Drug Development and Industrial Pharmacy, 28(4), 413–422 (2002)

RESEARCH PAPER

Stability Study of Flutamide in Solid State and in Aqueous Solution

A. Miranda, I. Caraballo,* and M. Millán

Departamento de Farmacia y Tecnologíá Farmacéutica,
Facultad de Farmacia, Universidad de Sevilla, 41012 Seville, Spain

ABSTRACT

A high-performance liquid chromatography (HPLC) assay has been developed for the determination of flutamide and its degradation products. Using this method, the influence of important formulation factors on the stability of flutamide has been estimated. The stability studies have been carried out in solid state as well as in aqueous solution. The results obtained have shown a good stability for flutamide in solid state. This drug remained practically unchanged after a four-month assay in adverse temperature and humidity conditions. On the other hand, the results obtained from the stability study in solution during 12 days have shown that flutamide in aqueous solution underwent a clear degradation at mean or high temperature (22°C, 37°C) and acidic pH conditions (1.1). With respect to the influence of ionic strength, it has been found that the presence of sodium chloride prevents the degradation of flutamide in aqueous solution. The second-order kinetics model provides the best fit for highly degraded solutions.

Key Words: *Degradation kinetics; Flutamide; Liquid chromatography; Stability*

INTRODUCTION

Flutamide (3′-trifluoromethyl-4′-nitromethyl propionylanilide) is a nonsteroidal pure antiandrogen indicated for palliation of advanced prostate cancer. It acts by inhibiting the uptake and/or binding of dihydrotestosterone to the target cell receptor, thus interfering with androgen action (1).

Flutamide is well absorbed orally and extensively metabolized; its active metabolite, 2-hydroxyflutamide, is formed rapidly and excreted almost entirely by the kidneys (1–4).

*Corresponding author.

The usual adverse effects are gynecomastia and mild diarrhea when given as a single agent, but with the advantage of preserving libido and sexual potency (1,5).

Its pharmacokinetics and dosage characteristics (usually three doses per day of 250 mg each) (6) make it a suitable candidate for the design of controlled-release delivery systems.

Drug stability in solid state as well as in solution is especially important for drugs included in controlled-release dosage forms, due to the long time in contact with the physiological fluids (7). Nevertheless, no stability study of flutamide has been found in the literature. For this reason, a preformulation study of flutamide has been performed, including stability studies in solid state and in solution.

The main objective of the present paper is to study the influence of the different factors on the stability of flutamide in solid state and in solution. For this reason, a specific high-performance liquid chromatography (HPLC) method (8) has been developed and validated to quantify flutamide in the presence of some of its main decomposition products. This method has been used to determine the drug in different humidity, temperature, pH, and ionic strength conditions.

MATERIALS AND METHODS

Materials

The following chemicals were used as received: flutamide (which was a gift of Laboratories Inibsa, Barcelona, Spain), sodium chloride and hydrochloric acid (Panreac, Barcelona, Spain), and methanol and acetonitrile (Merck, Darmstadt, Germany). The methanol and acetonitrile used were HPLC grade and the water was freshly distilled.

Methods

Liquid Chromatography Analysis

The HPLC system consisted of a constant flow pump (Kontron Instruments, type 420), a rotatory valve injector (Rheodyne type 7125) equipped with a 20-μL loop, an ultraviolet (UV) detector (Kontron Instruments, type 432), and an integrator (Konic Instruments, type DataJet 4600). The column used (Licrospher RP-18) was packed with silica particles bonded with octadecylsilane.

A flow rate of 1 mL/min for the mobile phase (acetonitrile/water/methanol 30 : 45 : 25 v/v/v) was employed, and the wavelength was set at 299 nm.

Each peak area was computed automatically by the integrator. Elution was carried out isocratically under conditions at ambient temperature (20±2°C).

Calibration Curve

The standard solutions were prepared by diluting the stock solution (20 ppm) with water to the desired concentrations (1.81×10^{-6}, 3.62×10^{-6}, 1.81×10^{-5}, 3.62×10^{-5}, and 5.43×10^{-5} M).

The molar extinction coefficient, ε, was determined by linear regression between the values of absorbance, measured at 299 nm, and the corresponding concentrations.

Validation Study

The molar extinction coefficient, ε, was determined by linear regression between the absorbance at 299 nm and the corresponding concentrations.

The method was validated by analyzing standard solutions of six different concentrations of flutamide in five replicates on the same day. Furthermore, these solutions were analyzed in triplicate on five different days. The intra-assay precision was determined from the coefficients of variation (CV) of the obtained values. Inter-assay data were calculated using the mean value of the three measurements performed on each day.

Stability in Solid State

Nine lots of 100 mg of flutamide were subjected to different conditions of temperature and humidity (see Table 1). High humidity conditions were obtained using an NaCl-saturated solution that provides 75% relative humidity (RH). Low humidity conditions were obtained by placing the samples into a desiccator containing silica gel.

These lots were analyzed by HPLC at the following times: $t = 0$, 1, 2, 4, 8, 16, 35, 66, and 120 days. On each working day, solutions of each lot were prepared by dissolving 5 mg of the powder accurately weighed into 1000 mL of purified water. Determinations were carried out at least in triplicate.

Stability in Solution

Twelve flutamide solutions of 1.81×10^{-5} M were prepared. The solvents used were purified water, HCl solution (pH 1.1), and simulated gastric fluid without enzymes, prepared according to USP 23, 1995.

The composition, pH, and temperature for each solution are shown in Table 2.

These solutions were analyzed by HPLC at the following times: $t = 0$, 1, 2, 4, 8, and 12 days. Furthermore, standard flutamide solutions of 1.81×10^{-5} M were injected on each working day. Determinations were carried out at least in triplicate.

Table 1

Temperature and Humidity of the Lots Corresponding to the Stability Study in Solid State

Lot	Temperature (°C)	Humidity[a]
1	22	A
2	22	L
3	22	H
4	40	A
5	40	L
6	40	H
7	5	A
8	5	L
9	5	H

[a]A: ambient humidity (60±5% RH); H: high humidity (75% RH); L: low humidity (silica gel desiccator).

RESULTS AND DISCUSSION

Liquid Chromatography Analysis

A good linearity is shown by the calibration curve obtained from 1.81×10^{-6} to 7.24×10^{-5} M flutamide concentration: $y = [(332.97 \times 10^7 \pm 577.25 \times 10^5)x - (618.10 \pm 2338.62)]$ with correlation coefficient $r = 0.995$ ($n = 30$) and Snedecor ratio $F = 3327.23$ ($P = 1.19 \times 10^{-30}$).

The specificity of the method is illustrated in Fig. 1, where complete separation was observed for flutamide and one degradation product. A 30 : 45 : 25 acetonitrile/water/methanol ratio was selected as mobile phase, providing a retention time of 8 min for flutamide.

The results of the validation study are shown in Table 3. As can be observed, acceptable precision and accuracy values were obtained from 3.62×10^{-6} to 5.43×10^{-5} M flutamide solutions. It must be taken into account that 7.24×10^{-5} M solution is very close to the water solubility of flutamide (1.03×10^{-4} M). In this sense it is not surprising to obtain worse precision and accuracy values due to different error sources, such as partial precipitation or uncompleted solubilization.

Table 2

Composition, pH, and Storage Temperature of the Solutions Assayed

Solution	Flutamide (M)	NaCl (g/L)	HCl (pH 1.1)	Neutral pH	Temperature (°C)
1	1.81×10^{-5}		+		22
2	1.81×10^{-5}		+		37
3	1.81×10^{-5}		+		5
4	1.81×10^{-5}	2	+		22
5	1.81×10^{-5}	2	+		37
6	1.81×10^{-5}	2	+		5
7	1.81×10^{-5}			+	22
8	1.81×10^{-5}			+	37
9	1.81×10^{-5}			+	5
10	1.81×10^{-5}	2		+	22
11	1.81×10^{-5}	2		+	37
12	1.81×10^{-5}	2		+	5

Stability in Solid State

In the present work, the influence of the temperature and humidity on flutamide stability in solid state was studied.

Nine lots of 100 mg of flutamide were subjected to different conditions of temperature and humidity (see Table 1). The lots were analyzed as described in the previous section. The amount of flutamide remaining was plotted vs. time, as can be observed in Fig. 2. The profiles obtained show that the lots studied have not undergone important degradation.

To assess this point, we have carried out a two-way analysis of variance (ANOVA) based on the flutamide concentration values of the nine lots studied. The main effects of this ANOVA were temperature (5°C, 22°C, 40°C) and humidity (high, environmental, low), whereas the time was considered as a covariate. The main parameters of this analysis are shown in Table 4.

The analysis shows that the temperature and humidity have no statistically significant influence on the degradation rate of flutamide in solid state. The results obtained show a good stability of flutamide in solid state. This drug remained practically unchanged after a four-month assay in adverse temperature and humidity conditions.

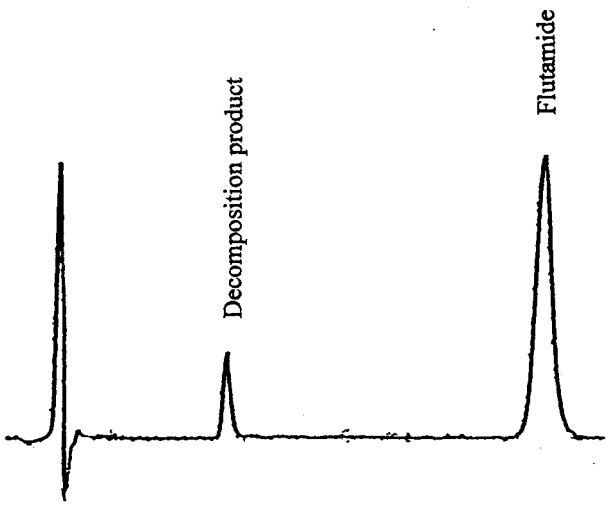

Figure 1. Chromatogram corresponding to a degraded flutamide solution.

Stability in Solution

To study the stability of flutamide in solution, 12 solutions have been prepared and subjected to different conditions of temperature, pH, and ionic strength.

The composition, pH, and storage temperature of each solution are shown in Table 2.

Samples of 20 μL were withdrawn from each solution at different times during 12 days. These samples were injected without filtration into the chromatograph and the concentration of flutamide was determined as described in the previous section.

Influence of the Formulation Factors

To assess the influence of the studied factors on the stability of flutamide in aqueous solution, a three-way ANOVA has been carried out, based on the flutamide concentration values of each solution.

Table 3

Intra- and Inter-assay Precision and Accuracy of the Chromatographic Method

Theoretical Concentration (M)	Intra-assay ($n=5$)			Inter-assay ($n=5$)		
	Mean Area	Precision[a] (%)	Accuracy[b] (%)	Mean Area	Precision[a] (%)	Accuracy[b] (%)
1.81×10^{-6}	—	—	—	—	—	—
3.62×10^{-6}	10,870.8	5.7619	2.5141	11,631.18	7.8763	−2.2904
1.81×10^{-5}	56,854.6	5.4489	4.0894	58,226.94	4.8938	2.2107
3.62×10^{-5}	123,245.6	1	0.0236	118,571.37	6.9650	7.7325
5.43×10^{-5}	169,748	3.5100	4.5628	162,831.47	10.8106	9.2525
7.24×10^{-5}	242,484.4	4.0416	−0.4222	221,646.17	19.0184	7.9351

[a]Precision = CV = (SD×100)/mean.

[b]Accuracy = percentage deviation = [(measured − theoretical)/theoretical] × 100.

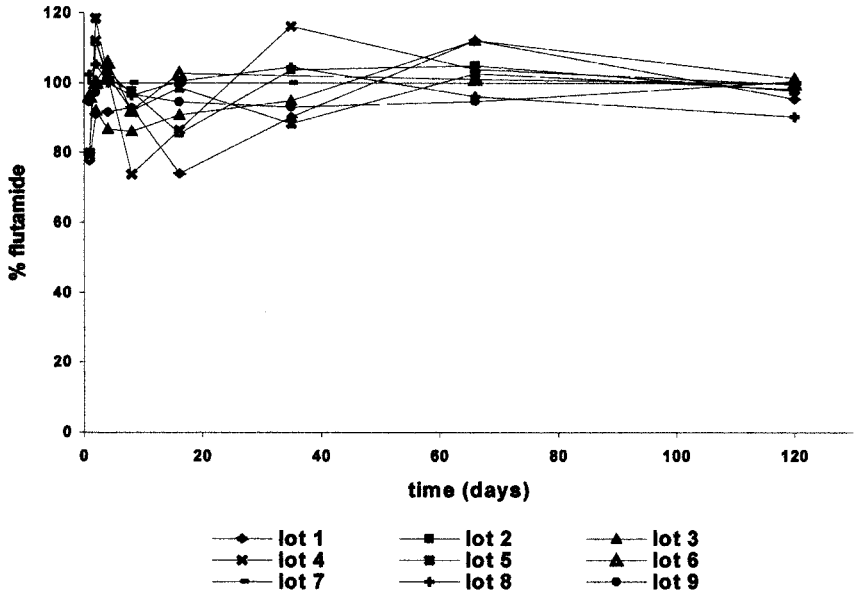

Figure 2. Degradation profiles corresponding to the stability study of flutamide in solid state.

Table 4

Multifactorial Analysis Showing the Influence of the Temperature and Humidity on the Stability of Flutamide in Solid State

Source[a]	DF	Sum of Squares	Mean of Squares	*F*	*P*
T	1	255.751	255.751	2.418	0.125
A	2	404.576	202.288	1.912	0.156
B	2	166.198	83.099	0.786	0.460
AB	4	149.045	37.261	0.352	0.842
Global	9	975.570	108.397	1.025	0.431
Residual	62	6558.928	105.789		
Total	71	7534.498	106.120		

[a]T: time; A: temperature; B: humidity.

The main effects of this ANOVA were pH (acidic, neutral), temperature (5°C, 22°C, 37°C), and sodium chloride addition (+, −), whereas the time was considered as a covariate. The main parameters of this analysis are shown in Table 5.

The results obtained from this analysis show that the temperature and pH have a marked influence on the degradation rate of flutamide in aqueous solution. These results are in agreement with the concentration profiles obtained (see Fig. 3). The degradation rate of flutamide in aqueous solution increases with the storage temperature. This influence is illustrated in Fig. 4 for solutions prepared in acidic medium without sodium chloride, stored at 5, 22, and 37°C.

With respect to the influence of pH, solutions prepared in acidic media have always shown lower values of flutamide concentration than those prepared in neutral media. As an example, the degradation profiles of solutions stored at 37°C, prepared without sodium chloride, in acidic (lot 2) and neutral (lot 8) media, are shown in Fig. 5.

On the other hand, the results obtained from the analysis of variance showed that the

Table 5

Multifactorial Analysis Showing the Influence of the Temperature, pH and Ionic Strength on the Stability of Flutamide in Solution

Source[a]	DF	Sum of Squares	Mean of Squares	*F*	*P*
T	1	8,099.623	8,099.623	68.887	0.000
A	1	7,069.351	7,069.351	60.125	0.000
B	2	11,007.172	5,503.586	46.808	0.000
C	1	517.472	517.472	4.401	0.040
AB	2	4,177.329	2,088.655	17.764	0.000
AC	1	270.078	270.078	2.297	0.134
BC	2	282.652	141.330	1.202	0.307
ABC	2	995.941	497.970	4.235	0.019
Global	12	31,856.442	2,650.704	22.578	0.000
Residual	67	7,877.700	117.578		
Total	79	39,734.142	502.964		

[a]T: time; A: pH; B: temperature; C: ionic strength.

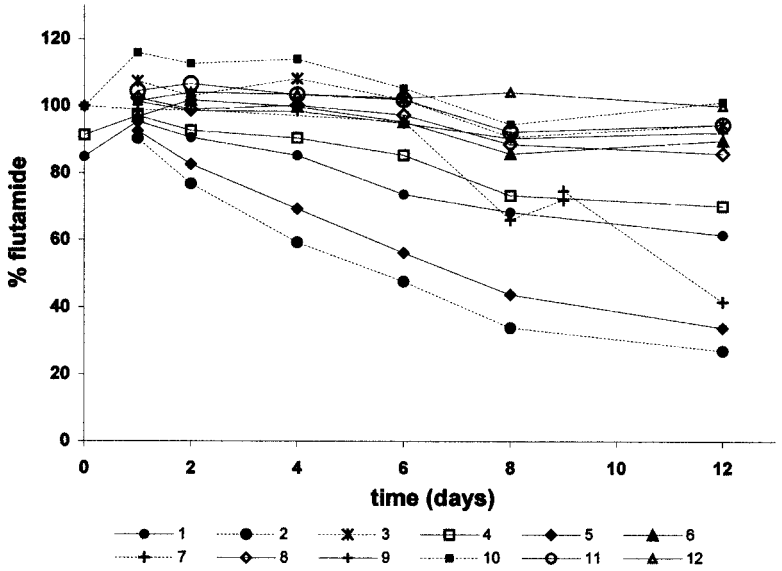

Figure 3. Degradation profiles corresponding to the stability study of flutamide in aqueous solution.

addition of sodium chloride has a lower influence than the temperature and pH on the stability of flutamide in solution. This influence acts in the sense of preventing the degradation of flutamide in aqueous solution. Figure 6 illustrates this effect for solutions prepared in acidic media and stored at 37°C.

Lastly, as can be observed in Fig. 6, lot 7 is out of trend for 5 and 37°C results, as well as those obtained for the NaCl solutions across the entire temperature range. The high decrease in the concentration found for lot 7 could be due to electronic displacement affecting the molar absorptivity of flutamide. This displacement could be due to weak chemical interactions, for example hydrogen bonds. As is well known, this kind of interaction is strongly affected by temperature and ionic strength. This hypothesis is mainly supported by the absence

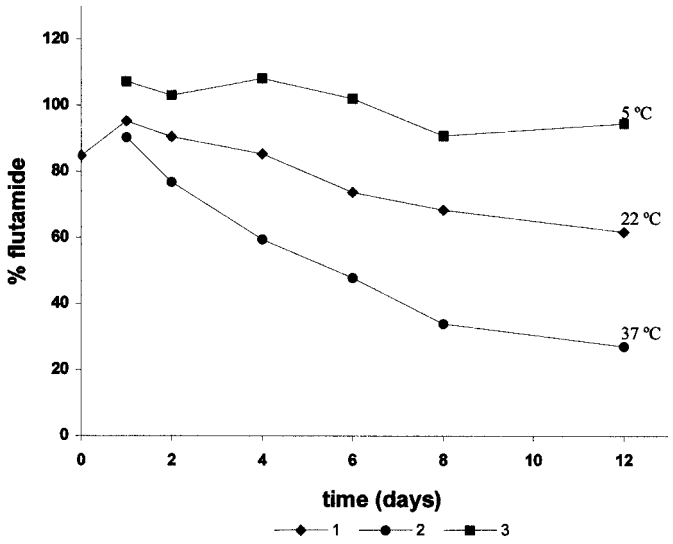

Figure 4. Degradation profiles of lots 1, 2, and 3, showing the influence of temperature on the stability of flutamide in aqueous solution.

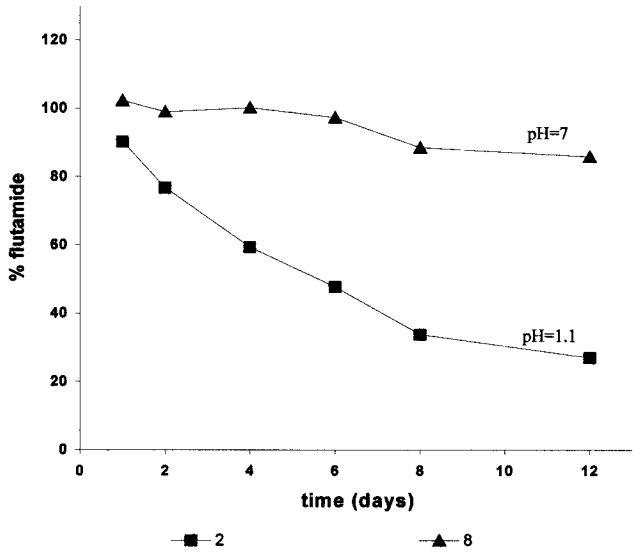

Figure 5. Degradation profiles of lots 2 and 8, showing the influence of pH on the stability of flutamide in aqueous solution.

of degradation products in the corresponding chromatograms.

Degradation Kinetics

As mentioned in the introduction, no stability study of flutamide has been found in the literature. Therefore, in addition to the formulation factors influencing the degradation rate of the drug, it is important to know the degradation kinetics followed by these reactions.

To investigate the degradation kinetics of flutamide in solution, the fit of the obtained degradation data to zero-, first-, and second-order

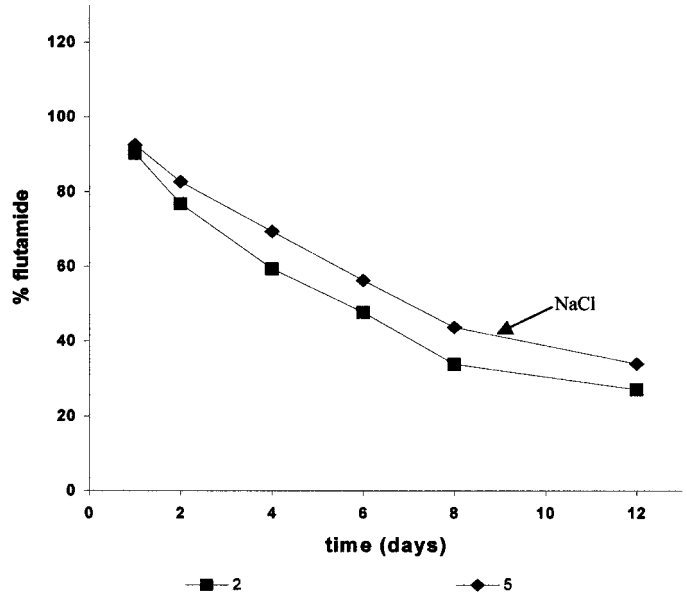

Figure 6. Degradation profiles of lots 2 and 5, showing the influence of ionic strength on the stability of flutamide in aqueous solution.

Table 6

Statistical Parameters from the Regression Analysis of the Degradation Profiles Obtained

	Kinetics Model[a]					
	Zero-Order		First-Order		Second-Order	
Lot	*r*	*F*	*r*	*F*	*r*	*F*
1	0.926	30.287	0.939	37.385	*0.948	44.874
2	0.956	42.654	0.985	139.185	*0.991	242.073
3	*0.794	6.848	0.791	6.707	0.0103	0.0004
4	0.936	35.641	0.9375	36.3368	*0.9376	36.359
5	0.977	84.112	*0.9944	357.447	0.9944	357.204
6	*0.763	5.607	0.760	5.487	0.756	5.345
7	*0.906	27.638	0.866	18.106	0.811	11.606
8	*0.93877	29.695	0.93876	29.695	0.9386	29.613
9	*0.880	13.777	0.878	13.479	0.875	13.154
10	*0.490	1.580	0.486	1.546	0.481	1.510
11	*0.863	11.756	0.859	11.342	0.855	10.921
12	0.386	0.703	0.390	0.718	*0.393	0.732

[a]Asterisks denote kinetics model showing the best fit.

kinetics was studied. The remaining concentration of flutamide (C), as well as its logarithm ($\ln C$) and inverse ($1/C$), were plotted vs. time and the fit was studied by linear regression. The obtained results are shown in Tables 6 and 7.

As can be observed, solutions with high degradation rates (lots 1, 2, and 4), corresponding to mean or high storage temperature and acidic pH, show the best fit to second-order kinetics (the regression line corresponding to lot 2 is shown in Fig. 7 as an

Table 7

Slope and Intercept of the Kinetics Model Showing the Best Fit

Lot	Slope	Intercept
1	106.766 ± 15.938 (M^{-1}/hr)	$58{,}486.95 \pm 2{,}353.53$ (M^{-1})
2	562.041 ± 36.123 (M^{-1}/hr)	$43{,}988.53 \pm 5{,}761.73$ (M^{-1})
3	$-1.01 \times 10^{-8} \pm 3.88 \times 10^{-9}$ (M/hr)	$1.96 \times 10^{-5} \pm 6.18 \times 10^{-7}$ (M)
4	76.129 ± 12.625 (M^{-1}/hr)	$56{,}592.96 \pm 1{,}864.35$ (M^{-1})
5	-0.0038 ± 0.0002 (hr^{-1})	10.924 ± 0.032 (M)
6	$-1.02 \times 10^{-8} \pm 4.33 \times 10^{-9}$ (M/hr)	$2.21 \times 10^{-5} \pm 6.91 \times 10^{-7}$ (M)
7	$-3.27 \times 10^{-8} \pm 6.22 \times 10^{-9}$(M/hr)	$1.92 \times 10^{-5} \pm 1.07 \times 10^{-6}$ (M)
8	$-1.15 \times 10^{-8} \pm 2.12 \times 10^{-9}$(M/hr)	$1.88 \times 10^{-5} \pm 3.38 \times 10^{-7}$ (M)
9	$-8.09 \times 10^{-9} \pm 2.18 \times 10^{-9}$(M/hr)	$2.20 \times 10^{-5} \pm 3.48 \times 10^{-7}$ (M)
10	$-7.03 \times 10^{-9} \pm 5.59 \times 10^{-9}$(M/hr)	$2.00 \times 10^{-5} \pm 8.25 \times 10^{-7}$ (M)
11	$-9.13 \times 10^{-9} \pm 2.66 \times 10^{-9}$(M/hr)	$1.94 \times 10^{-5} \pm 4.25 \times 10^{-7}$ (M)
12	3.249 ± 3.796 (M^{-1}/hr)	$53{,}383.18 \pm 605.61$ (M^{-1})

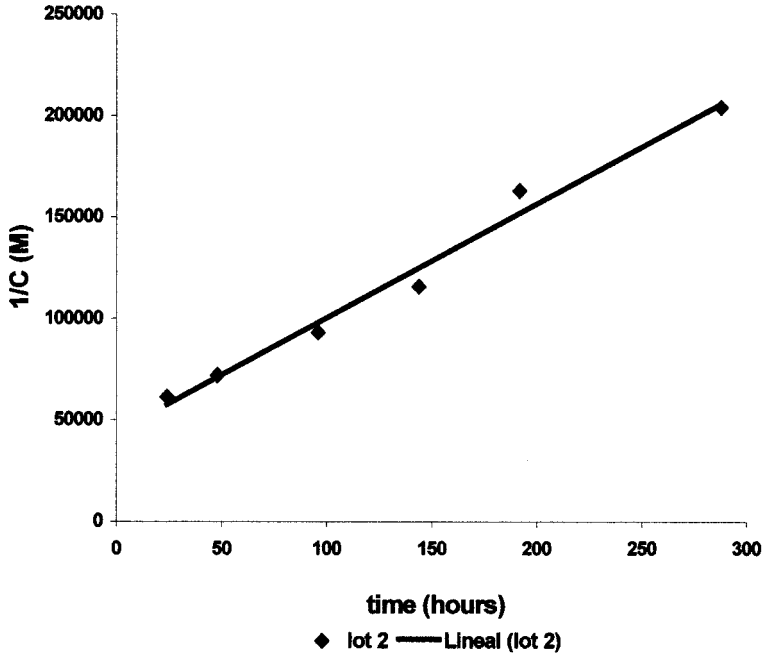

Figure 7. Regression line corresponding to the second-order kinetic model.

example). Only lot 5 fits slightly better to first-order kinetics.

Solutions exhibiting lower degradation rates show a better fit to the zero-order model. This fact could be attributed to the masking effect due to the low decrease in the concentration. The difficulty of identifying the kinetic model should be taken into account when the amount of drug degraded is very little.

In the light of the obtained results, flutamide did not show stability problems in solid state, even in adverse temperature and humidity conditions. On the other hand, the important degradation rate observed for flutamide solutions in physiological conditions suggests that a possible decrease in bioavailability due to stability problems must be investigated, especially for controlled-delivery systems.

REFERENCES

1. Goldspiel, B.R.; Kholer, D.R. Flutamide: An Antiandrogen for Advanced Prostate Cancer. Drug Intelligence and Clinical Pharmacy, **1990**, *24*, 616–623.
2. Schulz, M.; Schmoldt, A.; Donn, F.; Becker, H. The Pharmacokinetics of Flutamide and Its Major Metabolites After a Single Oral Dose and During Chronic Treatment. Eur. J. Clin. Pharmacol. **1988**, *34*, 633–636.
3. Brogden, R.N.; Clissod, S.P. Flutamide. A Preliminary Review of Its Pharmacodynamic and Pharmacokinetic Properties, and Therapeutic Efficacy in Advanced Prostatic Cancer. Drugs **1989**, *38*, 185–203.
4. Aso, Y.; Akaza, H.; Koiso, K.; Kumamoto, Y.; Kawai, T.; Origasa, S.; Hosaka, M.; Yamanaka, H.; Shimazaki, J.; Fuse, H. Phase I Study of Flutamide, a Nonsteroidal Antiandrogen, in Patients with Prostatic Cancer. Hinyokika Kiyo **1993**, *39*, 381–389.
5. Brogden, R.N.; Chrisp, P. Flutamide. A Review of Its Pharmacodynamic and Pharmacokinetic Properties, and Therapeutic Use in Advanced Prostatic Cancer. Drugs Aging **1991**, *1*, 104–115.
6. Chabner, B.A.; Allegra, C.J.; Curt, C.A.; Calabresi, P. Fármacos Antineoplásicos. In *Las Bases Farmacológicas de la Terapéutica*, 9th Ed.; Goodman, A., Hardman, J.G., Limbird, L.E., Molinoff, P.B., Ruddon, R.W., Eds.; McGraw-Hill Interamericana: Mexico, 1996; 1309–1367.
7. Caraballo, I.; Millán, M. Percolation Thresholds in Ultrasound Compacted Tablets. J. Contr. Rel. **2000**, *69*, 345–355.
8. Caraballo, I.; Fernández-Arévalo, M.; Holgado, M.A.; Vela, M.T.; Rabasco, A.M. A Rapid HPLC Method for the Quantification of Tyrotrhicin, Menthol and Benzocaine in Pharmaceutical Formulations. J. Pharm. Sci. **1994**, *83*, 1147–1149.
9. U.S. Pharmacopeia, Vol. XXIII, U.S. Pharmacopeial Convention, Rockville, MD, 1995; 2053.