## MATERIALS AND METHODS

### Chemicals and Reagents

The standard mefloquine HCl (Ro 21-5998/001) and a major carboxylic acid mefloquine metabolite, 2,8-bis(trifluoromethyl)-4-quinolinecarboxylic acid (Ro 21-5104) were kindly donated from F-hoffmann-La Roche Ltd., Basel, Switzerland. Mefloquine (Larium®, 250 mg/tablet, Lot No.B230) and rifampicin (Rifagen®, 300 mg/capsule, Lot No. 98558) were purchased from F-hoffmann-La Roche Ltd., Bangkok, Thailand and General Drugs House Co. Ltd., Bangkok, Thailand, respectively. An HPLC grade of acetonitrile and methanol were bought from J.T. Baker Inc., Phillipsburg, USA. Anhydrous sodium sulfate (analytical grade), 85% phosphoric acid (analytical grade), and zinc sulphate (pro analytical grade) were purchased from Merck Darmstadt, Germany and Carlo Erba, Italy, respectively. Water was purified for HPLC by the Milli Q Water Purification System (Millipore, Milford, MA, USA.)

### Instrumentation

The HPLC system consisted of a Jasco PU-980 pump, the Waters 717 plus Autosampler (Waters Associates, Milford, MA., USA.) and a Jasco UV 975 detector. Detection was done with the variable-wavelength UV detector set at 222 nm and peak area was measured by a Jasco 807-IT integrator (Tokyo, Japan). A Jasco recorder attenuation was set at 16 mV.F.S. and chart speed was 2 mm/min. Separation was achieved on a reversed-phase Novapak $C_{18}$ column (3.9 mm x 150 mm HPLC column, particle size 4 μm) (Waters

Associates, Milford, MA., USA.). A guard-pak precolumn module was used to obviate the effect of rapid column degeneration.

## Methods

### 1. Subjects

Seven Thai male volunteers aged 24-35 yr, weighing 57-72 kg were enrolled in this study. None were smokers. The subjects were considered to be healthy based on medical history, physical examination, and essential laboratory tests (complete blood count, renal and liver function tests). All subjects were asked to avoid from taking any other drugs, smoking, alcoholic beverages and coffee at least 1 week prior to and during an entire course of the study. Each volunteer was given a detailed explanation of the purpose and protocol of this study, and each gave a written consent which was approved by the Ethics Committee, Faculty of Science, Prince of Songkla University, Hat Yai, Thailand.

### 2. Protocol

The study was an open-label, two separated phases with a 2-month washout period.

### Phase 1

In the morning after an overnight fast, each subject received a single oral dose of 500 mg mefloquine (2 tablets of 250 mg mefloquine tablet). The drug was administered with a glass of water (200 ml) under supervision. No food was taken at least 2 hours after ingestion of the drug.

A catheter was inserted into a forearm vein for the collection of blood samples, and was maintained patent using 1 ml of a dilute heparin solution

(100 units/ml) after each sample. The catheter was maintained in a forearm vein only for 24 hours. Venous blood samples (5 ml) were collected in heparinized tubes before drug administration and at 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12 and 14 hours, and 1, 2, 3, 4, 7, 14, 21, 28, 35, 42, 49 and 56 days post-drug administration. Samples were centrifuged not later than 30 minutes after collection, and the plasma was separated and stored at -20 °C until analysis.

**Phase 2**

After 2 months of being drug free, all subjects received rifampicin capsule at an oral dose of 600 mg (2 capsules of 300 mg rifampicin capsule) once daily before breakfast for 7 days prior to mefloquine administration. In the morning of day 1 (after rifampicin pretreatment for 7 days), after an overnight fast, all subjects ingested a 600 mg rifampicin 2 hours before an oral administration of 500 mg mefloquine. Venous bloods samples were collected at the time interval before and after mefloquine administration as previously done in phase 1. However, rifampicin was continued to be orally administered 600 mg daily before breakfast from day 1 to day 7 and then 600 mg twice-weekly from day 8 to day 56.

**3. Sample Analysis**

The plasma mefloquine and mefloquine metabolite concentrations were measured by a high performance liquid chromatographic (HPLC) method (Bergqvist, 1988) with some modification as follows.

**3.1 Mobile Phase**

The mobile phase consisted of 50 mmol/L sodium sulfate-methanol-acetonitrile (50 : 34 : 16 vol/vol/vol) and adjusted to pH 3.07 with 85% phosphoric acid. The mobile phase was freshly prepared daily and was filtered

through 0.45 micropore filtered paper (Nyron 66), then degases by sonification for 10 minutes before using. The flow rate was 1 ml/min. All analysis were performed at room temperature (about $25 \pm 1$ °C)

## 3.2 Stock Standard Solution

A stock standard solution at a concentration of 400 μg/ml was prepared by dissolving 4 mg of standard mefloquine in 10 mmol/l hydrochloric acid and standard mefloquine metabolite dissolved in 10 mmol/l sodium hydroxide solution. All solutions were adjusted to 10 ml in a 10 ml volumetric flask. The stock solutions were stable for at least 6 months at 4 °C (Edstein, 1991). Working standard solutions used to prepare a calibration curve day by day were prepared by appropriate dilution of the stock standard solution with a blank plasma.

## 3.3 Calibration Curve

Calibration curves were prepared by adding a standard mefloquine and mefloquine metabolite solution to blank human plasma so that the final concentrations in plasma were 62.5, 125, 250, 500, 1,000 and 2,000 ng/ml. The calibration curves for mefloquine and mefloquine metabolite were linear in the range 62.5 to 2,000 ng/ml. The average coefficient of variations (CV) should be less than 10%. The lower detection limit for mefloquine and mefloquine metabolite was 50 ng/ml.

### 3.3.1 Recovery

Potential loss of mefloquine and metabolite during the precipitation by acetonitrile was determined by comparing the peak area of mefloquine and metabolite precipitated from plasma samples in the range of 62.5-2,000 ng/ml

and the equal concentration of standard mefloquine prepared in mobile phase. The percent recovery was calculated as following

$$\frac{\text{Peak area of standard mefloquine or metabolite in plasma}}{\text{Peak area of standard mefloquine or metabolite in mobile phase}} \times 100$$

### 3.3.2 Precision and Variability

To determine intra-day precision and variability, the standard mefloquine was spiked in blank plasma at low (125 ng/ml), medium (500 ng/ml) and high (2,000 ng/ml) concentrations and 10 replications of each were carried out on one day. All should be of ±10% of spiked value (actual value) and the CV (CV = SD/Mean normalised peak area ratio x 100) of each concentration should be < 10%.

To determine inter-day precision and variability, the standard mefloquine was spiked in blank plasma at low (125 ng/ml), medium (500 ng/ml) and high (2,000 ng/ml) concentrations and each concentration was carried out on 10 different days. Accuracy should be of ±10% of spiked value (actual value) and the CV of each concentration should be <10%.

### 3.4 Sample preparation

A 200 µl of plasma sample or spiked standard plasma was used and 50 µl of 0.2 M zinc sulphate solution was added dropwise to polypropylene tubes containing sample plasma or spiked standard plasma and vortex mixing for 30 seconds. A 500 µl volume of acetonitrile was then added dropwise during vortex-mixing for 30 seconds. After 15 minutes the tubes were centrifuged at 10,000 g for 7 minutes. The supernatant (600 µl) was transfered into polypropylene tubes and evaporated to dryness at 55 °C for 15-20 minutes under a stream of nitrogen. The residue was reconstituted in 300 µl of mobile

phase via ultrasonication (3 minutes) and 100 µl of the solution was injected onto the HPLC system. The chromatographic conditions used in this study were good to separate mefloquine and mefloquine metabolite from other endogenous substances in plasma.

## 4. Data Analysis

### 4.1 Pharmacokinetic Calculations

The following parameters were calculated by using Winnonlin$^{®}$ software program, 1995.

The maximum plasma mefloquine concentration ($C_{max}$), the time to reach $C_{max}$ ($T_{max}$), the absorption rate constant (Ka), the absorption half-life ($t_{1/2abs}$), the elimination rate constant (Ke), the elimination half-life ($t_{1/2}$) and the area under the concentration-time curve (AUC).

The apparent oral clearance (Cl/f) was calculated as dose/(AUC x body weights).

The apparent volume of distribution (Vd/f) was calculated as Cl/f divided by Ke.

### 4.2 Statistical Analysis

All results were expressed as means ± S.D. Differences in mefloquine and mefloquine metabolite pharmacokinetic parameters between control and treatment groups were tested for statistical significance by Student's *t* test with $P < 0.05$ taken as the minimum level of significant.