.1	126.02	126.87	100.672
	100.72	25.1	125.82	126.16	100.271
	101.22	25.1	126.32	127.53	100.953
50%	101.12	50.2	151.32	150.44	99.419
	100.92	50.2	151.12	149.01	98.600
	100.66	50.2	150.86	147.88	98.029
75%	100.59	75.3	175.89	173.99	98.92
	100.82	75.3	176.12	175.96	99.90
	100.69	75.3	175.99	174.26	99.02
				Mean ± SD	99.532 ± 0.5283

mobile phase, it was possible to separate small interfering peaks from the analytes, so that no interferences was observed in six different blank matrices around the retention times of flutamide and internal standard. Fig.2 shows the chromatograms of (a) a quality control standard with a flutamide concentration of 0.4 µg/ml and chromatogram of drug with internal standard.

4.3 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 found to be 8.697 x 10^{-3} µg/ml and the LOQ was determined as the lowest concentration was found to be 26.355 x 10^{-3} µg/ml in formulation.

4.4 .Robustness

The robustness were studied by the standard solutions with slight variations in the optimised conditions. They are ± 1 nm changes in excitation wavelength and emission wavelength and different sources of reagents and solvents (S.d. fine chemicals and Qualigens fine chemicals), ± 0.1 ml of the flow rate and ± 1% in the ratio of acetonitrile in the mobile phase and the results were presented in table 3.

Table 3: Robustness of Flutamide

Replicate	Wavelength (nm)		S.d. fine chemicals	Qualigens fine chemicals
	Excitation	**Emission**	**Response factor**	**Response factor**
1	254	374	1.5665	1.5691
2	255	375	1.5861	1.5883
3	256	376	1.5852	1.5867

4.5 Ruggedness

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 and the results were presented in table 4.

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

4.6 System Suitability

System suitability was performed by calculating the chromatographic parameters namely, capacity factor, separation factor, resolution, plate number and peak asymmetric factor. The results were presented in table 5.

Table 4: Ruggedness of Flutamide

Replicate	Analyst 1			Analyst 2		
	Weight taken (mg)	**Amount found (mg)**	**% Label Claim**	**Weight taken (mg)**	**Amount found (mg)**	**% Label Claim**
1	243.7	249.07	99.63	312.1	250.03	100.01
2	243.4	248.36	99.34	311.4	249.17	99.67
3	242.8	252.20	100.88	312.7	250.72	100.29
Mean		249.878	99.951		249.975	99.990
SD		2.0443	0.8177		0.7782	0.3113
RSD		0.8181	0.8181		0.3113	0.3113

Table 5: System Suitability data of Flutamide and Internal Standard (IS)

Parameters	Values obtained	
	Drug	**IS**
Retention time (t_R)	3.9 min	6.9
Capacity factor (or) Retention factor (K')	0.96	1.76
Separation factor (α)	-	1.833
Asymmetric factor (A_s)	1.31	1.0
Resolution (R_s)	-	5.375
Plate number (N)	2001	13862

Linearity of Flutamide

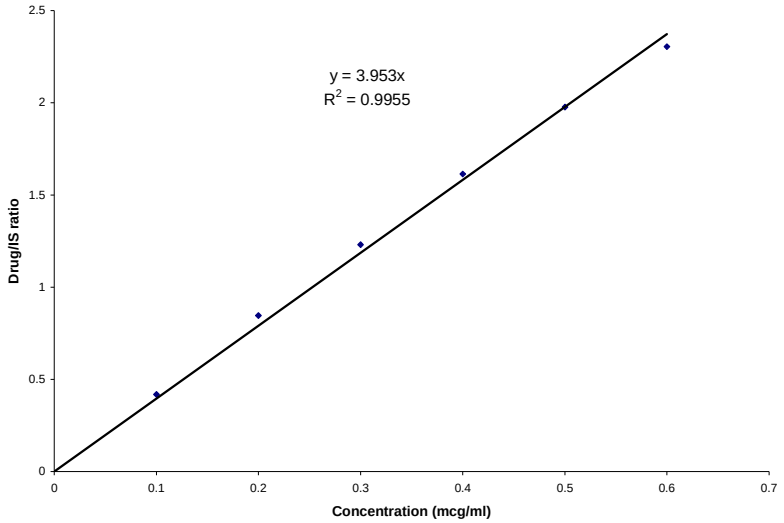

Figure 1: Regression analysis of the calibration curve for Flutamide showed a linear relationship between the peak-height ratio of flutamide and the internal standard concentration coefficients higher than 0.9955 in all the curves assayed.

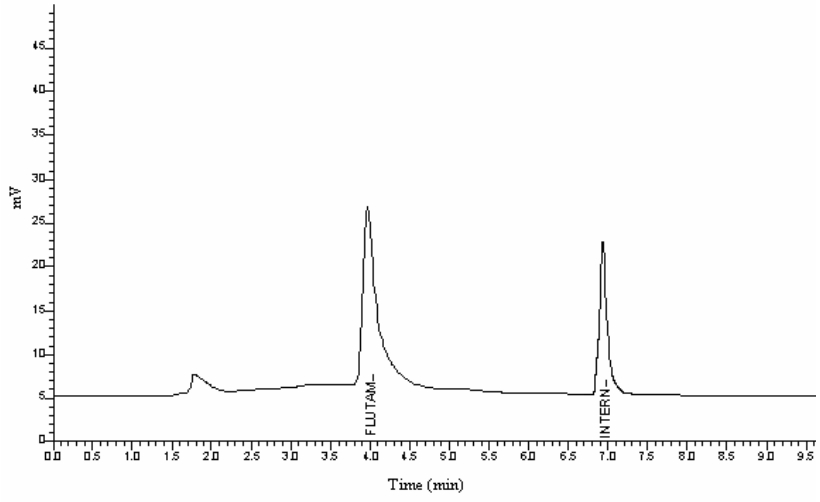

Figure 2: (a) Chromatogram of drug and internal standard

5. Conclusions

A liquid chromatographic method for quantifying flutamide in formulation has been developed and validated as per ICH guidelines. The developed method is selective, precise, accurate and linear. Linear relationship between response factor and concentration was obtained in the concentration range between 0.1 and 0.6 g/ml for the estimation of flutamide by HPLC with fluorescence detection. Calibration graph were constructed for the estimation of flutamide in tablet formulation. The correlation coefficient was found to be 0.9955 for the estimation of flutamide over the concentration range studied. The concentration of flutamide is as low as 26.355 x 10^{-3} µg/ml in formulation could be precisely quantified and LOD was approximately 8.697 x 10^{-3} µg/ml in formulation by the standard deviation of the response of Y intercept and the slope of the calibration curve.

The replicate analysis of the dosage form exhibited RSD by the proposed method was 0.7781, which are well within acceptance criteria of the ICH guidelines.The accuracy of the method was determined by the recovery study carried out by analysing the formulation spiked with the pure flutamide at three different concentration levels, 50%, 75% and 100%. The mean percentage recovery obtained from the method was 99.53%. Selectivity of the HPLC method would be confirmed by accuracy, small value of y intercept and precision.

The RSD value for the two sets of the data is less than 2.0% and it indicates that the method is rugged for analyst to analyst. The system suitability studies of HPLC analysis revealed that capacity factor, resolution, asymmetric factor, theoretical plates were within the acceptance limit and the results are given in table 5. The proposed method is accurate, precise, economical and less time consuming and can be employed in routine quality control of flutamide in tablet formulation.

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