Absorption of Phenobarbital from Tablets and Elixir

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Abstract □ A three-way crossover study was conducted with 24 healthy male volunteers to determine the relative bioavailability of four different 100-mg phenobarbital tablets compared with a reference elixir. Each subject received two of the tablets and the elixir at 30-d intervals. Blood samples were collected daily for 19 d after each dose. Plasma phenobarbital concentrations achieved with the five dosage forms differed by <20% within 2–3 h after dosing. The extent of absorption for all dosage forms, as determined from area under the plasma concentration-time profiles, were within 10% of each other. The peak plasma concentration was the greatest and the time to peak concentration was the shortest for the elixir. One of the tablets exhibited a time to peak concentration of 8.6 h, which was significantly longer than any of the other dosage forms. The time to peak concentration correlated with the percent of drug dissolved in 60 min, as determined in 0.1 M HCl, using the USP XX paddle method at 50 rpm.

Keyphrases □ Phenobarbital—relative bioavailability, tablets and elixir, pharmacokinetics in humans □ Bioavailability—relative, phenobarbital tablets and elixir, pharmacokinetics in humans □ Pharmacokinetics—phenobarbital tablets and elixir in humans, relative bioavailability

Phenobarbital, used as a sedative-hypnotic and as an anticonvulsant, has an aqueous solubility of only 1 mg/mL, and differences have been reported in the dissolution rates of different polymorphic forms of the drug (1). Because of its clinical indications and physicochemical properties, it is important to establish the bioavailability of dosage forms of phenobarbital. A recent brief clinical report indicated significantly different plasma phenobarbital concentrations in patients who were receiving 32-mg tablets from two manufacturers (2). However, a bioavailability study of a 30-mg tablet indicated better absorption from the tablet than from an intramuscular dose, which was only 80% absorbed (3). In another study (4) the absolute bioavailability of 60-mg tablets was determined, and the extent of absorption averaged 95%. In the most extensive study to date, five healthy subjects were given seven 100-mg tablets from different manufacturers (5). A noncrossover design was employed, and blood samples were obtained for only 64 h, although half-lives ranged from 41–220 h. Further, the doses were administered every 6 d, and no correction for residual drug from preceding doses was noted in the report. The time of maximum plasma concentration was the only parameter that exhibited statistically significant differences among the test products. There did not appear to be any correlation between the blood level-time profiles and the results of *in vitro* dissolution testing.

The present study employed 24 healthy subjects, four different 100-mg tablet dosage forms, and a reference elixir. Blood samples were obtained for 19 d following dose administration. Attempts were made to relate parameters obtained *in vivo* to those seen using an *in vitro* dissolution system.

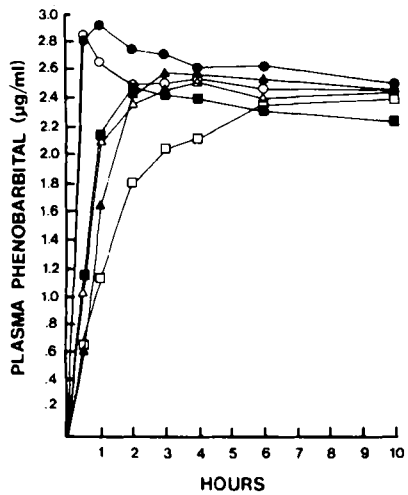

Figure 1—*Mean phenobarbital plasma concentrations during the initial 10 h after dosing* (n = 12). *Key: group I—product 1* (○); *product 2* (□); *product 3* (△); *group II—product 1* (●); *product 4* (■); *product 5* (▲).

EXPERIMENTAL

Dosage Forms—Four tablet products, containing between 97.2 and 100 mg of phenobarbital, were obtained from four manufacturers[1]. The products were selected from the numerous available sources on the basis of preliminary dissolution testing. An elixir[2] containing 100 mg of phenobarbital/25 mL was employed as a reference dosage form. Calculations were based on the labeled drug content of each of the five dosage forms.

Clinical Protocol—Twenty four male subjects (23–30 years, 53–100 kg) were randomly divided into two groups of 12. All subjects underwent urinalysis and hematological and blood chemistry[3] determinations, as well as a physical examination and an ECG, to ensure they were in good health. All subjects provided written informed consent. The subjects had not taken any known enzyme inducers for at least 3 months, any medication for 14 d, or any alcohol for 7 d prior to the start of the study.

Using a crossover design, group I received a 100-mg dose of the elixir (product 1) and two of the test tablets (products 2 and 3). The subjects in group II also received the elixir and the other two tablets (products 4 and 5). The three dosings were each separated by a 30-d period. The doses were administered after an overnight fast, along with 240 mL of water. No food or liquid

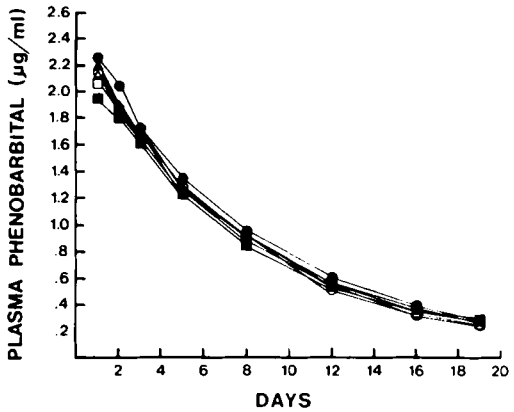

Figure 2—*Mean phenobarbital plasma concentrations 1–19 d after dosing* (n = 12). *Key: group I—product 1* (○); *product 2* (□); *product 3* (△); *group II—product 1* (●); *product 4* (■); *product 5* (▲).

[1] Parke-Davis, Lot WC187, 100 mg, Exp. 1/83 (product 2); Lannett, Lot 19806, 100 mg, Exp. 5/82 (product 3); Wyeth, Lot 1772455, 100 mg, Exp. 1/82 (product 4); West-Ward, Lot 42342, 97.2 mg, Exp. 12/82 (product 5).
[2] Parke-Davis, Lot YJ200, 20 mg/5 mL, Exp. 7/84 (product 1).
[3] SMA 18/90.

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Table I—Plasma Concentrations (μg/mL) at Each Sampling Time [a]

Group	Product No.	0.5	1 h	2 h	3 h	4 h	6 h	10 h	24 h	48 h	72 h	120 h	192 h	288 h	384 h	456 h
I	1	2.86	2.66	2.49	2.50	2.53	2.46	2.43	2.15	1.87	1.63	1.26	0.88	0.52	0.32	0.23
		(17)	(20)	(35)	(24)	(20)	(19)	(19)	(19)	(19)	(20)	(21)	(25)	(24)	(30)	(35)
	2	0.63	1.12	1.79	2.03	2.11	2.33	2.37	2.06	1.86	1.66	1.28	0.91	0.55	0.35	0.24
		(92)	(52)	(22)	(17)	(19)	(19)	(17)	(22)	(19)	(26)	(29)	(27)	(30)	(34)	(38)
	3	1.07	2.09	2.35	2.45	2.51	2.38	2.44	2.14	1.89	1.63	1.27	0.85	0.54	0.33	0.24
		(72)	(52)	(29)	(22)	(19)	(20)	(19)	(22)	(20)	(23)	(25)	(27)	(30)	(40)	(41)
Percent Difference [b]		78.0	57.9	28.1	18.8	16.6	5.3	2.9	4.2	1.6	1.8	1.6	6.6	5.5	8.6	4.2
II	1	2.81	2.93	2.75	2.71	2.61	2.62	2.48	2.26	2.05	1.72	1.35	0.96	0.61	0.40	0.28
		(25)	(19)	(18)	(18)	(19)	(20)	(20)	(21)	(21)	(24)	(26)	(43)	(61)	(72)	(80)
	4	1.14	2.14	2.48	2.42	2.38	2.29	2.21	1.95	1.80	1.61	1.23	0.84	0.55	0.38	0.29
		(62)	(32)	(23)	(20)	(21)	(26)	(23)	(25)	(20)	(21)	(22)	(30)	(46)	(57)	(60)
	5	0.60	1.64	2.43	2.57	2.55	2.51	2.42	2.19	1.88	1.68	1.27	0.89	0.53	0.35	0.27
		(96)	(61)	(24)	(14)	(15)	(18)	(16)	(18)	(15)	(17)	(24)	(28)	(37)	(48)	(56)
Percent Difference [b]		78.6	44.0	11.6	10.7	8.8	12.6	10.9	13.7	12.2	6.4	8.9	12.5	13.1	12.5	6.9

[a] Each value (μg/mL) represents the mean of the 12 subjects. The relative standard deviations are given in parentheses ($SD \times 100$/mean). [b] Percent difference = (100) (highest − lowest)/(highest).

other than water was permitted for 4 h following dosing. A standard meal[4] was provided 4 and 8 h after dosing. Ten-milliliter blood samples were obtained *via* a catheter or venipuncture just prior to the dose and 0.5, 1, 3, 4, 6, 10 h and 1, 2, 3, 5, 8, 12, 16, and 19 d after each dose using heparin as the anticoagulant. The blood samples were immediately centrifuged, and the plasma was stored frozen until the time of assay.

Plasma Assay—Plasma phenobarbital concentrations were determined using a slightly modified HPLC procedure previously employed in a study of ethotoin (6). A 1-μg/mL aqueous solution of phenytoin was used as the internal standard. The ether extraction of the plasma was adjusted to pH 11.2, and the chromatographic conditions were essentially as previously reported. The only modifications involved the reconstitution of the dried extract with 100 μL of mobile phase, the use of a 15-μL injection volume, and a mobile phase of 40% acetonitrile in 0.1 M sodium phosphate buffer (pH 7). Quantitation was accomplished with standard curves of peak height ratio (phenobarbital/phenytoin) *versus* phenobarbital concentration, prepared with pooled human plasma. The assay was linear over a concentration range of at least 0.25–3.0 μg/mL. The retention times for phenobarbital and phenytoin were 1.7 and 2.4 min, respectively. No interfering peaks were noted in blank plasma extracts. Duplicate determinations differed by <6% at the lowest standard concentration and by <3% at the highest value.

Data Analysis—The time of maximum plasma concentration (t_{max}) and maximum plasma concentration (C_{max}) were determined by inspection of individual subject data. The elimination rate constant (k) for each dose was determined by least-squares fitting of the postabsorption concentration–time data. The area under the plasma concentration–time curve (AUC) from 0 to 456 h was calculated using the trapezoidal method, while the $AUC_{0-\infty}$ was calculated by addition of the $AUC_{(456\ h-\infty)}$ to the $AUC_{(0-456\ h)}$. The $AUC_{(456\ h-\infty)}$ was determined by dividing the 456-h plasma concentration by k.

The statistical analysis was first performed separately on data from groups I and II. Analysis of variance was used to evaluate statistically significant differences ($p < 0.05$) at each sampling time, as well as values for t_{max}, C_{max}, and AUC. In cases where significant differences occurred, the Newman–Keuls *a posteriori* test was used to evaluate which subjects, treatment sequence, or dosage forms were different. To make comparisons of all four tablet formulations across both groups, a one-way analysis of variance and the Newman–Keuls *a posteriori* test were used to evaluate C_{max}, t_{max}, and relative $AUC_{(0-\infty)}$ for each tablet, adjusted for half-life differences. This latter value was determined as the product of the individual observed $AUC_{(0-\infty)}$ and k, divided by the product of $AUC_{(0-\infty)}$ and k for the elixir dose in each subject, to adjust for intrasubject differences in k. A power analysis (7) was used to evaluate the potential for statistical errors based on $\alpha = 0.05$ and $\beta = 0.2$. Because of an analytical failure, the data for one subject in group II (product 4) were lost. All concentrations and other parameters were estimated using a statistical method (8) based on the performance of the other two dosage forms in this subject and the data obtained for the other subjects within this group.

***In Vitro* Dissolution**—The dissolution of six tablets of each of the four tablet dosage forms was determined using the USP XX paddle method at 50 rpm. Deionized water and 0.1 M HCl at 37°C were employed as the dissolution media, using a volume of 1000 mL. Samples of the media were periodically withdrawn over a 90-min period.

[4] Content of each meal available on request.

RESULTS AND DISCUSSION

Although all subjects were instructed to avoid other drugs during the 3-month study, it was anticipated that some subjects might require minor medication. Thirteen of the subjects took at least one dose of some additional drug, with the majority of these being either acetaminophen for headache or antihistamines for rhinitis. Acetaminophen was previously shown not to interfere in the assay, and the basic antihistamines were not extracted during the assay. Further, inspection of time zero chromatograms and individual plasma concentration–time data did not reveal any effects of the other medications on the phenobarbital plasma concentration–time profiles.

Plasma Concentrations at Each Sampling Time—The mean plasma concentrations at each sampling time for both groups are summarized in Table I. The mean plasma concentrations at each sampling time from 0 to 10 h and from 24 to 456 h are illustrated in Figs. 1 and 2, respectively. Statistically significant differences among products in each study group are summarized in Table II. The rapidly absorbed elixir (product 1) resulted in the highest plasma phenobarbital concentrations for the first 6 h in group I and the highest concentrations at all but the 456-h time in group II. The actual difference among the products was <20% after the 2- and 1-h samples for the groups I and II studies, respectively. The intersubject variability, as reflected by the relative standard deviations, was much less for the elixir during the first hour, which is consistent with a rapidly absorbed dosage form.

The analysis of variance indicated that significant differences among the products occurred at 0.5, 1.0, 2.0, 3.0, and 4.0 h in group I and at 0.5 and 1.0 h in group II. In group I the Newman–Keuls *a posteriori* test (Table II) showed product 2 to have significantly lower concentrations than the elixir during the first 4 h. Product 2 was also significantly lower than product 3 in that group at 1, 3, and 4 h. Product 3 was significantly lower in concentration than the elixir at 0.5, 1, and 2 h. At the remainder of the sampling times product 2 had the lowest concentration at sampling times up to 48 h and slightly higher concentrations at the remainder of the sampling times.

In group II the Newman–Keuls *a posteriori* test indicated a significant difference among all the products at the 0.5-h sampling time, with product 5 having the lowest concentration and the elixir having the highest concentration. At 1 h the ranking of the products was identical to the ranking at 0.5 h, but the only significant difference was between the elixir and the two tablets. From 2 to 456 h no significant difference was observed among the three products.

Peak Concentration, Time of Peak Concentration, k, and AUC Values—

Table II—Newman-Keuls *a Posteriori* Test for Significant Product Differences

Observation	Product Ranking (Lowest to Highest) [a] Group I	Group II
Concentration		
0.5 h	2 3 1	5 4 1
1 h	2 3 1	5 4 1
2 h	2 3 1	5 4 1
3 h	2 3 1	4 5 1
4 h	2 3 1	4 5 1
C_{max}	2 3 1	4 5 1
t_{max}	1 3 2	1 4 5

[a] Dosage forms underlined by a common line show no significant differences ($p > 0.05$).

Table III—Phenobarbital Bioavailability Parameters [a]

Group	Product	C_{max}, μg/mL	t_{max}, h	$AUC_{(0-19d)}$, μg·d/mL	k, h^{-1}	$AUC_{(0-\infty)}$, μg·d/mL
I	1	3.01	1.0	17.54	0.00529	19.49
		(16)	(82)	(21)	(15)	(23)
	2	2.44	8.6	17.66	0.00522	19.73
		(16)	(68)	(24)	(15)	(26)
	3	2.70	2.6	17.46	0.00510	19.80
		(23)	(51)	(24)	(15)	(26)
	Percent Difference[b]	18.9	88.4	1.1	3.6	1.6
II	1	3.15	1.0	19.17	0.00500	22.16
		(17)	(75)	(34)	(22)	(46)
	4	2.57	3.2	17.27	0.00452	20.70
		(22)	(79)	(26)	(31)	(35)
	5	2.69	4.2	17.75	0.00478	20.53
		(14)	(59)	(22)	(23)	(29)
	Percent Difference[b]	18.4	76.2	9.9	9.6	7.4

[a] Each value represents the mean of the 12 subjects. The relative standard deviations are given in parentheses. [b] Percent difference = (100)(highest − lowest)/(highest).

Table III summarizes the mean values for the peak plasma concentration, time of peak concentration, $AUC_{(0-456\ h)}$, $AUC_{(0-\infty)}$, and the terminal elimination rate constant. The statistical analysis of differences among these values are given in Table II.

In group I the mean peak concentration (C_{max}) ranged from 3.01 μg/mL (product 1) to 2.44 μg/mL (product 2). This difference of 18.9% was significant ($p < 0.001$), and each product was significantly different from the other two products. The differences in C_{max} in group II was 18.4% with a range of 2.57 μg/mL (product 4) to 3.15 μg/mL (product 1). This difference was also significant ($p < 0.01$), with the elixir showing a higher concentration than the two tablets, which were not significantly different from each other.

The time of peak concentration (t_{max}) for the elixir (product 1) was 1 h in both groups. In group I, product 2 had a significantly longer t_{max} of 8.6 h compared with the elixir and product 3, which did not differ from each other. The significantly lower peak concentration and longer time to reach peak concentration for product 2 are indicative of a slower rate of absorption for this tablet. In group II, the elixir peaked sooner than the two tablets.

In group I the $AUC_{(0-456\ h)}$ ranged from 17.46 (product 3) to 17.66 μg·d/mL (product 2). This 1.1% difference was not significant. Extrapolation of the AUC to infinity resulted in AUC values ranging from 19.49 (product 1) to 19.80 μg·d/mL (product 3). Although extrapolation changed the ranking of the products, the 1.6% difference was not significant. The AUC values for the group II products were very similar to those in group I, with a range in $AUC_{(0-456\ h)}$ from 17.27 (product 4) to 19.17 μg·d/mL (product 1). The $AUC_{(0-\infty)}$ ranged from 20.53 (product 5) to 22.16 μg·d/mL (product 1). No significant differences in these values were noted ($p > 0.05$).

Sequence and Subject Differences—Significant differences among administration sequences (phases) were observed for several of the parameters. Generally, the ranking of the phases showed increases in plasma concentrations, with phase I < phase II < phase III. These differences were not significant ($p > 0.05$) for group II. Further, for group I the differences between phase I and phase III data were only ~10% for C_{max}, AUC, and k values. The slightly higher phenobarbital plasma concentrations obtained during phase III indicated that one dose per month did not result in any enzyme induction. Further, there was no progressive change in the apparent first-order elimination rate constant (k). Others have similarly found no apparent autoinduction of phenobarbital metabolism (4, 9). A decrease in the apparent volume of distribution over the 3-month testing period cannot be ruled out as a potential explanation for the slightly elevated plasma concentrations during phase III.

Significant subject differences were noted for all the parameters except the 0.5-h concentration and t_{max} in group I, and all but the 0.5-, 1.0-, 3.0-, 4.0- and 10.0-h concentrations and t_{max} in group II. Among the subjects, the C_{max} values ranged from 2.06 to 3.90 μg/mL and the mean $AUC_{(0-\infty)}$ ranged from 12.72 to 40.30 μg·d/mL. Mean terminal elimination rate constants for the subjects ranged from 0.0025 to 0.0064 h^{-1}, with an overall mean elimination rate constant for all dose administrations of 0.0050 h^{-1}.

Power Analysis—A power analysis (7) was also conducted utilizing an α level of 0.05 and a β level of 0.2. In group I, 12 subjects were sufficient to detect a 20% difference in products as being significant for the majority of the parameters. In those instances where >12 subjects were necessary, the actual difference in the products was greater than the percent difference required for significance to be detected. The statistical power for group II was not as great as in group I. However, it was sufficient to detect a 20% difference among the products in terms of 8 of the 15 sampling times, the C_{max}, and AUC values.

Comparisons Across Groups I and II—Table IV summarizes the one-way analysis of variance for comparison of products 2–5 in terms of C_{max}, t_{max}, and relative $AUC_{(0-\infty)}$. This table also includes the Newman–Keuls analysis of these parameters. The time of peak concentration ranged from 2.6 (product 3) to 8.6 h (product 2). Product 2 showed a significantly longer time to reach peak than the other three tablet products, which were not significantly different from each other. The peak plasma concentrations, which ranged from 2.44 to 2.70 μg/mL for the four tablet products, were not significantly different ($p > 0.05$). The relative $AUC_{(0-\infty)}$ ranged from 88 to 100% for the four tablets relative to the elixir. This difference among products was not significant ($p > 0.05$).

***In Vivo–In Vitro* Relationships**—Using the USP XIX basket method at 50 rpm, with pH 1.2 simulated gastric fluid as the dissolution media, Sylvestri

Table IV—Summary of Statistical Analysis Across Groups

Parameter	Mean Values: Product 2	Product 3	Product 4	Product 5	p Value
t_{max} (h)	8.60	2.60	3.20	4.20	<0.001
C_{max} (μg/mL)	2.44	2.70	2.57	2.69	0.532
Relative[a] $AUC_{(0-\infty)}$	1.01	0.98	0.88	0.95	0.515

Newman–Keuls Ranking[b]

Parameter	Product Ranking (Lowest to Highest)
t_{max}	3 4 5 2
C_{max}	2 4 5 3
Relative $AUC_{(0-\infty)}$	4 5 3 2

[a] See text for calculation. [b] Products underlined by a common line show no significant difference ($p > 0.05$).

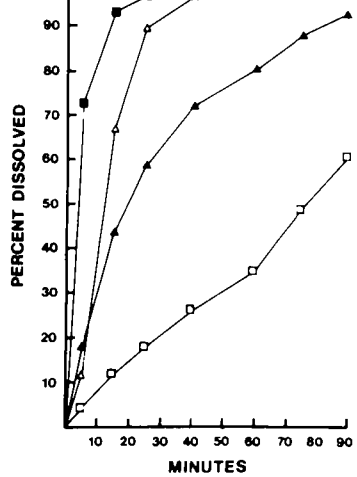

Figure 3—*Dissolution of four phenobarbital tablets in 0.1 M HCl using the USP XX paddle method at 50 rpm. Each value represents the mean of six determinations. Key: product 2 (□); product 3 (△); product 4 (■); product 5 (▲).*

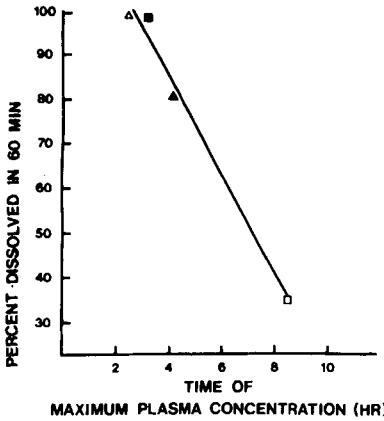

Figure 4—In vitro-in vivo *correlation for four phenobarbital tablets. Each* t_{max} *value represents the mean of 12 subjects and each* in vitro *value is the mean of six determinations. Key: product 2* (□); *product 3* (△); *product 4* (■); *product 5* (▲).

and Ueda (5) did not observe any correlation between the dissolution properties and the *in vivo* performance of seven different 100-mg phenobarbital tablets. Two of the products were different lots of products 2 and 4 employed in the present study. The results of the present study are shown in Fig. 3 for dissolution in 0.1 M HCl. Product 2 dissolved much slower than the other three tablets. The results were similar using deionized water, except for product 3 which dissolved slightly more slowly than product 5 in water. Since significant differences were noted among the products in terms of t_{max}, which reflects the rate of drug absorption, attempts were made to correlate the dissolution in acid with the mean values of t_{max} for the four tablets. Figure 4 illustrates the good relationship ($r = -0.995$) found between t_{max} and the percent of drug dissolved at 60 min. Whether the previously reported (5) lack of *in vitro–in vivo* correlation was due to differences between the two studies in the *in vivo* study design, in the *in vitro* methodology, and/or in the test tablets cannot be determined from the available data. In the present study there were no significant differences among the four tablets in terms of AUC, and the C_{max} values differed by <10%. Thus, it was not unexpected that the observed differences in dissolution rates could not be related to these *in vivo* parameters.

Clinical Implications—Since there were no significant differences in either C_{max} or AUC values, it can be concluded that the extent of absorption of phenobarbital was similar for the elixir and the four tablet products. However, there were significant differences among the tablet products in terms of rate of absorption, with product 2 showing the longest t_{max}. Because of the long half-life for phenobarbital in humans, differences in the rate of absorption should not have much effect on the steady-state phenobarbital plasma concentrations when the drug is employed on a chronic basis as an anticonvulsant. Projections of steady-state concentrations using a one-compartment model with first-order absorption suggest only minor differences in maximum and minimum plasma concentrations, comparing two dosage forms with an elimination half-life of 130 h and with absorption rate constants differing 10-fold. However if the drug is employed on an acute basis as a hypnotic, it is possible the onset of action could be delayed with product 2. In such an instance a patient could potentially ingest additional doses in an attempt to achieve the desired effect. Depending on the number of doses taken, there exists the possibility of excessive ingestion of the more slowly absorbed product resulting in undesirable side effects or toxicity. Thus, on the basis of rate of absorption, the four tablet products cannot be considered bioequivalent.

REFERENCES

(1) J. A. Clements and D. Stanski, *Can J. Pharm. Sci.*, **6**, 9 (1971).

(2) N. Alvarez, E. Hartford, and E. Cavalleri, *Ann. Neurol.*, **9**, 309 (1981).

(3) C. T. Viswanathan, H. E. Booker, and P. G. Welling, *J. Clin. Pharmacol.*, **18**, 100 (1978).

(4) E. Nelson, J. R. Powell, K. Conrad, K. Likes, J. Byers, S. Baker, and D. Perrier, *J. Clin. Pharmacol.*, **22**, 141 (1982).

(5) M. F. Sylvestri and C. T. Ueda, *Intl. J. Clin. Pharmacol. Biopharm.*, **17**, 492 (1979).

(6) M. C. Meyer, B. J. Holcombe, G. J. Burckart, G. Raghow, and M. K. Yau, *Clin. Pharm. Ther.*, **33**, 329 (1983).

(7) E. Purich, in "Drug Absorption and Disposition: Statistical Considerations," K. S. Albert, Ed., American Pharmaceutical Association, Washington, D.C., 1980, p. 123.

(8) W. G. Cochran and G. M. Cox, "Experimental Designs," 2nd ed., Wiley, New York, N.Y., 1957, p. 125.

(9) C. T. Viswanathan, H. E. Booker, and P. G. Welling, *J. Clin. Pharmacol.*, **19**, 282 (1979).

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