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Effect of Formulation and Process Variables on Bioequivalency of Nitrofurantoin II: *In Vivo–In Vitro* Correlation

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Abstract □ Based on preliminary *in vitro* evaluation, six formulations presenting a broad range of dissolution rates were selected for bioequivalency determination in a randomized complete block crossover. *In vitro–in vivo* correlations were developed relating cumulative percent dissolved to cumulative percent excreted. These correlations appear to be useful for comparing different formulations as well as different batches of the same formulation.

Keyphrases □ Nitrofurantoin—various formulations, bioavailability in humans correlated to dissolution rate *in vitro* □ Bioavailability—various formulations of nitrofurantoin in humans, correlated to dissolution rate *in vitro* □ Dissolution rate, *in vitro*—various formulations of nitrofurantoin correlated with bioavailability in humans □ Antibacterials, urinary—nitrofurantoin, various formulations, bioavailability in humans correlated to dissolution rate *in vitro*

Numerous reports (1–5) provided support for the contention that not all commercially available products meeting compendial requirements necessarily exhibit equivalent bioavailability. Nitrofurantoin exhibits this problem (3–5). The Food and Drug Administration included nitrofurantoin on its list (6) of drugs requiring bioavailability testing for market preclearance, and the American Pharmaceutical Association included it in their bioavailability monograph project (7).

Previous studies on the bioinequivalence of nitrofurantoin utilized commercially available products for testing without regard to formulation and process variables that might affect bioequivalency. A preliminary study (8) concerned the development and screening of 52 nitrofurantoin products having controlled variables in formulation and processing. This screening on the basis of *in vitro* test procedures led to the selection of six final formulations for bioequivalency testing and attempts at correlation with *in vitro* test results.

Table I—Final Formulations of Nitrofurantoin Tablets and Capsules

Formulation[a]	IA	IG	IIA	IIM	IIIA	IIIC
Nitrofurantoin crystals USP, %	6.67	6.67	16.67	16.67	9.52	11.36
Lime flavor, %	0.67	0.67	—	—	—	—
Citric acid monohydrate, powdered USP, %	1.67	1.67	—	—	—	—
Saccharin sodium USP, %	1.00	1.00	—	—	—	—
Magnesium stearate USP, %	0.50	0.50	0.50	0.50	1.00	1.00
Compressible sugar, %	89.5	—	82.83	—	89.48	—
Lactose, anhydrous USP, %	—	30.0	—	27.67	—	29.30
Mannitol, granular USP, %	—	59.5	—	55.16	—	58.59

[a] IA = nitrofurantoin chewable tablets, IG = nitrofurantoin chewable tablets, IIA = nitrofurantoin swallow tablets, IIM = nitrofurantoin swallow tablets, IIIA = nitrofurantoin swallow capsules, and IIIC = nitrofurantoin swallow capsules.

Table II—Experimental Design for Nitrofurantoin Bioavailability Evaluation [a]

Subject	Day 1	4	7	10	13	16	19
1	IG	CTL	IIA	IIM	IIIA	IIIC	IA
2	CTL	IIA	IIM	IIIA	IIIC	IA	IG
3	IIA	IIM	IIIA	IIIC	IA	IG	CTL
4	IIM	IIIA	IIIC	IA	IG	CTL	IIA
5	IIIC	IA	IG	CTL	IIA	IIM	IIIA

[a] Each item within the matrix corresponds to a specific formulation as described in Table I; CTL = control.

EXPERIMENTAL

Formulations—Based on preliminary dissolution data (8), six formulations (Table I) were selected to provide a broad range of dissolution rates with the expectation that this range would lead to a wide variation in bioavailability. The six formulations consisted of three dosage forms

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Table III—*In Vitro* Data Summary for Nitrofurantoin Solid Dosage Forms

Test	Formulation[a]						
	CTL[b]	IA	IG	IIA	IIM	IIIA	IIIC
Disintegration, min	9	10	5	13	7	—	—
Hardness[c], SCU	18.0	8.8	8.9	8.8	9.1	—	—
Dissolution, mg/min							
Phosphate buffer	0.0057	0.0040	0.0423	0.0011	0.0032	0.0196	0.0381
Acid buffer	—	—	—	—	—	0.0030	0.0090

[a] For description of formulations, see Table I. [b] Control. [c] Mean of 20 determinations.

(chewable tablet, swallow tablet, and hard gelatin capsule) produced with two diluent excipients [compressible sugar[1] and mannitol[2]–lactose[3] (2:1)]. All dosage forms were prepared using commercially available nitrofurantoin crystals[4] by direct blending–granulation and, for tablets, at the lowest compression force necessary to make physically satisfactory tablets. A commercially available nitrofurantoin tablet[5] was used as the control.

Bioavailability Protocol—Five male volunteers[6] between the ages of 20 and 40 years and with a weight range of 43–75 kg were given a blood chemistry analysis[7] to ensure inclusion of only those subjects in good health. All subjects were instructed to refrain from taking any other medication during the study. Each subject was given a 100-mg dose (one tablet or two capsules) of a nitrofurantoin product once every 72 hr until all dosage forms were administered in accordance with the experimental design (Table II).

Since bioavailability has been shown to be greater if nitrofurantoin is taken with food (9), the dose was taken in the morning after a light breakfast of 240 ml of milk and 30 g of cold cereal[8]. Since urinary excretion studies are the method of choice (approximately 40% of the drug is excreted in the urine as unchanged drug following oral administration) and since clearance is independent of urinary pH (10), urine samples were collected at 0, 0.5, 1.0, 1.5, 2.0, 3.0, 6.0, 8.0, 12.0, and 24.0 hr. A volume of 150 ml of water was provided at 0, 0.5, 1.0, 2.0, 3.0, and 4.0 hr to ensure adequate urine volume.

The volume of each urine sample was recorded along with the time of collection, and an aliquot was taken. Preliminary experimentation indicated that nitrofurantoin frozen in urine exhibited excellent stability for several days. Therefore, the samples were frozen to provide convenience in analysis and were allowed to thaw immediately before use.

Urinalysis—An aliquot (0.5 ml) of each urine sample was acidified with 2.0 ml of 0.2 *N* HCl. Nitromethane (5.0 ml) was added, mixed, and centrifuged. From the nitromethane layer, 3.0 ml was removed and transferred to a test tube. A quaternary ammonium hydroxide[9] solution (0.5 ml of 0.04 *M*) was added, and the solution was mixed and allowed to stand for at least 2, but not more than 30, min. Absorbance was determined spectrophotometrically[10] at 400 nm against a blank, and concentration was determined from a previously constructed standard curve.

Correlation Methodology—The urinary excretion data obtained from the randomized complete block experimental design were analyzed according to a one-compartment open model using the following relationship (11):

$$X_t = 100\left[\left(1 - \frac{1}{K_a - K}\right)(K_a e^{-Kt} - K e^{-K_a t})\right] \quad \text{(Eq. 1)}$$

where X_t is the percent of drug excreted to time t, K_a is the absorption rate constant, and K is the elimination rate constant.

Initial estimates of the absorption and elimination rate constants were obtained graphically by a semilog plot of the excretion rate *versus* time. With the initial estimates of K and K_a, the data were subjected to digital computer least-squares iterations using the program NONLIN (12) to obtain the best estimates.

A two-way analysis of variance was performed on the cumulative amount excreted, the absorption rate, the peak excretion rate, the peak excretion time, and the elimination rate. Where significant f ratios were

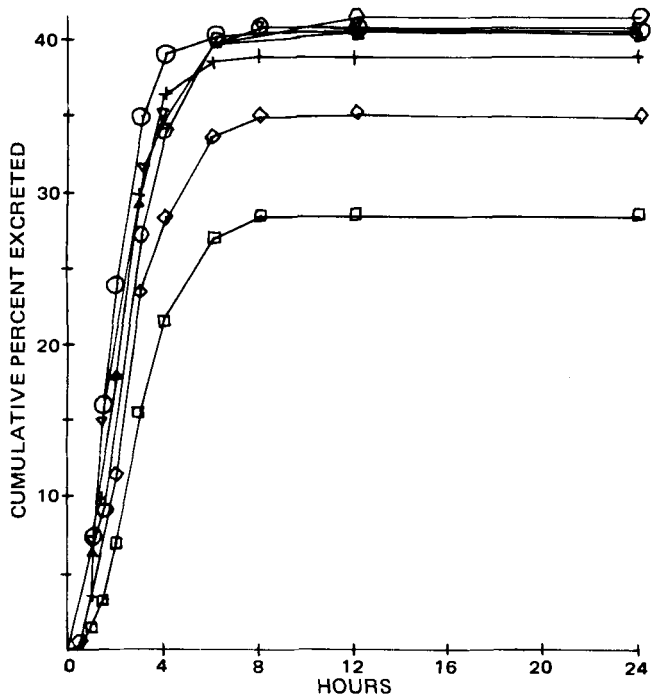

Figure 1—*Mean cumulative percent of nitrofurantoin excreted following the oral administration of nitrofurantoin products. Each data point is the mean cumulative percent excreted for all five subjects. Key: ○, Formulation CTL; ▽, Formulation IG; △, Formulation IA; ○, Formulation IIIC; □, Formulation IIA; ◇, Formulation IIM; and +, Formulation IIIA.*

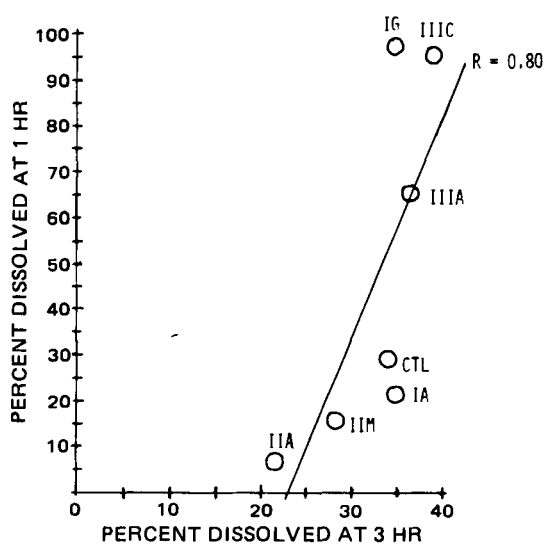

Figure 2—*Linear correlation of excretion in urine after 3 hr* versus *dissolution in phosphate buffer after 1 hr for seven nitrofurantoin formulations.*

[1] Nu-Tab, lot BD917M, Specialty Products by SuCrest, Pennsauken, N.J.
[2] Granular, lot 1219, ICI America, Wilmington, Del.
[3] Anhydrous, lot 4NM10, Sheffield Chemical Co., Union, N.J.
[4] Lot 12060, Berry and Withington Co., Cambridge, Mass.
[5] Furadantin, lot 810315, Eaton Laboratories, Norwich, N.Y.
[6] Each subject gave written informed consent.
[7] SMA 18/60.
[8] Product 19, Kellogg Co., Battle Creek, MI 49016.
[9] Hyamine.
[10] Model DB-GT, Beckman Instrument Co., Fullerton, Calif.

Table IV—Bioavailability Parameters for Nitrofurantoin Tablets and Capsules

Formulation[a]	Cumulative Amount[b], mg	Absorption Rate Constant[b], hr^{-1}	Peak Excretion Rate[b], mg/hr	Peak Excretion Time[b], hr	Duration min	
					30 μg/ml Maintained[b]	75 μg/ml Maintained
CTL	41.48	0.6900	13.37	2.40	272	127
	(2.01)	(0.1729)	(4.02)	(1.23)	(112)	(73)
IA	40.74	0.8270	12.99	1.73	256	101
	(2.44)	(0.1630)	(3.34)	(0.50)	(49)	(79)
IG	40.63	1.0559	14.20	1.55	235	107
	(2.99)	(0.2746)	(3.69)	(0.45)	(113)	(88)
IIA	28.30	0.6313	8.49	2.85	257	64
	(1.24)	(0.2385)	(2.31)	(1.11)	(77)	(76)
IIM	35.74	0.8299	11.60	2.42	265	171
	(1.02)	(0.2583)	(2.76)	(0.65)	(109)	(159)
IIIA	38.81	0.9667	14.40	2.25	228	85
	(3.22)	(0.2005)	(2.81)	(0.53)	(60)	(93)
IIIC	40.10	0.9826	17.44	1.70	226	135
	(3.54)	(0.1735)	(3.80)	(0.54)	(63)	(73)

[a] For description of formulations, see Table I. [b] Mean values of five subjects with standard deviation in parentheses.

detected, linear contrasts were utilized to elicit the specific factors related to their cause.

To derive *in vitro–in vivo* correlations, two approaches were taken. The cumulative percent excreted at various times was compared to the cumulative percent dissolved at various times using the Spearman rank correlation method. The times giving best correlation by this method were then plotted using simple linear regression analysis.

Next, to determine the correlation for each formulation, the percent excreted at various times was compared to the cumulative percent dissolved at various times. When dissolution was very rapid, it was necessary to change the conditions to produce the best correlation.

RESULTS AND DISCUSSION

Bioequivalency—Pertinent *in vitro* evaluative data for the six formulations and the control are presented in Table III. The mean hardnesses of the four tablet formulations were essentially identical, ranging from 8.8 to 9.1 Strong-Cobb units (SCU), while that of the control was significantly higher at 18. Disintegration time ranged from a low of 5 to a high of 13 min, with the control at 9 min. This result obviously precluded any correlation between hardness and disintegration time. The dissolution rate in phosphate buffer ranged from a low of 0.0011 to a high of 0.0423 mg/min. The significant differences among the tablet formulations precluded any correlation with tablet hardness. The dissolution profiles for the six formulations and the control in phosphate buffer were presented previously (8).

Nonlinear regression indicated that the urinary excretion data analyzed according to a one-compartment open model gave a correlation coefficient of at least 0.98. Table IV lists the means of the computer estimates of each bioavailability parameter for each formulation. Two-way analysis of variance, followed by linear contrast, revealed significant differences among the formulations with respect to all parameters. However, for all except cumulative amount excreted, the intersubject variability exceeded the interformulation variability.

Figure 1 shows the profiles for cumulative amount excreted as a function of time. Linear contrasts indicated that the chewable tablets and hard gelatin capsules were not significantly different ($p < 0.05$) from the control. The swallow tablets prepared with mannitol–lactose had a slightly lower (84.5%) bioavailability than the control, while the swallow tablets made with compressible sugar had a significantly lower bioavailability (68%).

The urine concentration of nitrofurantoin was also examined. A previous study (5) indicated that a urine concentration of 30 μg/ml should be effective against 90% of the strains of *Escherichia coli*, although levels of 75 μg/ml may be required against some strains. The urinary concentration of nitrofurantoin at each sampling time for each product in each subject was examined. Formulation differences were insignificant, but subject variability was significant. This result was due to extreme differences in the urine volume for each subject. It was concluded that a determination of this type would require a large number of subjects, preferably actual patients with urinary tract infections.

***In Vitro–In Vivo* Correlation**—The Spearman rank correlation test for the 3-hr cumulative excretion and 1-hr cumulative dissolution (Table V) produced a correlation coefficient of 0.93 and a t value of 5.59. Both values indicate good rank-order correlation between the parameters. When the data relative to these ranks were subjected to linear regression analysis, a correlation coefficient of 0.80 resulted (Fig. 2). This value was judged to be relatively acceptable for overall correlation of *in vitro–in vivo* data for seven different formulations.

For the individual tablet formulations, when the cumulative percent excreted at each available sample time was plotted against the cumulative percent dissolved at each sample time (0, 0.5, 1, 1.5, 2, 3, and 4 hr *versus* 0.5, 10, 15, 20, 30, and 40 min, respectively), excellent correlation was achieved ($R > 0.94$) (Fig. 3). Data for the six possible excretion sampling

Table V—Spearman's Rank Correlation for All Formulations

Formulation[a]	1-hr Dissolution Rank	3-hr Excretion Rank
IIA	1	1
IIM	2	2
IA	3	4
CTL	4	3
IIIA	5	5
IIIC	6	7
IG	7	6
	$R_s = 0.93$	
	$t = 5.59$	
	Critical $t_{0.975}$ (5 df) = 2.57	

[a] For description of formulations, see Table I.

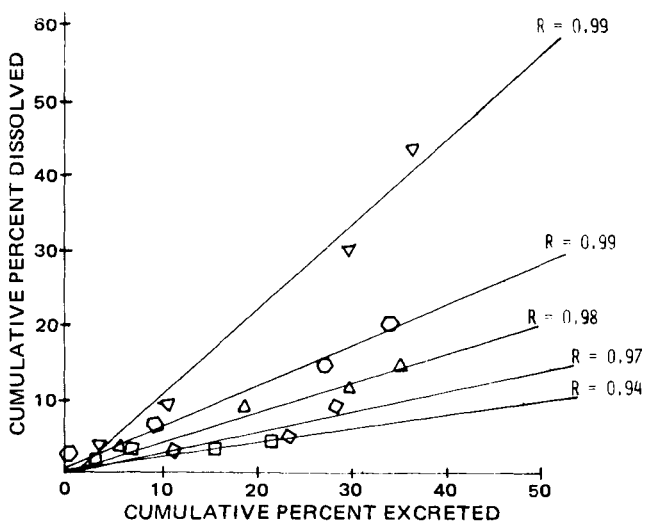

Figure 3—*Correlation of mean cumulative percent of nitrofurantoin excreted in 0, 0.5, 1.0, 1.5, 2.0, 3.0, and 4.0 hr following the oral administration of nitrofurantoin products with mean cumulative percent dissolved in 0, 5, 10, 15, 20, 30, and 40 min in phosphate buffer. Key: △, Formulation IA; ▽, Formulation IIIA; ○, Formulation CTL; □, Formulation IIA; and ◇, Formulation IIM.*

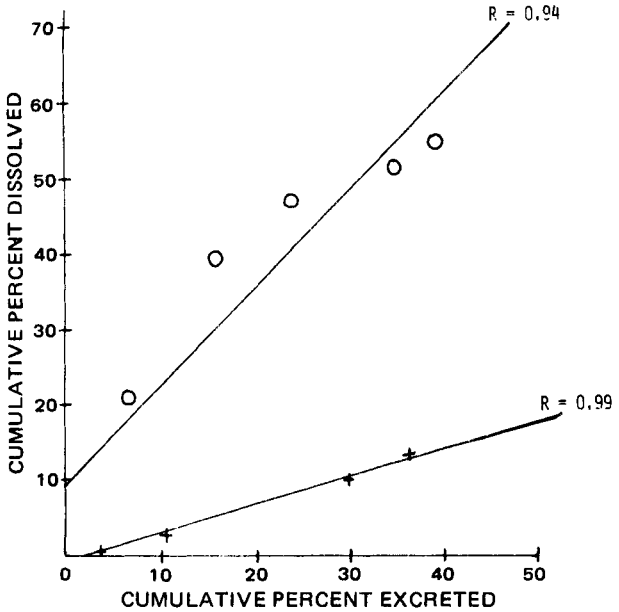

Figure 4—*Correlation of mean cumulative percent of nitrofurantoin excreted in 0, 0.5, 1.0, 1.5, 2.0, 3.0, and 4.0 hr following the oral administration of nitrofurantoin products with mean cumulative percent dissolved in 0, 5, 10, 15, 20, 30, and 40 min in hydrochloric acid buffer. Key: O, Formulation IIIC; and +, Formulation IIIA.*

times were not always available for all subjects because of individual variability in urination time. For any sampling time where data for all subjects were not available for a given formulation, the period was omitted from consideration to minimize error due to intersubject variability.

For the capsule formulations, correlation was not as good because of their very rapid dissolution. Therefore, the dissolution test conditions were modified to bring about rates more closely aligned with those for the tablets. The dissolution medium was changed to pH 1.2 hydrochloric acid buffer, and the stirring rate was reduced from 100 to 50 rpm. These changes produced dissolution rates for the capsules that were of a similar magnitude as the tablet dissolution rates while at the same time strongly differentiating between the two capsule formulations. Based on the dissolution rates in acid buffer, good correlation ($R > 0.94$) was found (Fig. 4). This correlation method appears useful for batch-to-batch testing on a given formulation or product.

All formulations in which at least 25% dissolved in 60 min were bioequivalent. Formulations that produced less than 25% dissolution exhibited significantly inferior bioavailability. Therefore, the existing USP specifications (13) appear to be adequately capable of eliminating formulations with a potential for poor bioavailability.

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