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COMPARATIVE BIOAVAILABILITY STUDIES OF FOUR COMMERCIAL NITROFURANTOIN PRODUCTS

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Summary

The relative bioavailability of nitrofurantoin in four commercial products marketed in Greece was examined in 4 subjects using a Latin square design. The urinary excretion method was used and the data obtained were analysed according to a one-compartment open model. Analysis of variance was performed on all parameters calculated. Statistically significant differences were found between the products examined. Dissolution tests were performed and *in vitro - in vivo* correlations were developed. These findings prove that the tested products can not be utilized interchangeably.

Key words : Bioequivalence, in vitro - in vivo correlation, analysis of variance.

Introduction

It is well known that the compliance of commercially available products with the compendial requirements does not necessarily imply their bioequivalence [1-5]. This problem is most acute in Greece where a list similar to that of Food and Drug Administration [6] for the drugs requiring bioavailability testing has not been issued. Consequently, bioequivalence regulations are not applied and relevant studies have not been reported. It was therefore of primary importance to perform a bioequivalence study for products marketed in Greece to provide the indispensable information for appropriate prescription.

Nitrofurantoin was chosen as a model drug since it exhibits bioavailability problems [7,8] and a total of 46 dosage forms (27 companies) are commercially available on the Greek market. Four formulations (3 tablets, 1 capsule) were examined and dissolution tests were undertaken to establish any correlation of *in vitro - in vivo* data.

Materials and Methods

Nitrofurantoin products were obtained directly from the manufacturers prior to

December, 1981. Specifications of the formulations are given in Table I. Analysis of 6 tablets or capsules of each brand detected no significant difference in nitrofurantoin content.

Dissolution test

The rotating basket method was used for the dissolution studies. One tablet or capsule was placed into a 250 mesh screen basket and the whole was immersed in 1 lit. beaker containing 400 ml of the dissolution medium (HCl 0.1 N or phosphate buffer pH 7.2). The basket was rotated at 60 rpm at 3 cm distance from the bottom of the beaker. The whole assemply was maintained at 37° in a constant temperature bath. Samples of 5 ml were collected at 5,10,20,30,40,50,60, and 70 min. Five milliliters of dissolution medium maintained at 37° was added to the beaker after each sample was removed. Each 5 ml sample was filtered (millipore 0.45 μm) and 1.0 ml aliquot was diluted to 10.0 ml with purified water. Absorbance was read at 380 nm against a blank of the corresponding diluted dissolution medium.

Bioavailability study

Two male and two female volunteers took part in the study. Their ages ranged from 28 to 35 years with a weight range 49-70 kg. Each subject gave a written informed consent. The volunteers were instructed to refrain from taking any other drug a week before and during the trials. Each subject ingested a 100 mg tablet or capsule of a nitrofurantoin product every 72 hr in accordance with the assigned schedule (Table I).

TABLE I : Latin square design for the evaluation of nitrofurantoin bioavailability.

	Day			
Subject	1	4	7	10
1	A	B	C	D
2	D	C	A	B
3	C	D	B	A
4	B	A	D	C

Key: A = Funit, tablets lot 81010, Ladrug Laboratories, Athens. B = Furonitran tablets, lot 14, Defarm Laboratories, Athens. C = Macrodantin capsules (nitrofurantoin in macrocrystals), lot 105. D = Furadantin tablets lot 103. Brands C and D are prepared in Greece by N. Petsiavas A.E. under the supervision of Morton Norwich Products Inc.

Because the biovailability of nitrofurantoin is greater when taken with food[9] but many patients have a tendency not to take their medications as prescribed by the physician, the dose was administered in the morning 1 h after a light breakfast

of 150 ml milk and 50 g of bread. Since the urinary excretion method has been suggested[10] as the preferred method for the determination of nitrofurantoin bioavailability, urine samples were collected at 0,1,2,3,4,6,8,12,24 h, ensuring each time complete emptying of the bladder. In order to stimutate urine output, 150 ml of water were ingested after each urine collection. All samples were frozen to ensure stability of drug and convenience of analysis. The samples were allowed to thaw immediately before use. The ammounts of drug in urine were determined according to the method of Conklin and Hollifield[11] as modified by Mendes et al[12].

Results and Discussion

Dissolution studies. The results of the dissolution tests are presented in Fig 1 and 2. For the plotting a correction factor[13] was taken into account. At both pH used the products A and B showed remarkably higher dissolution profiles to those obtained from C and D. The rapid dissolution of the products A and B was in accordance with their fast disintegration[14] (< 1 min) in 0.1 N HCl in comparison to the 7.5 min. found for D. As far as the product C is concerned the low dissolution rate was expected since the drug is in macrocrystalline form. The change of the

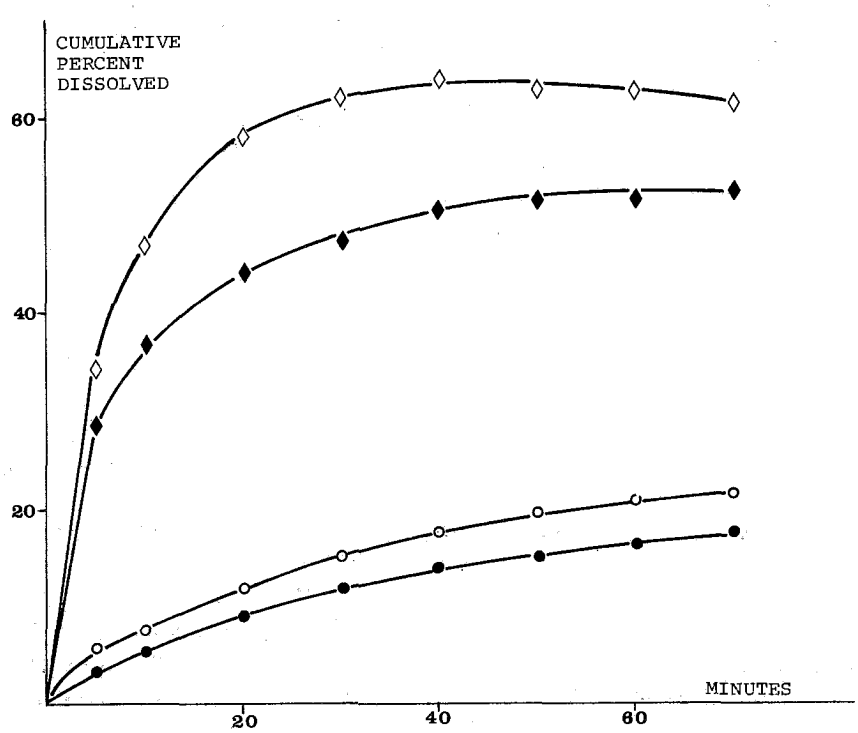

FIG. 1 : *Dissolution profiles for nitrofurantoin at pH 1.0. Each data point is the mean of three determinations.* Key : ◊ *brand A,* ◆ *brand B,* ○ *brand C,* ● *brand D.*

dissolution profile for brands C and D depicted in Fig 1 and 2 is probably attributed to formulation factors.

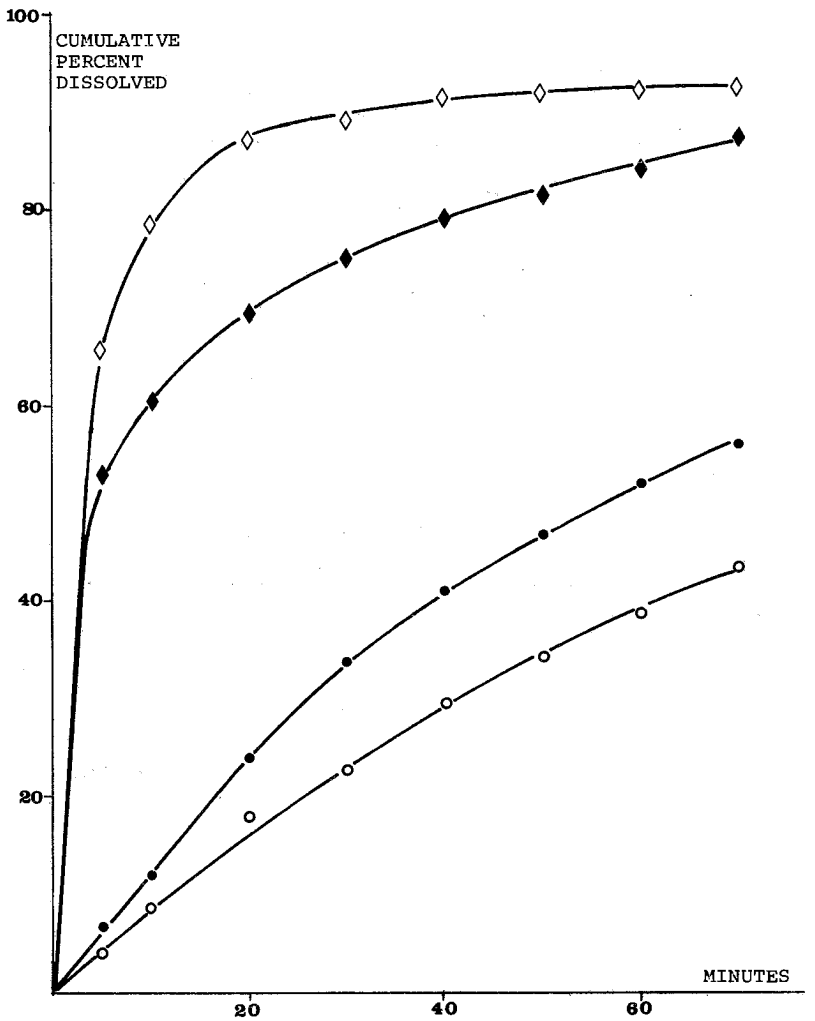

FIG. 2 : *Dissolution profiles for nitrofurantoin at pH 7.2. Each data point is the mean of three determinations.* Key : *see Fig. 1.*

Bioequivalency. Since no appreciable amount of drug was found in the 24 h specimen for all treatments and subjects the total amount excreted in urine over 12 h was proved sufficient to describe the extent of nitrofurantoin bioavailability. Figure 3 presents the cumulative amount of nitrofurantoin recovered as unchanged drug in urine versus time. It can be seen that the product C has a lower biovailability than the other formulations. In parallel, Fig 4 shows the mean

excretion rate profile of the formulations. Product C is the only exhibiting a sort of sustained release action.

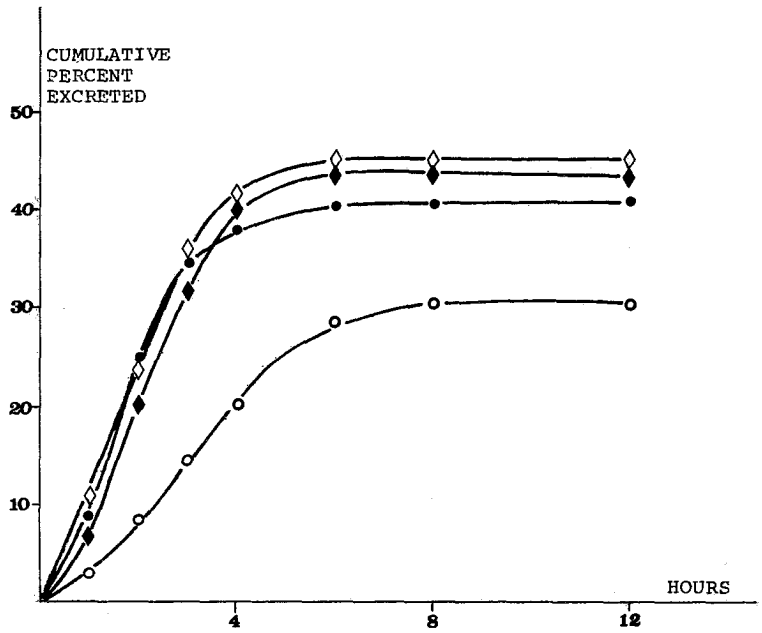

FIG. 3 : *Mean cumulative percent of nitrofurantoin excreted versus time. Each data point is the mean cumulative percent excreted for all four subjects.* Key : *see Fig. 1.*

The experimental results obtained were further analyzed according to a one-compartment open model using the equation (2) which is valid instead of the known relationship [15] (1) for the special case $k = k_a$ (see Appendix),

$$X_t = F\left[1 - \frac{1}{k_a - k}\left(k_a e^{-kt} - k e^{-k_a t}\right)\right] \quad (1)$$

$$X_t = F\left[1 - (1 + Kt)\, e^{-Kt}\right] \quad (2)$$

where X_t is the percent of drug excreted in time t, F is the percentage of total drug excreted, k_a is the absorption rate constant, k is the elimination rate constant, and K is the common value of the two rate constants e.g. $k = k_a = K$. The use of the simpler equation (2) was made for two reasons. Firstly, nitrofurantoin exhibits rapid absorption and excretion while the elimination half life is essentially independent of the dosage form [10]. In addition, the results quoted by Mendes et al [12] and the excretion rate profile given by Mattok et al [7] indicate the validity of approximation $k = k_a$. Secondly, equation (1) involves three parameters F, k, k_a not identifiable using the method of non-linear least squares. Admitedly each

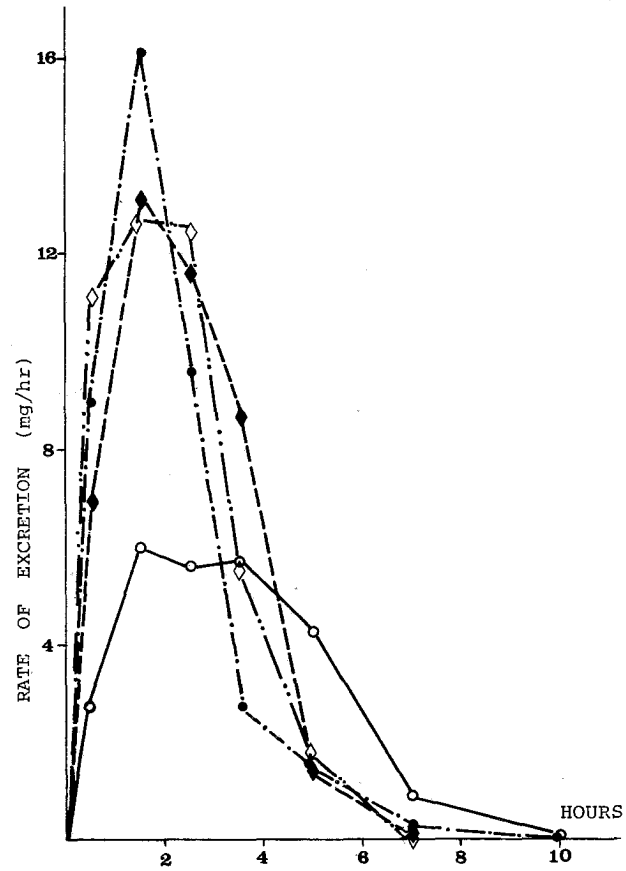

FIG. 4 : *Mean excretion profiles of nitrofurantoin products.* Key : *see Fig. 1.*

parameter can be separately calculated from the experimental data, namely, F from the cumulative amount excreted (Fig. 3) while k or k_a by standard graphing procedures. However, each calculation is accomplished with an accuracy involving a standard error s_F, s_k, s_{k_a} for each parameter. If one of the three parameters, say F, is chosen and imposed on the model, it follows that s_F will be transfered to the other two parameters. Therefore, to avoid biased estimation of the parameters the approximation quoted was used as a better simulation of the *in vivo* process.

The experimental data were subjected to computer non-linear least square method of analysis using the program NONLIN[16] to obtain the best estimates for the model described by equation (2). The values obtained for F and K as well as for the peak excretion time t_{max} (eq.3) and the peak excretion rate Q (eq. 4)

$$t_{max} = \frac{1}{K} \qquad (3) \ , \qquad Q = \frac{D.F.K}{e} \qquad (4)$$

where D represents the dose, are given in Table II.

TABLE II : Values of the parameters of nitrofurantoin formulations.

Formulation	F^a, %	K^a, hr^{-1}	t^a_{max}, hr	Q^a, mg/hr
A	47.05(4.94)	1.10(0.56)	1.12(0.57)	18.62(8.81)
B	44.98(5.65)	0.94(0.42)	1.19(0.41)	14.99(4.72)
C	32.24(6.92)	0.54(0.11)	1.90(0.40)	6.45(1.80)
D	41.61(4.06)	1.07(0.31)	1.02(0.40)	16.09(3.87)

a : Mean values of four subjects with standard deviation in parentheses.

Analysis of variance was performed on the four parameters estimated and the results obtained are summarized in Table III. The tabulation of variance reveals that the formulations are different in terms of F, t_{max} and Q. Also, the subject variability seems to be significant for t_{max} while no period effect was observed.

TABLE III : Analysis of variance (ANOVA). Critical Fisher's f for 3 and 9 degrees of freedom at p:0.05 is 3.86.

	Variance - f test			
Source of variation	F	K	t_{max}	Q
Formulations (3)[a]	171.6 (7.187)	0.263(2.689)	0.635(5.354)	111.8 (4.775)
Subjects (3)[a]	49.15(2.059)	0.307(3.145)	0.461(3.885)	48.87(2.087)
Residual (9)[a]	23.87	0.098	0.119	23.42

a : Degrees of freedom.

Since significant f ratios were detected a further analysis of variance was applied comparing all possible couples of formulations. Significant difference ($p = 0.05$) in drug parameters calculated for the brands studied was found, Table IV. Brand C was significantly different from D for all parameters calculated, and from A for the parameters F and t_{max}. In addition brands A and D were significantly different in terms of F. The differences for the parameter F observed between the pairs of brands C-D and A-C could be attributed either to formulation factors or to a different extent of food influence on the bioavailability of drug. However, the dissolution profiles depicted in Fig. 1 and 2 indicate that the differences between brands A and C as well as between A and D, most likely arise from formulation factors. It has been proved[9] that food enhances the bioavailability of nitrofurantoin more intensively when ingested in macrocrystalline than in microcrystalline form. Since similar dissolution profiles were found for brands C and D (Fig. 1 and 2), a

TABLE IV. Significant differences in drug parameters for the brands studied.

Parameter	Brands					
	A and B	A and C	A and D	B and C	B and D	C and D
F	N.S	*	*	N.S	N.S	*
K	N.S	N.S	N.S	N.S	N.S	*
t_{max}	N.S	*	N.S	N.S	N.S	*
Q	N.S	N.S	N.S	N.S	N.S	*

Key : * = significant at 0.05 level, N.S = not significant at 0.05 level.

study was undertaken coadministering brands C or D with breakfast to find out if bioavailability of brand C can be attributed to the 1h non-compliance interval. The bioavailability design was the standard, two period crossover for four subjects and two formulations. All other conditions and methods were indentical with those described above. Examination of the results obtained for period, intra-and inter-subject variability did not reveal any statistically significant difference. The experimentally calculated mean cumulative percent of drug excreted for brands C and D was 33.87% and 44.10% respectively. These values show a similar increase,

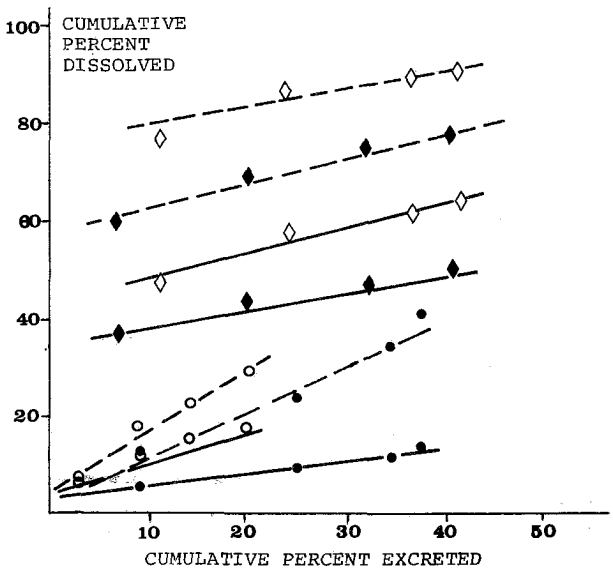

FIG. 5 : *Correlation of mean cumulative of nitrofurantoin excreted in 1.0, 2.0, 3.0 and 4.0 h following the oral administration of nitrofurantoin brands with mean cumulative percent dissolved in 10, 20, 30 and 40 min in pH 7.2 (---) or pH 1.0 (—).* Key : *see Fig. 1.*

namely 5% and 6% respectively when compared with the corresponding values given in Table II. It is concluded therefore that bioavailability differences between brands C and D are caused by formulation factors and not from variations in drug food interaction. Accordingly, the dissolution test did not ideally reflect the rate of drug absorption. Thus, product D appears to have been made available much more rapidly for absorption than product C (Fig. 3) but this could not have been predicted from the *in vitro* test, (Fig. 1,2).

Nevertheless, the multiple point *in vitro-in vivo* correlation depicted in Fig. 5 was excellent ($R > 0.95$) for all brands. It should be noted however, that the intercepts on the *in vitro* axis for brands A and B are 76% and 57% respectively at pH 7.2 while for C and D are near zero. Since intensity factors or lag time corrections were not employed and *in vitro* test conditions were indentical for all brands studied, the high intercepts on the abscissa for brands A and B are not due to improper agitation. It is quite apparent therefore that the rapid release of drug from brands A and B is atributable to formulation factors.

An overall correlation for the percentage of total drug excreted versus the cumulative drug dissolved at 1h, resulted in a correlation coefficient of 0.90 (Fig. 6). This value can be considered as reasonable in terms of the small number of brands utilized and their diversity in manufacturing procedures.

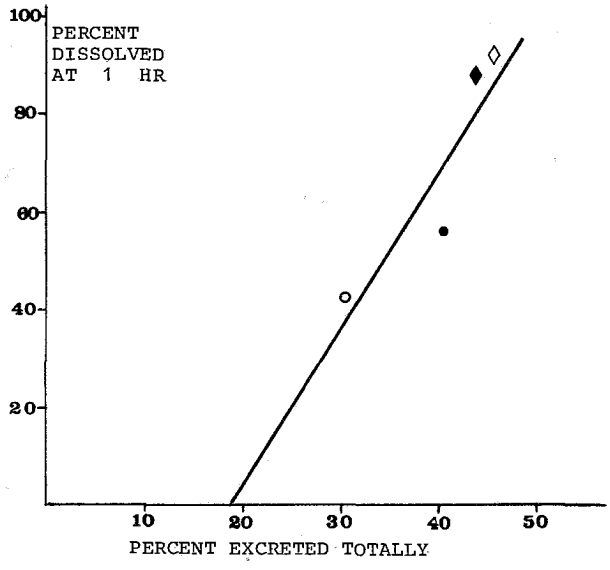

FIG. 6 : *Correlation of percent excreted totally in urine versus the percent dissolved in pH 7.2 at 1 hr for the four nitrofurantoin brands studied.* Key : *see Fig. 1.*

The results obtained gave a clear view of the remarkable differences between the brands studied. Thus, brands A and B did not meet the dissolution specification for nitrofurantoin of USP XIX: "Between 25% and 60% of the labelled amount in the tablets dissolves at one hour". Though, USP XX simply states that not less than

25% of the drug dissolves in 60 minutes. In the light of the official recommendations, the reformulation of brands A and B is advisable. Moreover, brand C just passed the *in vitro* test (Fig. 2) which was reflected in a significantly lower bioavailability than brands A and D (Table IV). Brand D is in accordance with the *in vitro* test criteria even though the dissolution tests (Fig. 1 and 2) anticipated for a lower bioavailability. The latter was found to be 41.61%, quite similar to 41.48% reported previously[12] for the same product marketed in USA. However, the analysis of variance (Table IV) proved that brands C and D can not be used interchangeably. The same is apparently applied to the pairs of brands A and C as well as A and D (Table IV).

Appendix

The amount of intact drug in plasma (as percentage of the total drug) X_p under the conditions of $k = k_a = K$ is described[17] as:

$$X_p = FKte^{-Kt}$$

while the urinary excretion rate is given by the equation:

$$\frac{dX}{dt} = KX_p = K^2 Fte^{-Kt}$$

The last equation can be integrated for $t = 0$, $X = 0$ and $t = t$, $X = X_t$ to yield,

$$X_t = K^2 F\left[-e^{-Kt}\frac{(Kt+1)}{K^2}\right]_0^t \quad \text{or} \quad X_t = F[1-(1+Kt)e^{-Kt}]$$

Περίληψη

Συγκριτική μελέτη της βιοδιαθεσιμότητας τεσσάρων σκευασμάτων Νιτροφουραντοϊνης.

Η Νιτροφουραντοϊνη είναι ένα από τα φάρμακα που παρουσιάζει κυμαινόμενη βιοδιαθεσιμότητα μεταξύ χημικά ισοδυνάμων σκευασμάτων. Στη μελέτη αυτή προσδιορίστηκε με τη μέθοδο της ουρικής απέκκρισης η βιοδιαθεσιμότητα τεσσάρων σκευασμάτων νιτροφουραντοϊνης που κυκλοφορούν στην Ελληνική αγορά. Τα πειραματικά δεδομένα αναλύθηκαν με τη μέθοδο των μη γραμμικών ελαχίστων τετραγώνων βασισμένη σε ένα απλοποιημένο μονοδιαμερισματικό μοντέλο. Οι παράμετροι που υπολογίστηκαν, υποβλήθηκαν σε ανάλυση της ποικιλίας (ANOVA) αποκαλύπτοντας την βιο-ανισοϋδυναμία (bio-inequivalence) για τρία ζεύγη σκευασμάτων.

Συσχετισμοί *in vitro - in vivo* έδωσαν υψηλούς συντελεστάς συμμεταβολής.

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