

Determination of Flutamide in Tablets by High-Performance Liquid Chromatography

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SUMMARY. Flutamide is a potent antiandrogen used for the treatment of prostatic cancer. A simple, sensitive and accurate high-performance liquid chromatographic (HPLC) method is presented for quantitative determination of flutamide in tablets, using a reversed-phase technique and UV detection at 240 nm. The isocratic elution was used to quantify the analyte. The samples were chromatographed on Luna-C18 column and the mobile phase was 0.05 M phosphate buffer pH 4.0 - acetonitrile (50:50, v/v). The method was linear between 2.9 - 11.6 mg L⁻¹. Over the tested concentration range the intra-day relative standard deviation for replicate analysis in tablets ranged from 0.44 to 0.78%. It was also found that the excipients in the commercial tablets did not interfere with the method.

RESUMEN. "Determinación de Flutamida en Tabletas por Cromatografía Líquida de Alta Resolución (HPLC)". La Flutamida es un antiandrógeno potente usado para el tratamiento del cáncer de próstata. Se presenta aquí un método simple, sensible y seguro de cromatografía de alta resolución (HPLC) para la determinación cuantitativa de flutamida en comprimidos, utilizando una técnica de fase inversa y detección UV a 240 nm. Se usó elución isocrática para cuantificar al analito. Las muestras fueron cromatografiadas sobre una columna Luna C-18 y la fase móvil fue buffer fosfat 0.05 M de pH 4, - acetonitrilo (50:50, v/v). El método resultó lineal entre 2,9 y 11,6 mg.L⁻¹. Por encima del rango de concentraciones ensayadas la desviación estándar relativa intradiaria para análisis duplicados en comprimidos varió del 0,44 al 0,78%. También pudo comprobarse que los excipientes de los comprimidos comerciales no interfieren con el método.

INTRODUCTION

Prostate cancer is the most common cancer in men in Western countries and it is the second leading cause of cancer death ¹. Flutamide (Fig. 1) is a potent nonsteroidal pure androgen receptor antagonist used clinically (250 mg 3 times daily) for the management of metastatic carcinoma of the prostate ²⁻⁴.

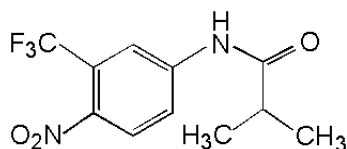


Figure 1. Chemical structure of flutamide (CAS number 13311-84-7).

Chemically, flutamide is a 2-methyl-N-[4-nitro-3(trifluoromethyl- phenyl)] propamide, and it is official in some pharmacopeias. It is soluble in organic solvents as acetone, ethyl alcohol, polypropyleneglicol 400, ethyl acetate, methyl alcohol and DMSO ⁵⁻⁶. The literature has reported analytical methods for determination of flutamide and flutamide metabolites such as visible spectrophotometry ⁷⁻¹¹, UV spectrophotometry ^{6,12}, high performance liquid chromatography (HPLC) ^{5,13-16}, gas liquid chromatography (GLC) ¹⁷, polarography ¹⁸ and non aqueous volumetry ¹⁹.

Therefore, the purpose of this investigation was to develop a HPLC method that could be used for the determination of flutamide in tablets. In the present paper a simple, rapid,

KEYWORDS: Analysis in tablets, Flutamide, High-performance liquid chromatography (HPLC), Pharmaceutical analysis.

PALABRAS CLAVE: Análisis de Comprimidos, Análisis Farmacéutico, Cromatografía Líquida de Alta Resolución (HPLC), Flutamida,

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sensitive, precise, accurate and specific HPLC assay is described that has been applied to quality control of drugs which required high sensitivity and selectivity. The results of the analysis were validated by statistical methods and recovery tests.

EXPERIMENTAL

Chemicals and reagents

Flutamide reference substance (assigned purity 99.9%) was kindly supplied by Schering-Plough do Brasil. Flutamide raw material and tablets were kindly supplied from Farmácia-Escola - FCF-Araraquara (São Paulo, Brasil) and Amaral Carvalho Hospital from Jaú (São Paulo, Brasil), respectively. Each flutamide tablet contained 250 mg of the active drug. Acetonitrile and methanol, both of HPLC grade were purchased from Merck (Brasil). All chemicals and solvents used were of analytical grade.

Equipment

The HPLC analyses were conducted using a Chromatograph System Varian (Model 2510), a UV detector (Varian Model 2550), a mobile phase constituted of 0.05 M phosphate buffer pH 4 and acetonitrile (50:50, v/v) pumped at a flow-rate of 1.0 mL min⁻¹ through a Luna C18 analytical column (100 mm x 4.6 mm i.d.), particle size 5 µm (Phenomenex, CA, USA) and a manual injector Rheodyne (Model 7125). The injection volume was 20 µL and peaks were detected at 240 nm. The integrator attenuation was 8 and the chart speed was 0.5 cm min⁻¹. The total run time for an assay was approximately 7 min. All assays were performed at ambient temperature.

Standard solution

The flutamide standard solution was prepared by accurately weighing 60.0 mg of flutamide which was transferred to a 100 mL volumetric flask and the volume was completed with methanol. Final concentration was 600 µg mL⁻¹. The stock solution of flutamide was then successively diluted with methanol in order to obtain solutions with concentrations of 2.9, 5.8, 8.7 and 11.6 µg mL⁻¹.

Sample preparation

For quantitative determination of flutamide in tablets, twenty tablets were weighed to obtain the average tablet weight. The tablets were grounded up and 60.0 mg were transferred to a 100 mL volumetric flask; 50 mL of methanol

were added and the flask was shaken for 20 min by mechanical stirrer followed by addition of methanol. The volume was completed with the same solvent. The samples were filtered and then successively diluted with methanol to obtain 10.0 mg mL⁻¹ of flutamide. All determinations were conducted in triplicate.

The most powerful statistical tool to verify the internal validity of an analytical procedure, a criterion of accuracy, is the analysis of variance (ANOVA). The data were analyzed by linear simple regression by the least-squares method using Excel 5.0.

The recoveries were determined by adding known amounts of flutamide reference substance (2.0, 4.0 and 6.0 µg mL⁻¹) to the samples at beginning of the process. A recovery exercise was then performed.

The precision and accuracy of the assay, as well as the linearity of the calibration curve were determined for intra- and inter-day on three different days. Precision and accuracy were assessed by performing replicate analyses of quality control samples against calibration standard. The precision and accuracy of the method were calculated as the relative standard deviation (RSD) and the percentage deviation of observed concentration from theoretical concentration, respectively. The statistical data were calculated by ANOVA.

The specificity study was assessed during all the steps of the analytical method. The ability of the assay to quantify flutamide accurately in the presence of the excipients was confirmed through the analysis of blanks and spiked quality control samples.

RESULTS AND DISCUSSION

Flutamide can be analyzed by using the proposed HPLC method both as a raw material and in tablets. The aim of this study was to develop a chromatographic procedure for the analysis of flutamide in raw material and tablets. Methanol and acetonitrile were chosen as the organic solvents because of flutamide solubility and a reversed phase mode chromatography was used to perform the assay. The USP modified method was used because the column specified in the monograph is not available in the laboratory. The column used in this research (100 mm x 4.6 mm) showed a high-resolution separation. Moreover the mobile phase was modified. Methanol and 0.05 phosphate buffer (7:4, v/v) [4] was substituted by acetonitrile and 0.05 M phosphate buffer pH 4 (5:5, v/v). The influence

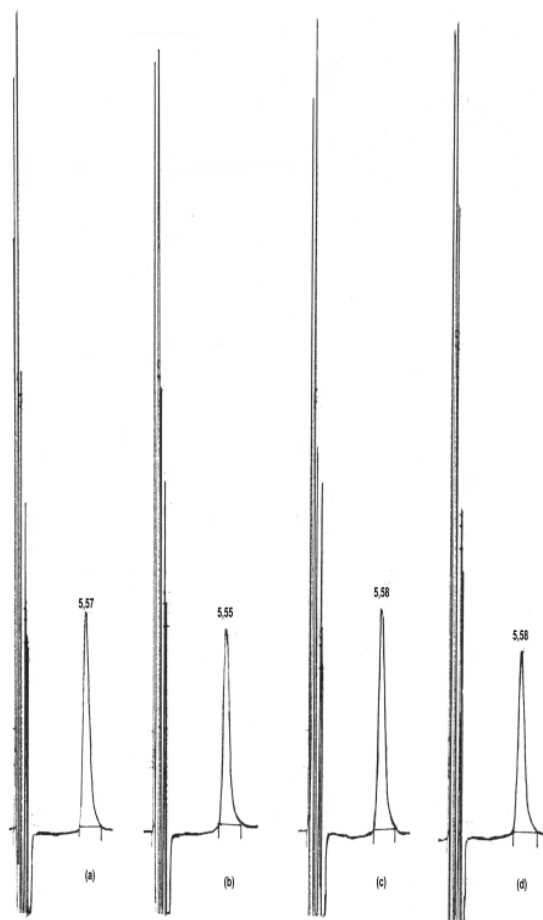


Figure 2. Typical chromatograms of flutamide. Conditions: Luna C18 (100 mm x 4.6 mm i.d.), particle size 5 μm column; mobile phase: 0.05 M phosphate buffer pH 4 and acetonitrile (50:50, v/v); flow-rate: 1.0 mL min^{-1} ; UV detector at 240 nm; RT 5.57 min; ambient temperature; concentration: 10 mg mL^{-1} .

of different organic solvents in the mobile phase was studied aiming to establish the best experimental conditions for the analysis of flutamide. Fig. 2 shows the chromatograms of flutamide using the proposed and validated chromatographic method.

A standard calibration curve of flutamide was constructed by plotting area versus concentration (Fig. 3). The linear regression equation for flutamide was calculated by the equation: $y = 2523.66x - 27.5$ where x and y are concentration and area, respectively. The procedure for raw material purity determination was the same as described for flutamide determination in tablets. The purity of flutamide raw material was found to be 100.5%, according to as pharmacopeial specifications⁵.

The UV absorption spectrum of flutamide was monitored at 240 nm. The retention time

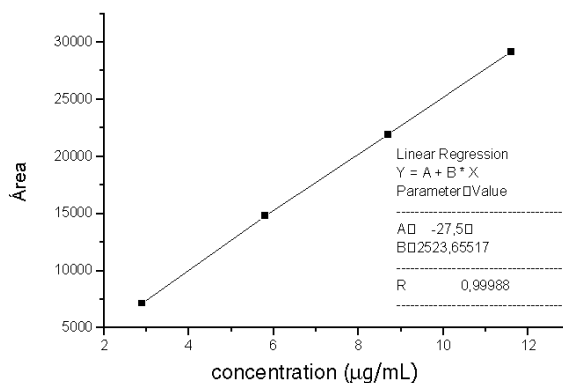


Figure 3. Standard calibration curve of flutamide (2.9 to 11.6 $\mu\text{g}\cdot\text{mL}^{-1}$) at 240 nm.

for flutamide was less than 6 min. Agreement with Beer's law was evident in the concentration range from 2.9 to 11.6 $\mu\text{g}\cdot\text{mL}^{-1}$. A total of 20 μL volume of each sample was then injected onto the column. The calibration curve was constructed by plotting the peak height ratios of flutamide vs. the respective drug concentration. The correlation coefficient obtained was 0.99998, indicating excellent linearity. The experimental results obtained in the determination of flutamide in tablets are shown in Table 1. The method had excellent reproducibility for the standard solution. The average purity (%) reached was 99.53%.

The recovery test is an experimental design to verify the relationship between the amount of substance added and the amount quantified. In this test the observed concentrations of flutamide reference substance in the powdered tablets were not significantly different from the stated concentrations (99.6%, $n=3$). Results of recovery tests are shown in Table 2.

	Amount found (mg/tablet) *	Average \pm S. D. (mg \pm S.D.)	RSD (%)
1	248.3		
2	251.15	249.75 \pm 1.94	0.78
3	249.79		
4	248.10		
5	249.30	248.23 \pm 1.01	0.44
6	247.30		
7	247.77		
8	250.15	248.52 \pm 1.42	0.57
9	247.63		

Table 1. Results of flutamide determination in tablets (250 mg/tablet) using a proposed HPLC method.

*Average of three determinations; S.D. standard deviation; R.S.D. relative standard deviation.

	Amount of standard ($\mu\text{g.mL}^{-1}$)		Recovery ^a (%)	RSD (%)
	Added	Recovered		
R1	2.0	1.98	99.00	
R2	4.0	4.07	101.7	
R3	6.0	5.88	98.00	

Table 2. Experimental values obtained in the recovery test for flutamide injection by HPLC. ^a Mean of three replicate analysis.

The precision of the chromatographic analysis in tablets was determined by analyzing three concentrations of flutamide. The relative standard deviations were obtained by repeating procedure three times for each sample (Table 1). No interfering peaks were found in the chromatograms due to the tablet excipients. Flutamide was shown to be stable during all the procedure.

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CONCLUSIONS

The described method involves a simple, rapid and specific assay for the determination of flutamide in raw material and tablets. Method validation yields good results and presented good precision and accuracy. It was also found that the excipients in the commercial tablet preparation did not interfere with the assay. Therefore, the proposed HPLC method is precise and accurate, and can be used for quantitative determination of flutamide in tablets.

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