# RESULTS

## Assay Validation

The assay validation of our experimental method showed that the standard curve was linear in the mefloquine and metabolite concentration range of 62.5-2,000 ng/ml (Figure 1-2). The correlation coefficient (r) and the coefficient of variation (CV) were 0.999 (Figure 1-2) and 3.69-8.06% (Table 1--4), respectively. The recovery of mefloquine and metabolite in plasma was 99.05-110.50 (Table 5) and 94.63-105.16% (Table 6), respectively. The chromatograms showed that a peak of mefloquine and its metabolite were well separated from the other peaks in plasma (Figure 3-5). Neither peak of rifampicin interfered with this analytical method.

The mean plasma concentrations of mefloquine and its metabolite after receiving mefloquine alone declined monoexponentially and were fitted to a one compartment open model (Figure 6). The summary of pharmacokinetic data of mefloquine in the present study were compared to other published data in human volunteers (Table 13). The pharmacokinetic parameters ($C_{max}$, $T_{max}$, Vd/f, Cl/f and AUC) of mefloquine in subjects receiving mefloquine alone were similar to those previous reports.

## Adverse Effects

Seven adult male healthy Thai volunteers were enrolled and completed this study. No serious side effects were observed after taking 500 mg of mefloquine and through the study. One subject reported gastrointestinal discomfort and one subject reported headache during rifampicin

coadministration. The symptoms occurred for a few day, and were not required a specific treatment. However, all subjects were well tolerated to all drugs throughout the study. No marked laboratory abnormalities occurred in any subjects, and physical examinations revealed no abnormal findings at the end of the study.

**Pharmacokinetics**

The mean plasma concentration-time profile of mefloquine and its metabolite were shown in Figure 7. The pharmacokinetic parameters (mean ± S.D.), estimated from the plasma concentration-time data of mefloquine were presented in Table 7-9. The results showed that AUC, Ke, $t_{1/2}$, $C_{max}$ and Cl/f were significantly different between parameters of mefloquine alone and mefloquine after rifampicin treatment while there were no significant difference in Ka, $t_{1/2}$(abs), $T_{max}$ and Vd/f. The values (mean ± S.D.) of $C_{max}$, $T_{max}$, $t_{1/2}$, Vd/f, Cl/f and AUC in subjects receiving mefloquine alone were 855.63 ± 168 ng/ml, 8.15 ± 2.91 hr, 1 ± 0.43 hr, 9.44 ± 1.87 l/kg, 0.0214 ± 0.0038 l/hr/kg and 373.73 ± 57.47 mg/l.hr, respectively ; in rifampicin treated subjects they were 695.67 ± 56.63 ng/ml, 8.67 ± 3.92 hr, 113.43 ± 49.71 hr, 10.89 ± 1.36 l/kg, 0.08 ± 0.03 l/hr/kg and 119.77 ± 54.94 mg/l.hr, respectively.

The pharmacokinetic data of mefloquine metabolite were summarized in Table 10-12, the $C_{max}$, $T_{max}$ and $t_{1/2}$ were significantly different between those groups receiving mefloquine alone and mefloquine after rifampicin treatment but the Vd/f, Cl/f and AUC were not significantly different. The values (mean ± S.D.) of $C_{max}$, $T_{max}$, $t_{1/2}$, Vd/f, Cl/f and AUC of mefloquine

metabolite in subjects receiving mefloquine alone were 813.16 ± 297.96 ng/ml, 220.62 ± 69.75 hr, 506.66 ± 127.64 hr, 8.12 ± 3.45 l/kg, 0.0116 ± 0.0051 l/hr/kg and 786.42 ± 285.40 mg/l.hr, respectively ; in rifampicin treated subjects they were 1,194.45 ± 249.10 ng/ml, 52.48 ± 28.81 hr, 307.45 ± 56.90 hr, 6.28 ± 1.87 l/kg, 0.01486 ± 0.00607 l/hr/kg and 549.88 ± 170.32 mg/l.hr, respectively. In addition, a large inter-individual variation in $C_{max}$, $T_{max}$, $t_{1/2}$ and AUC were also observed.

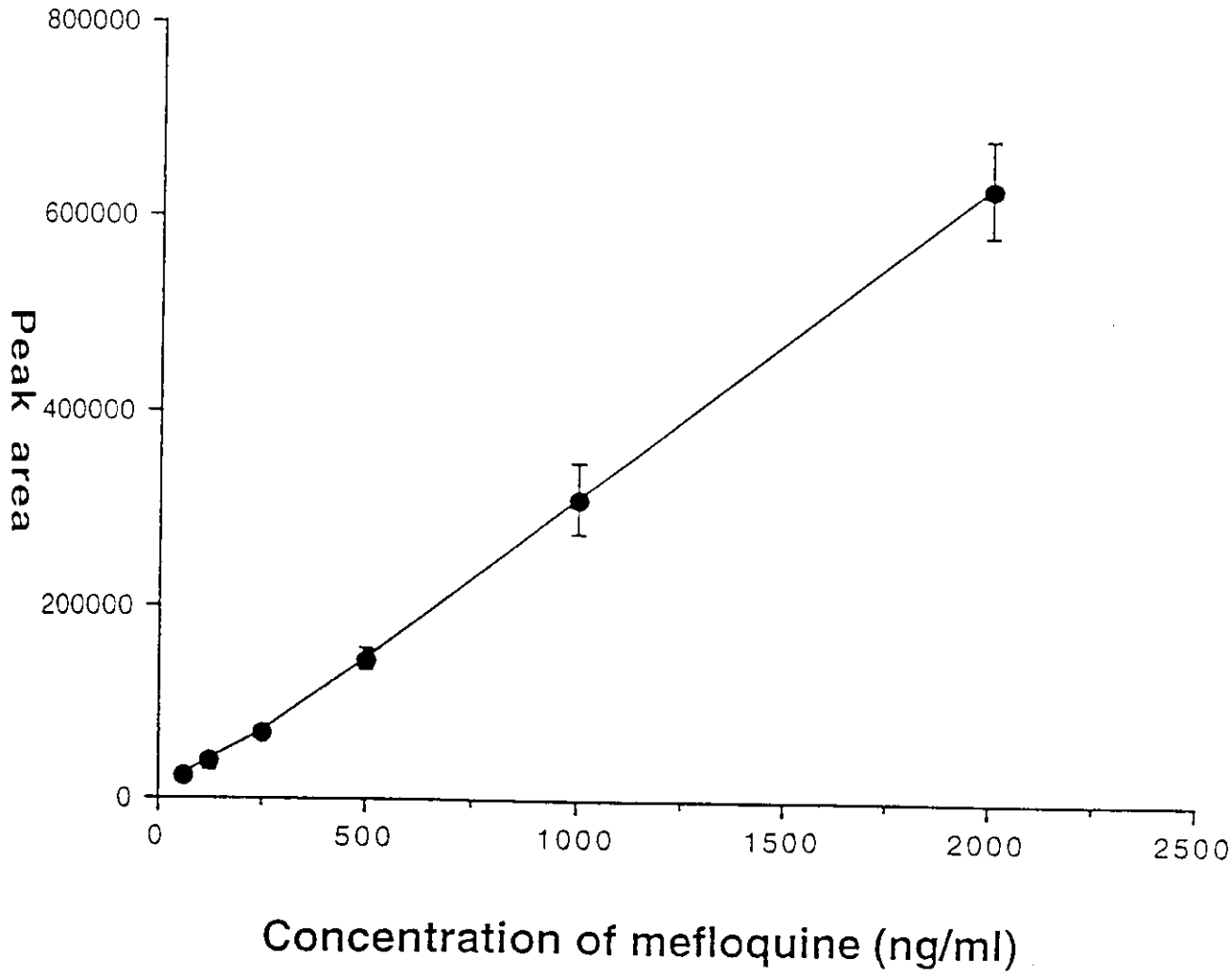


**Figure 1** Mean calibration curve of standard mefloquine in plasma, correlation coefficient (r) = 0.999

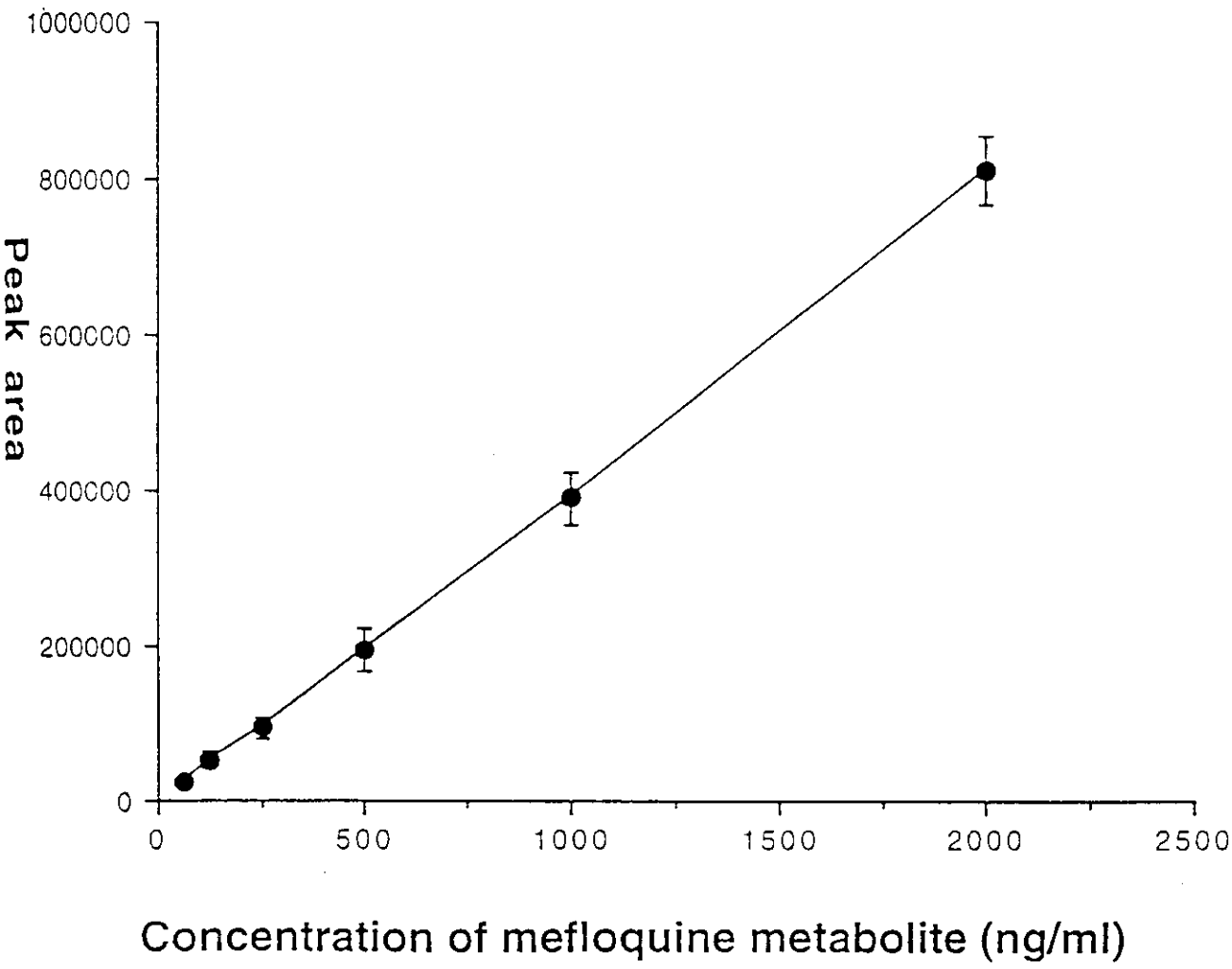


**Figure 2** Mean calibration curve of standard mefloquine metabolite in plasma, correlation coefficient (r) = 0.999

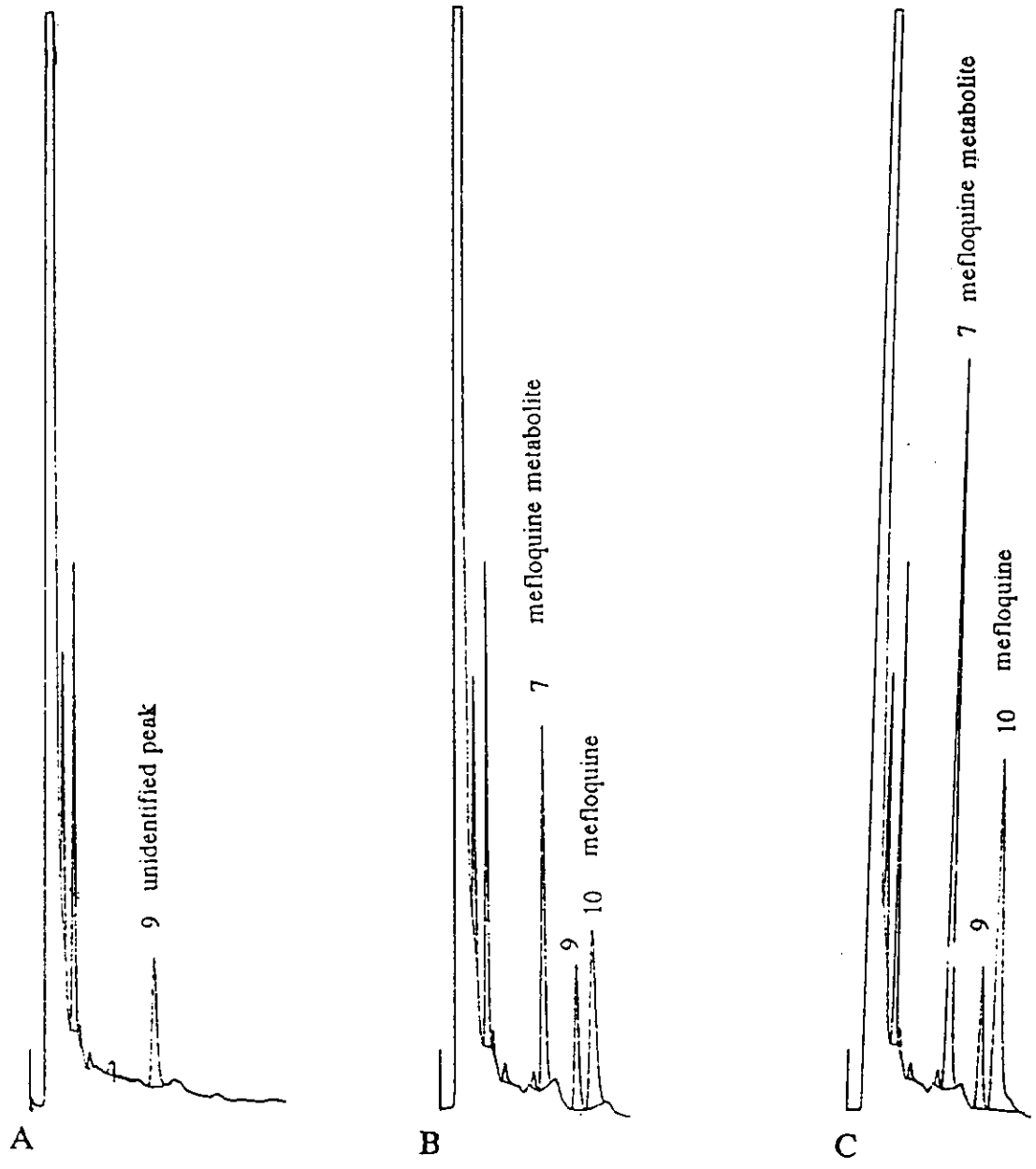


**Figure 3** Representative chromatograms of 100 µl human plasma samples. Key : (A) blank human plasma ; (B) spiked with standard mefloquine and mefloquine metabolite, 500 ng/ml ; (C) spiked with standard mefloquine and mefloquine metabolite, 1,000 ng/ml. The mobile phase consisted of 50 mmol/L sodium sulfate-methanol-acetonitrile (50 : 34 : 16 vol/vol/vol) pH 3.07 at a flow rate of 1.0 ml/min. Chart speed and attenuation were 2 mm/min and 32 mV F.S., respectively.

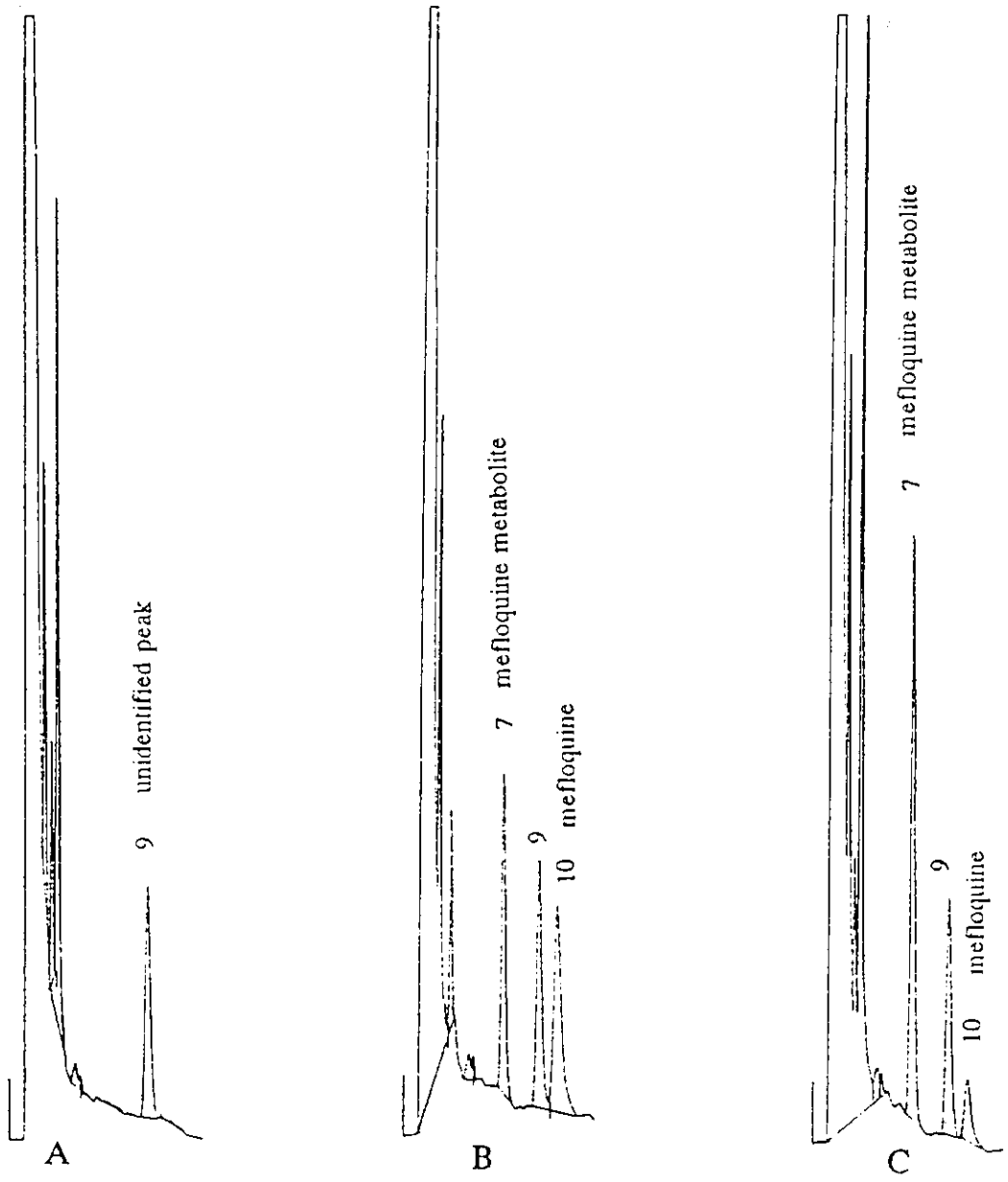


**Figure 4** Representative chromatograms of 100 µl human plasma samples. Key : (A) blank human plasma ; (B) and (C) plasma obtained from a subject without rifampicin treatment, 48 and 672 hr, respectively after an oral administration of 500 mg mefloquine. The mobile phase consisted of 50 mmol/L sodium sulfate-methanol-acetonitrile (50 : 34 : 16 vol/vol/vol) pH 3.07 at a flow rate of 1.0 ml/min. Chart speed and attenuation were 2 mm/min and 32 mV F.S., respectively.

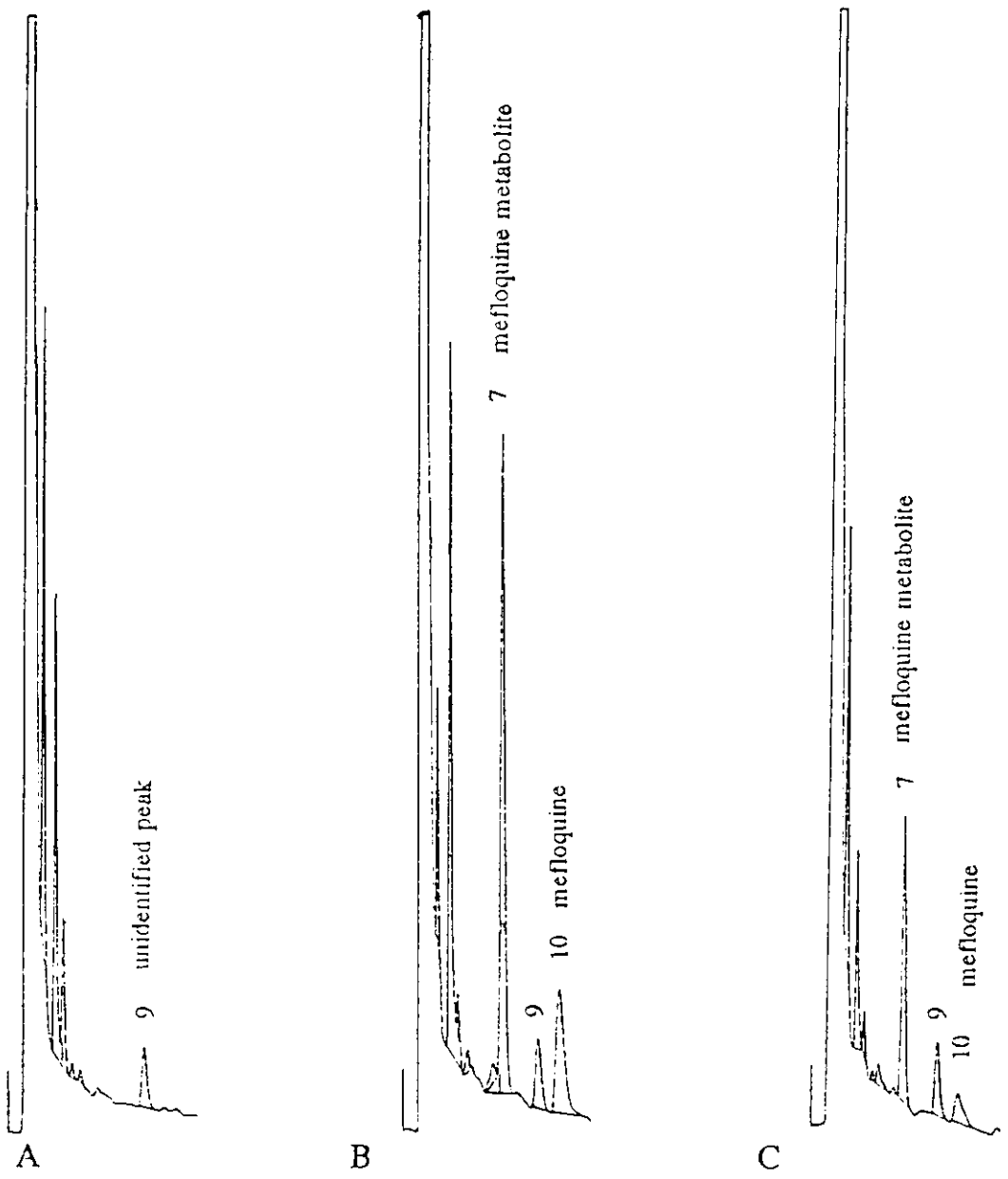


**Figure 5** Representative chromatograms of 100 µl human plasma samples. Key : (A) blank human plasma ; (B) and (C) plasma obtained from a subject treatment with rifampicin, 48 and 672 hr, respectively after an oral administration of 500 mg mefloquine. The mobile phase consisted of 50 mmol/L sodium sulfate-methanol-acetonitrile (50 : 34 : 16 vol/vol/vol) pH 3.07 at a flow rate of 1.0 ml/min. Chart speed and attenuation were 2 mm/min and 32 mV F.S., respectively.

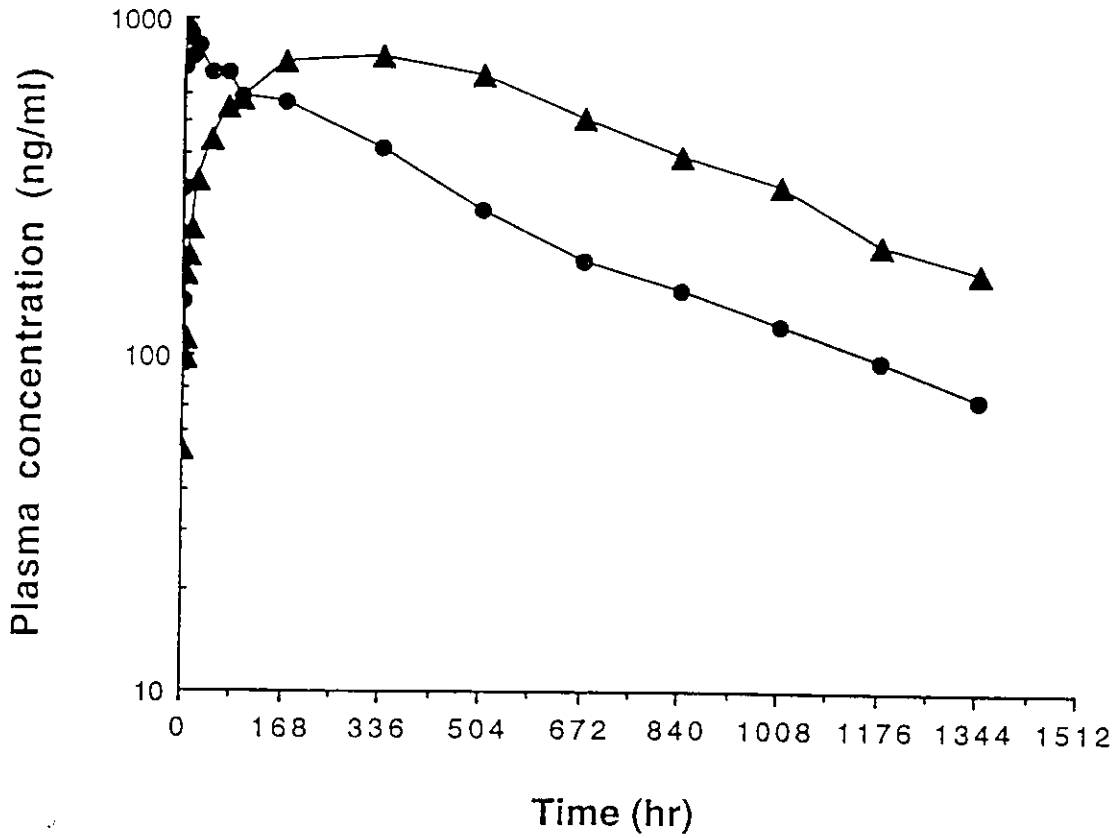


**Figure 6** The representative semi-logarithmic mean plasma mefloquine (●—●) and mefloquine metabolite (▲—▲) concentration-time profile after a single oral administration of 500 mg mefloquine.

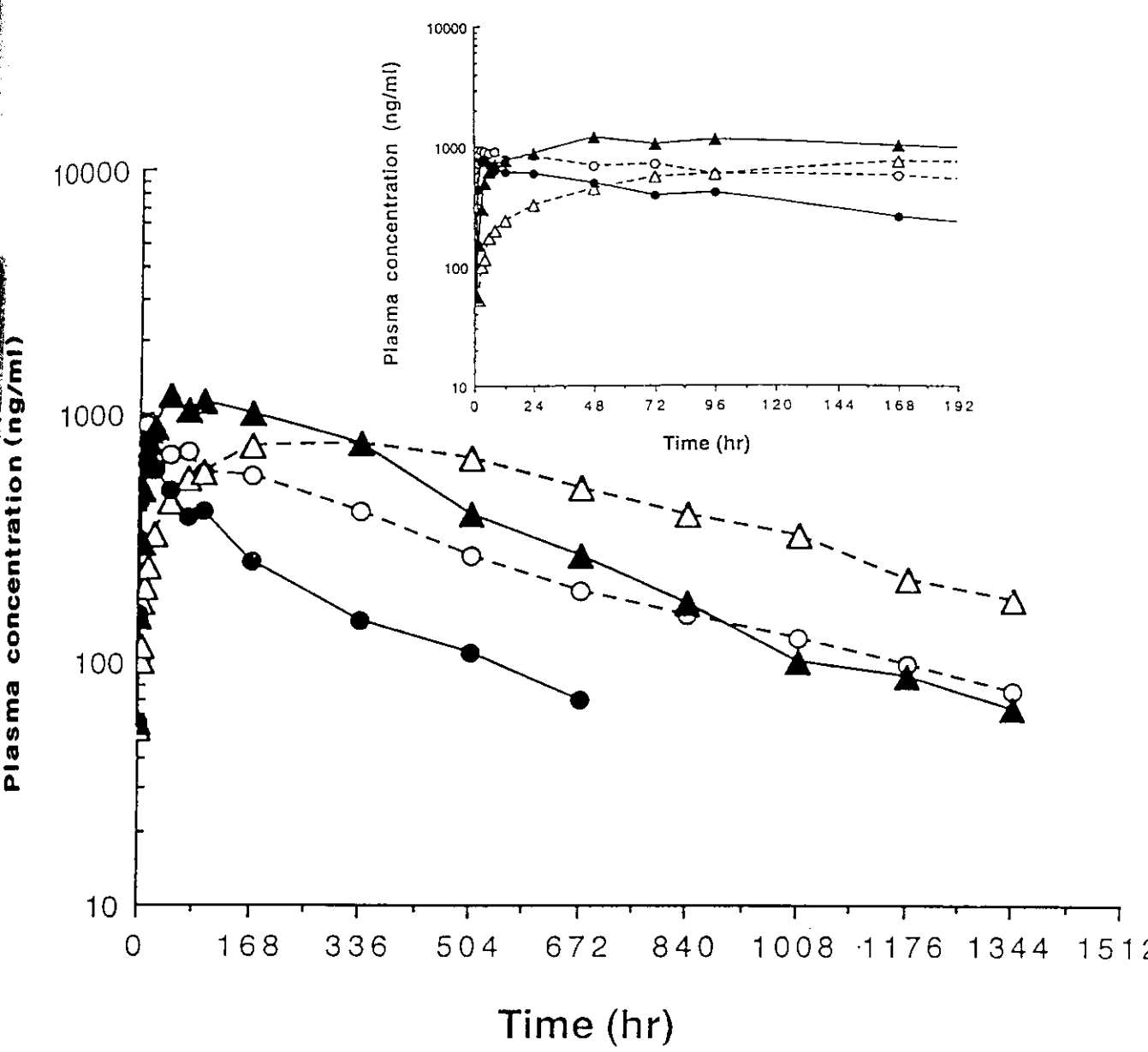


**Figure 7** Semi-logarithmic mean plasma mefloquine and mefloquine metabolite concentrations after a single oral dose of 500 mg mefloquine administration alone (O- - -O : Mefloquine; Δ- - -Δ : Mefloquine metabolite) and after rifampicin coadministration (●——● : Mefloquine + Rifampicin; ▲——▲ : Mefloquine metabolite + Rifampicin) in 7 normal volunteers.

**Table 1** The intra-assay variance of three different mefloquine concentrations in plasma[a]

| Concentration (ng/ml) | Mean peak area ± S.D. (n=10) | CV (%)[b] |
|---|---|---|
| 125 | 38921.30 ± 1965.68 | 5.05 |
| 500 | 168109 ± 10238.19 | 6.09 |
| 2,000 | 499670.50 ± 26723.31 | 5.35 |

[a]Various concentrations of standard mefloquine were added to drug-free human plasma samples prior to precipitation as described in the text

[b]Standard deviation divided by mean, expressed in percent

**Table 2** The intra-assay variance of three different mefloquine metabolite concentrations in plasma[a]

| Concentration (ng/ml) | Mean peak area ± S.D. (n=10) | CV (%)[b] |
|---|---|---|
| 125 | 48458.70 ± 2916.45 | 6.02 |
| 500 | 201760.70 ± 8832.44 | 4.38 |
| 2,000 | 761501.50 ± 28131.88 | 3.69 |

[a]Various concentrations of standard mefloquine metabolite were added to drug-free human plasma samples prior to precipitation as described in the text

[b]Standard deviation divided by mean, expressed in percent

**Table 3** The inter-assay variance of three different mefloquine concentrations in plasma[a]

| Concentration (ng/ml) | Mean peak area ± S.D. (n=10) | CV (%)[b] |
|---|---|---|
| 125 | 43194.00 ± 2997.319 | 6.94 |
| 500 | 158723.40 ± 12795.86 | 8.06 |
| 2,000 | 693740.80 ± 41991.50 | 6.05 |

[a]Various concentrations of standard mefloquine were added to drug-free human plasma samples prior to precipitation as described in the text

[b]Standard deviation divided by mean, expressed in percent

**Table 4** The inter-assay variance of three different mefloquine metabolite concentrations in plasma[a]

| Concentration (ng/ml) | Mean peak area ± S.D. (n=10) | CV (%)[b] |
|---|---|---|
| 125 | 51517 ± 3494.20 | 6.78 |
| 500 | 196899.30 ± 13552.92 | 6.88 |
| 2,000 | 859808 ± 60323.96 | 7.02 |

[a]Various concentrations of standard mefloquine metabolite were added to drug-free human plasma samples prior to precipitation as described in the text

[b]Standard deviation divided by mean, expressed in percent

**Table 5** Relative percent recovery of standard mefloquine in human plasma

| concentration (ng/ml) | peak area in mobile phase[a] (mean ± SD) (n=5) | peak area in plasma[b] (mean ± SD) (n=5) | % Recovery[c] |
|---|---|---|---|
| 125 | 53996.2 ± 2648.62 | 53606.0 ± 2651.27 | 99.05 |
| 500 | 171408.4 ± 26825.18 | 183195.86 ± 39554.86 | 105.99 |
| 2,000 | 667511.0 ± 55517.27 | 736840.11 ± 66430.56 | 110.50 |

[a]Various concentrations of standard mefloquine in mobile phase were directly injected.

[b]Various concentrations of standard mefloquine were added to drug-free human plasma samples prior to precipitation

[c]Mean peak area in plasma divided by mean peak area in mobile phase, expressed in percent

**Table 6** Relative percent recovery of standard mefloquine metabolite in human plasma

| concentration (ng/ml) | peak area in mobile phase[a] (mean ± SD) (n=5) | peak area in plasma[b] (mean ± SD) (n=5) | % Recovery[c] |
|---|---|---|---|
| 125 | 69871.80 ± 8069.35 | 65171.12 ± 5687.77 | 94.63 |
| 500 | 284487.20 ± 34753.64 | 284061.34 ± 18930.93 | 100.48 |
| 2,000 | 1137291.60± 49516.12 | 1194742.56 ± 98092.11 | 105.16 |

[a]Various concentrations of standard mefloquine metabolite in mobile phase were directly injected

[b]Various concentrations of standard mefloquine metabolite were added to drug-free human plasma samples prior to precipitation

[c]Mean peak area in plasma divided by mean peak area in mobile phase, expressed in percent

**Table 7** Pharmacokinetic parameters (mean ± S.D.) of mefloquine in seven subjects receiving a single oral dose of 500 mg mefloquine alone and during rifampicin coadministration.

| Parameter | Mefloquine alone | Mefloquine + Rifampicin | Paired student's *t*-test |
|---|---|---|---|
| Age (yr) | 32 ± 3.96 | 32 ± 3.96 | - |
| Weight (kg) | 63 ± 5.13 | 63 ± 5.13 | - |
| AUC (mg/l.hr) | 373.73 ± 57.47 | 119.77 ± 54.94 | $p < 0.01$ |
| Ka ($hr^{-1}$) | 0.79 ± 0.26 | 0.62 ± 0.30 | NS |
| Ke ($hr^{-1}$) | 0.00231 ± 0.00034 | 0.00693 ± 0.00239 | $p < 0.01$ |
| $t_{1/2}$ (abs)(hr) | 1.00 ± 0.43 | 1.41 ± 0.80 | NS |
| $t_{1/2}$ (hr) | 305.31 ± 47.15 | 113.43 ± 49.71 | $p < 0.01$ |
| $T_{max}$ (hr) | 8.15 ± 2.91 | 8.67 ± 3.92 | NS |
| $C_{max}$ (ng/ml) | 855.63 ± 168.00 | 695.67 ± 56.63 | $p < 0.05$ |
| Vd/f (l/kg) | 9.44 ± 1.87 | 10.89 ± 1.36 | NS |
| Cl/f (l/hr/kg) | 0.0214 ± 0.0038 | 0.08 ± 0.03 | $p < 0.01$ |

NS represents non-significant difference ($P > 0.05$)

**Table 8** Pharmacokinetic parameters of mefloquine in subjects receiving a single oral dose of 500 mg mefloquine alone.

| Subject No. | Age (yr) | Weight (kg) | AUC (mg/l.hr) | Ka ($hr^{-1}$) | Ke ($hr^{-1}$) | $t_{1/2}$(abs) (hr) | $t_{1/2}$ (hr) | $T_{max}$ (hr) | $C_{max}$ (ng/ml) | Vd/f (l/kg) | Cl/f (l/hr/kg) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 26 | 58 | 401.95 | 0.946 | 0.0018 | 0.73 | 385.00 | 6.58 | 747.98 | 11.38 | 0.02 |
| 2 | 34 | 70 | 342.85 | 0.392 | 0.0020 | 1.77 | 346.50 | 13.50 | 674.87 | 10.3 | 0.02 |
| 3 | 35 | 61 | 434.48 | 0.811 | 0.0024 | 0.85 | 288.75 | 7.19 | 1028.24 | 7.84 | 0.02 |
| 4 | 27 | 57 | 365.67 | 0.796 | 0.0028 | 0.87 | 247.50 | 7.13 | 995.95 | 8.64 | 0.02 |
| 5 | 32 | 69 | 384.92 | 0.982 | 0.0022 | 0.71 | 315.00 | 6.24 | 822.41 | 8.69 | 0.02 |
| 6 | 36 | 61 | 264.67 | 1.092 | 0.0025 | 0.63 | 277.20 | 5.56 | 667.03 | 12.12 | 0.03 |
| 7 | 34 | 65 | 421.60 | 0.484 | 0.0025 | 1.43 | 277.20 | 10.87 | 1052.89 | 7.11 | 0.02 |
| x ± S.D. | 32 ± 3.96 | 63 ± 5.13 | 373.73 ± 57.47 | 0.79 ± 0.26 | 0.0023 ± 0.0003 | 1.00 ± 0.43 | 305.31 ± 47.15 | 8.15 ± 2.91 | 855.63 ± 168.00 | 9.44 ± 1.87 | 0.0214 ± 0.00038 |

**Table 9** Pharmacokinetic parameters of mefloquine in subjects receiving a single oral dose of 500 mg mefloquine during rifampicin coadministration.

| Subject No. | Age (yr) | Weight (kg) | AUC (mg/l.hr) | Ka ($hr^{-1}$) | Ke ($hr^{-1}$) | $t_{1/2}$(abs) (hr) | $t_{1/2}$ (hr) | $T_{max}$ (hr) | $C_{max}$ (ng/ml) | Vd/f (l/kg) | Cl/f (l/hr/kg) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 26 | 58 | 67.80 | 0.457 | 0.011 | 1.52 | 63.00 | 8.40 | 663.52 | 11.88 | 0.13 |
| 2 | 34 | 70 | 127.59 | 0.229 | 0.005 | 3.03 | 130.75 | 16.75 | 628.60 | 10.38 | 0.056 |
| 3 | 35 | 61 | 106.90 | 1.150 | 0.007 | 0.60 | 100.43 | 4.47 | 724.55 | 10.97 | 0.077 |
| 4 | 27 | 57 | 91.52 | 0.603 | 0.007 | 1.15 | 97.61 | 7.44 | 620.75 | 13.4 | 0.096 |
| 5 | 32 | 69 | 237.35 | 0.782 | 0.003 | 0.89 | 216.56 | 7.06 | 742.01 | 9.55 | 0.031 |
| 6 | 36 | 61 | 108.02 | 0.42 | 0.007 | 1.65 | 99.00 | 9.87 | 734.69 | 10.38 | 0.076 |
| 7 | 34 | 65 | 99.20 | 0.666 | 0.008 | 1.04 | 86.63 | 6.71 | 755.59 | 9.65 | 0.078 |
| x ± S.D. | 32 ± 3.96 | 63 ± 5.13 | 119.77 ± 54.94 | 0.62 ± 0.30 | 0.00693 ± 0.00239 | 1.41 ± 0.80 | 113.43 ± 49.71 | 8.67 ± 3.92 | 695.67 ± 56.63 | 10.89 ± 1.36 | 0.08 ± 0.03 |

**Table 10** Pharmacokinetic parameters (mean ± S.D.) of mefloquine metabolite in seven subjects receiving a single oral dose of 500 mg mefloquine alone and during rifampicin coadministration.

| Parameters | Mefloquine alone | Mefloquine + Rifampicin | Paired student's *t*-test |
|---|---|---|---|
| Age (yr) | 32 ± 3.96 | 32 ± 3.96 | - |
| Weight (kg) | 63 ± 5.13 | 63 ± 5.13 | - |
| AUC (mg/l.hr) | 786.42 ± 285.40 | 549.88 ± 170.32 | NS |
| Ka ($hr^{-1}$) | 0.012 ± 0.006 | 0.09 ± 0.05 | $p < 0.01$ |
| Ke ($hr^{-1}$) | 0.00143 ± 0.00029 | 0.00232 ± 0.00044 | $p < 0.01$ |
| $t_{1/2}$ (abs)(hr) | 72.96 ± 39.47 | 10.89 ± 7.80 | $p < 0.01$ |
| $t_{1/2}$ (hr) | 506.66 ± 127.64 | 307.45 ± 56.90 | $p < 0.01$ |
| $T_{max}$ (hr) | 220.62 ± 69.75 | 52.48 ± 28.81 | $p < 0.01$ |
| $C_{max}$ (ng/ml) | 813.16 ± 297.96 | 1194.45 ± 249.10 | $p < 0.05$ |
| Vd/f (l/kg) | 8.12 ± 3.45 | 6.28 ± 1.87 | NS |
| Cl/f (l/hr/kg) | 0.0116 ± 0.0051 | 0.01486 ± 0.00607 | NS |

NS represents non-significant difference ($P > 0.05$)

**Table 11** Pharmacokinetic parameters of mefloquine metabolite in subjects receiving a single oral dose of 500 mg mefloquine alone.

| Subject No. | Age (yr) | Weight (kg) | AUC (mg/l.hr) | Ka ($hr^{-1}$) | Ke ($hr^{-1}$) | $t_{1/2}$(abs) (hr) | $t_{1/2}$ (hr) | $T_{max}$ (hr) | $C_{max}$ (ng/ml) | Vd/f (l/kg) | Cl/f (l/hr/kg) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 26 | 58 | 703.99 | 0.006 | 0.0016 | 105.00 | 433.13 | 285.38 | 709.93 | 7.72 | 0.012 |
| 2 | 34 | 70 | 549.95 | 0.019 | 0.0009 | 36.47 | 770.00 | 161.81 | 465.12 | 13.08 | 0.013 |
| 3 | 35 | 61 | 796.05 | 0.021 | 0.0015 | 33.00 | 462.00 | 135.93 | 983.94 | 6.78 | 0.010 |
| 4 | 27 | 57 | 1011.93 | 0.017 | 0.0013 | 41.01 | 533.08 | 163.07 | 1079.35 | 6.55 | 0.009 |
| 5 | 32 | 69 | 839.30 | 0.009 | 0.0013 | 79.65 | 533.08 | 253.47 | 799.93 | 6.45 | 0.009 |
| 6 | 36 | 61 | 370.70 | 0.009 | 0.0017 | 77.00 | 407.65 | 224.29 | 439.41 | 12.57 | 0.022 |
| 7 | 34 | 65 | 1232.99 | 0.005 | 0.0017 | 138.60 | 407.65 | 320.42 | 1214.42 | 3.68 | 0.006 |
| x ± S.D. | 32 ±3.96 | 63 ± 5.13 | 786.42 ± 285.40 | 0.012 ± 0.006 | 0.0014 ± 0.0003 | 72.96 ± 39.47 | 506.66 ± 127.64 | 220.62 ± 69.75 | 813.16 ±297.96 | 8.12 ± 3.45 | 0.012 ± 0.005 |

**Table 12** Pharmacokinetic parameters of mefloquine metabolite in subjects receiving a single oral dose of 500 mg mefloquine during rifampicin coadministration.

| Subject No. | Age (yr) | Weight (kg) | AUC (mg/l.hr) | Ka ($hr^{-1}$) | Ke ($hr^{-1}$) | $t_{1/2}$(abs) (hr) | $t_{1/2}$ (hr) | $T_{max}$ (hr) | $C_{max}$ (ng/ml) | Vd/f (l/kg) | Cl/f (l/hr/kg) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 26 | 58 | 553.94 | 0.055 | 0.0019 | 12.72 | 364.74 | 63.03 | 970.35 | 7.84 | 0.016 |
| 2 | 34 | 70 | 581.98 | 0.119 | 0.0023 | 5.82 | 301.30 | 33.76 | 1229.81 | 5.38 | 0.012 |
| 3 | 35 | 61 | 572.67 | 0.165 | 0.0028 | 4.21 | 251.09 | 25.23 | 1474.33 | 5.19 | 0.014 |
| 4 | 27 | 57 | 433.44 | 0.072 | 0.0029 | 9.63 | 238.97 | 46.03 | 1124.34 | 6.8 | 0.020 |
| 5 | 32 | 69 | 886.05 | 0.026 | 0.0019 | 27.07 | 364.74 | 109.66 | 1366.51 | 4.31 | 0.008 |
| 6 | 36 | 61 | 325.39 | 0.124 | 0.0026 | 5.57 | 266.54 | 31.64 | 790.23 | 9.54 | 0.025 |
| 7 | 34 | 65 | 831.87 | 0.062 | 0.0019 | 11.18 | 364.74 | 58.01 | 1405.61 | 4.91 | 0.009 |
| x ± S.D. | 32 ± 3.96 | 63 ± 5.13 | 549.88 ± 170.32 | 0.09 ± 0.05 | 0.0023 ± 0.0004 | 10.89 ± 7.80 | 307.45 ± 56.90 | 52.48 ± 28.81 | 1194.45 ± 249.10 | 6.28 ± 1.87 | 0.015 ± 0.006 |

**Table 13** Mefloquine pharmacokinetic data were compared to other published data

| Data | Sources | | | | | |
|---|---|---|---|---|---|---|
| | Karbwang 1991 | Karbwang 1992 | Crevoisier 1997 | Mansor 1989 | Sunbhanich 1997 | Present study[a] |
| Subjects | 8 men | 8 men | 20 men | 10 men | 10 men | 7 men |
| Age (yr) | 24-51 | 25-52 | - | - | 20-40 | 24-35 |
| Dose (mg) | 750 | 750 | 750 | 500 | 500 | 500 |
| Route | oral | oral | oral | oral | oral | oral |
| $C_{max}$ (ng/ml) | 1228 ± 223 | 1161 ± 120 | 868 ± 204 | 1010 | 555.46 ± 117.41 | 855.63 ± 168 |
| $T_{max}$ (hr) | 6 ± 3 | 5.6 ± 2.8 | 36 ± 30 | 5.7 | 12.54 ± 7.35 | 8.15 ± 2.91 |
| $t_{1/2}$ (days) | 17.7 ± 2.51 | 19.7 ± 3.2 | 17.17 ± 4.58 | 16.1 | 9.6 ± 1.45 | 12.72 ± 1.96 |
| Vd/f (l/kg) | 19.4 ± 3.03 | 19.6 ± 4.0 | - | 17.6 | 15.58 ± 3.56 | 9.44 ± 1.87 |
| Cl/f (l/hr/kg) | 0.032 ± 0.005 | 0.029 ± 0.004 | - | 0.032 | 0.051 ± 0.026 | 0.0214 ± 0.0038 |
| AUC (mg/l.hr) | 446.4 ±51.36 | 480 ± 91.2 | 461 ± 123 | - | 225.18 ± 109.76 | 373.73 ± 57.47 |

[a]Data obtained from subjects receiving mefloquine alone