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Steady-state hydroxyflutamide plasma levels after the administration of two dosage forms of flutamide

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Summary. A bioavailability study of randomized crossover design was carried out in eight volunteers who were given a 48-h flutamide treatment consisting of 250-mg tablets three times daily or 400-mg sustained-release tablets twice daily, followed 3 weeks later by the alternative dosage form. Just before the last dose and 15 times during the subsequent 24 h, blood samples were obtained for the determination of plasma hydroxyflutamide (the active metabolite of flutamide) levels by high-performance liquid chromatography. No statistically significant differences between the two dosage forms were found for the lag time, rate of initial increase in concentration, peak plasma concentration, mean hydroxyflutamide concentration within one dosing interval or 24-h AUC value. One subject presented mild and transient nausea during both treatment periods. After the first treatment period (250-mg tablets), an increase in serum bilirubin was observed in another volunteer, who was withdrawn from the study. It may be concluded that both dosage forms were bioequivalent.

Introduction

Flutamide is used in several countries in the treatment of metastatic prostate cancer [4]. It is considered to be a prodrug that is rapidly metabolized to hydroxyflutamide which is both its active form and its major metabolite [6].

The currently recommended dose of flutamide is 250 mg p.o. given three times daily; higher doses do not appear to produce a better therapeutic response and may be associated with a higher incidence and degree of gynecomastia [1]. Flutamide is generally well tolerated, but some side effects have been reported, including nausea, diarrhea, gynecomastia, cholestatic hepatitis and hypersensitivity [9].

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A sustained-release dosage form may enhance patients' compliance by lowering the number of tablets to be taken daily and may result in a reduction in the incidence of local side effects such as nausea and diarrhea, but it must be bioequivalent to the conventional dosage form. Therefore, the aim of this randomized crossover study was to compare the steady-state plasma levels of the active metabolite hydroxyflutamide in healthy volunteers after a 48-h flutamide treatment consisting of 250-mg tablets given three times daily or 400-mg sustained-release tablets given twice daily, as a prior step to the evaluation of the efficacy and safety of the new dosage form as well as patients' compliance.

Subjects and methods

In vitro tablet-dissolution test. A dissolution test in simulated intestinal fluid containing 1% Tween-80 was performed as described in the 21st edition of the United States Pharmacopoeia [13]. Sampling was carried out at 0.5 and 1 h for both dosage forms and thereafter at every hour up to 8 h for the 400-mg sustained-release tablets; the results were expressed as undissolved drug in milligrams per tablet. Each experiment was repeated nine times for each dosage form. Nine tablets of each form were assayed for flutamide content and the results were taken as zero-time values.

Volunteers' characteristics. The health status of the eight volunteers, whose age, weight and height are shown in Table 1, was assessed by a clinical interview, a thorax roentgenogram, a standard electrocardiogram and the following laboratory tests: RBC, hemoglobin, hematocrit, WBC, erythrosedimentation rate, blood urea nitrogen (BUN), fasting serum glucose, serum cholesterol, serum creatinine, serum albumin and total protein, serum bilirubin, serum aspartate aminotransferase, serum alanine aminotransferase, serum alkaline phosphatase, plasma prothrombin and urinalysis.

Drug treatment and experimental design. If there was no evidence of disease, written consent was obtained from each volunteer. Subjects were then randomly assigned to a crossover 2×2 block design. First they received a 48-h flutamide treatment consisting of either 250-mg tablets given three times daily or 400-mg sustained-release tablets given twice daily. Both dosage forms were prepared using the same batch of flu-

Table 1. Volunteers' characteristics

First treatment	Volunteer	Age (years)	Weight (kg)	Height (cm)	Body surface (m^2)
250 mg/8 h (standard tablets)	E. S.	29	102	187	2.2
	A. E. B.	40	66	168	1.75
	L. P.	22	89	180	2.09
	J. C. C.	45	102	160	2.03
400 mg/12 h (sustained release)	G. S.	27	95	180	2.15
	R. C.	23	68	174	1.82
	V. G.	21	80	175	1.96
	J. E. R.	39	62	174	1.75

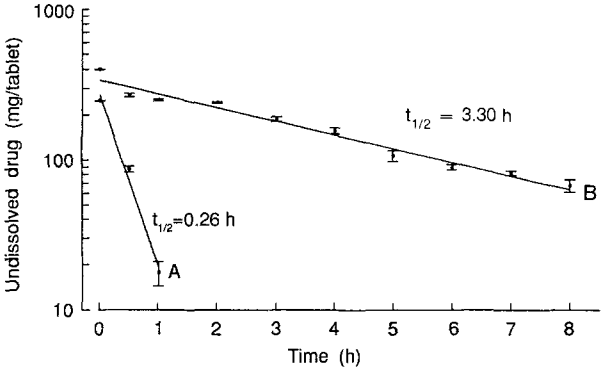

Fig. 1. In vitro dissolution kinetics of two dosage forms of flutamide. Bars represent the mean ±1 SD of 9 independent determinations. *A*, 250-mg tablets; *B*, 400-mg sustained-release tablets

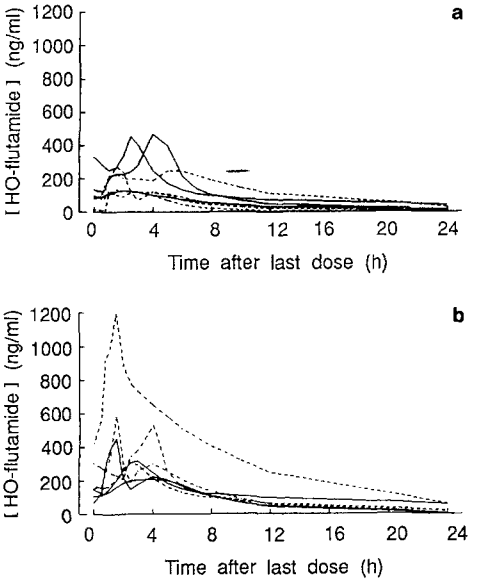

Fig. 2 a, b. Individual hydroxyflutamide plasma profiles in subjects receiving **a** 250-mg flutamide tablets and **b** 400-mg sustained-release tablets. ———, volunteers who initially received 250-mg tablets; – – – –, volunteers who initially received 400-mg sustained-release tablets. *0 h* represents the blood sample obtained immediately before intake of the last tablet

tamide. After a 3-week washout period, the alternative treatment was given and the same procedure was applied. Between both treatment periods and after the last treatment, the clinical interview and all laboratory tests were repeated for the detection of adverse drug reactions.

Blood sampling. Just before the last dose and at 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 10, 12 and 24 h after it, 10-ml venous blood samples were drawn using heparinized disposable syringes and 21-gauge needles. Each sample was centrifuged within 15 min of sampling and plasma was separated and stored at –20°C until the time of hydroxyflutamide assay.

Hydroxyflutamide assay. The hydroxyflutamide assay was performed by reverse-phase high-performance liquid chromatography (HPLC). A 2-ml plasma sample and 3 ml dichloromethane were mixed, shaken by a vortex for 1 min and then centrifuged at 3,500 rpm for 5 min. The organic phase was separated with a syringe, transferred to a conic tube, evaporated to dryness, and redissolved in 100 µl methanol. A 20-µl sample of this solution was injected into the HPLC system.

HPLC was carried out under the following conditions: column, 150×4.6 mm (Chrompack); stationary phase, Chromsphere C18; mobile phase, methanol: water 60:40 (v/v); flow rate, 1 ml/min; and retention time, 4.2 min. Absorbance was measured at 365 nm by a Waters UV detector. The detection limit was 5 ng/ml plasma, and intra- and interassay coefficients of variation were 7.2% and 10.1%, respectively. Peak heights were used for quantification against an external standard of hydroxyflutamide in methanol (10 ng/µl). Calibration curves were constructed by adding 1, 2, 5, 10, 25, 35, 50 and 100 µl standard solution to the HPLC system.

Pharmacokinetic and statistical analysis. Non-compartmental pharmacokinetic analysis was carried out using KINPAK software [3]. AUCs for one dosing interval were estimated by the software. Because the two dosage forms were given over different dosing intervals, comparisons were made using the 24-hour AUC value (i.e., 3 times the AUC of standard 250-mg tablets vs 2 times the AUC of 400-mg sustained-release tablets).

The pharmacokinetic parameters of both dosage forms were compared using Grizzle's analysis of variance for 2×2 crossover block design [5]. Plasma concentrations obtained before and after the dosing period for each dosage form were compared using Student's paired *t*-test. A type I error of 0.05 was chosen for assessment of statistical significance.

Results

In vitro dissolution studies

Both dosage forms released flutamide to the simulated intestinal fluid [13] according to first-order kinetics. Drug-release half-time was 0.26 h for the standard 250-mg tablets and 3.30 h for the 400-mg sustained-release tablets (Fig. 1).

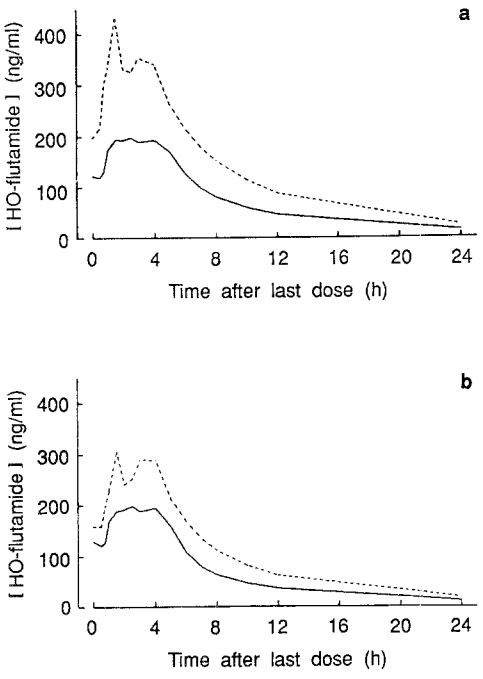

Fig. 3 a, b. Mean hydroxyflutamide plasma profiles **a** in all subjects ($n = 7$) and **b** in all volunteers except subject V. G., who was excluded due to his outlier plasma profile following treatment with sustained-release flutamide tablets (Fig. 2). ———, 250-mg tablets; – – – –, 400-mg sustained-release tablets

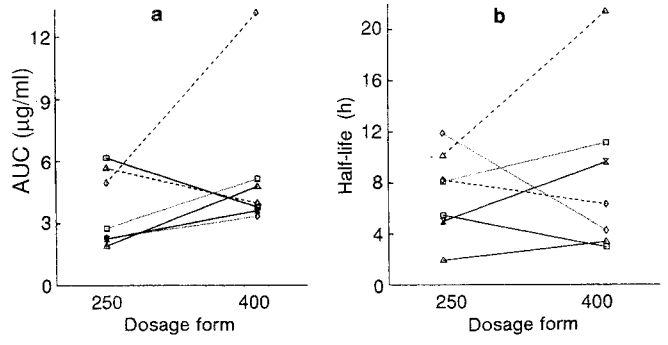

Fig. 4 a, b. Hydroxyflutamide pharmacokinetic parameters. **a** 24-h AUC. **b** Terminal half-life. Each line represents one volunteer. *250*, Standard tablets containing 250 mg flutamide; *400*, sustained-release tablets containing 400 mg flutamide

Pharmacokinetic studies

Individual pharmacokinetic profiles of the seven subjects who completed both treatment periods are shown in Fig. 2. One volunteer (V.G.) showed an outlier profile during treatment with the 400-mg sustained-release tablets.

Profiles of the mean hydroxyflutamide plasma concentrations showed a higher AUC value for the 400-mg tablets (Fig. 3 a). When the data of volunteer V. G. were excluded, the AUC for the 400-mg treatment remained greater than that obtained for 250-mg tablets, but the difference became smaller (Fig. 3 b). The difference in the 24-h AUC obtained for the two dosage forms was not significant (Fig. 4 a).

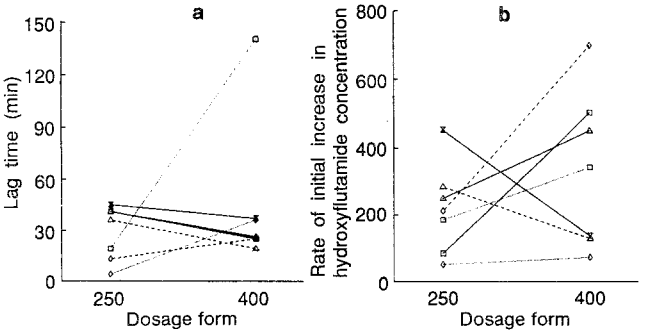

Fig. 5. a Lag time and **b** rate of initial increase in hydroxyflutamide concentration ($ngml^{-1}h^{-1}$) in subjects treated with two dosage forms of flutamide. Each line represents one volunteer. *250*, Standard tablets containing 250 mg flutamide; *400*, sustained-release tablets containing 400 mg flutamide

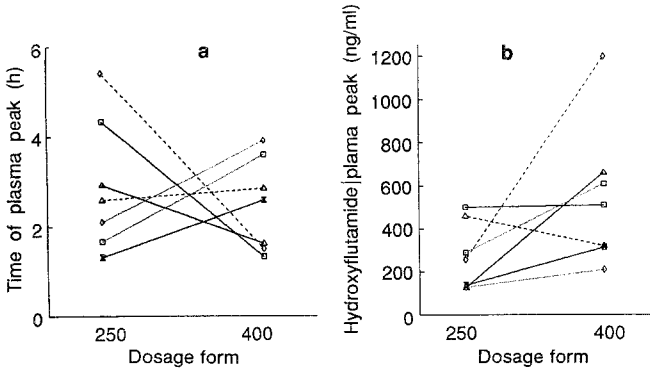

Fig. 6. a Time elapsed between intake of the last tablet and the achievement of peak plasma hydroxyflutamide concentrations and **b** peak plasma hydroxyflutamide concentrations obtained in subjects treated with two dosage forms of flutamide. Each line represents one volunteer. *250*, Standard tablets containing 250 mg flutamide; *400*, sustained-release tablets containing 400 mg flutamide

The lag time was 4–45 min except in the case of volunteer R.C., whose lag time after treatment with the sustained-release tablets was 140 min (Fig. 5 a). The rates of the initial increase in hydroxyflutamide concentration ranged between 50.5 and 698 ng ml^{-1} h^{-1} (Fig. 5 b). Of the 14 terminal half-lives obtained, 13 varied between 1.9 and 11.8 h for both dosage forms, but that of volunteer J.C.C. was 21.3 h after treatment with the 400-mg tablets (Fig. 4 b).

Hydroxyflutamide peak plasma concentrations of 126–608 ng/ml were observed at 1.3–5.4 h after dose intake in 13/14 curves. Volunteer V.G. showed a peak of 1,193 ng/ml at 1.5 h (Fig. 6). Mean hydroxyflutamide plasma levels within one dosing interval ranged between 94.3 and 257 ng/ml, except in the case of volunteer V.G., whose value was 546 ng/ml after treatment with the sustained-release tablets (Fig. 7 a). Minimal plasma levels (concentrations at the end of the dosing interval plus lag time) were 17.1–94.3 ng/ml for both dosage forms in six subjects. Volunteer V.G. showed concentrations of 188 and 240 ng/ml after treatment with 250- and 400-mg tablets, respectively (Fig. 7 b).

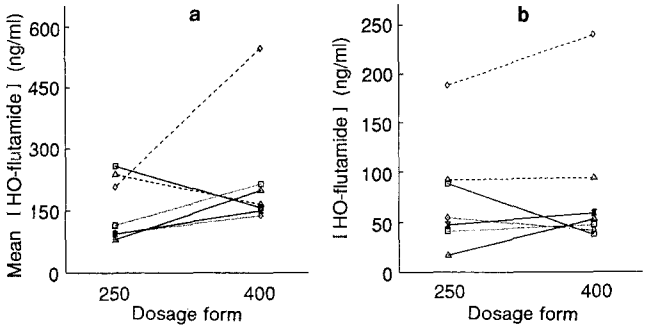

Fig. 7. a Mean hydroxyflutamide plasma concentrations within one dosing interval and **b** concentrations estimated for the end of one dosing interval plus lag time in subjects treated with two dosage forms of flutamide. Each line represents one volunteer. *250*, Standard tablets containing 250 mg flutamide; *400*, sustained-release tablets containing 400 mg flutamide

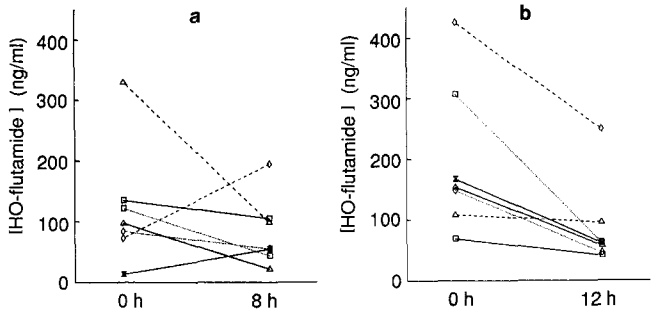

Fig. 8 a, b. Hydroxyflutamide plasma levels measured at the beginning and end of the dosing interval in subjects treated with **a** 250-mg flutamide tablets and **b** 400-mg sustained-release tablets. Each line represents one volunteer. *0 h* represents the blood sample obtained immediately before intake of the last tablet. *8 h* represents the sample taken after one dosing interval for 250-mg tablets, and *12 h* represents that obtained after one dosing interval for 400-mg sustained-release tablets. $P < 0.05$ for the difference between values obtained at 12 h vs 0 h

For all of the above parameters, the differences between treatment sequences, treatment periods and dosage forms were not statistically significant.

Plasma levels just prior to the ingestion of the last tablet were similar for both treatment periods (Fig. 8). No statistically significant differences were found between hydroxyflutamide plasma concentrations at the beginning vs the end of the dosing interval for 250-mg tablets (Fig. 8a), but a statistically significantly lower concentration was observed at the end of the dosing interval for sustained-release tablets ($P < 0.05$; Fig. 8b).

Adverse drug reactions

Volunteer J.C.C. presented mild and transient nausea during both treatment periods, which disappeared after treatment was completed. After the first treatment period (standard 250-mg tablets), volunteer E.S. showed an increase in conjugated bilirubin to 0.52 mg/dl (normal, up to 0.2 mg/dl) and an increase in total bilirubin to 1.89 mg/dl (normal, up to 1 mg/dl). Although both values spontaneously returned to pretreatment values within 2 weeks and other parameters of liver function remained within normal limits, this volunteer was withdrawn from the study for ethical reasons. Neither clinical nor laboratory evidence of adverse drug reactions were observed in the other six volunteers.

Discussion

After the administration of a single dose of 200 mg flutamide, Katchen and Buxbaum [6] identified 11 plasma metabolites, of which hydroxyflutamide was the main one. Hydroxyflutamide represented about one-third of the total extractable radioactivity, whereas the parent drug accounted for <4% of the total radioactivity at 1, 2, 4, 6 and 8 h after flutamide intake. Accordingly, hydroxyflutamide plasma levels were >10 times higher than flutamide levels at any time in 12 geriatric volunteers treated with 250 mg flutamide and evaluated on treatment days 1, 6 and 9 [10]. Furthermore, Schulz et al. [12] failed to detect unmetabolized flutamide at 8 h after a 250-mg dose, and hydroxyflutamide tissue concentrations were up to 70 times higher than flutamide tissue levels in rats at 6 h after dosing [8]. Both flutamide and hydroxyflutamide have antiandrogenic activity, with one or the other being more potent according to the assay system used [4]. Taken together, these data indicate that hydroxyflutamide is the active metabolite through which flutamide produces its effects in vivo. Therefore, we focused the present study on hydroxyflutamide levels, as has also been done by others [2].

Our data showed a rather large interindividual variation; this was also observed in the kinetics studies discussed above. Radwanski et al. [10] reported coefficients of variation ranging between 34% and 182% for hydroxyflutamide levels in 12 volunteers.

The pharmacokinetic profiles obtained for hydroxyflutamide after the administration of 400-mg sustained-release tablets were what would have been expected following a higher dose rather than those normally expected after treatment with a sustained-release tablet (Figs. 2 and 3), and differences between the times at which peak plasma levels were obtained did not reach statistical significance (Fig. 6a). However, the half-time of in vitro drug-release from the 400-mg sustained-release tablets was >12 times that of the standard 250-mg tablets (Fig. 1). The multiple metabolic pathways of flutamide and its metabolites [6] and differences between the drug's in vitro and in vivo dissolution kinetics might explain the plasma profiles observed.

We applied a non-compartmental pharmacokinetic analysis to avoid the artifactual assumptions involved in model kinetics. The KINPAK software was chosen because it fits individual curves without user intervention and because it is flexible enough to be applied even to multiple peak curves [3]. Grizzle's analysis of variance [5] enables the separation of sequences, periods and treatment effects. The finding that no statistically significant differences occurred between sequences or between periods indicates that the sequence of treatments was not a determinant of the results observed.

AUCs within dosing intervals were clearly higher after treatment with the 400-mg tablets, and the levels measured at 8 h after administration of the 250-mg tablets and at 12 h following the sustained-release tablets were similar (Fig. 3), as were minimal plasma levels (concentrations at the end of the dosing intervals plus lag time; Fig. 7b). Because the two dosage forms were given over different dosing intervals, the daily AUCs (i.e., 3 times the AUC of standard 250-mg tablets and 2 times that of 400-mg sustained-release tablets) were compared, but no statistically significant difference was found (Fig. 4a). The terminal half-lives of hydroxyflutamide found for our volunteers were similar to the beta half-lives reported previously [10].

A lag time was observed after the administration of both dosage forms. Flutamide is a basic drug; therefore, no absorption through gastric mucosa is to be expected. On the other hand, we did not study the levels of parent drug but those of its main metabolite. Thus, gastric emptying time and the time required for statistically significant metabolite production, as well as the time required for dissolution of the drug in intestinal medium, may explain the lag time.

Of the 14 half-lives obtained, 13 were <12 h and 10 of these were <8.3 h. Thus, in all but one case the sampling period began at ≥ 4 half-lives after the beginning of treatment. Consequently, it can reasonably be assumed that sampling was performed at steady state. This is further supported by the accumulation curves reported by Schulz et al. [12]. Levels determined immediately before the intake of the last tablet in the present study (0 h in Fig. 8) were similar for both dosage forms. After one dosing interval, levels were similar to the 0-h values following treatment with the standard tablet, but were lower than the 0-h values after treatment with sustained-release tablets ($P < 0.05$; Fig. 8). It is difficult to explain these lower levels. If steady state were not achieved, one would expect higher rather than lower levels. For other drugs, circadian variations in bioavailability and metabolism have been reported [7, 11], and it is reasonable to assume that a longer availability for absorption of the parent drug (Fig. 1) may result in a longer exposure of the liver to drug, which is more suitable for unmasking circadian variations.

The linearity of flutamide kinetics can be questioned, but this was not the aim of the present study and could not be clarified by a review of the literature. Schulz et al. [12] studied doses of 250 (two patients) vs 500 mg (three patients), but they used different patients for each dose; thus, no valid conclusion can be drawn about the dose dependence of flutamide pharmacokinetics.

The adverse drug reactions seen in two of our subjects have previously been reported [9]. Nausea occurred in one subject after treatment with both dosage forms. Hyperbilirubinemia was observed after the lower-dose treatment, and the subject was withdrawn from the study. Due to the small number of subjects studied, no general conclusion could be drawn as to the safety of the new dosage form. Considering that the 24-h AUCs were similar after treatment with both dosage forms and that no statistically significant differences were found in any of the pharmacokinetic parameters studied, it may be concluded that the sustained-release tablets and the standard-dosage tablets were bioequivalent.

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References

1. Airhart RA, Barnett TF, Sullivan JW, Levine RL, Schlegel JU (1978) Flutamide therapy for carcinoma of the prostate. South Med J 71: 798
2. Bélanger A, Giasson M, Couture J, Dupont A, Cusan L, Labrie F (1988) Plasma levels of hydroxy-flutamide in patients with prostatic cancer receiving the combined hormonal therapy: an LHRH agonist and flutamide. Prostate 12: 79
3. Betzien F, Kaufmann B, Schneider B, Ritschel WA (1985) KINPAK – a new program package for standardized evaluation of kinetic parameters. Arzneimittelforschung 35 (I) and 35 (II): 14
4. Brogden RN, Clissold SP (1989) Hormonal manipulation in the treatment of metastatic prostatic cancer. Drugs 38: 185
5. Grizzle JE (1965) The two-period change-over design and its use in clinical trials. Biometrics 21: 467
6. Katchen B, Buxbaum S (1975) Disposition of a new, nonsteroid, antiandrogen, α,α,α-trifluoro-2-methyl-4′-nitro-*m*-propiono-toluidide (flutamide), in men following a single oral 200 mg dose. J Clin Endocrinol Metab 41: 373
7. Lucena MI, Gil-Extremera B, Mira F, Valiente R, Sánchez de la Cuesta F (1984) Serum theophylline concentrations during twice daily administration of a slow release preparation in chronic airflow obstruction. Abstract 1305P, IUPHAR 9th International Congress on Pharmacology, London 30 July–3 August
8. Neri R (1989) Pharmacology and pharmacokinetics of flutamide. Urology 34 [Suppl]: 19
9. Neri R, Kassem N (1984) Biological and clinical properties of antiandrogens. In: Bresciani F, King RJB, Lippmann E, Namer N, Baynaud P (eds) Progress in cancer research and therapy, vol 31. Raven Press, New York, p 507
10. Radwanski E, Perentesis G, Symchowicz S, Zampaglione N (1989) Single and multiple dose pharmacokinetic evaluation of flutamide in normal geriatric volunteers. J Clin Pharmacol 29: 554
11. Scheving LE (1980) Chronobiology – a new perspective in pharmacology and chemotherapy. Trends Pharmacol Sci 1: 303
12. Schulz M, Schmoldt A, Donn F, Becker H (1988) The pharmacokinetics of flutamide and its major metabolites after a single oral dose and during chronic treatment. Eur J Clin Pharmacol 34: 633
13. USP XXI (1985) Physical tests. Dissolution. United States Pharmacopeial Convention Inc., Rockville Md. p 1243