International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304
Vol.8, No.7, pp 146-153, 2015

Development of Analytical Methods for the Determination of Flutamide in Bulk Drug and its Pharmaceutical Formulation

Mohamed Zerein Fathima[1], T.S.Shanmugarajan[1], I.Somasundaram[1]

[1]Department of Pharmaceutics, School of Pharmaceutical Sciences, Vels University (VISTAS), Pallavaram, Chennai, Tamilnadu, India.

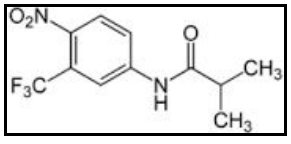
Abstract: A method was developed for estimation of flutamide in bulk drug and its tablet dosage form by using methanol as a solvent and shows absorbance maxima at 330 nm and 410 nm respectively.This present work deals with the development of two spectrophotometric methods. The first method is based on diazotization and coupling method using phloroglucinol (METHOD I). The second one is based on formation of schiff's base method using vanillin (METHOD II). In the both methods we are used Perkin Elmer EZ301- UV visible double beam spectrophotometer. Validation study reveals that the methods are specific. Flutamide obey Beer's law in the concentration ranges used for the methods. Validation studies are statistically significant as all the statistical parameters are within the acceptance range (% COV< 1.0 and S.D. <1.0) for both accuracy and precision study. The methods are simple, rapid, accurate, precise, reproducible, and economic and can be used for routine quantitative analysis of Flutamide in pure and tablet dosage form.
Keywords: UV Visible Spectroscopy, Flutamide, Beer's Law, Schiff's Base Method, Diazotization Method.

Introduction

Flutamide is an oral non steroidal antiandrogen drug primarily used to treat prostate cancer. Flutamide is a α,α,α – trifluro – 2 – methyl- 4- nitro- m- propionotoluidide. Its also known as 2- methyl-N-[4-nitro-3-(trifluro methyl) phenyl] propanamide. It completes with testosterone and its powerful metabolite, dihydro testosterone (DHT) for binding to androgen receptors in the prostate gland. By doing so, It prevents them from the prostate cancer cells to grow. Flutamide has been largely replaced by a newer member of this class Bicalutamide, due to the better side effects profile. Flutamide may also be used to treat excess androgen levels in women.[10,11]

Flutamide is a non steroidal anti androgen that blocks the action of both endogenous and exogenous testosterone by binding to the androgen receptor. And it is a potent inhibitor of testosterone stimulated Prostatic DNA synthesis. [1,3,5,6,] It is capable of inhibiting prostatic nuclear uptake of androgen. Several assay techniques have been described for quantitative determination of Flutamide in pure and tablet dosage forms. The LC determination, HPLC determination, HPTLC determination.[12,13,14,14]

Chemical Structure of Flutamide



Experimental Methods and Materials

Instrument

Absorption spectral measurements were carried out with Perkin Elmer EZ301- UV visible double beam spectrophotometer.

Materials

Flutamide, Phloroglucinol(AR), Sodium Nitrite(AR), Vanillin(AR) all were purchased from sigma Aldrich, India and Methanol(AR), Zinc Powder(AR), Conc.HCL(AR), Ammonium Sulphamate(AR) all were purchased from sisco research laboratories pvt. Ltd,Mumbai and used as such. Water used was generated by double distillation.

Preliminary Solubility Study of Drug

Solubility of drug was determined. A small quantity of standard drug is dissolved in different solvents like distilled water, methanol, ethanol, acetonitrile, isopropyl alcohol, and choloroform. By the solubility studies we determined that the drug is dissolved in methanol without heat. But It also soluble in ethanol, acetone, ethyl acetate, chloroform, and ether but with heat.

Preparation of 4n HCL

It was prepared by dissolving 34ml of con.HCL in 100ml of distilled water.

Preparation of (0.1%W/V) Sodium Nitrite

It was prepared by dissolving 0.1gm of sodium nitrite in 100ml distilled water.

Preparation of (0.5%W/V) Ammonium Sulphamate

It was prepared by dissolving 0.5gm of ammonium sulphamate in 100ml distilled water.

Preparation of (0.5%W/V) Phloroglucinol

It was prepared by dissolving 0.5gm of phloroglucinol in 100ml distilled water.

Preparation of 2% Vanillin

It was prepared by dissolving 2gm of vanillin in 100ml distilled water.

Preparation of Standard Stock Solution

25mg of flutamide was accurately weighed and dissolved in 5ml methanol. The methanolic solution of flutamide was treated with 200mg of zinc powder and 2.5ml of 4N HCL and kept aside for 1 hour at room temperature. The solution was filtered and the volume was made upto 25 ml of methanol- I (1000µg/ml).

Method I- Spectrophotometric Determination –I

Spectrophotometric Determination of Flutamide by Diazotization Coupling Method using Pholoroglucinol

Determination of Λ Max:

Absorption Spectra of Flutamide for Method I

2.5 ml of standard stock solution I was pipette into 25 ml of volumetric flask with water solution II (100µg/ml). From this 2 ml was pipette into 10 ml volumetric flask followed by the addition of 1 ml of 4N HCL and 1 ml of 0.1% sodium nitrite and kept aside for 5 minutes at room temperature. An aqueous solution of 1 ml of 0.5% ammonium sulphamate and 1 ml 0.5% phloroglucionl were added. Finally the volume was make upto

10ml with distilled water. The final concentration of the solution was 20µg/ml. The absorbance was scanned between 280-380nm against reagent blank. The maximum absorbance was measured at 330nm. And plotted graph - figure1.

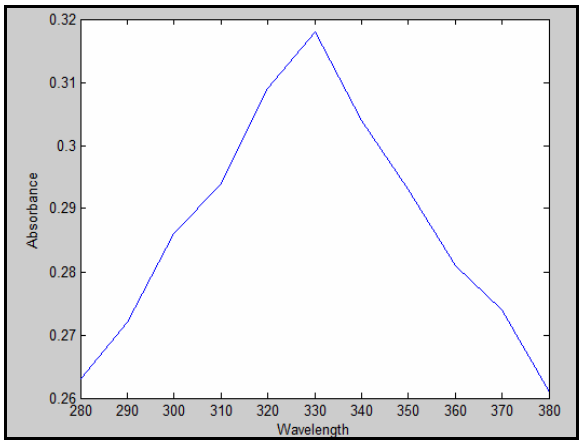


Figure 1 - Absorption Spectral of Flutamide for Method I

Effect of Reagent Concentration

Choosing the correct reagent concentration was an important aspect in the spectrophotometric determination. The optimum concentration of reagent was choosen by adding 1ml of reagent of different concentration i.e, 0.5%,1%,1.5% of pholoroglucionl solution in water with series concentration of drug solution. The calibration curve for each reagent concentration was prepared by using drug in the concentration range of 10-30mcg/ml and absorbance was measured at 330nm against reagent blank. The readings were recorded in the table1 and graphically plotted in graph figure2. The optimum concentration was found to be 0.5% since it exhibits the linearity.

Table1 : Data For Calibration Curve Plot With Different Pholoroglucinol Reagent Concentration

Drug Concentration (mcg/ml)	Absorbance of Reagent Concentration		
	0.25%	**0.5%**	**1%**
10	0.062	0.158	0.166
15	0.095	0.238	0.228
20	0.128	0.317	0.291
25	0.159	0.396	0.353
30	0.192	0.475	0.416

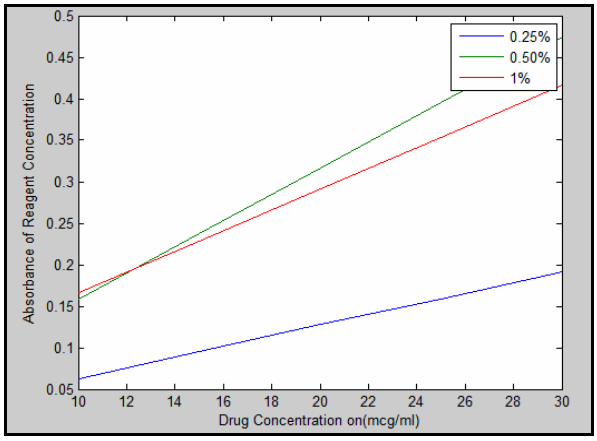


Figure 2 - Calibration Curve for Flutamide for Method I
Efffect of Reagent Concentration

Effect of Reagent Amount

Addition of correct amount of reagent was an important aspect in this experiment. The optimum amount of reagent was fixed by construting calibration curve. The calibration curve was prepared by employing the drug concentration range of 10-30mcg/ml with different amount of reagent i.e, 0.5ml,1ml,1.5ml with same reagent concentration. The readings were recorded in the table 2 and graphically plotted in graph figure3. The optimum amount of reagent was found to be 1 ml Beer's law was obeyed in the concentration range of 10-30µg/ml and plotted in graph figure4.

Table 2 Data for the Calibration Curve Plot with Different Amount of Pholoroglucinol Reagent

Drug Concentration (mcg/ml)	Absorbance Of Reagent Concentration		
	0.5ml	**1ml**	**1.5ml**
10	0.104	0.162	0.132
15	0.158	0.245	0.200
20	0.210	0.325	0.268
25	0.268	0.406	0.332
30	0.314	0.488	0.400

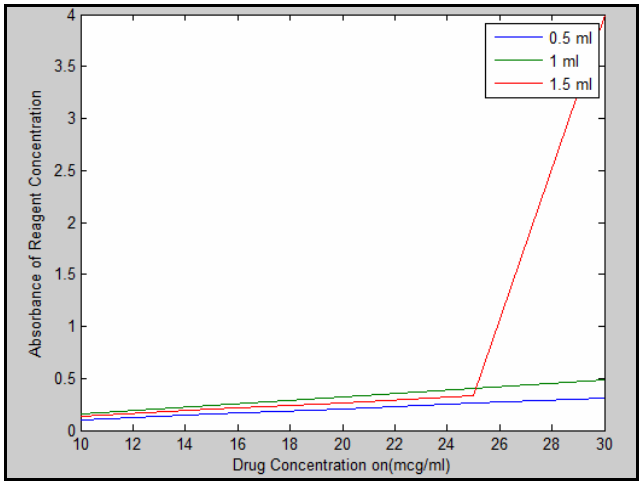


Figure 3 - Calibration Curve for Flutamide for Method I
Effect of Reagent Amount

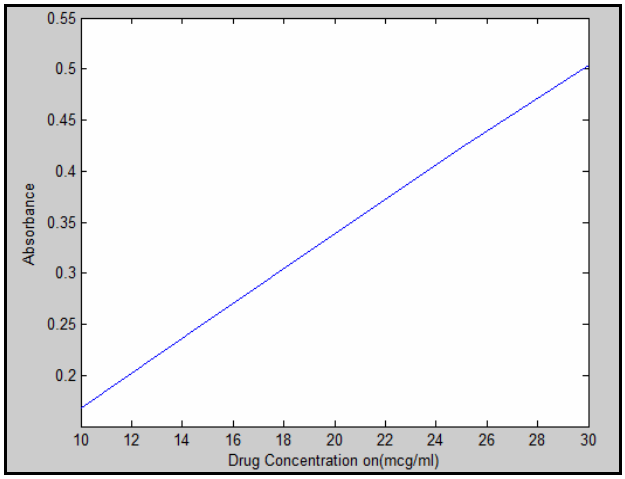


Figure 4 - Beer's Law Plot for Method I

Method II- Spectrophotometric Determination –II

Spectrophotometric Determination of Flutamide by Schiff's Base Method using Vanillin

Determination of Λ Max:

Absorption Spectra of Flutamide for Method Ii

25mg of flutamide was accurately weighed and dissolved in 5ml methanol. The methanolic solution of flutamide was treated with 200mg of zinc powder and 2.5ml of 4N HCL and kept aside for 1 hour at room temperature. The solution was filtered and the volume was made upto 25 ml of methanol- I (1000µg/ml). 2.5 ml of standard stock solution I was pipette into 25 ml of volumetric flask with water solution II (100µg/ml). From this 1 ml was pipette into 10 ml volumetric flask followed by the addition 3ml of 2% vanillin and 2ml of conc.Hcl where added then heated and kept aside for 10 minutes. The volume was made upto 10ml with methanol. The final concentration of the solution was 10mcg/ml. the absorption was measured between 380-450nm against reagent blank. Readings were shown in the following table and plotted in the graph – figure5. The maximum absorbance was measured at 410 nm. Beer's law was obeyed in the concentration range of 5-25µg/ml. The results shows that excipients have no effect in the absorption charecteristics of the drug.

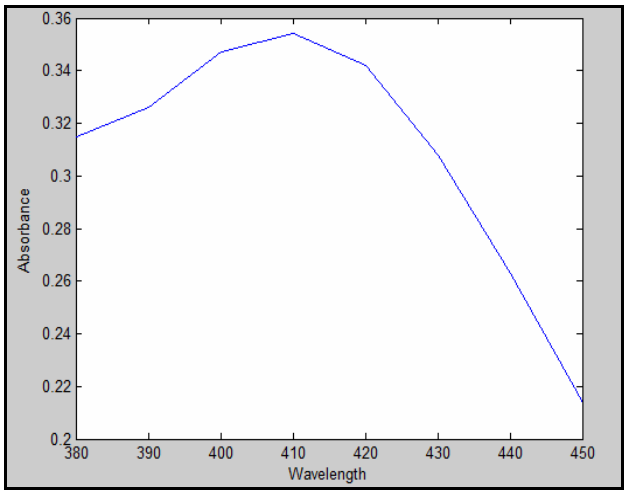


Figure 5 - Absorption Spectra of Flutamide for Method II

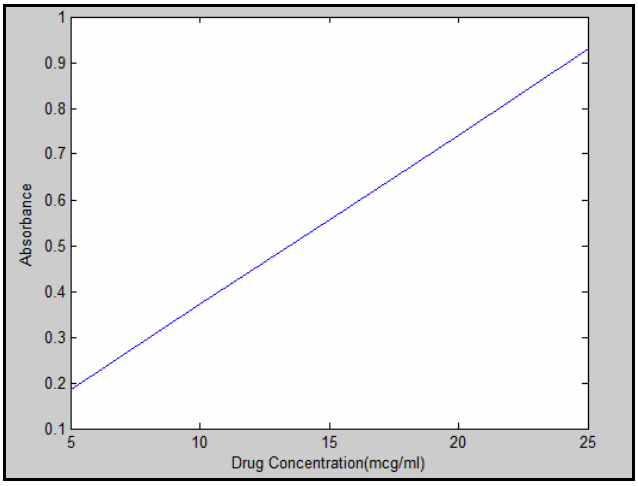


Figure 6 - Beer's Law Plot for Method II

Table 3 Data for Assay of Tablet – Label Claim 250mg

S. no	Brand Name	Avg.wt of tablet (mg)	Wt.of std drug (mg)	Std absorbance	Wt. of tablet powder (mg)	Test Absor Bance	Drug content in tablet mg	% of Drug content in tablet mg	Avg. Content (mg)	% of avg. content (mg)
1	Cytomid (Cipla)	75.024	25	0.358	75.0	0.360	249.3	99.7	249.66	99.864
					75.3	0.358	250.2	100.0		
					75.0	0.363	251.7	100.6		
					76.2	0.368	251.7	100.7		
					75.7	0.359	249.4	99.7		
2	Flutide (samarth)	70.705	25	0.359	72.3	0.361	249.0	99.6	249.32	99.728
					70.9	0.356	250.6	100.2		
					72.0	0.360	249.0	99.6		
					72.6	0.364	249.0	99.6		
					71.0	0.355	249.0	99.6		

Table 4 Recovery Studies – Accuracy Data for the Recovery Study of Tablets – Label Claim 250gm

S. no	Brand Name	Wt. of The std Drug (mg)	Std absorbance	Avg.wt of the tablet powder (mg)	Wt. of the tablet powder (mg)	Amount of pure drug added (mg)	Absorbance of Recovered sample	% of recovery
1	Cytomid (cipla)	25	0.356	75.0	75.0	5	0.426	98%
					75.3	5	0.428	
					75.6	5	0.429	
					75.4	5	0.432	
					75.9	5	0.436	
2	Flutide (samarth)	25	0.358	70.7	70.7	5	0.426	99%
					70.8	5	0.428	
					72.0	5	0.432	
					72.1	5	0.441	
					72.2	5	0.439	

Table 5 - Precision Data for the Assay Precision

S.no	Brand name	Std deviation	Co- efficient variation
1	Cytomid (cipla)	0.1195	0.4772
2	Flutide (samarth)	0.0715	0.2869

Table6 - Assay of Tablet Data for Assay of Tablet – Label Claim 250mg

S. no	Brand Name	Avg.wt of tablet (mg)	Wt.of std drug (mg)	Std absorbance	Wt. of tablet powder (mg)	Test Absor Bance	Drug content in tablet mg	% of Drug content in tablet mg	Avg. Content (mg)	% of avg. content (mg)
1	Cytomid (Cipla)	75.024	25	0.363	70.7	0.359	247.0	98.8	249.66	99.864
					72.0	0.364	246.0	98.4		
					72.6	0.368	246.0	98.4		
					71.4	0.360	245.0	98.0		
					71.8	0.361	244.7	97.8		
2	Flutide (samarth)	70.705	25	0.363	75.0	0.358	246.5	98.6	249.32	99.728
					75.6	0.360	245.9	98.3		
					76.2	0.364	246.7	98.6		
					76.8	0.367	246.8	98.7		
					74.8	0.357	246.5	98.6		

Table 7 - -Recovery Studies - Accuracy Data for The Recovery Study of Tablets – Label Claim 250gm

S. no	Brand Name	Wt. of The std Drug (mg)	Std absorbance	Avg.wt of the tablet powder (mg)	Wt. of the tablet powder (mg)	Amount of pure drug added (mg)	Absorbance of Recovered sample	% of recovery
1	Cytomid (cipla)	25	0.363	75.0	75.0	5	0.428	99.798%
					75.6	5	0.434	
					76.2	5	0.435	
					76.8	5	0.437	
					74.8	5	0.427	
2	Flutide (samarth)	25	0.363	70.7	70.7	5	0.428	99.518%
					72.0	5	0.436	
					71.0	5	0.440	
					71.4	5	0.430	
					71.9	5	0.427	

Table - 8 Precision- Data for the Assay Precision

S.no	**Brand name**	**Std deviation**	**Co- efficient variation**
1	Cytomid (cipla)	0.0195	0.3725
2	Flutide (samarth)	0.0348	0.1413

Table - 9 Data for Interference Studies

Name of the Excipients	Absorbance at 330nm
Talc	0.001
Lactose	0.002
Starch	0.001
Magnesium stearate	0.003

Results and Disscussion

Flutamide demonstrates potent antiandrogenic effects. It exerts its anti androgenic action by inhibiting androgen uptake and / or by inhibiting binding of androgen in target tissues and used to treat prostate cancer. Two wavelengths 330nm and 410nm were selected for analysis of the drugs in methanol.

In the first method the absorption spectral analysis showed the maximum wavelength at 330nm and beer's law obeyed in the drug concentration range 10-30 mcg/ml. The percentage recovery value was 98% and 99% and the result of interference studies shown that the excipients have no effect in the absorption of the drug in this method. The RSD value was 0.4772 and 0.2869 which is below 2% indicated that proposed method is précised.

In the second method the absorption spectral analysis showed the maximum wavelength at 410 nm and beer's law obeyed in the drug concentration range 5-25 mcg/ml. The percentage recovery value was 99.798% and 99.518% and the result of interference studies shown that the excipients have no effect in the absorption of the drug in this method. The RSD value was 0.3725 and 0.1413 which is below 2% indicated that proposed method is précised.

All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical formulation of flutamide. From this, it was concluded that two methods developed was simple, sensitive, accurate, precise and cost effective spectrophotometry which can be used for the determination of flutamide in bulk drug as well as in its pharmaceutical formulation.

References

1. Salgado,Menezes and Storti, Determination of flutamide in tablets by high performance liquid chromatography, Acta Farm, Banaerense. 2005,volume 24(2),. Page no :246-9.
2. P.Nagaraja,K.R.Sunitha and M.F.Silwadi, New spectrophotometric method for the determination of flutamide in pharmaceutical preparations, 24 august 2000, pubmed.
3. Anton smith.A, manavalan.R, kannan.K and rajendiran.N. Improved Liquid Chromatographic Method For The Determination Of Flutamide In Pharmaceutical Formulations. International journal of pharma tech research, 2009, volume I (2),.page no 360-364.
4. K.M.Reddy,K.Suvardhan, K.suresh, S.Prabhkar and P.Chiranjeevi. New Spectrophotometric Method For The Determination Of Flutamide In Pharmaceutical Preparation Using Choromotropic Acid As A Coupling Agent. 2003, page no 410-416.
5. Sharon hendershot. Davia koharski. Appropriate column configurations for the rapid analysis and semipreparative purification of the radio labeled drug flutamide by high performance liquid chromatography. Journal of chromatography A, volume 914,2001, page no 23-27.
6. Paraskevas D.Tzanavaras, Demetrius G, themelis Automated Determination Of Flutamide By A Validated Flow Injection Method: Application To Dissolution Studies Of Pharmaceutical Tablets. Journal of pharmaceutical and bio medical analysis, 2007, volume 43,.page no 1820-1824.
7. Padmarajaiah nagaraja, Hassan R, arun kumar, ramanathapuraA, vasantha, hemmige S, yathirajan, Novel Reagents For The Sensitive Spectrophotometric Determination Of Flutamide, An Anti Cancer In Pharmaceutical Preparations, International journal of pharmaceutics, 2002, volume 325, page no 113-120.
8. Nazik elgindy, kadria elkhodairy, abdallah molokhia and ahamed elzoghby, Lyophophillization Monophase Solution Technique For Improvement Of The Physio Chemical Properties Of An Anti Cancer Drus Flutamide. European Journel Of Pharmaceutics And Biopharmaceutics. 2010,Volume 74,.page no 397-405.

9. Jabbar emami, mona tajeddin and fatemeh ahmadi. Preparation and vitro evaluation of sustained – release matrix tablets of flutamide using synthetic and natural occurring polymers. Iranian journel of pharmaceutical research, 2008, 7(4), Page no: 247-257.
10. N.Annika adamson, leon J.S. Brokken, Jorma Paranko, Jorma Toppari. In vivo and in vitro effects of flutamide and diethyl stilbestrol on fetel testicular steroidogenesis in the rat. Reproductive toxicology. 2008, Volume25,.page no:76-83.
11. Ioannis niopas, athanasios C.Daftsios, determination of 2-hydroxy flutamide in human plasma by high performance liquid chromatography and its application to pharmacokinetic studies. Journal of chromatographyB. 2001, Volume 759,. Page no: 179-183
12. R.nagewara rao, A. Narasa Raju, R. Narsimha. Isolation and charecterisation of process related impurities and degradation products of bicalutamide and development of RP-HPLC method for impurity profile study. Journal of pharmaceutical and bio medical analysis. 2008,Volume46, Page no:505-519.
13. G.saravanan, B.M.Rao, M. ravikumar, M.V.suryanarayana, N. Someswararao, P.V.R.Acharyulu, A stability – indicating LC assay method for Bicalutamide. Chromatographia. 2007, Volume 66,.page no: 219-222.
14. Roland Torok, adam Bor, Gyorgy orosz, Ferenc Lukacs, Daniel W.armstrong, antal peter. High performance liquid chromatographic enantioseperation of bicalutamide and its related compounds. Journal of chromatographyA, 2005, volume 1098, Page no:75-81.
15. Ajeet kumar singh, akash chaurasiya, gaurav K.jain, anshumali awasthi, dinesh asati, Gautham Misra, Roop.K.khar, rama mukherjee. High performance liquid chromatography method for the pharmacokinetic study of bicalutamide SMEDDS and suspension formulations after oral administration to rats. Talanta, 2009, volume 78,. Page no 1310-1314.

*****