4. RESULTS

1. Assay Validation

1.1 Linearity of the standard calibration curve

The standard calibration curve for mefloquine and mefloquine carboxylic metabolite at concentration of 62.5, 125, 250, 500 and 1,000 ng/ml was linear with the correlation coefficient (r) of 0.999 (Figure 4 - 5).

The equation of linear regression line of mefloquine was

$$y = -1.165E\text{-}03 + 2.208E\text{-}03x$$

The equation of linear regression line of mefloquine metabolite was

$$y = 1.942E\text{-}03 + 2.842E\text{-}03x$$

1.2 Precision

The intra-day assay was repeated 5 times per day and coefficients of variation (CV) for mefloquine and mefloquine metabolite was 1.65-9.07 % (Table1-2), whereas the inter-day assay was assessed on 10 different days; the CV was 3.51-10.21% (Table 3 - 4).

1.3 Accuracy

The recovery of mefloquine and metabolite in plasma were 83.19-97.30 % (Table 5) and 89.68 - 99.48% (Table 6), respectively. The results reflect that the method is obviously accurate and this ensures reliable result.

1.4 Limit of quantification

The limit of quantification (LOQ) of mefloquine and mefloquine metabolite in plasma was approximately 62.5 ng/ml by a %CV less than 10%

2. Chromatograms

The chromatograms showed that the peak of mefloquine and its metabolite were well separated from the other peaks in plasma (Figure 6). There was the peak of ketoconazole in this analytical method (Figure 8). The retention time for ketoconazole, mefloquine and, mefloquine metabolite were approximately 4, 8 and 15 minutes,

respectively.

3. The plasma concentration-time data of mefloquine and mefloquine metabolite

The mean plasma concentration-time profiles of mefloquine and mefloquine metabolite after receiving mefloquine alone and pretreatment with ketoconazole were shown in Figure 9 and 10.

4. The period and sequence of study

No significance of period and sequence effect affects this study. The interaction between period and sequence were evaluated with the use of two- way ANOVA analysis (Appendix-3).

5. Adverse Effects

Eight male healthy volunteers were enrolled and completed in this study. No serious side effects were observed after taking 500 mg of mefloquine. Two subjects reported mild headache during ketoconazole co-administration. The symptom occurred for a few day, and was not required a specific treatment. However, all subjects were well tolerated to all drugs throughout the study. No significant laboratory abnormalities occurred in any subject, and physical examinations revealed no abnormal findings at the end of the study.

6. Pharmacokinetics

6.1 Pharmacokinetics of an oral single dose of 500 mg mefloquine

After a single oral dose of 500 mg mefloquine alone in eight volunteers, the mean value of $AUC_{0\text{-last}}$, $AUC_{0\text{-}\infty}$, Ka, Ke, $t_{1/2}$, t_{max}, C_{max}, V_d/f and Cl/f of mefloquine were 159.66 ± 33.28 mg/l.hr, 205.22 ± 32.58 mg/l.hr, 0.323 ± 0.145 hr^{-1}, 0.0022 ± 0.0006 hr, 322.68+99.95 hr, 17.99±8.17 hr, 345.10±43.22 ng/ml, 1.40±0.20 l/kg and 0.0031 ± 0.0007 l/hr/kg, respectively (Table 7).

The mean values of $AUC_{0\text{-last}}$, $AUC_{0\text{-}\infty}$, Ka, Ke, $t_{1/2}$, t_{max}, C_{max}, V_d/f and Cl/f of mefloquine metabolite were 492.43 ± 141.66 mg/l.hr, 656.67 ± 193.24 mg/l.hr, 0.032 ± 0.020 hr^{-1}, 0.0012 ± 0.0006 hr, 679.08 ± 358.49 hr, 147.14 ± 110.16 hr, 606.11 ± 184.00 ng/ml, 0.77 ± 0.40 l/kg and 0.0007 ± 0.0002 l/hr/kg, respectively (Table 11).

6.2 Pharmacokinetics of an oral single dose of 500 mg mefloquine in subjects pretreated with 400 mg ketoconazole once daily for 10 days

Mefloquine

The mean values of $AUC_{0\text{-last}}$, $AUC_{0\text{-}\infty}$, Ka, Ke, $t_{1/2}$, t_{max}, C_{max}, V_d/f and Cl/f of mefloquine pharmacokinetic parameters after pretreatment with 400 mg ketoconazole were 286.05 ± 64.25 mg/l.hr, 360.49 ± 81.98 mg/l.hr, 0.453 ± 0.163 hr^{-1}, 0.0016 ± 0.0003 hr^{-1}, 448.41 ± 103.88 hr, 12.36 ± 3.00 hr, 567.65 ± 88.69 ng/ml, 1.00 ± 0.21 l/kg and 0.0015 ± 0.0003 l/hr/kg, respectively (Table 10).

Mefloquine pharmacokinetic parameters pretreated by ketoconazole demonstrated the significant increase of the mean $AUC_{0\text{-last}}$, $AUC_{0\text{-}\infty}$, $t_{1/2}$, and C_{max} when compared to mefloquine alone (phase 1) by 179.16% (286.05 ± 64.25 mg/l.hr vs 159.66 ± 33.28 mg/l.hr; $P<0.001$), 175.66%(360.49 ± 81.98 mg/l.hr vs 205.22 ± 32.58 mg/l.hr $P<0.001$), 138.96%(448.41 ± 103.88 hr vs 322.68 ± 99.95 hr; $P<0.05$) and 164.48%(567.65 ± 88.69 ng/ml vs 345.10 ± 43.22 ng/ml; $P<0.001$), respectively, whereas there were a significant decrease of the mean Ke, V_d/f and Cl/f by 72.72% (0.0016 ± 0.0003 hr^{-1} vs 0.0022 ± 0.0006 hr^{-1}; $P<0.05$), 71.42%(1.00 ± 0.21 l/kg vs 1.40 ± 0.20 l/kg; $P<0.05$) and 48.38%(0.0015 ± 0.0003 l/hr/kg vs 0.0031 ± 0.0007 l/hr/kg; $P<0.001$), respectively. There were no significant differences in Ka and t_{max} when compared to mefloquine alone (Table 7).

Mefloquine metabolite

The mean value of $AUC_{0\text{-last}}$, $AUC_{0\text{-}\infty}$, Ka, Ke, $t_{1/2}$, t_{max}, C_{max}, V_d/f and Cl/f of mefloquine metabolite pharmacokinetic data after pretreatment with ketoconazole were 352.29 ± 47.08 mg/l.hr, 504.15 ± 127.28 mg/l.hr, 0.051 ± 0.034 hr^{-1}, 0.0010 ± 0.0004 hr^{-1}, 775.03 ± 82.28 hr, 106.66 ± 46.80 hr, 419.65 ± 45.02 ng/ml, 1.09 ± 0.16 l/kg and 0.001 ± 0.0003 l/hr/kg, respectively (Table 12)

The pharmacokinetic data of mefloquine metabolite were summarized in Table 12, the $AUC_{0\text{-last}}$, and C_{max} were significantly decreased when compared to mefloquine

alone (phase 1) by 71.54%(352.29 ± 47.08 vs 492.43 ± 141.66 mg/l.hr; $P<0.05$) and 69.23%(419.65 ± 45.02 vs 606.11 ± 184.00 ng/ml; $P<0.05$), respectively. There were no significant differences in Ka, Ke, $t_{1/2}$, $t_{max,}$ and Cl/f when compared to mefloquine alone (Table 12).

,

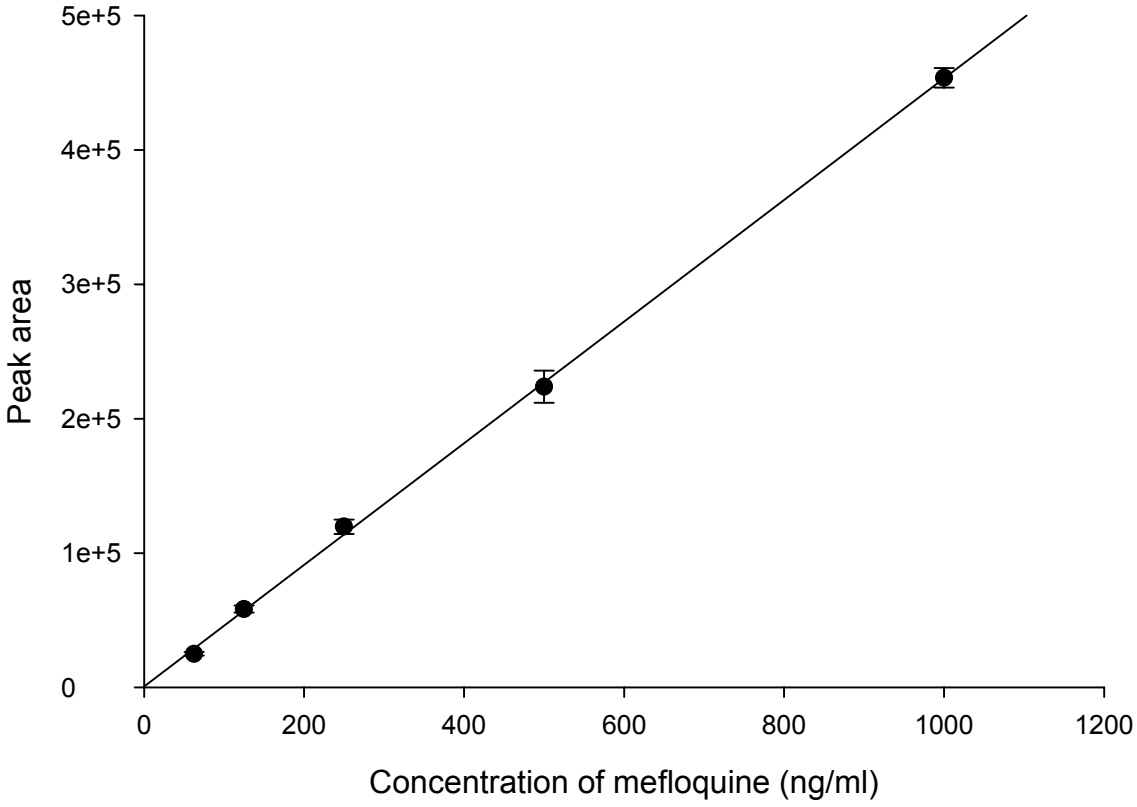


Figure 4 Mean calibration curve of standard mefloquine in plasma, correlation coefficient (r)=0.999

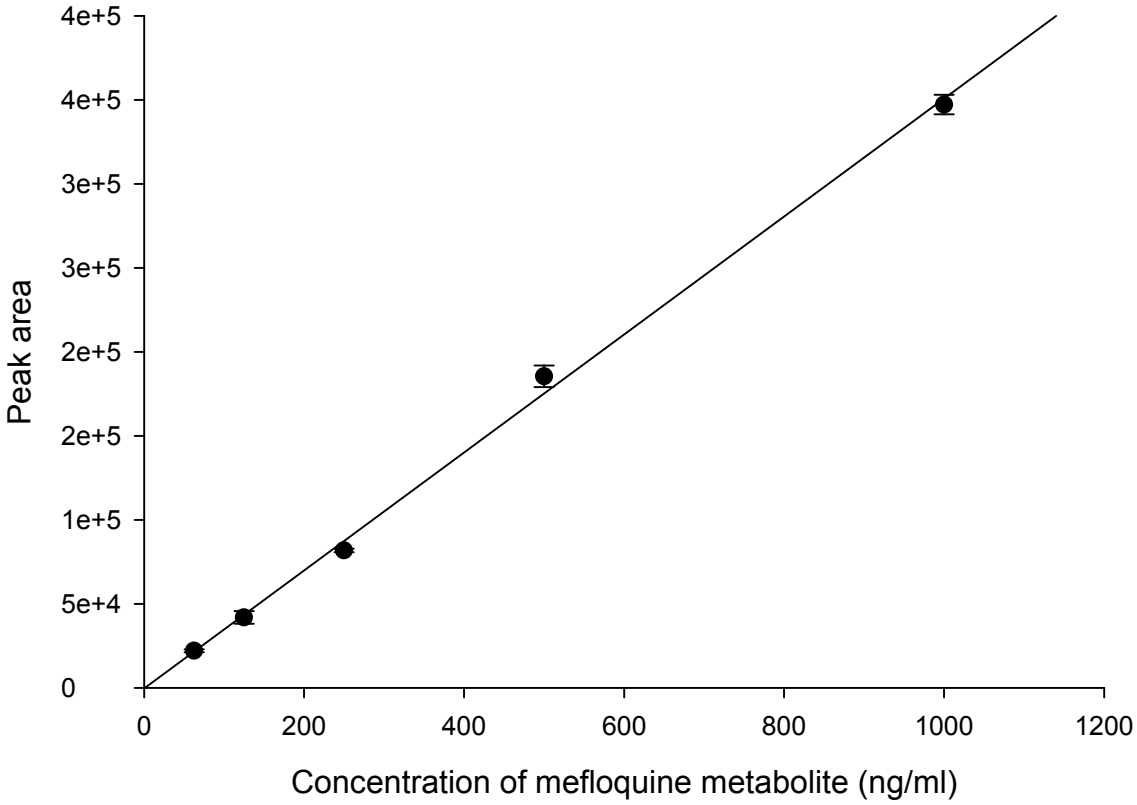


Figure 5 Mean calibration curve of standard mefloquine metabolite in plasma, correlation coefficient (r)=0.999

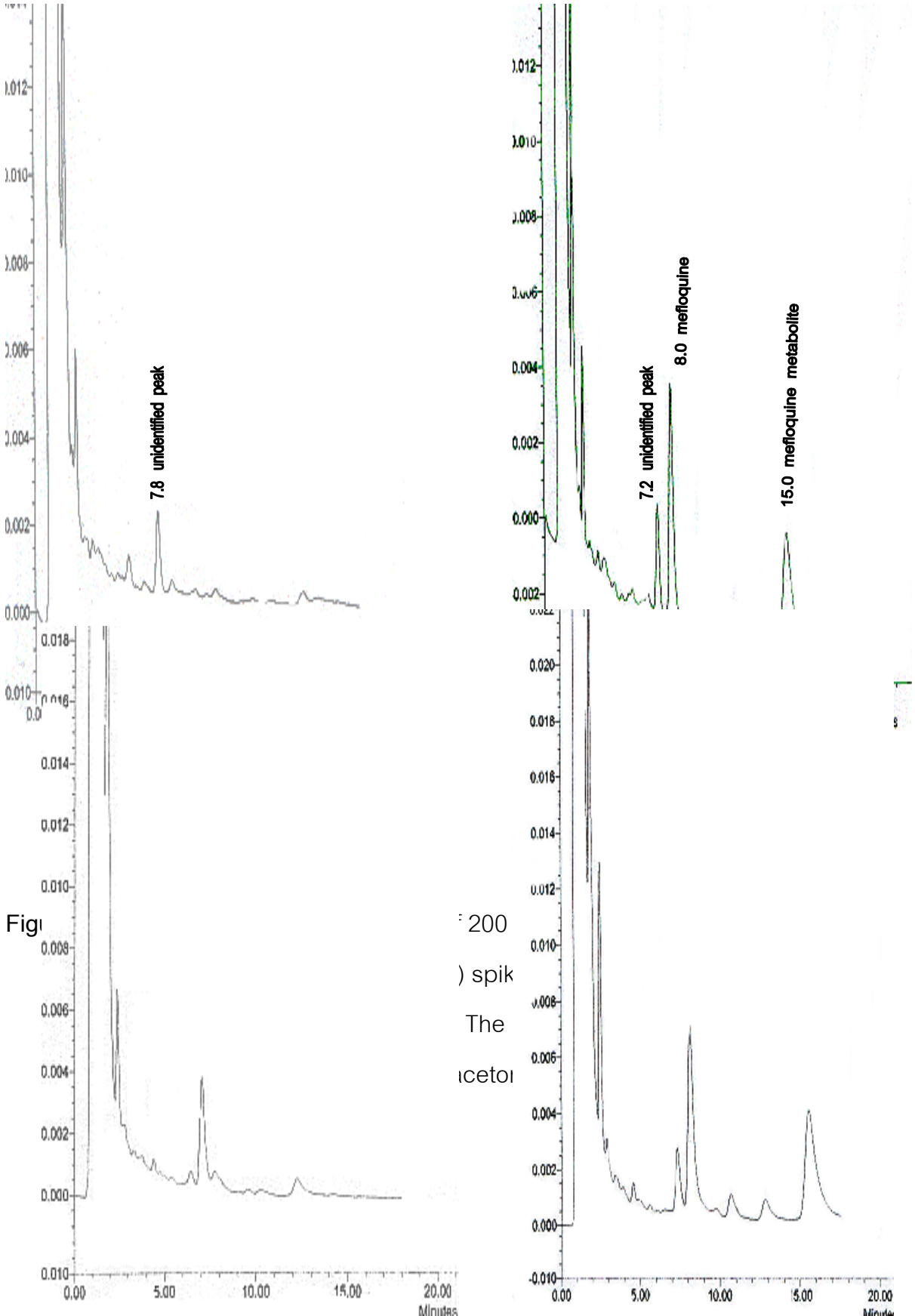
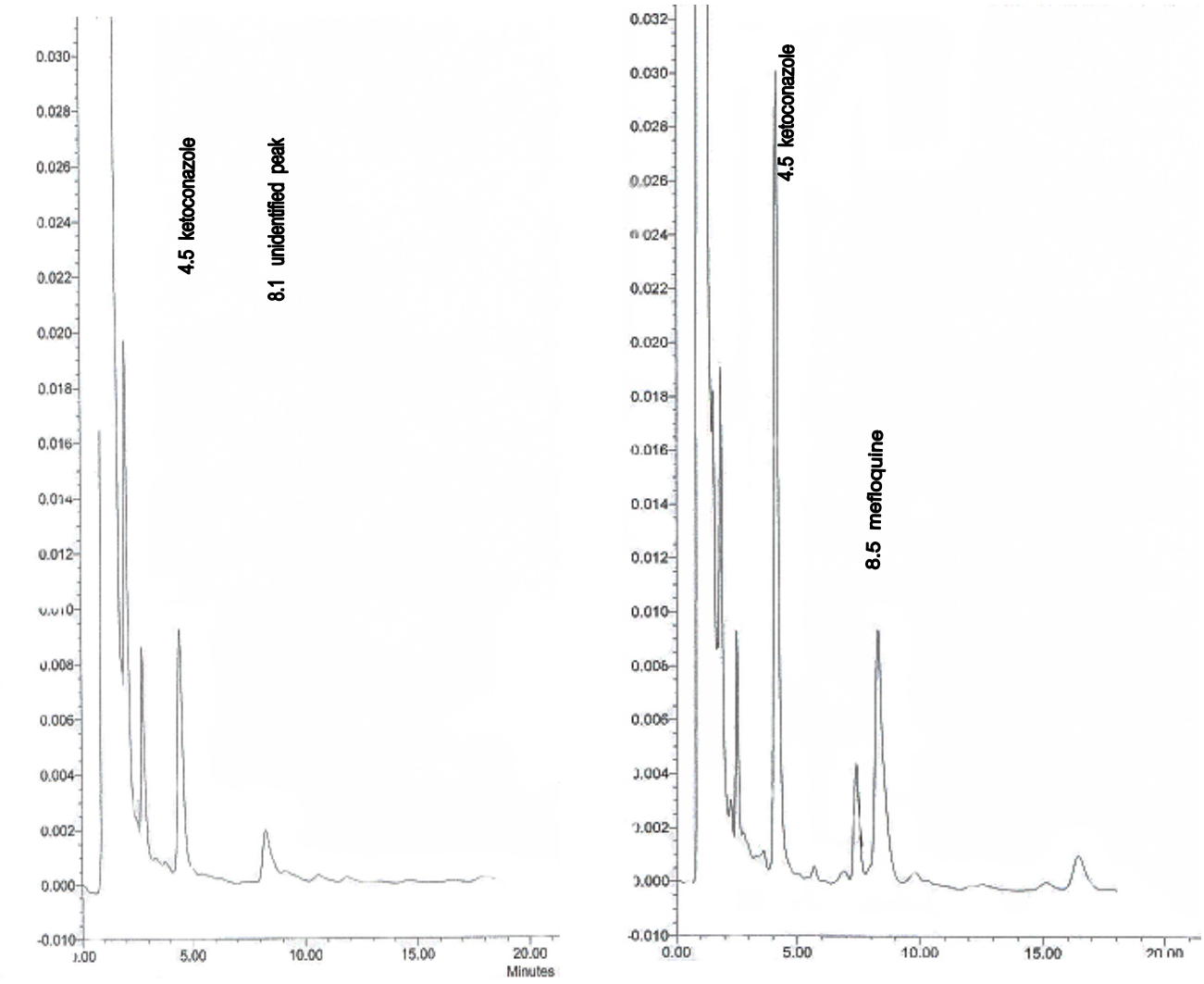


Fig : 200
) spik
The
acetor

A B

Figure 7 Representative chromatograms of 200 µl human plasma sample. Key: (A) Blank human plasma; (B) plasma obtained from a subject without ketoconazole treatment at day 3. The mobile phase consisted of 50 mM/L sodium sulfate: methanol: acetonitrile (50:34:16) pH 3.07 at a flow rate of 1.5 ml/min.



A B

Figure 8 Representative chromatograms of 200 µl human plasma sample. Key: (A) blank human plasma; (B) plasma obtained from a subject with ketoconazole treatment at day 3. The mobile phase consisted of 50 mmol/L sodium sulfate: methanol: acetonitrile (50:34:16) pH 3.07 at a flow rate of 1.5 ml/min.

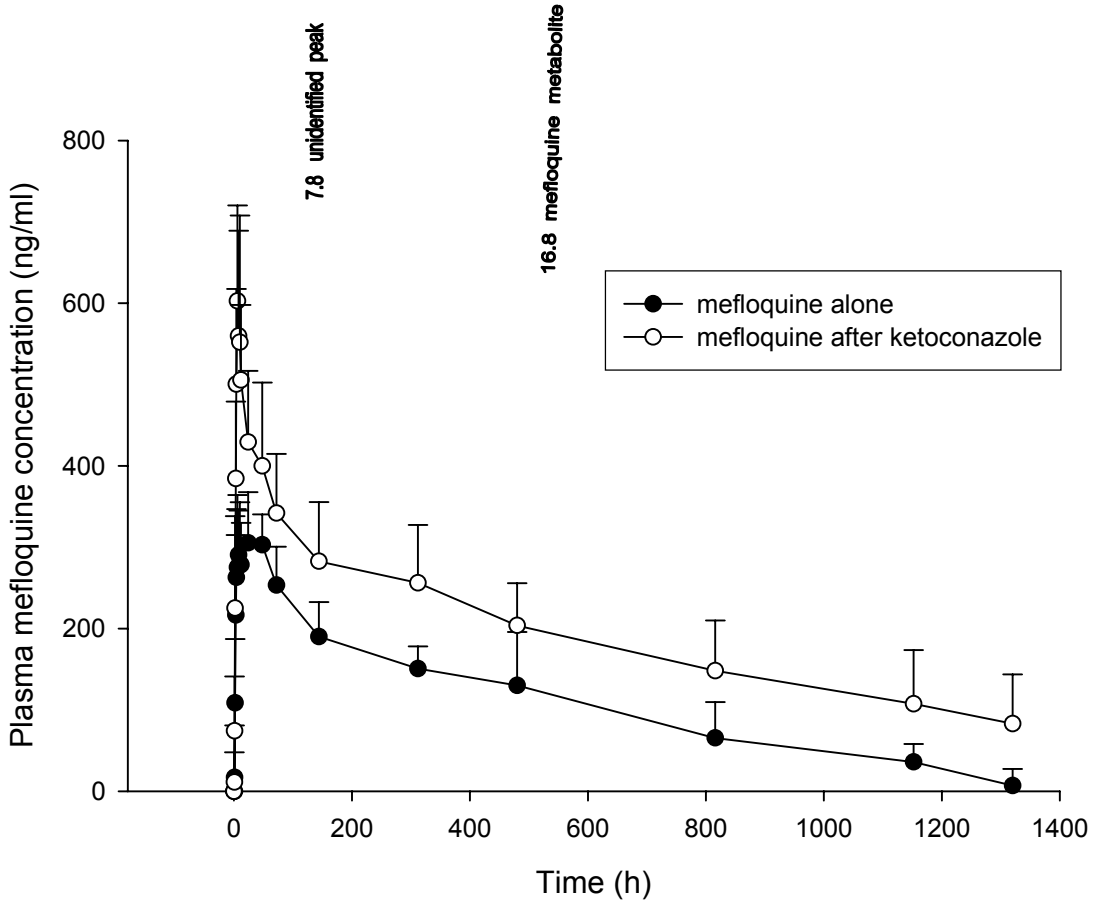


Figure 9 Mean plasma mefloquine concentrations after a single oral dose of 500

mg mefloquine administration alone and after pretreatment with 400 mg ketoconazole once daily for 10 days.

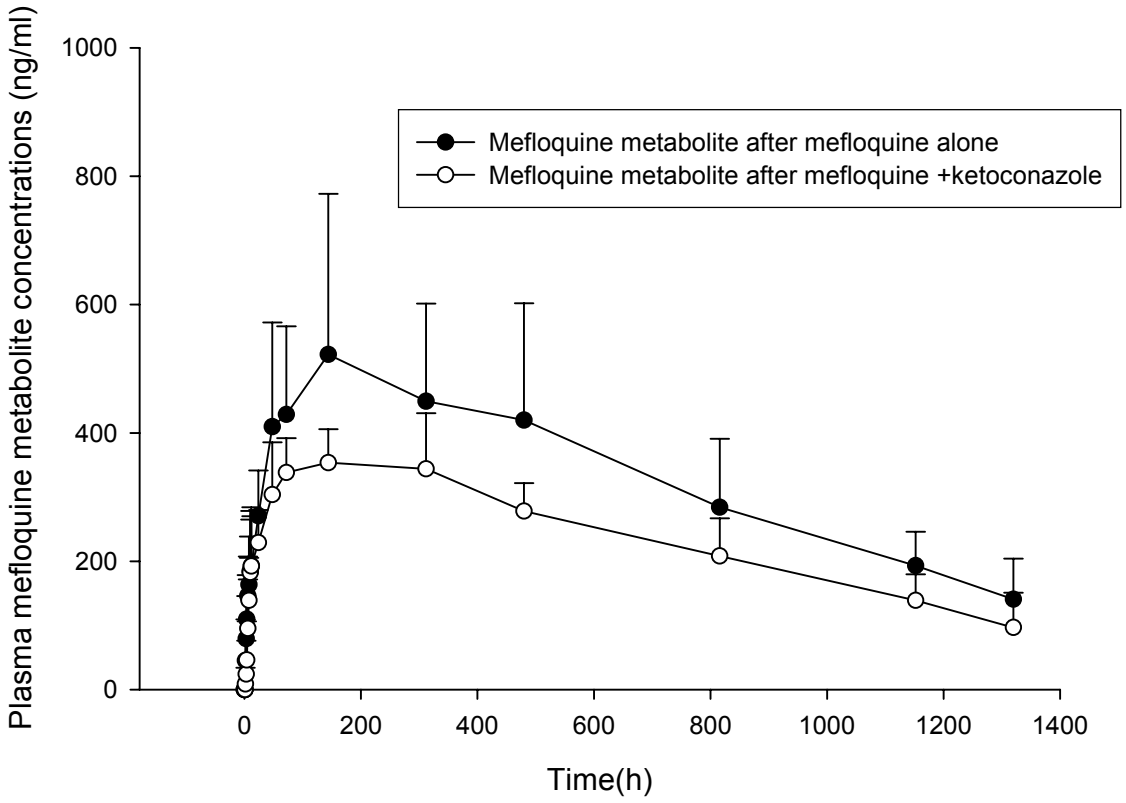


Figure 10 Mean plasma mefloquine metabolite concentrations after a single oral dose of 500 mg mefloquine administration alone and after pretreatment with 400 mg ketoconazole once daily for 10 days.

Table 7 Pharmacokinetic parameters (mean ± SD.) of mefloquine in eight subjects receiving a single oral of dose 500 mg mefloquine alone and during ketoconazole co-administration.

Parameter	Mefloquine alone	Mefloquine + ketoconazole	P-value
AUC $_{0\text{-last}}$ (mg/l/hr)	159.66 ± 33.28	286.05 ± 64.25	*P*< 0.001
AUC $_{0\text{-}\infty}$(mg/l/hr)	205.22 ± 32.58	360.49 ± 81.98	*P*< 0.001
Ka (hr^{-1})	0.323 ± 0.145	0.453 ± 0.163	NS
Ke (hr^{-1})	0.0022 ± 0.0006	0.0016 ± 0.0003	*P*< 0.05
$t_{1/2}$ (hr)	322.68 ± 99.95	448.41±103.88	P< 0.05
t_{max} (hr)	17.99 ± 8.17	12.36 ± 3.00	NS
C_{max} (ng/ml)	345.10 ± 43.22	567.65 ± 88.69	*P*< 0.001
Vd/f (l/kg)	1.40 ± 0.20	1.00 ± 0.21	*P*< 0.05
Cl/f (l/hr/kg)	0.0031 ± 0.0007	0.0015 ± 0.0003	*P*< 0.001

NS; no significant differences from control when compared to mefloquine alone (one-way ANOVA)

Table 8 Pharmacokinetic parameters (mean ± SD.) of mefloquine metabolite in eight subjects receiving a single oral of dose 500 mg mefloquine alone and during ketoconazole co-administration.

Parameter	Mefloquine alone	Mefloquine + ketoconazole	P-value
AUC $_{0\text{-last}}$ (mg/l/hr)	492.43 ± 141.66	352.29 ± 47.08	*P*<0.05
AUC $_{0\text{-}\infty}$(mg/l/hr)	656.67 ± 193.24	504.15 ± 127.28	NS
Ka (hr^{-1})	0.032 ± 0.020	0.051 ± 0.034	NS
Ke (hr^{-1})	0.0012 ± 0.0006	0.0010 ± 0.0004	NS
$t_{1/2}$ (hr)	679.08 ± 358.49	775.03 ± 82.28	NS
t_{max} (hr)	147.14 ± 110.16	106.66 ± 46.80	NS
C_{max} (ng/ml)	606.11 ± 184.00	419.65 ± 45.02	*P*<0.05
Vd/f (l/kg)	0.77 ± 0.40	1.09 ± 0.16	*P*<0.05
Cl/f (l/hr/kg)	0.0007 ± 0.0002	0.001 ± 0.0003	NS

NS; no significant differences from control when compared to mefloquine alone (one-way ANOVA)

