CHAPTER III

RESULTS AND DISCUSSION

In Vitro Studies

The results of the *in vitro* tests were summarized in Table 2. Each of the six commercial brands of praziquantel Tablets met the BP requirements for uniformity of weight and standard for content of active ingredient in tablet. Each brand had its own weight within the range of limit weight (±5 %). Assayed products indicated that each brand was within the 95-105 % limits. These data supported the assumption that all various brands were chemically equivalent.

Neither U.S.P. XXI nor B.P. 1980 contains a disintegration time specification for praziquantel tablets. However, disintegration time requirements are currently official for general film-coated tablets. In this study, five of the six brands of praziquantel tablets met the B.P. 1980 requirements for disintegration in distilled water at 37 °C within 60 minutes. Only brand D failed to disintegrate. Although 0.1 M hydrochloric acid was used as the test solvent, the product remainly disintegrated in acid medium over one hour.

Figures2 and 3 illustrated the dissolution profiles of all six brands of praziquantel tablets in simulated gastric fluid without enzyme [I] and in simulated intestinal fluid without enzyme [II], respectively. Numerous differences were observed for the rates and extent of dissolutions of the different drug products. As seen in

Table 2 Physical Characteristics of Six Commercial Brands of Praziquantel Tablets in Vitro Studies.

Brand	Weight[a] (gm)	Assay,%[b] of Labelled amount	Disintegration[c] Time (min)	Dissolution Rate[c] Percent Dissolved in Dissolution Medium after 60 min.	
				pH 1.2	pH 7.5
A	0.930±0.012	101.9±0.42	8.75±1.08	35.26±0.26	27.38±0.29
B	0.931±0.020	105.0±0.28	5.25±1.04	38.16±1.03	29.98±0.32
C	0.926±0.016	103.8±0.56	24.08±1.96	23.64±1.00	33.16±0.67
D	0.933±0.011	104.4±0.28	66.6±10.27	2.56±0.28*	7.33±3.89*
E	0.936±0.008	99.9±0.14	7.08±3.18	30.86±0.44	27.88±0.34
F	0.966±0.002	103.4±0.28	22.83±2.91	31.17±0.33	24.77±0.36

a Values are mean ± Standard deviation (n = 20)

b Values are mean ± Standard deviation (n = 2)

c Values are mean ± Standard deviation (n = 6)

* Calculated by extrapolating standard curve

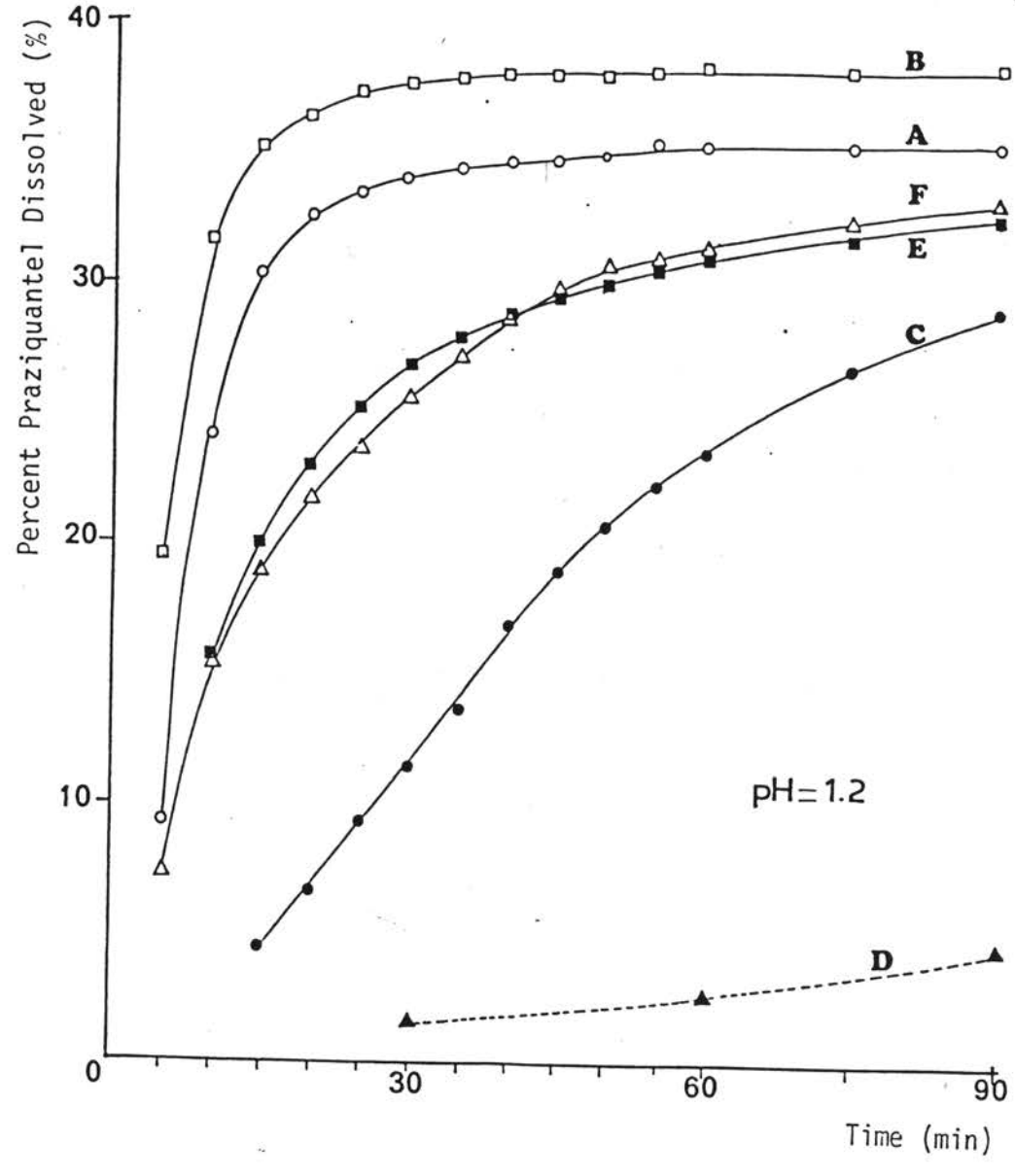

Figure 2 Dissolution profile of six commercial brands of praziquantel tablets in simulated gastric fluid without enzyme (pH 1.2) key : Brand A (○), Brand B (□), Brand C (●), Brand D (▲), Brand E (■) and Brand F (△).

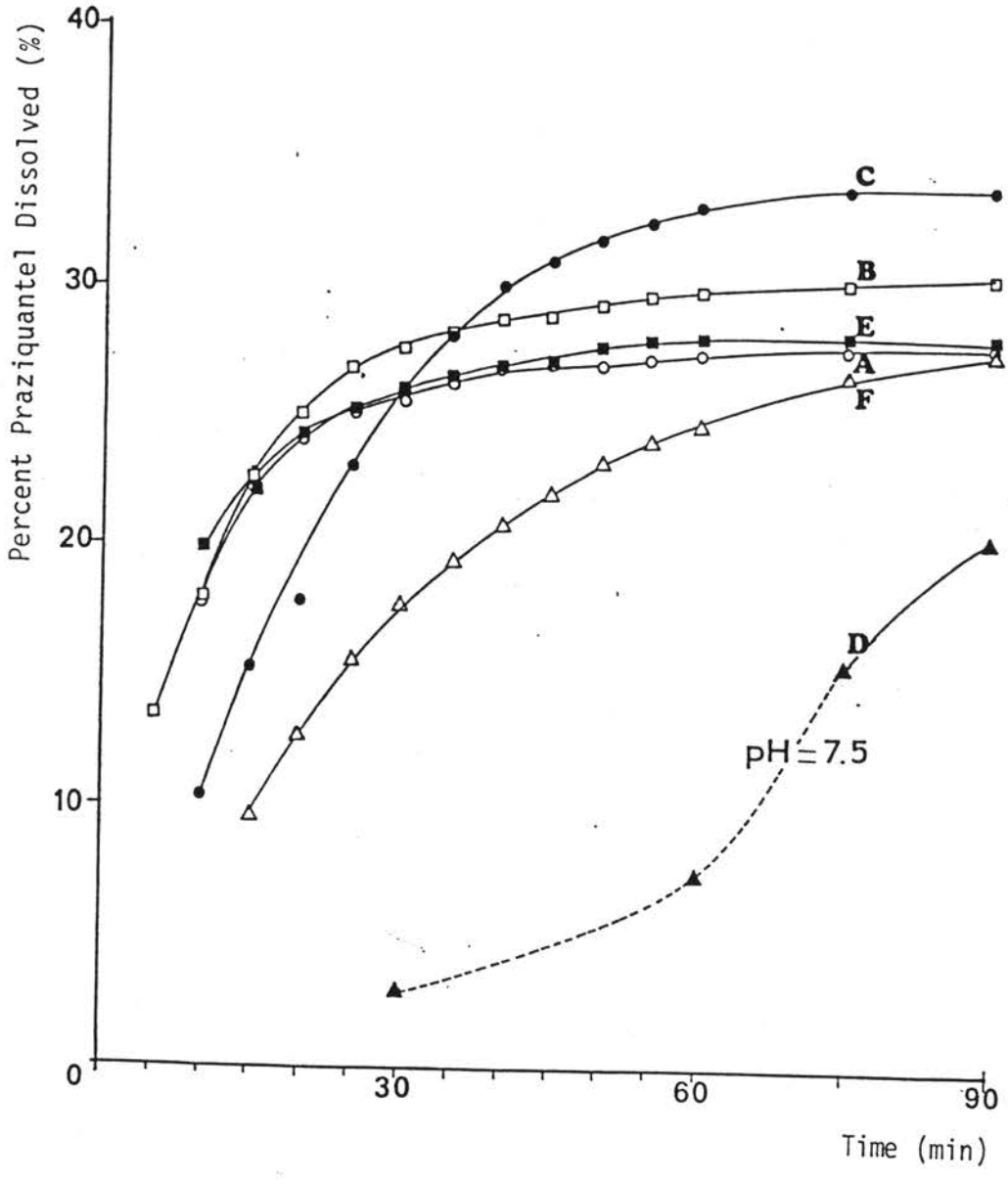

Figure 3 Dissolution profile of six commercial brands of praziquantel tablets in simulated intestinal fluid without enzyme (pH 7.5) key : Brand A (○), Brand B (□), Brand C (●), Brand D (▲), Brand E (■) and Brand F (△).

Table 2, the mean percent drug dissolved at 60 minutes ranged from 2.56 to 38.16 % and 7.33 to 33.16 % in [I] and [II], respectively. Rank orders of six brands in terms of mean percent dissolved in [I] after 60 minutes (maximum to minimum dissolved product)were : Brand B $>$ Brand A $>$ Brand F , Brand E $>$ Brand C $>$ Brand D (at $p < 0.05$). While the rank orders of dissolution in [II] were : Brand C $>$ Brand B$>$ Brand A, Brand E $>$Brand F $>$ Brand D (at $p < 0.05$) (see Appendix E). All products except brand D, were significantly greater than 20 % dissolved of drug after 60-minutes of sampling time in both media. However, there were large ranges of tablet dissolution rates as indicated in Table 2. These variations might be due to differences in manufacturing processes and/or sources of praziquantel supplied for use in tabletting. The possible interactions among components for tabetting might contribute to result in these different variations (47) Failure of the film-coated tablet of brand D to disintegrate may significantly delay the process of drug dissolution. This is due to limited surface area to expose to dissolution media. While the slower release rate in simulated gastric fluid for brand C was expected due to the poor solubility of film-coating materials in acid. The dramatic increase in the release rates when dissolution medium was changed to simulated intestinal fluid was not surprising(48).

In Vivo studies

Assay for Praziquantel in Serum

Typical chromatogram from serum containing both praziquantel and internal standard was shown in Figure 4. Retention times for praziquantel and internal standard were 6.83 and 8.61 min, respectively. The analytical procedure was highly specific and reproducible. Chromatographic response was readily for serum praziquantel concentrations ranging from 0.05 to 3 µg/ml (see Appendix C, Figure 11). The reproducibility of the method obtained using multiple replication (n=5) was within ± 3.23 % S.D. at the highest concentration and ± 15.94% S.D. at the lowest concentration. The sensitivity of praziquantel detection in human serum was 5 ng/ml. The variation in peak areas between these quantitations was only 5.14% (n=5).

Dose - Related Side Effects

Preliminary studies (6,7,8,9,10,11) with praziquantel indicated that the administration with high dose of drug may induce some side-effects, especially nausea and vomiting. To avoid these symptoms, each subject was permitted to have an identical standard breakfast 30-minutes prior to dose. In this study, side effects were noted following praziquantel administration and they were judged by the subjects. The side effects primarily originated within 1-4 hours postdose. They were generally typical manifestation of transient drowsiness and weakness. However,only few case were observed. None of the subjects had to break off the trials before completion.

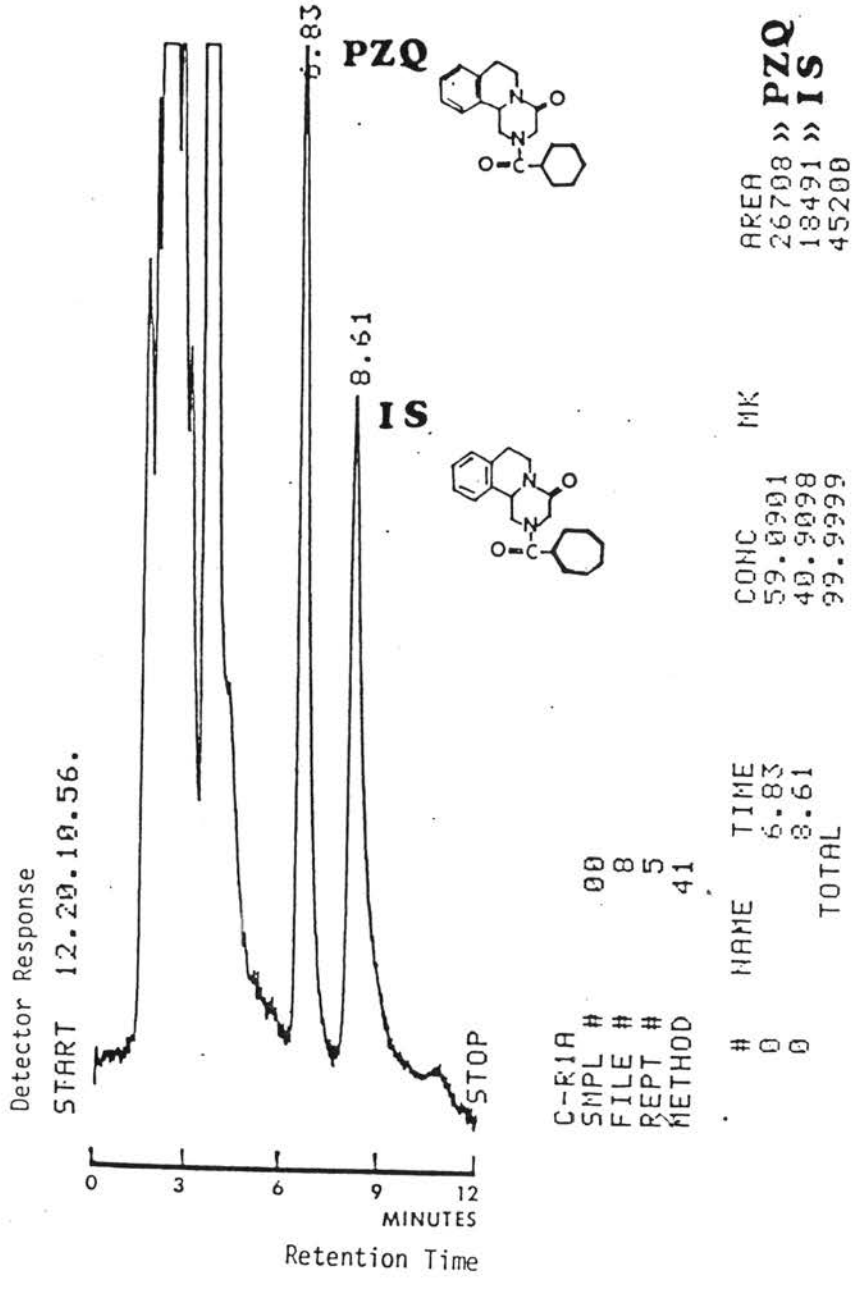

Figure 4 High pressure liquid chromatographic chromatogram[a] of praziquantel (PZQ) and internal standard (IS)

[a]Obtained from HPLC analysis of human serum containing 1 μg/ml of praziquantel and 0.8 μg/ml of internal standard

Serum Praziquantel Level

The individual praziquantel serum concentrations for each product at each appropriate sampling time from 0 to 8 hours were shown in table 3. The average values were illustrated graphically in Figure 5. Each point in the figure represents the mean value of 8 subjects and the bars represent the standard errors. Comparision among treatments were also summarized graphically in Figure 6.

Bioavailability of Praziquantel

The bioavailability of a drug from tablet dosage forms depends on both the rate and the extent of durg absorption into the general circulation (49). These factors can be evaluated by determining the pharmacokinetic parameters derived from blood level-time profiles for an unchanged drug. Bioequivalence is assured when the serum level-time curves for pharmaceutical equivalence of tablets are superimposable. Bioequivalence can also be established by comparing the peak serum concentrations of the drug, the times of the peak concentration, and the extent of absorption as reflected by the areas under the serum level-time curves.

In general, relative bioavailability is a relative amount of drug as compared to the most available from or compared to that of an original brand.

In this study, comparative bioavailability of praziquantel tablets was evaluated by comparing the selected local manufactured brands which passed *in-vitro* tests to that of original product (Brand A) with respect to the peak serum levels ($C_{p\ max}$), times to peak

Table 3 Individual Serum Praziquantel Concentrations from 8 Subjects Following Oral Administration of 40 mg/kg of Four Different Brands of Praziquantel Tablets

Brand	Time (hr)	Subject no. 1	2	3	4	5	6	7	8	Mean	SEM
A	0.5	0.207	0.005	0.364	1.817	0.019	0.133	0.053	0.274	0.358	0.213
	1.0	0.537	0.015	1.244	2.118	0.012	1.030	0.560	0.959	0.810	0.246
	1.5	1.127	0.140	1.362	1.778	0.114	1.306	1.000	1.401	1.028	0.212
	2.0	1.552	0.443	2.492	1.355	0.367	2.115	1.524	2.252	1.512	0.279
	3.0	1.233	0.661	0.904	0.657	1.456	1.071	1.064	1.433	1.060	0.110
	4.0	0.533	0.842	0.562	0.399	0.786	0.772	0.644	0.811	0.669	0.056
	5.0	0.268	0.376	0.240	0.215	0.418	0.435	0.278	0.379	0.326	0.115
	8.0	0.047	0.050	0.053	0.042	0.073	0.154	0.095	0.136	0.081	0.015
B	0.5	0.063	0.073	0.050	1.918	0.023	0.042	0.005	1.269	0.430	0.262
	1.0	1.446	0.096	0.302	2.443	0.021	0.125	0.268	2.102	0.850	0.350
	1.5	1.233	0.155	0.936	1.864	0.149	0.748	1.513	2.425	1.131	0.282
	2.0	1.106	0.515	1.466	1.350	0.720	1.548	1.191	1.905	1.225	0.159
	3.0	0.721	0.735	0.745	0.612	1.450	1.137	0.791	1.052	0.905	0.100
	4.0	0.422	0.505	0.426	0.357	0.874	1.002	0.553	0.522	0.582	0.082
	5.0	0.184	0.436	0.177	0.184	0.367	0.620	0.241	0.127	0.361	0.079
	8.0	0.094	0.141	0.057	0.025	0.097	0.062	0.137	0.062	0.084	0.014
C	0.5	0.133	0.088	0.075	0.085	0.029	0.021	0.005	0.063	0.062	0.015
	1.0	0.467	0.304	0.227	0.785	0.010	0.495	0.085	0.837	0.401	0.107
	1.5	1.415	0.290	0.850	0.825	0.106	1.629	0.325	1.495	0.867	0.211
	2.0	1.282	0.289	1.065	1.706	0.308	1.371	0.808	1.428	1.032	0.185
	3.0	0.806	0.574	0.687	0.873	1.241	0.885	1.296	1.183	0.943	0.094
	4.0	0.340	0.501	0.506	0.443	0.855	0.554	0.835	0.510	0.568	0.064
	5.0	0.162	0.548	0.377	0.214	0.527	0.251	0.557	0.240	0.360	0.064
	8.0	0.026	0.059	0.114	0.032	0.191	0.129	0.113	0.073	0.092	0.020
D	0.5	0.042	0.030	0.005	0.362	0.000	0.033	0.028	0.006	0.063	0.043
	1.0	0.052	0.033	0.019	1.275	0.009	0.342	0.033	0.028	0.211	0.143
	1.5	0.065	0.031	0.048	1.867	0.025	1.227	0.065	0.449	0.472	0.247
	2.0	0.161	0.047	0.746	1.307	0.084	1.450	0.267	0.493	0.569	0.195
	3.0	0.902	0.098	0.977	0.602	1.144	0.886	0.624	0.649	0.750	0.117
	4.0	0.574	0.208	0.777	0.316	0.790	0.575	0.809	0.792	0.605	0.083
	5.0	0.350	0.258	0.435	0.108	0.523	0.362	0.969	0.298	0.413	0.091
	8.0	0.082	0.080	0.121	0.050	0.154	0.105	0.243	0.076	0.114	0.022

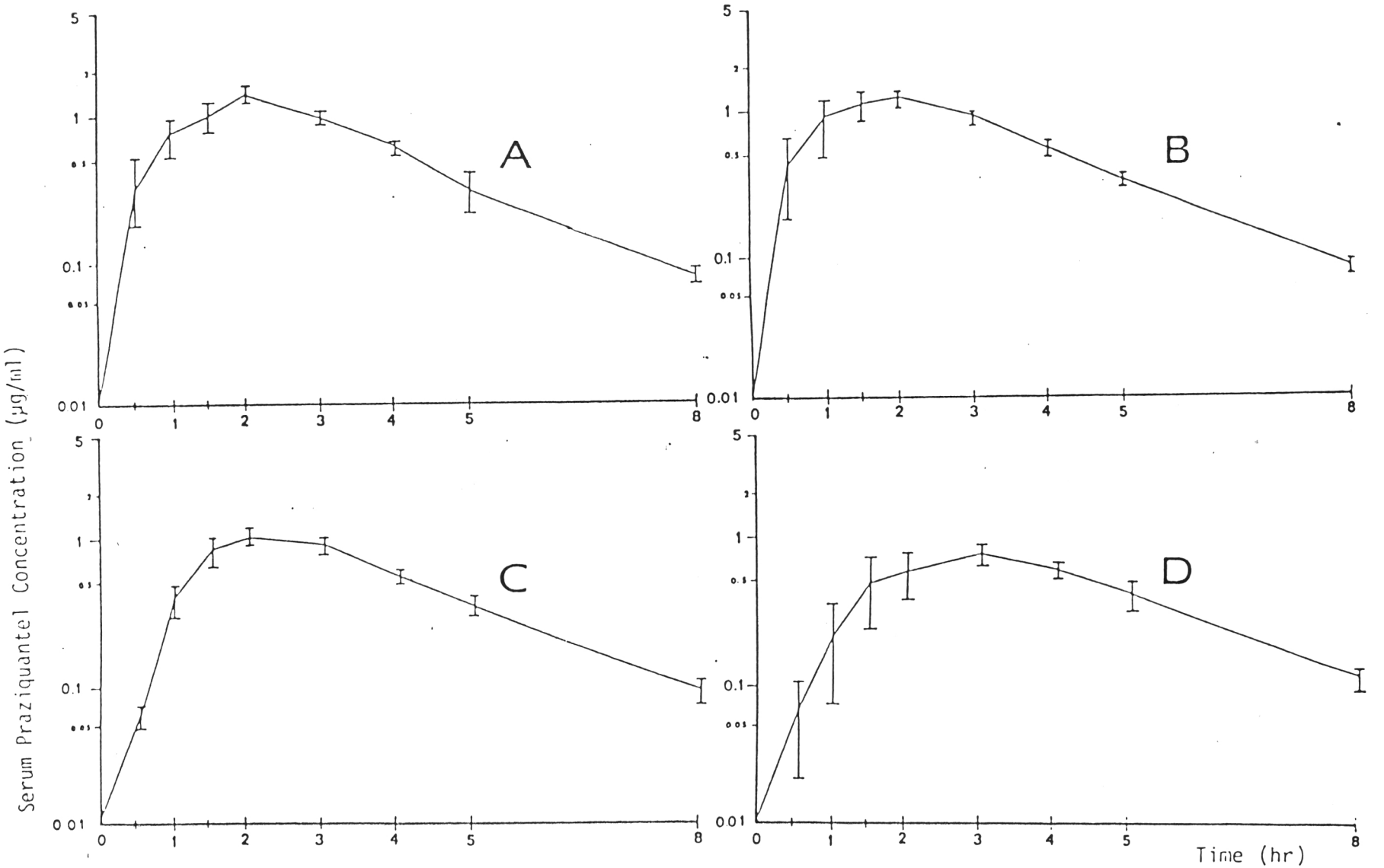

Figure 5 Serum praziquantel concentrations (Mean±SEM) from 8 subjects following oral administration of 40 mg/Kg of four different brands (Brand A, B, C and D)

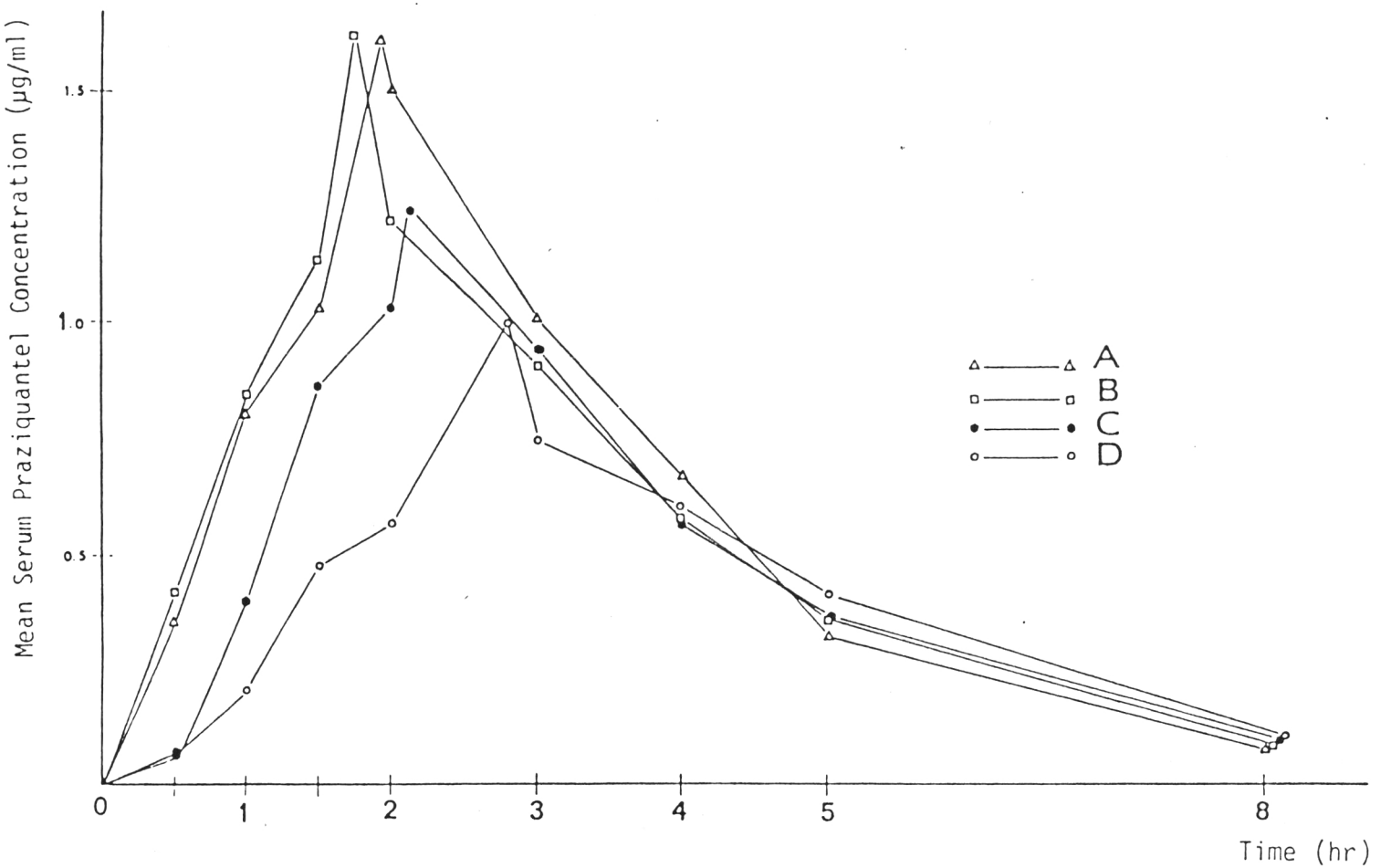

Figure 6 Comparision of mean serum praziquantel concentration-time curves from 8 subjects following oral administration of 40 mg/kg of four different brands of praziquantel tablets.

Key : Brand A (△), Brand B (□), Brand C (●), and Brand D (○).

(t_{max}), the area under the serum level-time curves [$AUC]_0^\infty$ and/or the absorption rate constants (K_a).

Table 4 showed the statistical comparison of the parameters obtained after oral administration of 4 diffent brands of praziquantel tablets in 8 subjects. There were significantly different from each other according to the one-way analysis of variance and the Student's t-test ($p < 0.05$) (Appendix E). The degree of uniformity of individual data within the group of subjects were expressed as the coefficient of variations (C.V.).

Peak Serum Concentration

Previous reports (13,15,16) indicated that the average peak serum levels achieved following oral administration of praziquantel could vary widely. Putter and Held (16) reported a peak plasma praziquantel level of 1.36 µg/ml for averaged data obtained in a study of five lactating women receiving a 50 mg/kg dose of drug, with individual peak level ranging from 0.515 to 2.81 µg/ml.

In the present study, the average of the peak levels for each of the four brands of praziquantel tablets, obtained by averaging individual peak level as calculated for each subject, was rangedfrom 0.240 to 2.535 µg/ml in 8 subjects following a single oral dose of 40 mg/kg. When comparing the mean peak values of individual serum concentration-time curves, statistically significant difference were observed. Peak serum levels of brands A and B were higher than that of brand D. While there were no statistically differences between brand A and B, A and C, and C and D. ($p > 0.05$).

Table 4 **Pharmacokinetic Parameters** for Praziquantel from 8 Subjects following Oral Administration **of** 40 mg/kg of Four Different Brands of Praziquantel Tablets

Brand	$C_{p\ max}$ (μg/ml)			t_{max} (hr)			$[AUC]_0^{\infty}$ ($hr.\mu g.ml^{-1}$)		
	Mean	SEM	CV	Mean	SEM	CV	Mean	SEM	CV
A	1.614	0.170	0.30	1.93	0.22	0.32	4.8298	0.3219	0.19
B	1.625	0.207	0.36	1.72	0.26	0.44	4.4072	0.3981	0.26
C	1.247	0.123	0.28	2.14	0.22	0.29	3.9099	0.1794	0.13
D	1.007	0.150	0.42	2.81	0.37	0.37	3.3743	0.3664	0.31
F-test[a]	$F_{3,28} = 3.31$			$F_{3,28} = 2.95$			$F_{3,28} = 3.69$		
Paired-t-test[a,b]	A > D NS between A,B,C			D > A NS between A,B,C			A > C,D NS between A,B		
% Difference	37.6 %			45.6 %			19.0 %, 30.1 % respectively		

$C_{p\ max}$ mean individual peak serum levels

t_{max} mean individual time to peak

$[AUC]_0^{\infty}$ mean area under the serum concentration-time curve

SEM standard error of the mean

CV coefficient of variation

a significant at $p < 0.05$

NS No significant diffence at $p > 0.05$

b Brand A was assigned as the reference standard against Brand B,C, and D

Time of Peak Serum Level

In each case, the time required to reach peak serum praziquantel level was 3 hours or less, indicating relatively rapid absorption of the drug following oral administration. The average peak times ranged from 1.72 to 2.81 hours. These values were in agreement with a mean value of 1.88 hours as reported previously (13).

From Table 4, the order of four brands in terms of peak time were : Brand D > Brand C > Brand A > Brand B. There were statistically significant ($p < 0.05$) diffences only brands A *vs* D and brands B *vs* D.

Area Under Serum Level-Time Curve

Table 4 summarized the estimated average area under the serum level-time curves. Results showed that the relative bioavailability of praziquantel for brands B,C and D were 91.25 %, 80.95 % and 69.86 %, respectively, with respect to brand A which was assigned as a reference brand. Statistical analysis of differences among $[AUC]_0^\infty$ values indicated that there were significant differences ($p < 0.05$) observed between brands A and C and brands A and D (Appendix E)

The values of the coefficient of variation were high in each parameters, indicating distinct interindividual variations. This may be due to praziquantel undergoes "first-pass-effect" prior to reach general circulation.

Bioequivalence Evaluation

Results in Table 4 showed that there were more than 20 percent differences in C_{pmax}, t_{max}, and $[AUC]_0^\infty$ values between brand D and brand A.

This suggested that brand D and brand A were bioinequivalent. In contrast, brands A and B yielded almost identical serum praziquantel level-time curve and there were no statistically difference ($p > 0.05$) between any of the products in terms of $C_{p\ max}$, t_{max} and $[AUC]_0^{\infty}$, indicating these products were bioequivalent. Although the statistical comparisions revealed no significant differences in t_{max} and C_{max} except $[AUC]_0^{\infty}$ after oral administration of brand A and brand C, these products were bioequivalent regarding to the rate of absorption.

In general observed differences in oral bioavailabilities may be due to differences in manufacturing process and/or formulations (50). Thus, the poor bioavailability of brand D may be due to unsatisfactory disintegration and/or dissolution, which may reflect in reduction of the amount of praziquantel available in those area of the upper gastro-intestinal tract where the most rapid absorption takes place (47).

Pharmacokinetic of Proziquantel Tablets

Based on the semilogarithmic plots of individual serum concentration-time data for 8 subjects, the data were assumed to follow the classical one-compartment model with or without a lag time (15). Using PCNONLIN (nonlinear estimation program) on a digital computer, the initial estimates of the parameters were obtained. The goodness of fit was tested by comparing the values of individual sum of squares of the deviations between experiment data and calcutated values. Results showed that one-comparment model with a lag time had a better fit (Appendix G). The average correlation coefficient observed for individual serum level data of the fit to one-compartment model was 0.97±0.04.

The pharmacokinetic data obtained from individual serum data of 8 subjects following oral administration of dose 40 mg/kg of four different brands of praziquantel tablets were summarized in Table 5. An analysis of variance and Student's t - test ($p < 0.05$) were performed for significant differences among or between related parameters.

The mean values of peak serum concentration (C_{pmax}) ranged from 1.007 to 1.625 µg/ml ; the mean time of the peak serum level (t_{max}) ranged from 1.72 to 2.81 hours, and the mean $[AUC]_0^\infty$ ranged from 3.3743 to 4.8298 $hr.\mu g.ml^{-1}$. These three parameters were in agreement with those reported previously for praziquantel by other investigators (13,15,16). Leopold *et al* (13) reported that the mean individual peak serum concentration of praziquantel after oral administration of the drug with 50 mg/kg was 1.319 µg/ml and reached at 1.88 hours after dosing. Also, the mean area under the serum concentration-time curve $[AUC]_0^\infty$ was 3.931 $hr.\mu g.ml^{-1}$. Tawatsin *et al* (51) demonstrated that praziquantel kill *Opisthorchis viverini* quickly at concentration of 0.1 - 2.0 µg/ml in *in-vitro* experiment.

The values of absorption rate constants (K_a), estimated using computer program, were 1.97, 3,96, 2.07, and 1.65 hr^{-1} for brands A,B, C, and D, respectively. No statistically significant differences among these values were observed ($p > 0.05$). The overall elimination rate constant (K_e) and the serum half-life ($t_{\frac{1}{2}}$) were 0.66 hr^{-1} and 1.15 hr. These were comparable to those 0.58 hr^{-1} and 1.19 hr as reported by Leopold *et al* (13). The mean apparent volume of distribution (V_d) was 18.24 L/kg with a range of 17.29-25.74 L/kg. There were no statistically significant differences from each others($p > 0.05$).The mean lag time(t_0) for all subjects and brands was 1.12 hours (range 0.13 to 2.71 hours).

Table 5 Estimated Pharmacokinetic Parameters (Mean ± SEM) for Praziquantel from 8 subjects following Oral administration of 40 mg/kg of Four different Brands of Praziquantel Tablets

Parameter	Brand				Statistical[a] Significance
	A	B	C	D	
Peak serum concentration, $C_{P\ max}$ ($\mu g/ml$)	1.614 ± 0.170	1.625 ± 0.207	1.247 ± 0.123	1.007 ± 0.150	A > D B > D
Time to peak concentration, t_{max} (hr)	1.93 ± 0.22	1.72 ± 0.26	2.14 ± 0.22	2.81 ± 0.37	A < D B < D
Area under the serum concentration-time curve from the time zero to infinity, $[AUC]_0^{\infty}$ ($hr.\mu g.ml^{-1}$)	4.8298± 0.3219	4.4072± 0.3981	3.9099± 0.1794	3.3743± 0.3664	A > C,D B > D
Delay of absorption or the lag time, t_o (hr)	0.84 ± 0.21	0.94 ± 0.20	1.08 ± 0.14	1.61 ± 0.28	NS
Absorption rate constant, K_a (hr^{-1})	1.97 ± 0.96	3.96 ± 1.65	2.07 ± 0.60	1.65 ± 0.45	NS
Overall elimination rate constant, K_e (hr^{-1})	0.75 ± 0.04	0.65 ± 0.10	0.64 ± 0.08	0.60 ± 0.08	NS
Serum half-life, $t_{\frac{1}{2}}$ (hr)	0.94 ± 0.05	1.22 ± 0.18	1.20 ± 0.14	1.25 ± 0.11	NS
Apparent volume of distribution, V_d ($L_{/kg}$)	11.88 ± 1.50	17.29 ± 3.05	18.03 ± 2.42	25.74 ± 6.17	NS

a significant level at $p < 0.05$

NS no significant difference at $p > 0.05$

This value was greater than that reported by Putzschke *et al.* (15) by 0.3 hour. This may be due to the effect of foods in delaying the absorption process of praziquantel (13).

The pharmacokinetic parameters obtained from this study were slightly different from those reported by other investigators (13,15, 16). The factors possibly responsible to the differences were, ie, the subjects participated in the studies, the differences in their races, ages, weights and normal habits, the mathemetical model applied and assumptions used to interpret the data.

In Vitro-In Vivo Correlations

Table 6 summarized the relationships observed between various *in vitro* and *in vivo* parameters. Good correlations were significantly found between disintegration times and dissoluion rates in both dissolution media, indicating disintegration times of tablets might be rate-limiting step of praziquantel dissolution. Excellent correlations were found between *in vivo* pharmacokinetics parameters($C_{p\ max}$, t_{max}, and $[AUC]_0^{\infty}$) and the mean percent drug dissolved at 60 min in simulated gastric fluid without enzyme, with correlation coefficients of 0.97, -0.99, and 0.92, respectively. Likewise, the correlations of the same *in vivo* pharmacokinetic parameters with the mean *in vitro* disintegration time were -0.94, 0.99, and -0.90, respectively. However the latter correlations were not as good as the values mentioned above. This may support the fact that the correlations between bioavailability and dissolution rate of solid dosage forms were more meaningful than those with disintegration times for certain drug (52).

Table 6 *In Vitro - In Vivo* correlations

Correlation	Degree of[a] Freedom	Correlation coefficient	t-Value	p Level
Disintegration[b] *versus* dissolution[c]	4 (4)	-0.97 (-0.88)	7.98 (3.70)	$p < 0.05$ ($p < 0.05$)
Disintegration *versus* peak serum level	2	-0.94	3.90	$p < 0.1$
Disintegration *versus* time of peak	2	0.99	9.92	$p < 0.05$
Disintegration *versus* $[AUC]_0^{\infty}$	2	-0.90	2.92	$p < 0.1$
Dissolution *versus* peak serum level	2 (2)	0.97 (0.69)	7.98 (1.35)	$p < 0.05$ (NS)
Dissolution *versus* time of peak	2 (2)	-0.99 (-0.49)	9.92 (0.79)	$p < 0.05$ (NS)
Dissolution *versus* $[AUC]_0^{\infty}$	2 (2)	0.92 (0.61)	3.32 (1.24)	$p < 0.1$ (NS)

a Degree of Freedom = number of pairs - 2 (53)

b Dissintegration time (min) in water at temperature 37 °C

c Percent drug dissolved after 60 min. in simulated gastric fluid without enzyme (simulated intestinal fluid without enzyme)

NS no significant at $p > 0.1$

An attempt to correlate the mean percent drug dissolved at 60 min in simulated intestinal fluid without enzyme with each *in vivo* pharmacokinetic parameters were made. Poorly correlative values were obtained with correlation coefficients of 0.69, -0.49, and 0.66, respectively.

These results indicated that the bioavailability of praziquantel from oral tablets was dissolution-rate controlled. The rate and extent of praziquantel absorption increased with an increasing dissolution rate in simulated gastric fluid without enzyme. In the present study, the prediction of *in vivo* bioavailability may be sufficiently precise to permit applications of the *in vitro* testing procedure to evaluate the products.