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Nehal F. Farid, Nada S. Abdelwahab

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Two Different Spectrophotometric Determinations of Potential Anticancer Drug and its Toxic metabolite

Nehal F. Farid*, Nada S. Abdelwahab

Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Benisuef University, Benisuef, Egypt.

*** Corresponding author**

Name: Nehal Fayek Farid

Tel: +201277950994

Email: nehalfayek@gmail.com

ABSTRACT

Flutamide is a hormone therapy used for men with advanced prostate cancer. Flutamide is highly susceptible to hydrolysis with the production of 3-(trifluoromethyl)aniline, which is reported to be one of its toxic metabolites, impurities and related substances according to BP and USP. Flutamide was found to be stable when exposed to oxidation by 30% hydrogen peroxide and direct sunlight for up to four hours. Two accurate and sensitive spectrophotometric methods were used for determination of flutamide in bulk and in pharmaceutical formulations.

Method (I) is the area under curve (AUC) spectrophpotometric method that depends on measuring the AUC in the wavelength ranges of 275-305 nm and 350-380 nm and using Cramer's Rule. The linearity range was found to be 1-35 µg/mL and 0.5-16 µg/mL for the drug and the degradate, respectively. In method (II), combination of the isoabsorpative and dual wavelength spectrophotometric methods was used for resolving the binary mixture. The absorbance at 249.2 nm (λ_{iso}) was used for determination of total mixture concentration, while the difference in absorbance between 232 nm and 341.2 nm was used for measuring the drug concentration. By subtraction, the degradate concentration was obtained. Beer's law was obeyed in the range of 2-35 µg/mL and 0.5-20 µg/mL for the drug and its degradate, respectively.

The two methods were validated according to USP guidelines and were applied for determination of the drug in its pharmaceutical dosage form. Moreover AUC method was used for the kinetic study of the hydrolytic degradation of flutamide. The kinetic degradation of flutamide was found to follow pseudo-first order kinetics and is pH and temperature dependent. Activation energy, kinetic rate constantsand $t_{1/2}$ at different temperatures and pH values were calculated.

Keywords: flutamide; area under curve; isoabsorpative; dual wavelength; kinetic study; degradation.

1. INTRODUCTION

Flutamide is chemically known as 2-methyl-N[4-nitro-3-(trifluoromethyl)pheneyl]propamide [1]. It is a nonsteroidal component with anti-adrenergic properties used for treatment of prostatic carcinoma [2]. Flutamide is susceptible to hydrolytic degradation with the production of 3-(trifluoromethyl)aniline which is stated to be one of the impurities and related substance of flutamide according to BP [3] and USP [4].The resulted degradate was also reported to be one of the main toxic metabolites of flutamide [5].

Flutamide was determined in BP [3] by UV spectrophotometric method through measuring the absorbance at 295 nm of methanolic solution. On the other hand USP [4] determined the drug in pure form and in capsules by RP-HPLC method using acetonitrile: water (1:1, v/v) as a mobile phase.

Several methods were reported for analysis of the studied drug including electrochemical methods [6], different spectrophotometric methods [6-11] and chromatographic methods [6, 12-15].

Reaction kinetics is the study of chemical change and the way by which this change is influenced by variable conditions such as concentration of reactant, the present chemicals and temperature. It provides information that permits a rational approach to the stabilization of drug products, prediction of shelf life and optimization of storage conditions [16-19]. Many environmental conditions such as heat, light, hydrolysis or oxidation can take part in pharmaceutical stability [19]. Hydrolysis is one of the most common degradation routes, since water exists for most drugs and excipients as a solvent or moisture in the air [21].

Most reported spectrophotometric methods included the formation of colored products that suffered from various drawbacks and tedious sample preparation. On the other hand, none of the reported methods described the degradation behavior or mechanism of flutamide. The work in this manuscript aimed to study the kinetic of the hydrolytic degradation of the anticancer flutamide under acidic and alkaline conditions using different temperatures through the development of stability indicating spectrophotometric methods. Characterization of the produced degradate (flutamide toxic metabolite) was carried out by IR and mass spectroscopic analyses. Other degradation pathways as oxidation and exposure to direct

sunlight were tried and the drug was found to be stable under these conditions. The developed methods are easy to be applied, need simple sample preparation and data manipulation, hence they can be used in quality control and stability studies of the proposed drug.

2. EXPERIMENTAL

Instruments

-A double beam UV-Visible spectrophotometer (SHIMADZU, Japan), model UV-1601 PC with 1cm path length quartz cell is used and it is connected to IBM compatible computer. The software was UVPC personal spectroscopy software version 3.7.

-UV lamp with short wavelength 254 nm (USA).

Materials

-Pure samples

-Flutamide sample was purchased from Sigma-Aldrishchemie GmbH., Germany, its purity was found to be 99.25%.

-Pharmaceutical formulation

-Cytomed® tablets is labeled to contain 250 mg flutamide and manufactured by CIPLA LTD. INDIA.

-Chemicals and reagents

All chemicals and reagents used throughout this work were of analytical grade and were used without further purification.

Methanol and glacial acetic acid [(Sigma-Aldrich, Chromasolv®, Germany).

Sodium hydroxide (0.05 N,0.1 N and 0.2N aqueous solution), HCl (0.3N, 0.5N and 1Naqueous solution), hexane, ethylacetate (El-Nasr Pharmaceutical Chemicals Co., Abu-Zabaal, Cairo, Egypt).

-Preparation of flutamide degradation product

0.2 g of flutamide was dissolved into 10mL methanol and then refluxed at 80^0C with 40mL of either 1N HCl or 0.1N NaOH or 30% hydrogen peroxide for 3 hours. The drug solution was also left for 4 hours in direct sunlight. The degradation process was followed by TLC

using a developing system consisting of hexane: ethyl acetate: glacial acetic acid (8: 3: 0.1, by volume). After complete degradation, the produced yellow solution was extracted with chloroform (3X10mL) and the extracted degradate was dried at 60^0C and then identified by IR and mass spectroscopic analyses.

Solutions:

Stock solutions of flutamide and degradate(1mg/mL) were prepared by accurately weighing 0.1 gm flutamide and the prepared degradate in two separate 100-mL volumetric flasks and dissolving in methanol.

Working solution of flutamide and degradate (0.1mg/mL) were prepared by transferring 10 mL from their respective stock solutions(1 mg/mL) into two separate 100-mL volumetric flasks and then diluting with methanol.

For kinetic investigations, standard solutions of flutamide (2.9×10^{-3} and 4.3×10^{-3} M) were prepared by dissolving the corresponding amount of flutamide in 10mL methanol and then completing the volume to 25-mL with either HCl (0.3, 0.5 or 1N) or NaOH (0.05, 0.1 or 0.2N).

Preparation of laboratory prepared mixtures

Different aliquots of flutamide and its degradation product working solutions (0.1mg/mL) were transferred to a set of 10-mL volumetric flasks and the volume was completed with methanol to prepare laboratory prepared mixtures containing from 5% to 90% of the degradation product.

Application to pharmaceutical formulation

Ten tablets of cytomed® were grinded well and then an accurately weighted amount was transferred into 100-mL volumetric flask. 75 mL methanol was added and the solution was ultra-sonicated for 30 minutes, filtered and then the volume was completed with the same solvent to prepare sample stock solution (1mg/mL). Sample working solution (0.1mg/mL) was then prepared.

Procedure

Spectral characteristics of flutamide and its degradation product

Zero order absorption spectra of 10µg/mL each of flutamide and its degradation product were recorded in the range of 200-450 nm using methanol as a blank.

Construction of calibration curves

Concentrations of flutamide in the range of 1-35µg/mL and of the degradation product in the range of 0.5-20µg/mL were prepared in methanol. The absorption spectra of the prepared solutions were recorded in the range of 200-450nm.

For method (I) [area under curve method, AUC]: the area under the curve in the range of 275-305 nm (λ_1-λ_2) and 350-380nm (λ_3-λ_4) were recorded for the prepared solutions each of flutamide and the degradation product. Then the absorptivity 'Y' values of each were calculated where Y = the recorded area under curve of each component (from 275 to 305 nm or 350 to 380 nm)/concentration of the component (in µg/mL). The concentrations of the studied components in the prepared solutions were determined by applying Cramer's rule and matrices in the following equations:

$A_1 = Y_{x1}C_x + Y_{z1}C_z$ (λ_1-λ_2)

$A_2 = Y_{x2}C_x + Y_{z2}C_z$ (λ_3-λ_4)

Where A_1, $A_2$are the areas under curve in the range of 275-305 nm and 350-380nm, respectively.Y_{x1}, Y_{x2} are the absorptivity values of flutamide at (λ_1-λ_2) and (λ_3-λ_4), respectively. $Y_{z1}$and Y_{z2} are the absorptivity values of the degradate at (λ_1-λ_2) and (λ_3-λ_4), respectively. C_x and C_z are the concentrations in µg/mL of flutamide and its degradate, respectively.

For method (II)[dual wavelength and isoabsorbtivepoint methods]: The absorbance values at 232, 341.2 nm(for flutamide) and at 249.2 nm (for the degradate) were recorded. Concentrations of flutamide were measured by plotting the absorbance difference at the selected wavelengths (zero difference for the degradate) versus the corresponding concentrations of flutamide from which the regression equation was computed. On the other hand the absorbance at 249.2 nm (λiso) was used for construction of the calibration curve of the degradation product.

Analysis of laboratory prepared mixtures

The absorption spectra of the previously prepared mixtures were recorded in the range of 200-450nm. For method (I), the area under curve in the range of 275-305nm (λ1-λ2) and 350-380nm (λ3-λ4) were recorded then by substituting in the corresponding equations, concentrations of the drug and the degradate could be obtained. For method (II), the

absorbance difference at 232nm and 341.2 nm was used for measuring flutamide concentrations while the absorbance values at 249.2 nm were used for measuring the total mixture concentration and by subtraction, concentrations of the degradation product could be calculated.

Analysis of pharmaceutical formulation

Dilutions of the prepared sample working solution (0.1mg/mL) were performed to prepare concentrations within the linearity range of the studied drug. Procure illustrated under construction of the calibration curve for each method was followed. Moreover, recovery studies were carried out at three levels (80, 100 and 120%) to access accuracy of the method.

Kinetic study

a- Effect of drug concentration

Two concentrations of flutamide (2.9×10^{-3} and 4.3×10^{-3} M) were prepared once in 0.5N HCl (set-1) and the other in 0.1N NaOH (set-2). The prepared solutions were rapidly inserted into thermostatic water baths set at 80 ^{0}C (for set-1) and 50^{0}C (for set-2).At the specified time intervals, 0.25 mL of each sample was accurately transferred into 10-mL measuring flask, 0.25mL of either 0.5N NaOH (for set-1) or 0.1N HCl (for set-2) was added to stop the reaction and then the volume was completed with methanol. Immediately the absorption spectra of the prepared solutions were recorded and the remaining drug concentration was determined by applying method (I). The kinetic parameters [order of the reaction, degradation rate constant (K) and half-life of the reaction ($t_{1/2}$)] were calculated by plotting the logarithm of the remaining flutamide concentration versus time in minutes.

b- Effect of HCl and NaOH strength on the reaction rate

Solutions of 2.9×10^{-3}M flutamide were prepared once in 0.3N, 0.5N and 1N HCl (set-1) and the other in 0.05N, 0.1N and 0.2N NaOH (set-2). The solutions were then placed in thermostated water baths set at 80^{0}C (for set-1) or at 50^{0}C (for set-2). The procedure illustrated under (the effect of drug concentration) was then followed.

c- Effect of temperature on the reaction rate

Solutions of 2.9×10^{-3}M flutamide were prepared one in 0.5N HCl (set-1) and the other in 0.1N NaOH (set-2). The solutions were then placed in thermostated water baths set at 70^{0}C, 80^{0}C and 90^{0}C (for set-1) or at 40^{0}C, 50^{0}C and 60^{0}C (for set-2). The procedure illustrated under (the effect of drug concentration) was then followed. Temperature effect on drug

degradation rate was studied by plotting of Arrhenius plot (the logarithm of the reaction rate constant (log K) versus the reciprocal of the absolute temperature (1/T) in kelvin) from which the activation energy (Ea) was calculated (Slope= -Ea (KJ/mol)/R, R=8.314J/Kmol).

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3- RESULTS AND DISCUSSION

Prostate cancer is one of the most common cancers in many industrialized nations and among the leading causes of cancer death [22]. It may be less common in developing countries, but its rate and mortality has been on the increase [23]. Flutamide is widely used to treat advanced (metastatic) prostate cancer. Till now, no report has been found in the literature for studying the stability and the degradation kinetic of the anticancer flutamide. Moreover, the produced degradate is considered to be toxic metabolite, impurity and related substance of the main drug. Due to the pharmaceutical importance of the studied drug, it is important to develop sensitive, selective and accurate methods for its analysis and to study its degradation behavior. Studying the reaction kinetics plays a vital role in providing information about the chemical reaction rate, $t_{1/2}$, thermodynamic effects as well as the effect of various process variables.

-Degradation behavior of flutamide

Flutamideis an amide containing drug that is highly susceptible to alkaline and acidic hydrolysis. Upon refluxing the cited drug with either 1N HCl or 0.1N NaOH at 80^0C for 3 hours, complete drug degradation was observed with the formation of a yellow degradate. Structural elucidation of the resulted degradation product has been carried out by IR and mass spectroscopic analyses.

The IR spectrum of flutamide, **Fig. (1)** showed a sharp peak at 1715 $cm^{-1}$and a peak at 3360cm^{-1} corresponding to C=O and NH groups which disappeared in the IR spectrum of the degradate and a new forked peak at 3371.92-3479.92 $cm^{-1}$was appeared indicating the cleavage of the amide linkage and the formation of a primary amine (NH_2) containing compound, **Fig. (1).**The electron impact of the degradate showed mass ion peak at 206m/z corresponding to molecular weight of the expected degradation product, **Fig. (1).**

Flutamide was found to be stable to oxidation by 30% hydrogen peroxide and light for up to four hours (no spots on TLC plate other than that of flutamide were observed).

-Methods development and optimization

The developed area under curve, dual wavelength and isoabsorptive point spectrophotometric methods are sensitive, simple and selective with minimum sample preparation and data manipulation comparing to all the reported spectrophotometric methods [3, 6-11] that are of low selectivity and need tedious sample preparation steps.On the other hand the reported chromatographic methods [6, 12-15] need expensive and sophisticated apparatus. Zero order absorption spectra of flutamide and itsdegradate, **Fig. 2** showed severe spectral overlap which impeded their direct determinations. By applying the proposed methods, the drug and the degradate spectral overlap could be successfully resolved. The developed area under curve method (AUC)(method I) was used for simultaneous determination of the drug and the degradate. In the second method (method II), dual wavelength method was applied for determination flutamide while the isoabsorpitive method developed by Erram and Tipinis [24-26] has been used measuring the total mixture concentration and by subtraction, concentration of the degradate could be obtained.

In the area under curve method (AUC), selection of wavelength ranges is an important step during method optimization hence it affects selectivity of the method. Different wavelength ranges were tested where the wavelength ranges of 275-305nm and 350-380nm were the most suitable ranges regarding selectivity for the drug and the degradate. On the other hand,the developed dual wavelength method requires the careful selection of two wavelengths where the interfering compound shows equal absorbance while the analyte of interest shows significant difference in absorbance with concentration. Different wavelength pairs were tested and good results were obtained when using the wavelength pair of 232nm and 341.2 nm for selective determination of flutamide where the degradate showed zero difference in absorbance.

-Methods validation

Method validation was carried out following USP[4] guideline.

- Linearity

a- Area under curve method (method I): In order to check the linearity of the developed area under curve method, different concentrations of flutamide and the degradate in the range of 1-35 µg/mL and 0.5-20µg/mL were prepared. Then area under curve in the wavelength

ranges of 275-305nm and 350-380nm was recorded, **Fig. 2.** By using the absorptivity values at the chosen ranges and applying cramer's rule and the following equations, concentrations of the drug and itsdegradate could be calculated.

$$A_1 = 0.858C_{drug} + 0.366C_{deg} \qquad (\lambda_1\text{-}\lambda_2)$$

$$A_2 = 0.201C_{drug} + 1.270C_{deg} \qquad (\lambda_3\text{-}\lambda_4)$$

Where A_1, A_2 are the areas under curve in the range of 275-305 nm and 350-380nm, respectively.C_{drug} and C_{deg} are the concentrations in µg/mL of flutamide and itsdegradate, respectively.

*b- Dual wavelength and isoabsorptive point method(method II):*the absorbance difference of flutamide between 232nm and 341.2 nm was found to be proportional to its concentration in the range of 2-35µg/mL. While the degradate showed linear relationship with the absorbance at 249.2 (λiso) inthe range of 0.5-16µg/mL.Calibration curves were constructed from which the following regression equations were computed:

A= 0.0245C+ 0.0098 r= 1 absorbance difference between 232nm& 241.2nm

A= 0.0174C+ 0.0028r=0.9999 For the total mixture at 249.2 nm (λ iso)

Where, A is the absorbance at the specified wavelengths, C is the concentration in µg/mL and r is the correlation coefficient.

- Accuracy

Accuracy of the methods was tested by applying the methods for determination of different three concentrations (n=3X3) of pure drug and degradate and then calculating the mean percentage recoveries. The methods showed good accuracy, **Table (1)**, where the obtained percentage recoveries were found to be 100.03 and 101.46 (method I) for the drug and the degradate, respectively and 100.10100.2646 (method II) for the drug and the degradate, respectively. Accuracy was further confirmed by application of standard addition technique where good results were obtained, **Table (2)** which confirmed that tablets excepients did not interfere.

- Precision:

It measures the closeness between the measured values and was proved by testing method repeatability and Intermediate precision. Repeatability was tested by analyzing three concentrations of pure flutamide (10, 15 and 20µg/mL) and degradate (5,10 and 15µg/mL) in

triplicates on the same day. While intermediate precision was assessed by assaying the prepared solutions of the above mentioned concentrations in triplicates in three successive days. Mean recoveries and RSD% were then calculated for the two components and given in **Table (1).**

- Specificity

It was tested by application of the proposed methods for determination of the dug and the degradate in different laboratory prepared mixtures containing the degradate in the ratio from 5 to 90%. Good percentage recoveries were obtained, **Table 3**, confirming the selectivity of the proposed methods.

- Limits of detection (LOD) and quantification (LOQ)

They were calculated by using the lower parts of the linear range of the calibration curves and then applying in the following equations:

$$LOD = 3.3 \times N/B \qquad LOQ = 10 \times N/B$$

Where N is the standard deviation of the intercept and B is the slope of the corresponding calibration curve. The methods showed good sensitivities and results are presented in **Table (1).**

-Application of the methods

The methods were applied for measuring flutamide concentration in the marketed pharmaceutical preparation where no interference from tablets excepients were observed and good results were obtained (**100.86 ± 1.751**%), **Table (2).** Results of recovery study presented in **Table (2)** confirmed the good accuracy of the developed methods **Table (2).**

-Statistical analysis

When the developed methods were compared with the official one [3] using F-value and student's t-test, no significant difference was found between them, **Table (2).**

-Kinetic study

As previously mentioned, no kinetic spectrophotometric method has been reported in the literature for analysis of flutamide. Hence, the developed area under curve method (method I) was used for measuring the kinetic parameters of flutamide hydrolytic degradation.

Hydrolytic degradation is a bimolecular reaction containing water (one of the reactant) in excess amount and concentration changes are negligible, hence this reaction follows pseudo first order reaction. To test the order of the reaction, experiment at initial flutamide concentration of $2.9X10^{-3}$mol/L, constant temperature and constant reagent (either HCl or NaOH) normality was performed. By plotting the logarithm of the remaining concentration of flutamide versus time (by minutes), straight line was obtained, **Fig. (3)**indicatingthat the hydrolytic degradation of flutamide followed pseudo first order reaction. The reaction rate constant (K) was calculated from the obtained curve (slope= -K/ 2.303) from which t1/2 was calculated (t1/2= 0.693/K), **Table (4)**.

Variables affecting the reaction rate such as flutamide concentration, reagent normality and temperature have been tested.

a- Effect of drug concentration

When the experiment was repeated on two initial concentrations of flutamide(2.9×10^{-3} and 4.3×10^{-3} M) at constant temperature and constant NaOH (0.1N) and HCl (0.5N) normality.On plotting the logarithm of the remaining flutamide concentration versus time (by minutes), two parallel lines were obtained, **Fig. (3),** that have nearly the same slopesand almost the same rate constant (K), hence t1/2 values near to each other. Results given in **Table (4)** assessed that the reaction rate is not affected by the initial drug concentration and hence followed pseudo first order reaction.

b- Effect of HCl and NaOH strength on the reaction rate

Flutamide degradation was found to be pH sensitive. As shown from **Fig. (4)**and results in **Table (4)**. K value is highly affected by normality of NaOH and HCl. Degradation with 0.2 NaOH possessed the highest K value and thehighest reaction speed.

c- Effect of temperature on the reaction rate

Hydrolysis of flutamide was significantly affected by temperature. As shown from the results in **Table (4),** K values increased with the increasing in temperature either in alkaline or acidic hydrolytic degradation indicating thatthe reaction rate (velocity) increases with increasing in temperature. Moreover, the effect of temperature on the reaction rate constant was studied by using Arrhenius plot, **Fig. (5)**from which activation energy was calculated and found to be 22097.38 KJ/Mole and 29032.49KJ/Mole for alkaline and acidic hydrolysis, respectively.

The values of activation energy indicated that flutamide acidic degradation reaction was slower than its alkaline degradation reaction.

4- CONCLUSION

This work concerned with the development and validation of two stability indicating spectrophotometric methods for determination of the anticancer, flutamide. The developed methods were the first developed stability indicating spectrophotometric methods for determination of the proposed drug. They were successfully applied for determination of flutamide and its hydrolytic degradation product in raw materials and laboratory prepared mixtures. Moreover, the developed area under curve method was utilized for kinetic study of alkaline and acidic degradation of the drug and factors affecting reaction rate constant (K) were studied. Flutamide was found to be highly degraded and its degradation was found to be pH and temperature dependent.

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Figure captions:

Figure 1.IR and mass spectra of flutamide and itsdegradate

Figure 2: Zero order aborption spectra of 10µg/mL each of flutamide ( ) and the degradate (-----).

Figure 3: Kinetic plot of (A) alkaline, (B) acidic degradation of 2.9X 10^{-3}mol/L and 4.3 X 10^{-3}mol/L of flutamide.

Figure 4: Plot for the effect of sodium hydroxide (A) and HCl (B) concentration on the rate of degradation of 2.9X 10^{-3}mol/L flutamide.

Figure 5: Arrhenius plot for alkaline (A) and acidic (B) degradation of2.9X 10^{-3}mol/L flutamide.

Table captions:

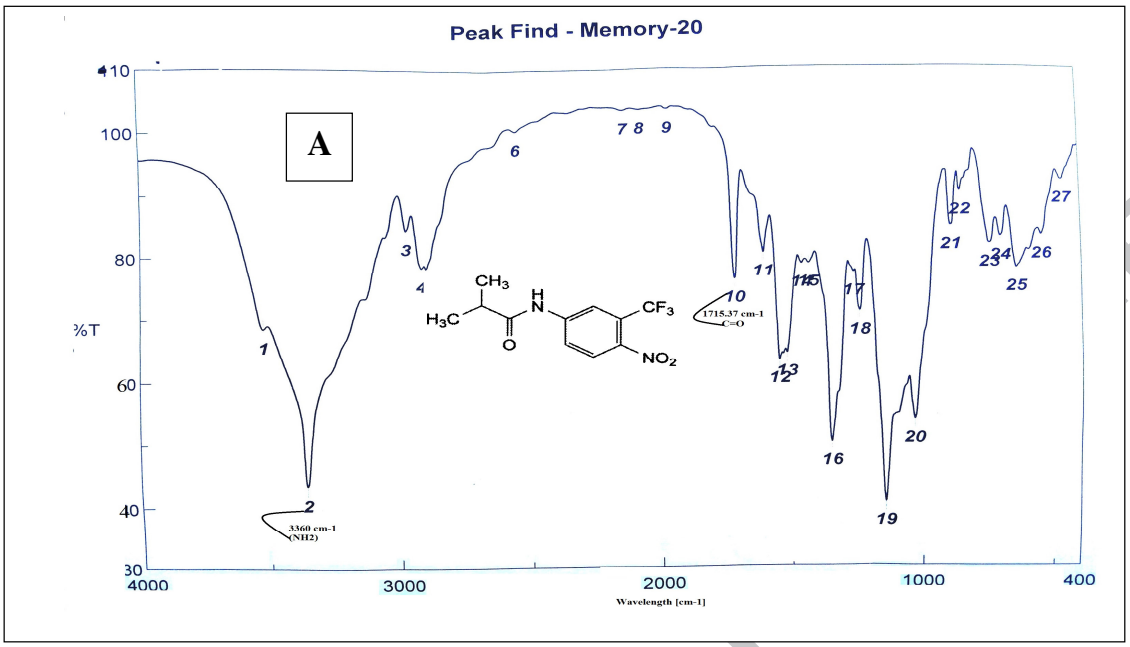
Table 1: Assay and method validation parameters for the determination of pure samples of flutamide and its degradate by the proposed methods.

Table 2: Determination of Flutamide in its pharmaceutical formulation by the proposed methods and application of standard addition technique.

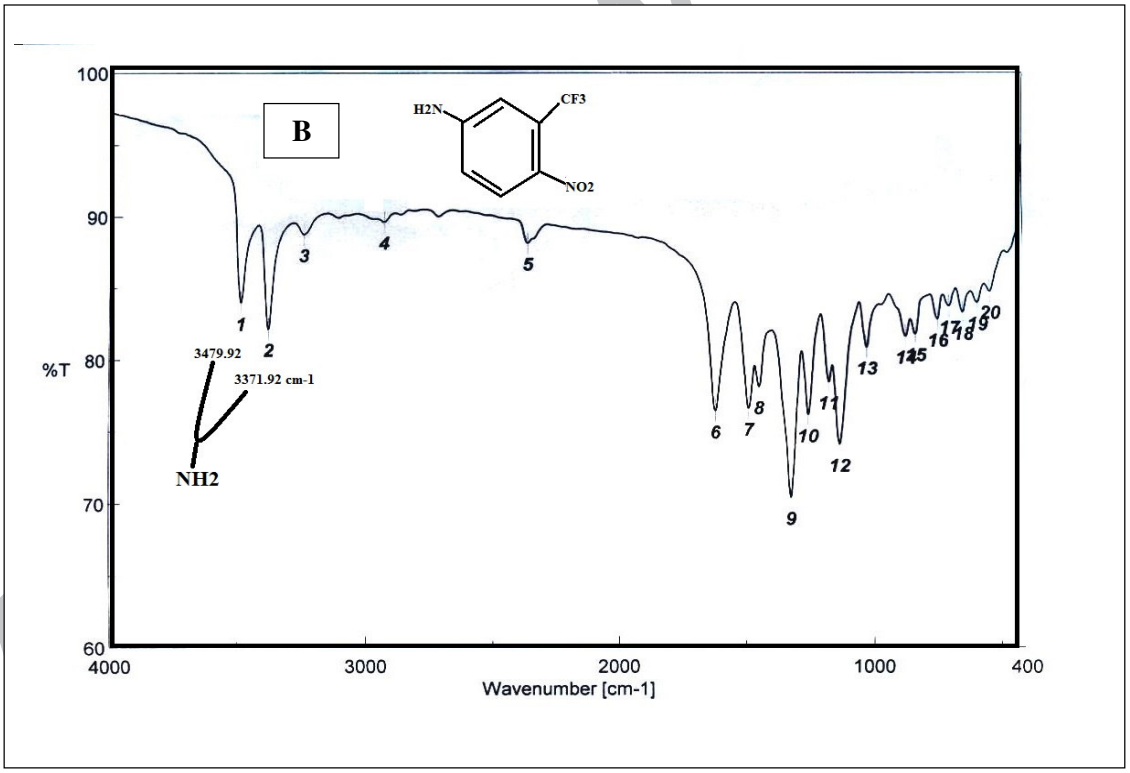
Table 3: Determination of Flutamide and itsdegradate in Laboratory prepared mixtures by the proposed methods.

Table 4: Kinetic data of flutamide alkaline and acidic hydrolysis

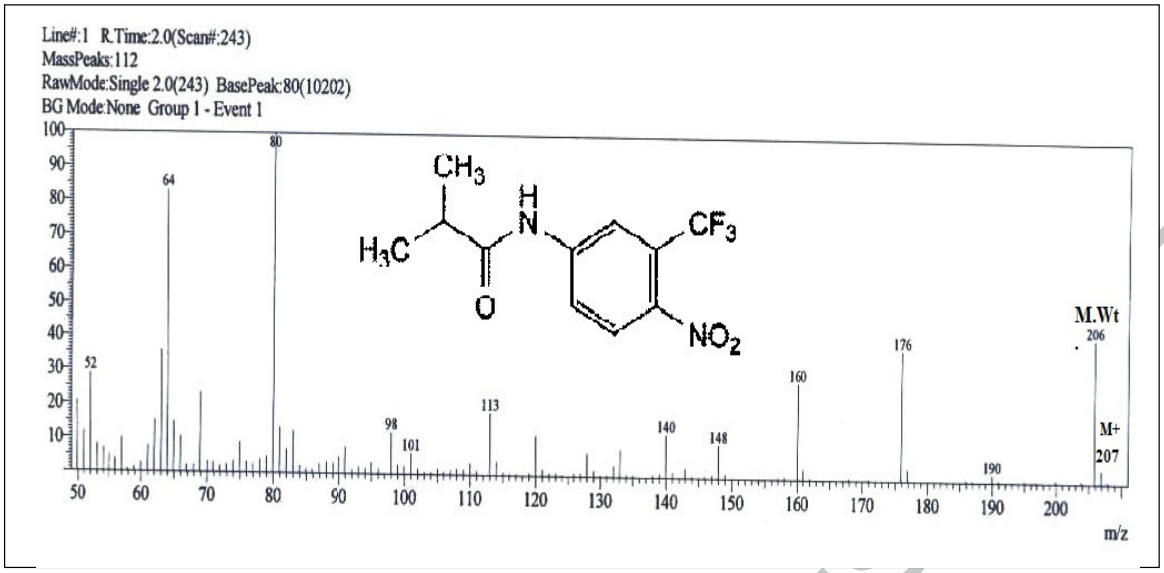
Figures



IR spectrum of the flutamide



IR spectrum of the degradate



Mass spectrum of the degradate

Figure 1.IR and mass spectra of flutamide and its degradate

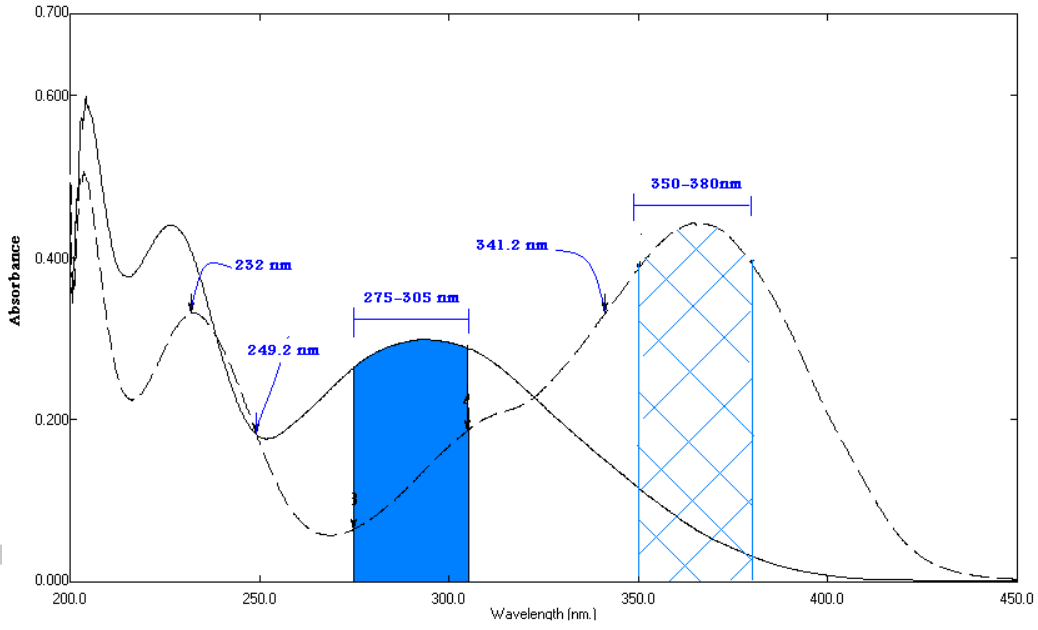
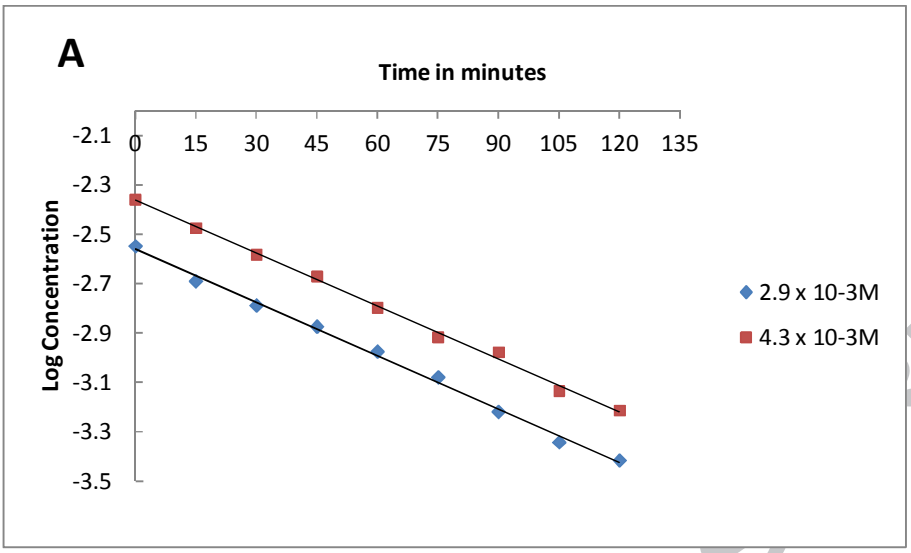
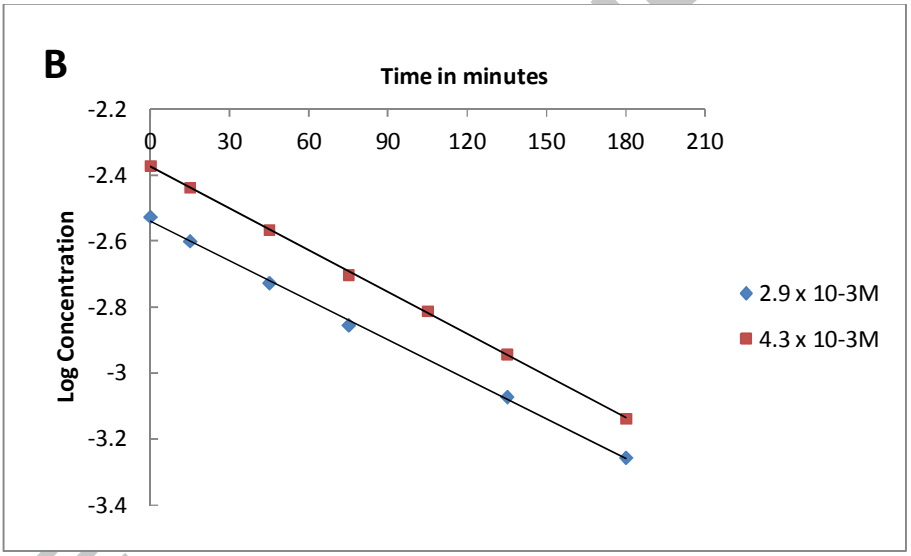


Figure 2: Zero order aborption spectra of 10µg/mL each of flutamide (——) and the degradate (------).

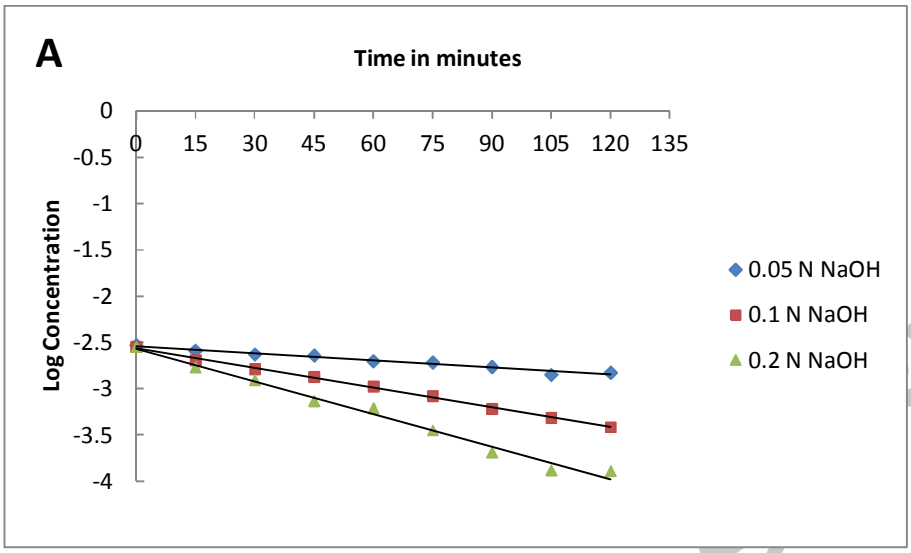


(A)

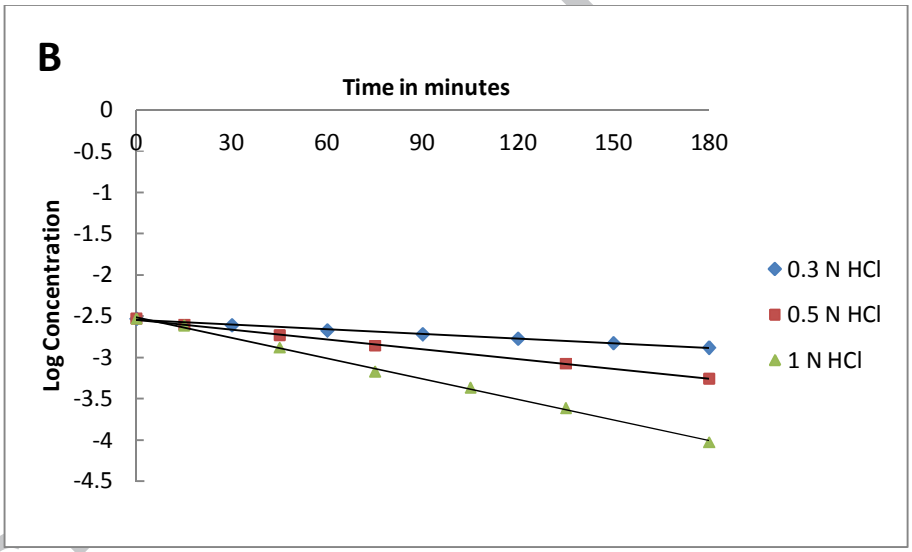


(B)

Figure 3: Kinetic plot of (A) alkaline, (B) acidic degradation of 2.9X 10^{-3}mol/L and 4.3 X 10^{-3}mol/L of flutamide.

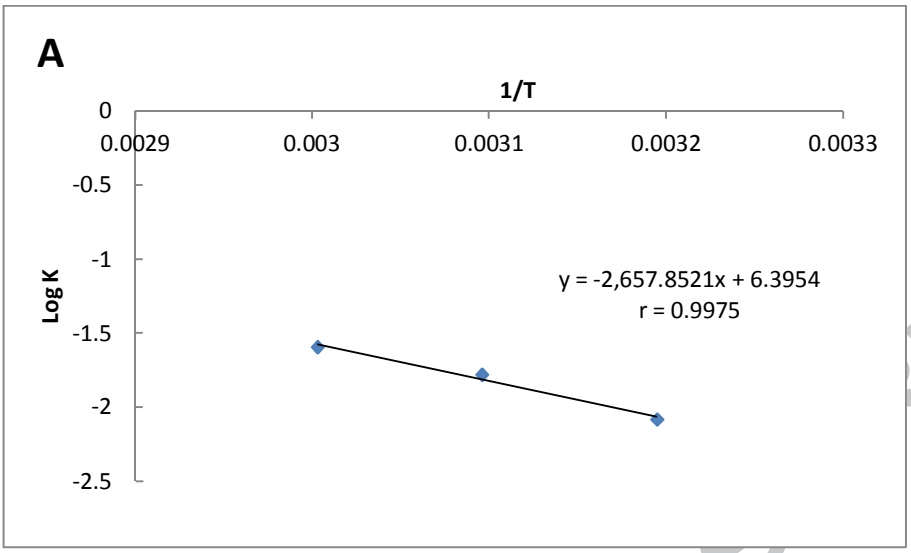


(A)

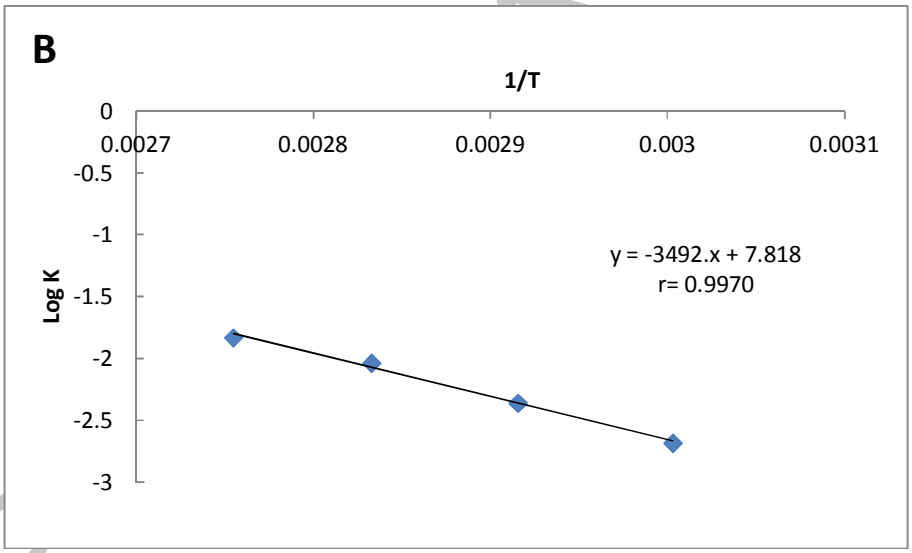


(B)

Figure 4: Plot for the effect of sodium hydroxide (A) and HCl (B) concentration on the rate of degradation of 2.9X 10^{-3}mol/L flutamide.



(A)



(B)

Figure 5: Arrhenius plot for alkaline (A) and acidic (B) degradation of2.9X 10^{-3}mol/L flutamide.

Tables

Table 1: Assay and method validation parameters for the determination of pure samples of flutamide and its degradate by the proposed methods.

Parameters	Flutamide		Degradate	
	AUC method	Difference between 232 – 341.2 nm	AUC method	Isoabsorptive point at 249.2 nm
Range (µg/mL)	1 – 35	1 – 35	0.5-20	0.5 – 16
Accuracy	100.03	100.10	101.46	100.26
Selectivity	98.55± 0.658	100± 1.556	98.65± 1.337	102.23± 1.229
Precision (%RSD)				
Repeatability [a]	1.338	1.068	1.071	1.340
Intermediate precision [b]	1.577	1.490	1.572	1.963
LOD (µg/mL)	0.31	0.30	0.15	0.16
LOQ (µg/mL)	0.95	0.90	0.45	0.48

[a] The intraday (n = 3), average of three different concentrations repeated three times within day.

[b] The interday (n = 3), average of three different concentrations repeated three times in three successive days.

Table 2: Determination of Flutamide in its pharmaceutical formulation by the proposed methods and application of standard addition technique.

Pharmaceutical formulation	Component	AUC method				Dual wavelength method			Reference method [3] [a]
		Taken (µg/mL)	Found [a] % ± RSD%	Added (µg/mL)	Recovery %	Found [a] % ± RSD%	Added (µg/mL)	Recovery %	
Cytomed® tablets labeled to contain 250 mg flutamide/tablet	Flutamide	15.00	100.86 ± 1.751	10.00	97.20	98.64 ± 1.473	10.00	99.80	100.43± 1.253
				15.00	100.47		15.00	103.00	
				20.00	100.10		20.00	103.40	
			Mean ± SD		99.26 ± 1.791		Mean ± SD	102.07 ± 1.973	
F-Test (6.388 [c])			1.951			1.381			
Student's t-test (2.306 [c])			0.424			2.074			

[a] Average of 5 determinations.

[b] Average of 3 determinations.

[c] The values between parenthesis are corresponding to the theoretical values of t and F ($P = 0.05$).

Reference method: direct spectrophotometric determination of methanolic solution of flutamide and measuring the absorbance at 295 nm.

Table 3: Determination of Flutamide and its degradate in Laboratory prepared mixtures by the proposed methods.

% degradate	Taken (µg/mL)		Found %**			
			AUC method		Dual wavelength method	Isoabsorptive point method
	Flutamide	Degradate	Flutamide	Degradate	Flutamide	Degradate
5%	30.00	1.50	98.07	100.67	98.8	102.67
20%	20.00	4.00	98.10	99.50	98.10	103.75
40%	25.00	10.00	99.20	102.85	101.28	100.00
60%	15.00	9.00	97.80	99.22	97.73	102.11
80%	10.00	8.00	98.70	98.63	98.00	102.38
90%	10.00	9.00	99.40	99.11	98.00	102.44
Mean± RSD%			**98.55± 0.658**	**100± 1.556**	**98.65± 1.337**	**102.23± 1.229**

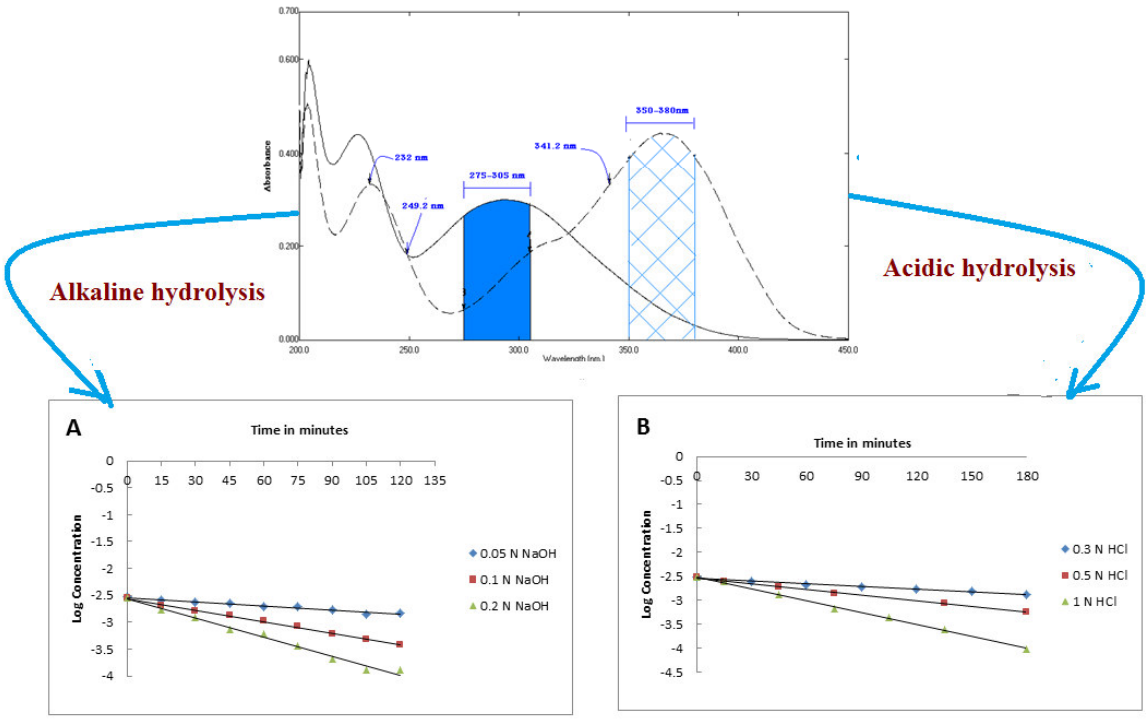
** Average of 3 determinations.

Table 4: Kinetic data of flutamide alkaline and acidic hydrolysis

Temperature (^oC)	Strength	Concentration (M/L)	$t_{1/2}$(min.)	K($min.^{-1}$)
Alkaline degradation				
40	0.1 N NaOH	2.9×10^{-3}	83.59	0.00829
50	0.05N NaOH	2.9×10^{-3}	115.73	0.00599
50	0.1N NaOH	2.9×10^{-3}	42.38	0.01635
50	0.1N NaOH	4.3×10^{-3}	41.79	0.01658
50	0.2 N NaOH	2.9×10^{-3}	25.719	0.02695
60	0.1 N NaOH	2.9×10^{-3}	27.11	0.02556
Acidic degradation				
70	0.5 N HCl	2.9×10^{-3}	158.38	0.00438
80	0.3N HCl	2.9×10^{-3}	158.38	0.00438
80	0.5N HCl	2.9×10^{-3}	75.23	0.0092
80	0.5N HCl	4.3×10^{-3}	71.65	0.0097
80	1N HCl	2.9×10^{-3}	36.25	0.01912
90	0.5N HCl	2.9×10^{-3}	48.53	0.0148

Graphical Abstract

Flutamide was highly sensitive to hydrolysis. Reaction kinetic was studied using AUC method and found to follow pseudo first order kinetics. Reaction rate constant (K) was found to be pH and temperature dependant.



High lights

1- No kinetic spectrophotometric study was published for assay of flutamide.
2- Developed methods were the first developed stability indicating ones for flutamide.
3- Degradation identification by IR and mass spectra analyses.
4- The drug degradation followed pseudo first order kinetics.
5- Degradation rate was highly sensitive to temperature and pH