DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 13(3), 501-516 (1987)

" IN VITRO "- " IN VIVO " CORRELATIONS OF EIGHT NITROFURANTOIN TABLET FORMULATIONS: EFFECT OF VARIOUS TECHNOLOGICAL FACTORS

Concheiro A.; Vila-Jato J.L.; Martinez-Pacheco R.; Seijo B. and Ramos T.

Departamento de Farmacia Galénica
Facultad de Farmacia
Santiago de Compostela (Spain)

ABSTRACT

The bioavailability of eight nitrofurantoin tablet formulations differing in technological respects has been evaluated and correlated with de dissolution curves obtained using USP XXI Ed methods I and II. Method I was found incapable of predicting bioavailability of formulations when Carbopol 934 is used as binder. The influence of the technological factors varied is discussed.

INTRODUCTION

Nitrofurantoin, which has been succesfully employed in the treatment of urinary infections for some 30 years is administered orally as tablets or suspensions, but various studies (1 - 3) have reported bioinequivalence among commercial preparations. The results of a bioequivalence study carried out in 1973 by McGilveray et al. (4) using 18 canadian brands of nitrofurantoin tablets revealed significant differences among them, both as regards the total quantity of nitrofurantoin excreted and the absorption rate. Meyer el al. (5) came to similar conclusions in a subsequent study of 14 commercial brands, finding significant

differences as regards the total quantity of nitrofurantoin excreted after 12 hours. These and other similar findings have led the FDA to class nitrofurantoin among drugs with bioequivalence problems and suggest that an " in vitro " dissolution rate study should be carried out on nitrofurantoin products, and that is possible these data should be related to " in vivo" data obtained using human subjects (6).

Most bioavailability studies of nitrofurantoin have emphasized the importance of particle size (7 - 11), but few have paid attention to the influence of other technological factors (12 - 14). The present article reports a study undertaken with the aim of correlating the " in vitro " behaviour of eight nitrofurantoin tablet formulations prepared in our laboratory with " in vivo " results.

MATERIALS, METHODS

Formulations assayed-. Eight nitrofurantoin tablet formulations containing 50 mg of nitrofurantoin per tablet (micronized nitrofurantoin batch V-0496: Liade Laboratories) were prepared. Table 1 shows the composition of the eight formulations (A; B; C; D; E; F; G and H) and the forces used to compress the tablets. For each of the binders used, the compression forces and the quantities of binder make up a 2 x 2 factorial dessign. All the formulations were prepared by the conventional wet granulation method and the granulates were compressed using a single-punch tablet machine (Korch- Erweka G.m.b.h. Germany) with a 9 mm punch.

Analytical methods-. Concentration of nitrofurantoin in samples obtained during dissolution studies were determined using the method described in USP XXI Ed. The method of Albert et al. (15) was used to determine nitrofurantoin in urine. Urine samples were stored in opaque flasks at -30ºC until analysed.

Clinical Protocol-. Fourteen healthy male and female volunteers aged between 20 and 30 years lacking any history of kidney disorder were used in a randomized balanced incomplete block dessign (16).

TABLE 1

Composition and compression forces applied to the 8 formulations studied

Formulation	mg Nitrofurantoin	mg Wheat Starch	mg Lactose	mg Compritol*	mg Carbopol 934	mg Gelatine	Compression Force (Nw)
A	50	140	60	1.25	0.625	-	2500
B	50	140	60	1.25	1.250	-	2500
C	50	140	60	1.25	-	0.210	2500
D	50	140	60	1.25	-	0.420	2500
E	50	140	60	1.25	0.625	-	4800
F	50	140	60	1.25	1.250	-	4800
G	50	140	60	1.25	-	0.210	4800
H	50	140	60	1.25	-	0.420	4800

* Compritol is a glyceryl behenate (Gattefossé.France)

Samples of urine were collected:1; 2; 3; 4; 5; 6; 8; 10 and 12 hours after administration of the formulation, which took place inmediately following a standard breakfast. A one-week elimination period was allowed to pass between succesive administration.

In a supplementary study of three of the formulations, 15 volunteers were used in a 3 x 3 Latin square design with 5 replicates.

Pharmacokinetic analysis-. Urinary excretion curves of nitrofurantoin were characterized by means of the statistical moments

proposed by Yamaoka et al (17) for plasma level analysis and adapted by Vila et al. (18): the total quantity of nitrofurantoin excreted in the 12 hours following administration (E^{12}), the mean residence time (MRT) and the variance of the residence time (VRT) measured in mg, h and h^2 respectively.

Dissolution rate studies-. The apparatus used (Dissolutest. Prolabo. France) complied with the specifications of USP XXI Ed. Tablets were subjected to two dissolution rate assays: Method I in accordance with the conditions laid down by USP XXI Ed. for nitrofurantoin tablets and USP XXI Ed. Method II at a speed of 50 rpm. Six tablets of each formulation were tested by each method and the dissolution curves obtained were characterized by the percentage of nitrofurantoin dissolved after 60 minutes (D_{60}).

Statistical analysis-. The E^{12}, MRT and VRT data were processed by a two-way multivariant analysis of variance (subjects and formulations) (18). Wilk´s test (19) was applied to the null hypothesis in the incomplete block study and the greatest eigenvalue test (20) in the Latin squares study. The D_{60} data were processed by one-way analysis of variance.

Quadrant analysis-. " In vivo "- " In vitro " correlations were stimated by applying the Fairweather´s method of quadrant analysis (21) to the graphs of D_{60} against Eff_α , α being the pharmacokinetic parameter involved (E^{12} or MRT) and Eff_α (f) the efficiency of formulation f with respect to α as measured by the percentage of subjects for whom α (f) lay within certain bounds similar to the " arbitrary guidelines " used by Yau et al. (22). Thus an acceptable lower limit of 0.75 x E^{12} (R) was set for E^{12} and an acceptable upper limit of 1.25 x MRT (R) for MRT, R being a reference formulation which for reasons set out below in the Results and Discussion was in fact the mean of the values of the eight formulations.

TABLE 2

Technological characteristics of the 8 nitrofurantoin tablet formulations studied

Formulation	Coefficient of variation mean weight(1)	Hardness(Kg) (2)	Friability (3)	Disintegration time (sec.) (4)	Coefficient of variation nitrofurantoin content (5)
A	1.14	3.6	2.37	25	4.8
B	1.86	3.6	0.84	23	4.2
C	1.10	3.7	0.74	11	3.2
D	1.33	3.9	0.45	10	2.0
E	0.63	7.9	0.89	24	2.0
F	0.61	8.1	0.77	15	2.0
G	0.59	6.8	0.07	31	3.7
H	1.36	6.3	0.19	27	4.0

(1) According to USP XXI Ed. Assay

(2) Mean of six determinations using an Erweka TB-24 Durometer

(3) % weight loss of 10 tablets after 15 min. in an Erweka Friabilometer

(4) According to USP XXI Ed. Assay

(5) According to USP XXI Ed. for nitrofurantoin tablets.

RESULTS AND DISCUSSION

Table 2 displays the characteristics of the eight formulations studied.

Figures 1 and 2 show the mean urinary excretion curves and Table 3 statistical moments characterizing the

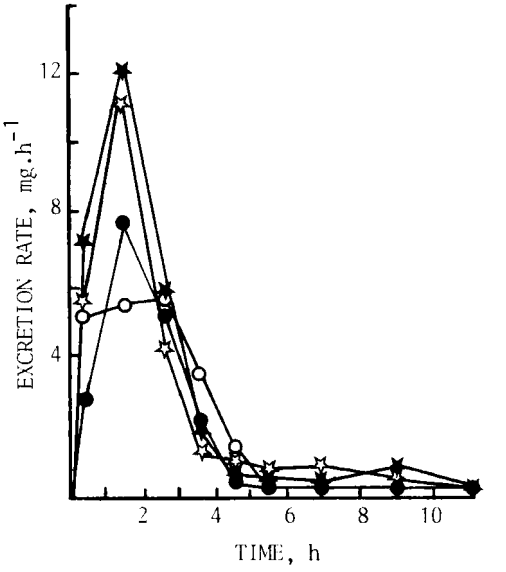

FIGURE 1-. Nitrofurantoin mean urinary excretion curves of A (●) B (○), E (☆) and F (★) formulations.

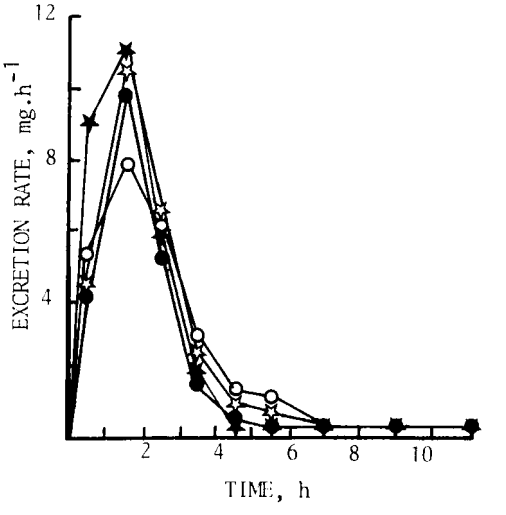

FIGURE 2-. Nitrofurantoin mean urinary excretion curves of C (○) D (●), G (☆) and H (★) formulations.

TABLE 3

Statistical moments of the urinary excretion curves of nitrofurantoin for each subject and formulation

Volunteers	Formulation	E^{12} (mg)	MRT (h)	VRT (h^2)	Formulation	E^{12} (mg)	MRT (h)	VRT (h^2)	Formulation	E^{12} (mg)	MRT (h)	VRT (h^2)	Formulation	E^{12} (mg)	MRT (h)	VRT (h^2)
1	A	22.84	3.14	2.25	B	20.84	3.10	0.96	C	17.81	2.07	1.52	D	22.53	1.73	2.31
2	E	39.33	1.46	2.44	F	34.69	1.28	1.09	G	25.51	1.75	0.61	H	30.98	1.56	0.87
3	A	19.67	3.32	3.92	B	18.40	3.50	5.48	G	16.33	2.22	3.03	H	25.97	1.69	0.67
4	C	25.08	2.41	3.81	D	20.59	2.71	0.76	E	22.42	10.23	23.13	F	30.21	1.45	0.50
5	A	31.27	1.41	0.66	C	35.62	2.28	1.42	F	29.96	1.67	0.59	H	28.56	1.30	1.26
6	B	17.76	1.53	1.79	D	18.54	2.00	1.71	E	15.75	1.57	0.83	G	28.70	1.61	0.64
7	A	16.85	2.13	0.63	D	18.04	1.90	2.79	F	16.87	2.16	1.41	G	20.44	2.65	4.97
8	B	21.89	1.67	0.40	C	24.14	1.84	1.23	E	29.52	1.49	1.85	H	26.76	1.59	2.05
9	A	28.66	1.56	0.82	B	15.24	2.27	1.46	E	29.81	1.96	1.85	F	41.19	2.03	0.89
10	C	30.98	4.32	4.23	D	34.16	4.29	10.57	G	33.79	5.10	13.24	H	28.99	3.35	9.14
11	A	20.91	2.65	1.80	C	16.67	2.30	1.36	E	27.51	4.50	8.02	G	23.52	1.96	1.05
12	B	23.92	1.44	0.53	D	26.15	1.85	2.00	F	33.76	0.95	0.59	H	32.59	1.45	2.70
13	A	22.20	2.64	5.62	D	23.10	2.52	4.02	E	28.15	1.71	0.73	H	21.50	3.40	2.28
14	B	24.50	1.72	2.71	C	15.86	2.70	3.49	F	24.00	3.69	3.57	G	29.13	5.30	7.73

TABLE 4

Results of the multivariant analysis of variance

Balanced Incomplete Block Design

Source of Variation	Matrix	Sum of squares and products		
Formulations	H	284.5533		
		14.5894	11.2815	
		31.3444	31.4045	91.9677
Subjects	S	1207.5620		
		- 21.9630	45.8070	
		129.8345	131.7907	466.0245
Error	E	714.5438		
		- 24.9935	57.1994	
		- 27.2127	131.0744	371.7078
Total	T	2206.6592		
		- 32.3671	114.2879	
		133.9661	294.2697	929.7001

the excretion of nitrofurantoin by each individual for each of the formulations administered. Table 4 shows the results of the multivariant analysis of variance and according to Wilk's test, the formulations studies are all bioequivalent ($\chi^2 = 0.53$ with 7 df; the critical value for $\alpha \leqslant 0.01$ being 7.24). In other words, the technological factors considered (type and proportion of binder and compression force) had no significant effect on the bioavailability of nitrofurantoin over the ranges employed.

Figures 3 and 4 present the mean dissolution curves of the formulations studied as obtained by Method I and Figures 5 and 6 the curves obtained by Method II and the mean percentages of nitrofurantoin dissolved after 60 minutes are listed in Table 5

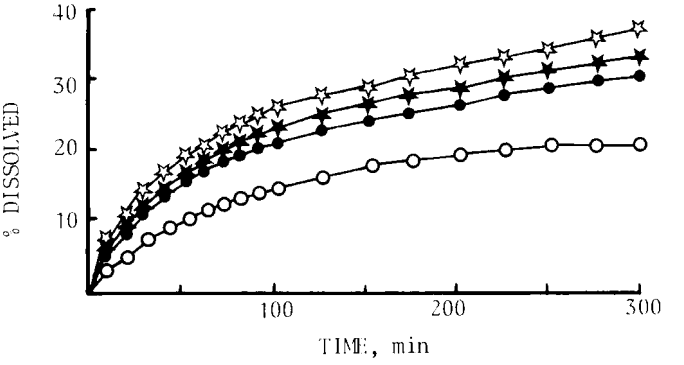

FIGURE 3-. Nitrofurantoin dissolution curves of formulations A (✱), B (●), E (☆) and F (O) obtained by Method I. Each point represents the mean of 6 determinations.

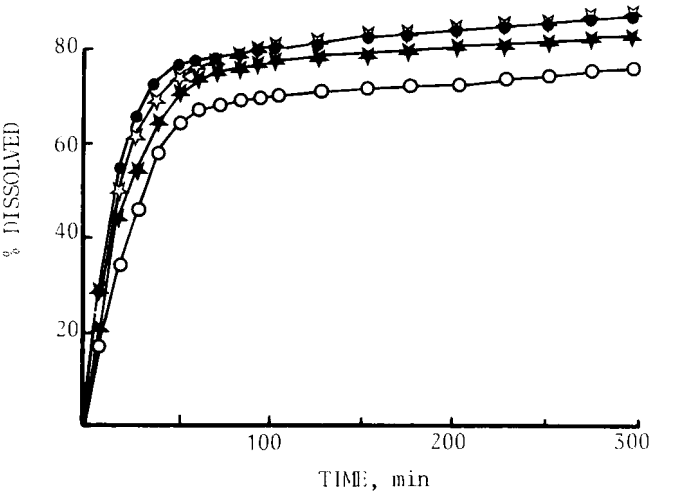

FIGURE 4-. Nitrofurantoin dissolution curves of formulations C (O), D (✱), G (☆) and H (●) obtained by Method I. Each point represents the mean of 6 determinations.

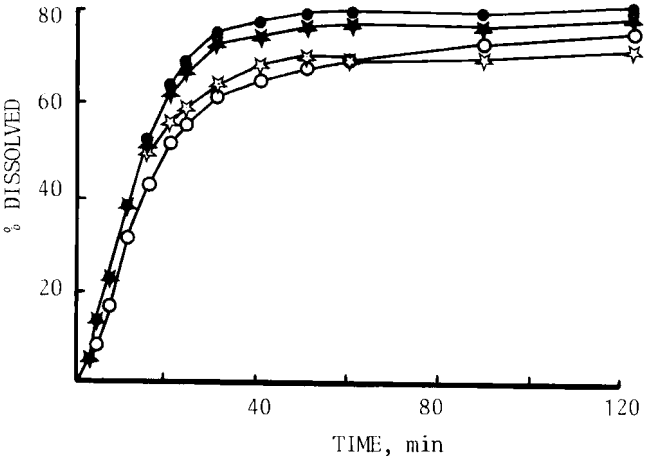

FIGURE 5-. Nitrofurantoin dissolution curves of formulations A (○), B (☆), E (★) and F (●) obtained by Method II. Each point represents the mean of 6 determinations

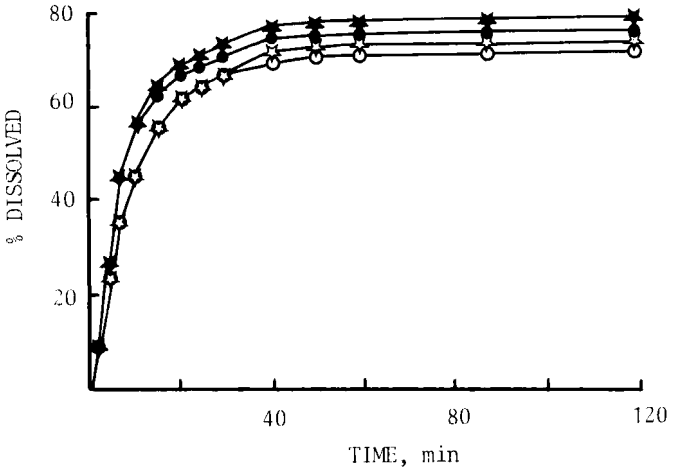

FIGURE 6-. Nitrofurantoin dissolution curves of formulations C (★), D (●), G (☆) and H (○) obtained by Method II. Each point represents the mean of 6 determinations.

TABLE 5

Mean percentages of nitrofurantoin dissolved after 60 minutes under Method I and II

	A	B	C	D	E	F	G	H
Method I	18.69	16.61	65.02	73.14	20.99	11.52	77.12	76.72
Method II	65.98	66.14	75.91	71.73	73.28	77.86	75.13	70.75

The results obtained using Method I differ markedly from those for Method II, and this endorsed by analysis of variance of the D_{60} data, which reveals significant differences among the formulations when Method I was used ($F = 134.3$ with 7/41 df), but not when Method II was used ($F = 2.9$ with 7/41 df). The source of the differences observed when Method I was employed is clearly the use of the binder Carbopol 934 in formulations A,B, E and F, whose quantities of nitrofurantoin dissolved failed to meet the USP XXI Ed. Assay.

The lack of significant differences among the bioavailability of the eight formulations makes it impossible to obtain " in vitro "- " in vivo " correlations of the conventional sort. It is nevertheless possible to carry out quadrant analysis to detect possible misclassifications of the formulations by the dissolution assays. To do so a reference formulation must be chosen (see Experimental Section), and the bioequivalence of all eight a logical choice. However, the larger number of formulations involved, the nature of the experimental design employed and the wide differences among the responses of different subjects might suggest that the absence of statistically significant evidence of bioinequivalent study is due more to the lower power of test to real bioequivalence of the formulations. We therefore carry out a supplementary bioequivalence study with formulations B,F and G, the formulations with respectively the lowest, highest and most approximately average values of E^{12} in the main study. Although

TABLE 6

Mean statistical moments for formulations B; G and F in the Latin square study

Formulation	E^{12} (mg)	MRT (h)	VRT (h^2)
B	21.46	2.62	4.24
G	19.94	2.72	2.77
F	22.83	2.41	2.25

TABLE 7

Results of the multivariant analysis of variance (MANOVA) of the Latin square study. The greatest eigenvalue of $H.E^{-1}$ is C_S = 0.6187 and $C_S/(1 + C_S)$ = 0.3822; the parameters of its distribution are: s = 2; m = 0 and n = 12. Null hypothesis is not rejected at α = 0.01 level.

Source of Variation	Matrix	Sum of squares and products		
Formulations	H	3.0317		
		2.3055	8.0742	
		2.5716	13.1201	21.9053
Subjects	S	211.1986		
		- 20.3948	13.3654	
		- 33.0094	18.1141	61.2983
Error	E	407.1074		
		- 53.7508	21.6947	
		- 143.6239	48.4120	175.6221
Total	T	621.3197		
		- 71.9391	43.1343	
		- 174.0617	79.6462	258.8257

the experimental dessign of this second study (fifteen volunteers in a 3 x 3 Latin square with five replicates) ensured its being sensitive enough to detect any real bioinequivalence, the results (Tables 6 and 7) conclusively rule out such bioinequivalence.

The use of the mean as reference value for quadrant analysis is thus fully justified. Figures 7 and 8 show the

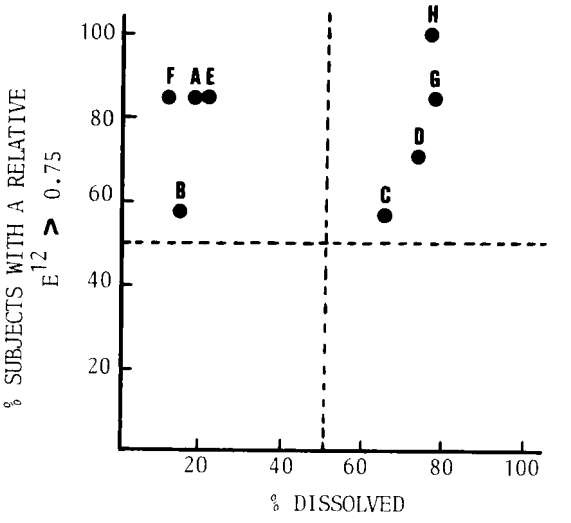

FIGURE 7-. Quadrant analysis of E^{12} against D_{60} (Method I) for the 8 formulations studied.

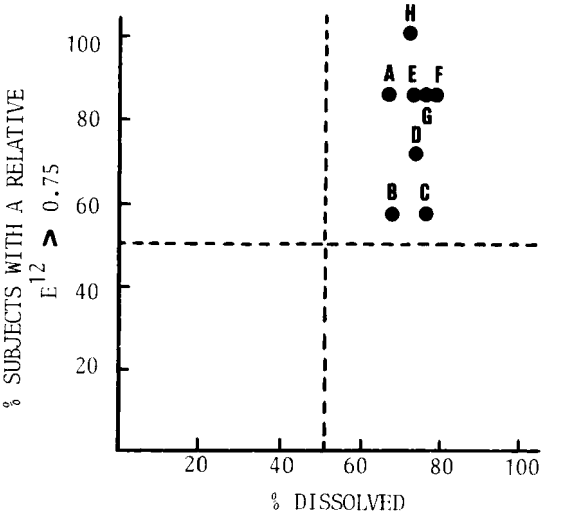

FIGURE 8-. Quadrant analysis of E^{12} against D_{60} (Method II) for the 8 formulations studied.

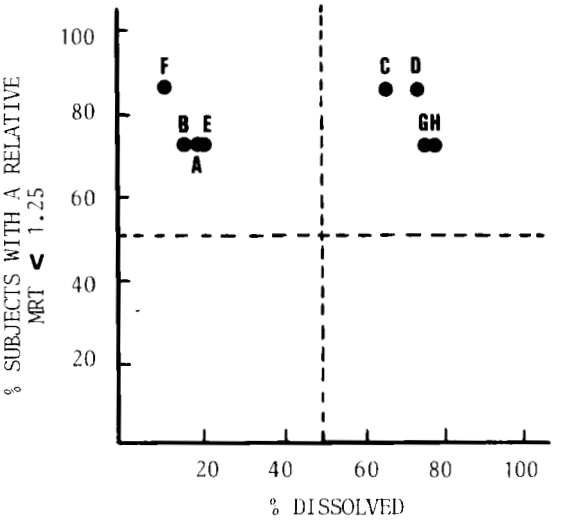

FIGURE 9-. Quadrant analysis of MRT against D_{60} (Method I) for the 8 formulations studied.

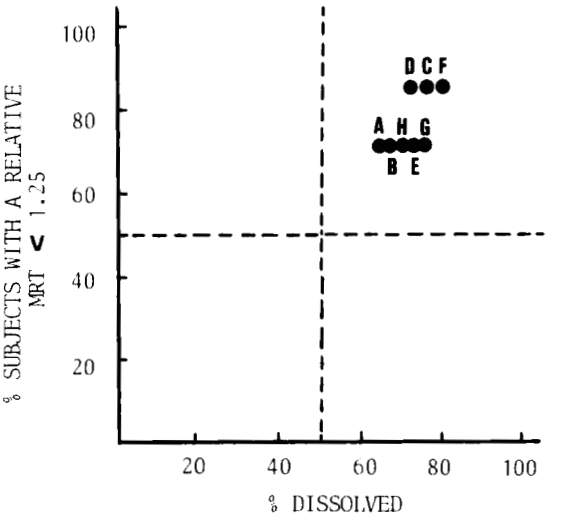

FIGURE 10-. Quadrant analysis of MRT against D_{60} (Method II) for the 8 formulations studied.

results of quadrant analysis of E^{12} and MRT for Method I and Figures 9 and 10 the results for Method II (analysis of the VRT data was found to add nothing new to the MRT results and is no shown). Method I systematically misclassified the formulations containing Carbopol 934 whereas Method II produced no misclassifications.

The bioequivalence of the formulations makes it impossible to draw any conclusions regarding the " in vitro " behaviour of deficiently absorbed formulations. It is, however possible to point out that the USP XXI Ed. Method I is not appropriate for assaying this kind of formulations when Carbopol 934 is used as binder. Other autor´s results for a variety of other formulations (22) indicate that the cause is the failure of the rotating basket to produce sufficient stirring for the tablets to break up, and this is reflected in quadrant analysis by the fact that as far as we know no formulation has been misclassified in quadrant IV; all misclassified formulations have been located in quadrant I. It is clear that further studies of this kind are required in order to establish technological conditions and limits for dissolution assays.

Supported by a grant 2777-83 from Comision Asesora de Investigación Cientifica y Técnica (Spain)

REFERENCES

(1)-. McGilveray I.J., Mattok G.L. and Hossie R.D.: J. Pharm. Pharmacol.: 23, 246S (1971)

(2)-. McGilveray L.J., Mattok G.L. and Hossie R.D.:" The comparison of the bioavailability of commercial nitrofurantoin tablets ": Canadian Association for Research and Toxicology Simposium ; Montreal, Canada Dec. 8-9 (1971)

(3)-. Mattok G.L., Hossie R.D. and McGilveray I.J.: Can. J. Pharm. Sci.: 7, 84 (1972)

(4)-. McGilveray L.J., Mattok G.L. and Hossie R.D.: Rev. Can. Biol.: 32, 99 (1973)

(5)-. Meyer M.C., Slywka G.W.A., Dann R.E. and Whyatt P.L.: J. Pharm. Sci.: 63, 1693 (1974)

(6)-. Bioequivalence Requirements and " in vivo " bioavailability procedure: Fed. Regist.: 42, 1621 (1977)

(7)-. Paul H.E., Kenyon J.H., Paul M.F. and Borgman A.R.: J. Pharm. Sci.: 56, 882 (1967)

(8)-. Bates T.R., Rosemberg H.A. and Tembo A.V.: J. Pharm. Sci.: 62, 2057 (1973)

(9)-. Bates T.R., Young J.M., Wu C.M. and Rosemberg H.A.: J. Pharm. Sci.: 63, 643 (1974)

(10)-.,Bron J. Vree T.B. and Damsma J.E.: Arzn. Forsch.: 29, 1614 (1979)

(11)-.Mendes R.W., Masih S.J. and Kammur R.P.: J. Pharm. Sci.: 67, 1613 (1978)

(12)-.Conte V., Colombo P., Caramella C. and La Manna A.: Il Farmaco Pract. Ed.: 34, 306 (1979)

(13)-.Capan Y.: Labo-Pharma Probl. Tech.: 31, 837 (1983)

(14)-.Eldem T. and Capan Y.: Proceedings of the 3rd International Conference on Pharmaceutical Technology. Chatenay-Malabry (France) 1983. Vol I pg 137

(15)-.Albert K.S., Sedman A.J., Wilkinson P., Stoll R.G. Murray W.J. and Wagner J.G.: J. Clin. Pharm.: 14, 264 (1974)

(16)-.Cochran W.G. and Cox G.: Diseños Experimentales. Ed. Trillas Mexico (1978). Pag 416-436

(17)-.Yamaoka K., Nakagawa T and Uno T.: J. Pharmacokin. Biopharm. 6, 547 (1978)

(18)-.Vila J.L., Martinez R., Gimenez I. and Llabres M.: J. Pharmacokin. Biopharm.: 8, 411 (1980)

(19)-.Kshirsagar A.M.: Multivariate Analysis: Marcel Dekker N.Y. (1978) pag.: 289-339

(20)-.Dagnielie P.: Analyse Statistique a Plusieurs Variables. Les Presses Agronomiques de Gembloux (1975) pag. 251-268

(21)-.Fairweather W.R.: J. Pharmacokin. Biopharm.: 5, 405 (1977)

(22)-.Yau M.K. and Meyer M.C.: J. Pharm. Sci.: 70, 1017 (1981).