CHAPTER 4

RESULTS

1. Method validation tests

1.1 Linearity of the standard calibration curve

The standard calibration curve for praziquantel at concentration of 12.25, 100, 200, 400 and 800 ng/ml was linear with the correlation coefficient (*r*) of 0.999 (Figure 6). The equation of linear regression line was $y = 5.587E\text{-}03 + 2.666E\text{-}03x$

1.2 Precision

In mobile phase, the intra-day assay was repeated 5 times per day and coefficients of variation (CV) for praziquantel were 6.061% at 12.25 ng/ml, 1.145% at 100 ng/ml, 1.498% at 200 ng/ml, 2.041% at 400 ng/ml and 0.429% at 800 ng/ml, whereas the inter-day assay was assessed on 10 individual days; the CV were 9.76% at 12.25 ng/ml, 3.33% at 100 ng/ml, 5.41% at 200 ng/ml, 4.05% at 400 ng/ml and 3.08% at 800 ng/ml. All results were shown in Table 1 and 2, respectively.

In human plasma, the intra-day assay was repeated 5 times per day and coefficients of variation (CV) for praziquantel were 7.50% at 12.25 ng/ml, 1.418% at 100 ng/ml, 0.930% at 200 ng/ml, 2.442% at 400 ng/ml and 1.684% at 800 ng/ml, whereas the inter-day assay was assessed on 10 individual days; the CV were 10.256 at 12.25 ng/ml, 6.086% at 100 ng/ml, 3.465% at 200 ng/ml, 3.333% at 400 ng/ml and 3.914% at 800 ng/ml. All results were shown in Table 3 and 4, respectively.

1.3 Accuracy

The mean absolute recovery values of the present HPLC method throughout the linear range were presented in Table 5. The results in Table 5 reflect that the method is obviously accurate and this ensures reliable results.

1.4 Limit of detection

The lower limit of detection (LOD) of praziquantel in plasma was approximately 12.25 ng/ml by considering a signal-to-noise of 3 :1.

2. Chromatograms

The chromatograms showed that a peak of praziquantel was well separated from the other peaks in plasma (Figure 7). There were no interference from the peak of ketoconazole or itraconazole in this analytical method. The retention time for praziquantel and diazepam (internal standard) were approximately 15 and 19 min, respectively. At figure 9, unidentified peak was not ketoconazole peak because we used ketoconazole solution (ketoconazole powder diluted with mobile phase after filtered through 0.45 micropore filtered paper [Nyron 66]) injected into the HPLC system. After injected had not any peak at time of unidentified peak in phase 2 so unidentified peak was not peak of ketoconazole.

3. The plasma concentration-time data of praziquantel

The mean plasma concentrations-time profiles of praziquantel after receiving praziquantel alone and pretreatment with ketoconazole or itraconazole were shown in Figure 11.

4. Adverse Effects

Ten male healthy volunteers were enrolled and completed in this study. In phase one, no side effects after taking 20 mg/kg praziquantel were reported. No serious side effects were observed after taking 400 mg of ketoconazole. However, two subjects reported mild nausea and vomiting, one subject reported mild GI disturbances and four subjects reported mild headache but did not need any medication. In phase 3, no serious side effects were observed after taking 200 mg of itraconazole. However, four subjects reported mild GI disturbances, two subjects reported mild nausea and vomiting and one subject reported mild headache but these side effects did not require any medication.

5. Pharmacokinetics

5.1 Pharmacokinetics of praziquantel alone in the single doses

After a single oral dose of 20 mg/kg praziquantel alone in ten subjects, the mean value of C_{max}, AUC_{0-12}, $AUC_{0-\infty}$, λ_z, $t_{1/2}$, T_{max} , Cl/f, V_z/f, *Ka* and MRT of praziquantel were 183.38 ± 138.82 ng/ml, 793 ± 895 ng/ml.hr, 956 ± 973 ng/ml.hr, 0.29 ± 0.22 hr^{-1}, 3.80 ± 3.03 hr, 2.05 ± 1.14 hr, 2.65 ± 2.03 l/kg/hr,10.75 ± 6.55 l/kg, 0.48 ± 0.40 hr^{-1} and 6.35 ± 3.20 hr, respectively (Table 7).

5.2 Pharmacokinetics of an oral single dose of 20 mg/kg praziquantel in subjects pretreated with 400 mg ketoconazole once daily for 5 day

The mean values of C_{max}, AUC_{0-12}, $AUC_{0-\infty}$, λ_z, $t_{1/2}$, T_{max}, Cl/f, V_z/f, *Ka* and MRT of praziquantel pharmacokinetic parameters after pretreatment with ketoconazole in ten subjects were 371.31 ± 141.10 ng/ml, 1563 ± 756 ng/ml.hr, 1843 ± 1064 ng/ml.hr, 0.23 ± 0.14 hr^{-1}, 4.05 ± 2.39 hr, 1.82 ± 0.92 hr, 1.11 ± 1.09 l/kg/hr, 4.42 ± 2.06 l/kg, 1.06 ± 0.41 $hr^{-1}$and 5.53 ± 2.44 hr, respectively (Table 8).

Praziquantel pharmacokinetic data pretreated by ketoconazole demonstrated the significant increase of the mean C_{max}, *Ka*, $AUC_{0-12,}$ and $AUC_{0-\infty}$ when compared with phase I by 102.48% (371.31 ± 141.10 ng/ml vs 183.38 ± 138.82 ng/ml; $P<0.01$), 119.96% (1.06 ± 0.41 hr^{-1} vs 0.481 ± 0.40 hr^{-1}; $P<0.01$), 97.09% (1563 ± 756 ng/ml.hr vs 793 ± 895 ng/ml.hr; $P<0.01$) and 92.78% (1843 ± 1064 ng/ml.hr vs 956 ± 973 ng/ml.hr; $P<0.01$), respectively, whereas significantly decreased Cl/f and V_z/f by 58.11% (1.11 ± 1.09 l/kg/hr vs 2.65 ± 2.03 l/kg/hr; $P<0.01$) and 58.88% (4.42 ± 2.06 l/kg vs 10.75 ± 6.55 l/kg; $P<0.05$), respectively. There were no significant difference in λ_z, $t_{1/2}$, T_{max} and MRT when compared with praziquantel alone (Table 6).

5.3 Pharmacokinetics of an oral single dose of 20 mg/kg praziquantel in subjects pretreated with 400 mg ketoconazole once daily for 5 day

The mean values of C_{max}, AUC_{0-12}, $AUC_{0-\infty}$, λ_z, $t_{1/2}$, T_{max}, Cl/f, V_z/f, *Ka* and MRT of praziquantel pharmacokinetic parameters after pretreatment with itraconazole in ten subjects were 312.80 ± 204.98 ng/ml, 1182 ± 826 ng/ml.hr, 2371 ± 3621 ng/ml.hr, 0.21

± 0.13 hours^{-1}, 10.55 ± 21.09 hr, 1.75 ± 1.17 hr, 2.19 ± 3.20 l/kg/hr, 11.90 ± 13.04 l/kg 0.88 ± 0.45 hours^{-1}and 14.26 ± 29.02 hr, respectively (Table 9).

Praziquantel pharmacokinetic data after pretreatment with itraconazole demonstrated significant increase the mean C_{max} and *Ka* by 70.58% (312.81 ± 204.98 ng/ml vs 183.38 ± 138.82 ng/ml; $P<0.05$) and and 83.38% (0.88 ± 0.45 hr^{-1} vs 0.48 ± 0.40 hr^{-1}; $P<0.01$) respectively. while there were no significant difference in AUC_{0-12}, $AUC_{0-\infty}$, λ_z, $t_{1/2}$, T_{max}, Cl/f, V_z/f and MRT when compared with praziquantel alone (Table 6).

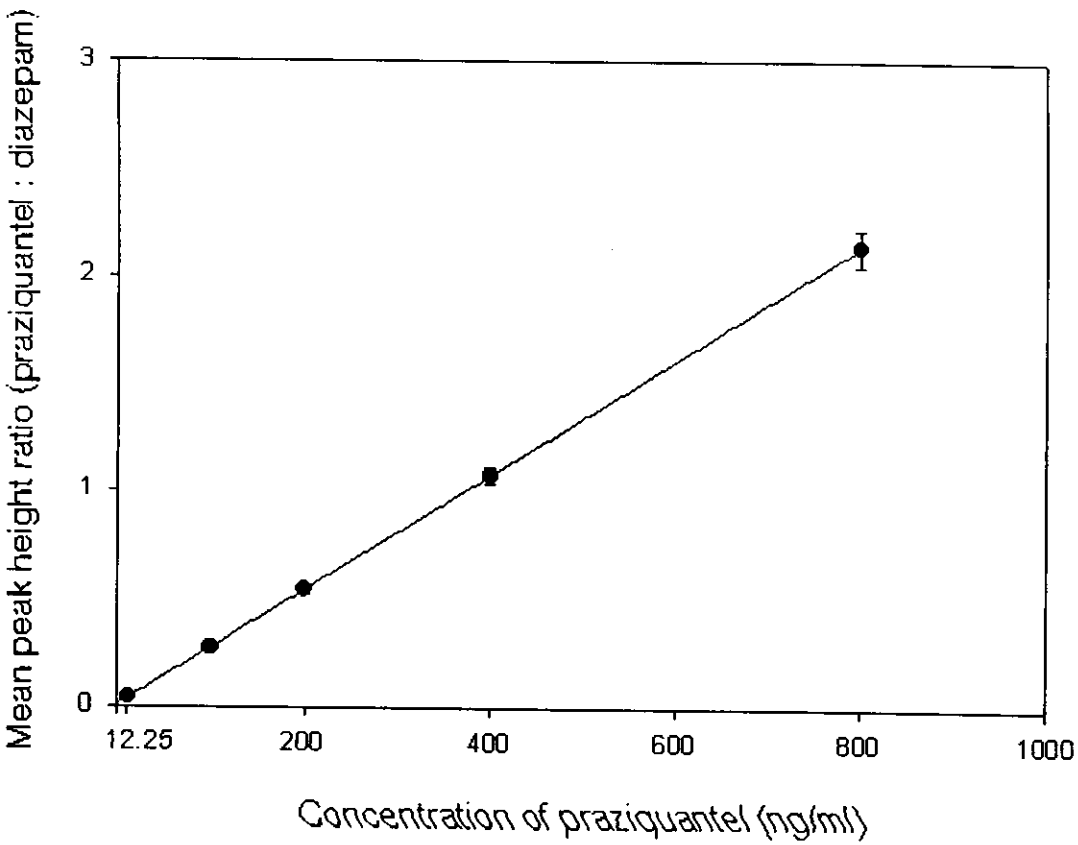


Figure 6 *Correlation between peak height ratio of praziquantel to diazepam* (internal standard) and concentration of praziquantel in plasma. The correlation coefficient (r) of regression line is 0.999999.

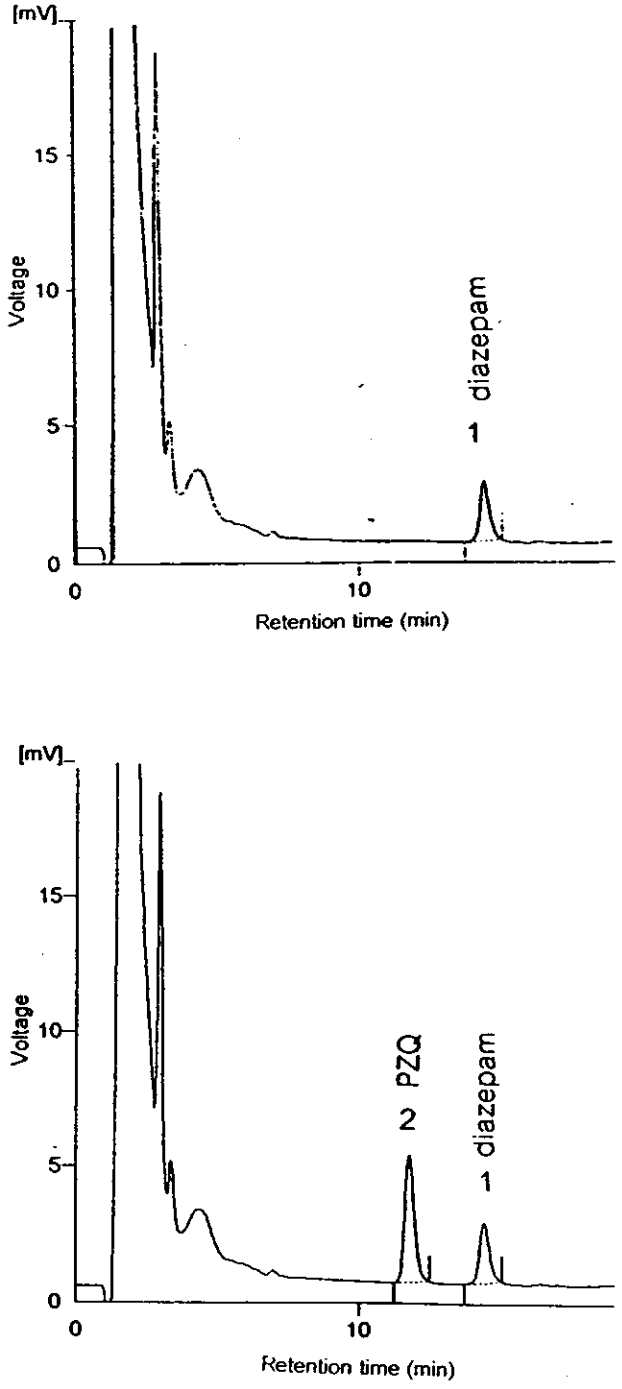
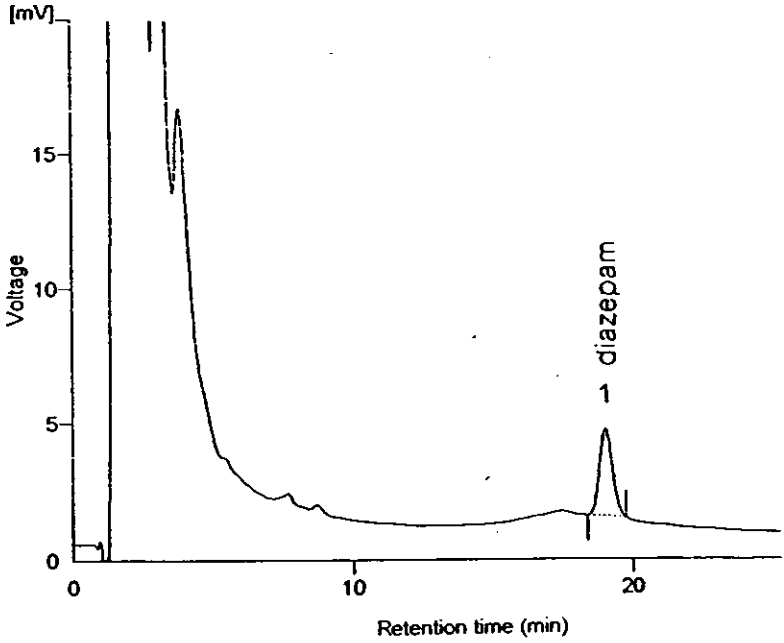
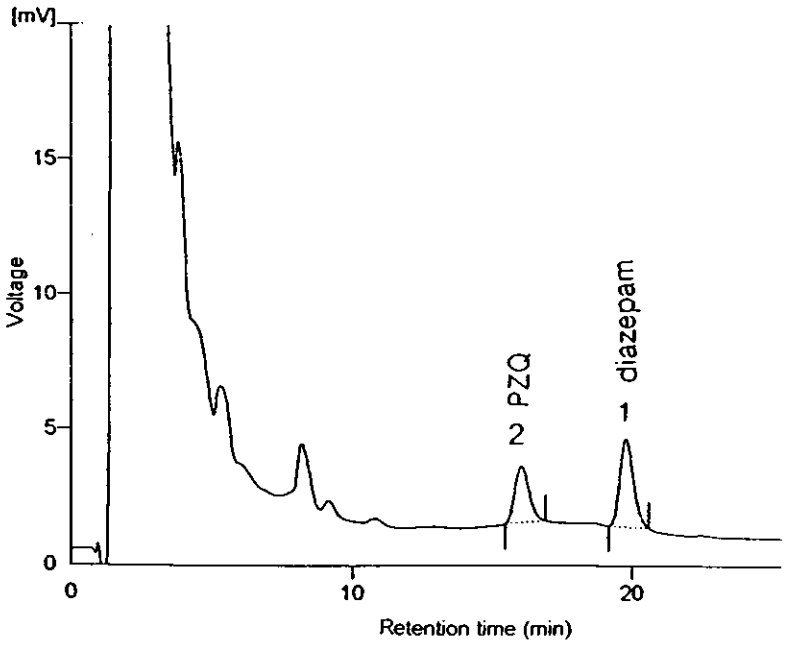


Figure 7 Representative chromatograms of 100 µl human plasma samples. Key : (A) blank human plasma spiked with internal standard diazepam; (B) spiked with standard praziquantel 800 ng/ml (1, internal standard diazepam; 2, PZQ (praziquantel)). The mobile phase consisted of deionized water-acetonitrile-methanol (56 : 34 : 10 vol/vol/vol) at flow rate of 1.5 ml/min.

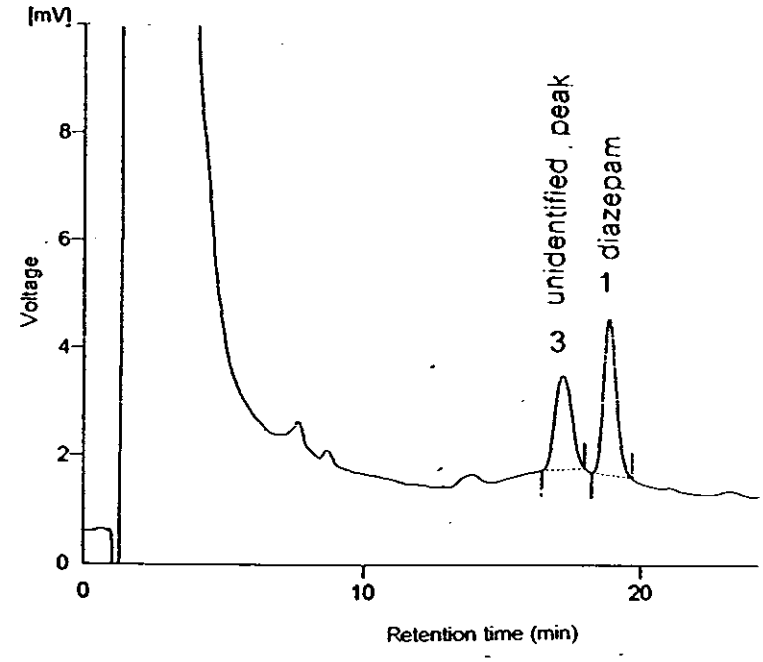


A

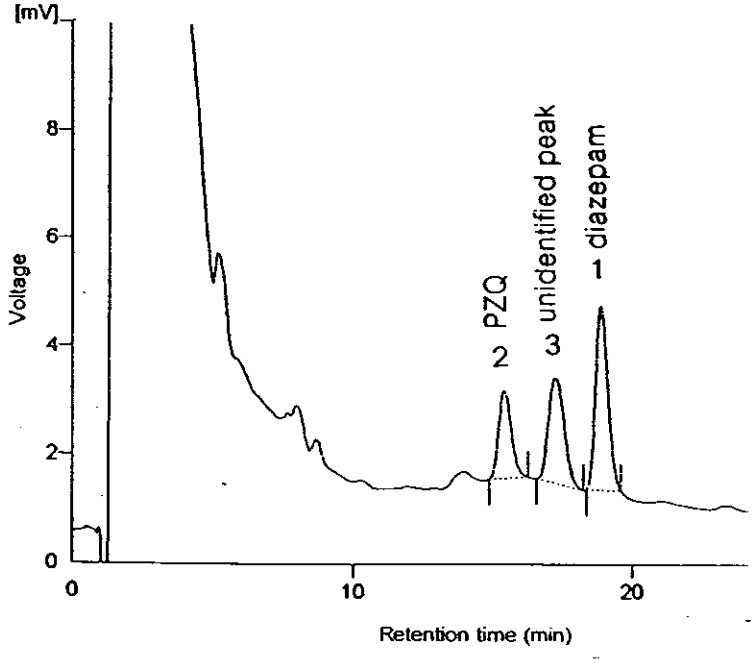


B

Figure 8 Representative chromatograms of 200 µl human plasma samples. Key : (A) blank human plasma spiked with internal standard diazepam; (B) plasma obtained from a subject receiving 20 mg/kg PZQ alone at 1.5 hours (1, internal standard diazepam; 2, PZQ). The mobile phase consisted of deionized water-acetonitrile-methanol (54 : 36 : 10 vol/vol/vol) at flow rate of 1.5 ml/min.



A



B

Figure 9 Representative chromatograms of 200 μl human plasma samples. Key : (A) human plasma after pretreatment with ketoconazole 5 days; (B) plasma obtained from a subject receiving 20 mg/kg PZQ single oral dose at 0.75 hours after pretreatment with ketoconazole (1, internal standard diazepam; 2, PZQ; 3, unidentified peak). The mobile phase consisted of deionized water-acetonitrile-methanol (56 : 34 : 10 vol/vol/vol) at flow rate of 1.5 ml/min.

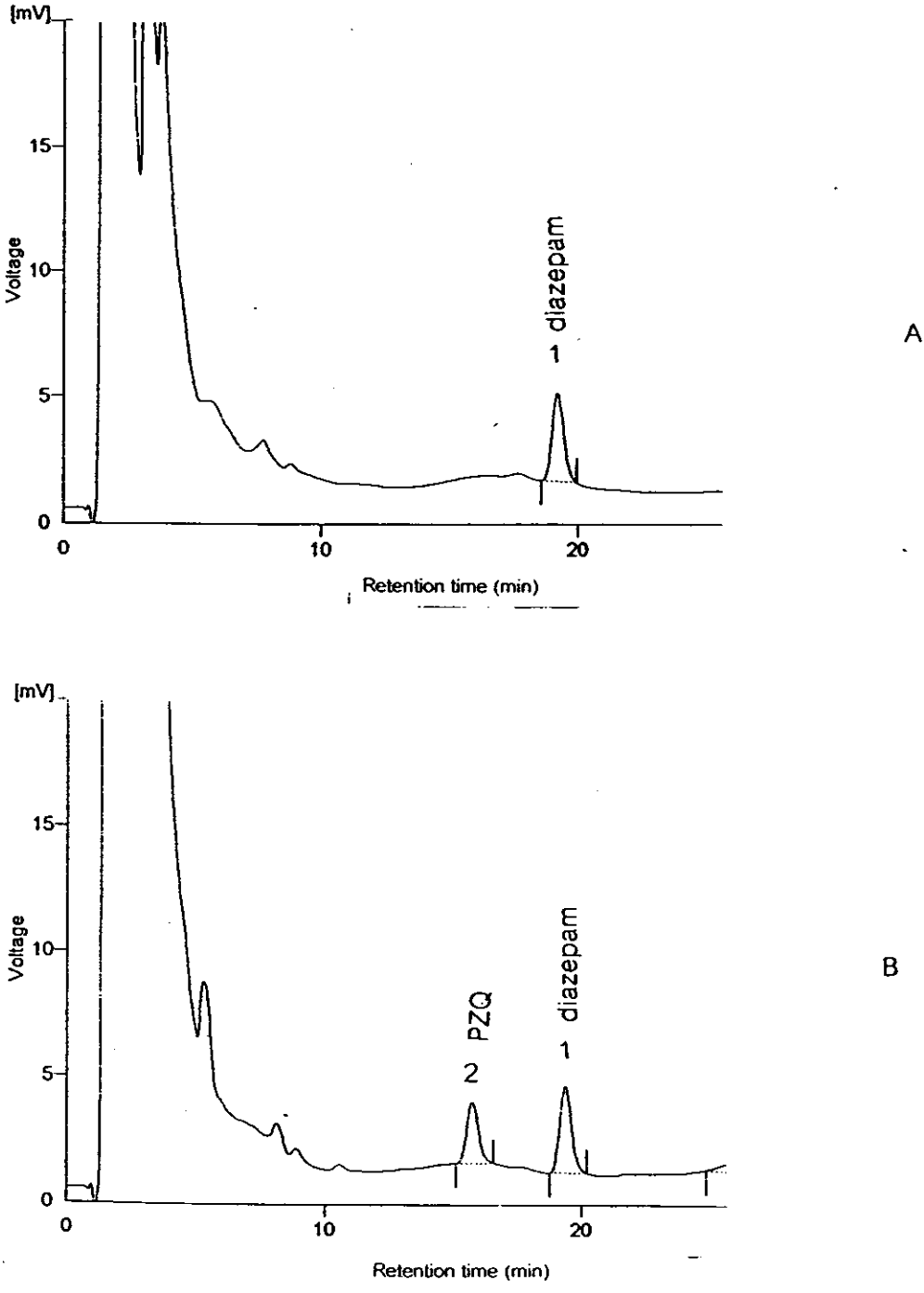


Figure 10 Representative chromatograms of 200 µl human plasma samples. Key : (A) human plasma after pretreatment with itraconazole 5 days; (B) plasma obtained from a subject receiving 20 mg/kg PZQ single oral dose at 0.75 hours after pretreatment with itraconazole (1, internal standard diazepam; 2, PZQ). The mobile phase consisted of deionized water-acetonitrile-methanol (56 : 34 : 10 vol/vol/vol) flow rate of 1.5 ml/min.

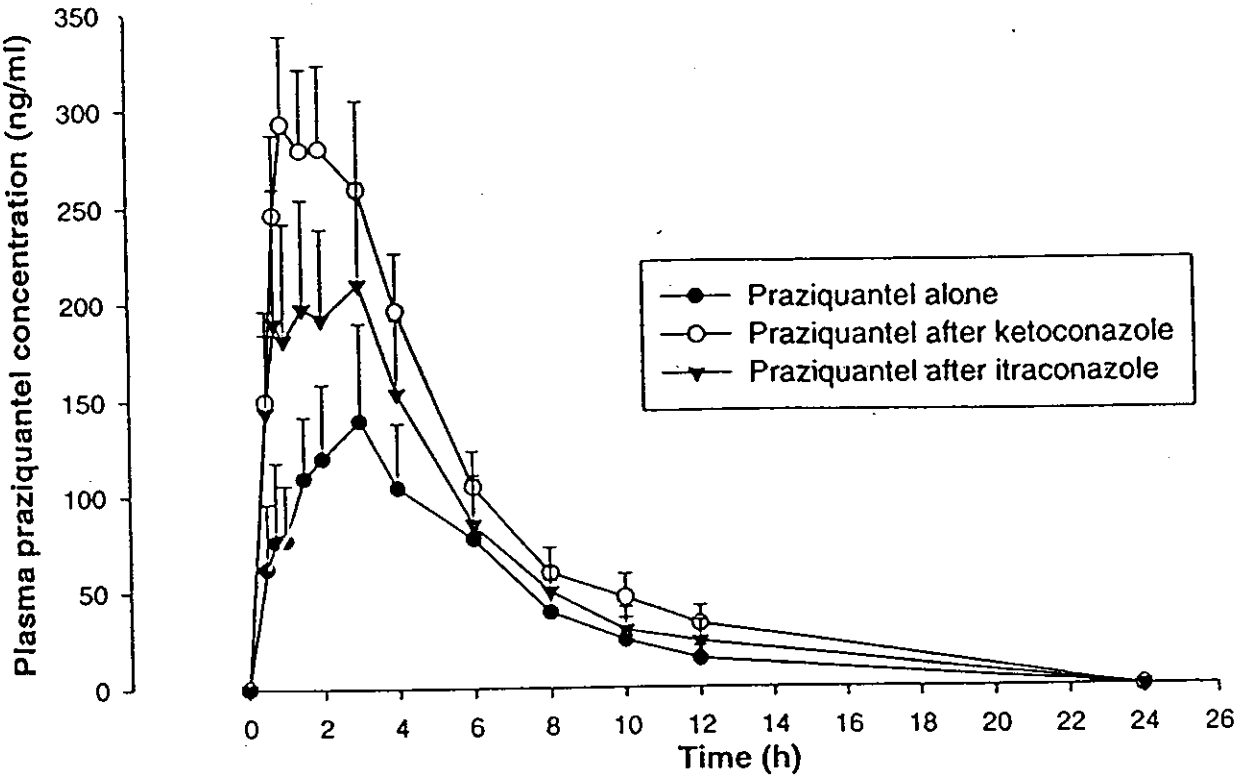


Figure 11 Mean plasma praziquantel concentrations after a single oral dose of 20 mg/kg praziquantel administration alone and after pretreatment with 400 mg ketoconazole or 200 mg Itraconazole once daily for 5 days. Each points were represented as mean and SEM for clarity.

Table 7 Pharmacokinetic parameters (mean ± S.D.) of praziquantel in subjects after receiving a single oral dose 20 mg/kg praziquantel alone and after pretreatment with 400 mg ketoconazole or 200 mg itraconazole orally for 5 days.

Parameters	PZQ alone	PZQ plus ketoconazole	*P*-value *t*-test	PZQ plus itraconazole	*P*-value *t*-test
C_{max} (ng/ml)	183.38 ± 138.82	371.31 ± 141.10	$P < 0.01$ (P=0.003)	312.81 ± 204.98	$P < 0.05$(P=0.0205)
$AUC_{(0-12)}$ (ng/ml.hr)	793 ± 895	1563 ± 756	$P < 0.01$ (P=0.0065)	1182 ± 826	NS (P=0.0705)
$AUC_{(0-\infty)}$ (ng/ml.hr)	956 ± 973	1843 ± 1064	$P < 0.05$ (P=0.008)	2371 ± 3621	NS (P=0.131)
λ_z (hr^{-1})	0.29 ± 0.22	0.23 ± 0.14	NS (P=0.255)	0.21 ± 0.13	NS (P=0.224)
Ka (hr^{-1})	0.48 ± 0.40	1.06 ± 0.41	$P < 0.01$ (P=0.006)	0.88 ± 0.45	$P < 0.01$(P=0.001)
$t_{1/2,Z}$ (hr)	3.80 ± 3.03	4.05 ± 2.39	NS (P=0.428)	10.55 ± 21.09	NS (P=0.181)
T_{max} (hr)	2.05 ± 1.14	1.82 ± 0.92	NS (P=0.2285)	1.75 ± 1.17	NS (P=0.2815)
Cl/f (l/kg/hr)	2.65 ± 2.03	1.11 ± 1.09	$P < 0.01$ (P=0.002)	2.19 ± 3.20	NS (P=0.257)
V_Z/f (l/kg)	10.75 ± 6.55	4.42 ± 2.06	$P < 0.01$ (P=0.006)	11.90 ± 13.04	NS (P=0.383)
MRT (hr)	6.35 ± 3.20	5.53 ± 2.44	NS (P=0.279)	14.26 ± 29.02	NS (P=0.214)

NS; no significant difference from control when compared with praziquantel (PZQ) alone.

Table 8 Pharmacokinetic parameters of praziquantel in each of ten subjects receiving a single oral dose of 20 mg/kg praziquantel alone.

Parameters	Subject number (S1-S10)											
	1	2	3	4	5	6	7	8	9	10	Mean	S.D.
Age (yrs)	24	22	37	38	30	39	30	33	29	27	30.90	5.82
Weight (kgs)	57	58	70	64	64	64	65	55	55	62	61.40	4.87
C_{max}(ng/ml)	90.6	206	528	101	288	169	48.9	152	95.3	155	183.38	138.83
$AUC_{(0-12)}$ (ng/ml.hr)	289.8	1176.5	3083.9	306.5	1245.3	406.9	165.99	740	314.9	202.3	793.21	895.19
$AUC_{(0-\infty)}$ (ng/ml.hr)	328.7	1322.7	3215.3	588.6	1982.5	407.2	205.9	869.8	416.1	222.6	955.94	973.07
λ_z (hr^{-1})	0.27	0.22	0.33	0.11	0.06	0.81	0.32	0.17	0.16	0.45	0.29	0.22
Ka (hr^{-1})	0.47	0.48	0.51	0.07	0.77	0.57	0.21	0.16	0.14	1.78	0.48	0.40
$t_{1/2,z}$ (hr)	2.58	3.09	2.08	6.25	11.2	0.86	2.1	4.02	4.3	1.53	3.08	3.03
T_{max} (hr)	2	4	3	1	3	1.5	3	1.5	1	0.5	2.05	1.14
Cl/f (l/kg/hr)	3.47	0.88	0.44	2.17	0.64	3.14	6.31	1.26	2.64	5.57	2.65	2.03
V_z/f (l/kg)	12.90	3.92	1.30	19.60	10.44	3.89	19.49	7.35	16.22	12.36	10.74	6.55
MRT (hr)	4.84	6.75	4.96	9.45	13.58	3.36	5.27	6.25	6.65	2.37	6.35	3.20

Table 9 Pharmacokinetic parameters of praziquantel in each of ten subjects receiving a single oral dose of 20 mg/kg praziquantel after pretreatment with 400 mg ketoconazole orally for 5 days.

Parameters	Subject number (S1-S10)											
	1	2	3	4	5	6	7	8	9	10	Mean	S.D.
Age (yrs)	24	22	37	38	30	39	30	33	29	27	30.90	5.82
Weight (kgs)	57	58	70	64	64	64	65	55	55	62	61.40	4.87
C_{max} (ng/ml)	523	320	463	446	395	524	81.1	239	444	278	371.31	141.10
$AUC_{(0-12)}$ (ng/ml.hr)	1474	1545	2502	1321	2026	2617	316	1438	1914	477	1563	756
$AUC_{(0-\infty)}$ (ng/ml.hr)	1576	1816	3350	1380	2211	3671	354	1577	2007	489	1843	1063
λ_z (hr^{-1})	0.19	0.13	0.08	0.26	0.17	0.09	0.38	0.22	0.28	0.53	0.23	0.14
Ka (hr^{-1})	0.73	1.57	1.12	0.44	1.24	1.02	1.85	0.96	0.79	0.86	1.06	0.41
$t_{1/2,Z}$ (hr)	3.6	5.3	8.51	2.71	4.03	7.55	1.82	3.22	2.48	1.30	4.05	2.38
T_{max} (hr)	1	3	1.5	0.75	3	3	2	2	1.5	0.5	1.82	0.92
Cl/f (l/kg/hr)	0.73	0.64	0.42	0.92	0.58	0.35	3.67	0.69	0.54	2.54	1.11	1.09
V_z/f (l/kg)	3.78	4.88	5.13	3.63	3.36	3.79	9.65	3.23	1.96	4.77	4.42	2.06
MRT (hr)	4.29	6.56	9.27	4.09	5.13	9.98	4.02	5.41	4.41	2.12	5.53	2.44

Table 10 Pharmacokinetic parameters of praziquantel in each of ten subjects receiving a single oral dose of 20 mg/kg praziquantel after pretreatment with 200 mg itraconazole orally for 5 days.

Parameters	Subject number (S1-S10)											
	1	2	3	4	5	6	7	8	9	10	Mean	S.D.
Age (yrs)	24	22	37	38	30	39	30	33	29	27	30.90	5.82
Weight (kgs)	57	58	70	64	64	64	65	55	55	62	61.40	4.87
C_{max} (ng/ml)	97.10	420	427	220	657	530	114	417	195	51	312.81	204.98
$AUC_{(0-12)}$ (ng/ml.hr)	346	2210	1845	782	2147	1916	167	1384	937	94	1182	825.96
$AUC_{(0-\infty)}$ (ng/ml.hr)	455	2375	2309	813	2544	12359	319	1467	956	117	2371	3620
λ_z (hr^{-1})	0.17	0.24	0.12	0.33	0.09	0.01	0.11	0.32	0.43	0.31	0.21	0.13
Ka (hr^{-1})	0.54	1.063	1.14	0.35	1.20	0.68	1.21	0.56	0.35	1.73	0.88	0.45
$t_{1/2,z}$ (hr)	4.11	2.84	5.87	2.11	8.03	70.28	6.21	2.16	1.62	2.25	10.54	21.09
T_{max} (hr)	1.50	4.00	0.75	1.50	0.75	3.00	0.50	1.50	3.00	1.00	2.27	0.60
Cl/f (l/kg/hr)	2.50	0.49	0.61	1.57	0.05	0.1	4.07	0.75	1.15	10.61	2.19	3.20
V_z/f (l/kg)	14.85	2.00	5.14	4.79	5.83	10.50	36.47	2.33	2.69	34.44	11.90	13.03
MRT (hr)	6.78	5.65	7.38	4.38	6.28	96.64	7.69	3.42	1.18	3.20	14.26	29.02