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Determination of the enantiomers of mefloquine in plasma and whole blood using a coupled achiral–chiral high-performance liquid chromatographic system

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ABSTRACT

A coupled achiral–chiral high-performance liquid chromatographic system has been developed for the determination of the enantiomers of mefloquine, (+)-MFQ and (−)-MFQ, in plasma and whole blood. The MFQ was separated from the interfering components in the biological matrix and quantified on a cyano-bonded phase, and the enantiomeric composition was determined on an (*S*)-naphthylurea chiral stationary phase. The two columns were connected by a switching valve equipped with a silica precolumn. The precolumn was used to concentrate the MFQ in the eluent from the achiral column before backflushing onto the chiral phase. The coupled-column

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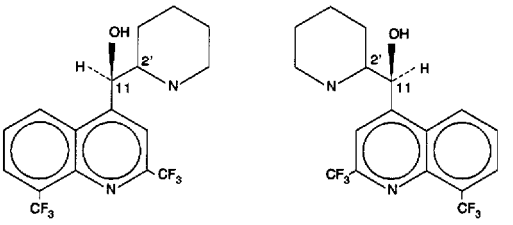
system was validated and applied to the analysis of a pilot study of the pharmacokinetics of (+)- and (−)-MFQ in plasma and whole blood.

INTRODUCTION

Mefloquine (MFQ) is a chiral molecule which is used for the treatment and prophylaxis of malaria. The agent is administered orally as a racemic mixture of the (+)-(11*R*,2′*S*) and (−)-(11*S*,2′*R*) forms (Fig. 1). Initial studies of MFQ pharmacokinetics have demonstrated that the drug has a long half-life of between 10 and 33 days [1–3]. However, these studies have not considered the plasma disposition and elimination of the MFQ enantiomers, (+)-MFQ and (−)-MFQ.

The potential importance of determining the pharmacological fate of the separate MFQ enantiomers was indicated by a study which found that (−)-MFQ is a more potent acetylcholinesterase and butylcholinesterase inhibitor than (+)-MFQ [4]. In addition, there have been conflicting studies regarding the differential accumulation of MFQ in erythrocytes versus plasma [5,6]. The enantioselectivity of this accumulation has not been studied.

The enantiomers of MFQ can be directly resolved on an (*S*)-naphthylurea chiral stationary phase using a hexane–2-propanol–methanol (82:4:14, v/v) mobile phase with a stereoselectivity factor (α) of 1.63. However, this separation could not be directly applied to plasma or whole blood samples due to interference from endogenous compounds. To overcome this problem, a coupled achiral–chiral high-performance liquid chromatographic (HPLC) system was developed (Fig. 2). In this system, the MFQ was separated from interferences in the biological matrix and quantitated on an achiral cyano-bonded phase. The eluent containing the MFQ was then transferred to a silica gel precolumn where the MFQ was concentrated. The target compound was then backflushed onto the column containing the (*S*)-naphthylurea chiral stationary phase where (−)- and (+)-MFQ were stereochemically resolved and the enantiomeric ratio determined.



(+) Mefloquine
11R,2'S

(-) Mefloquine
11S,2'R

Fig. 1. Structures of the enantiomers of mefloquine.

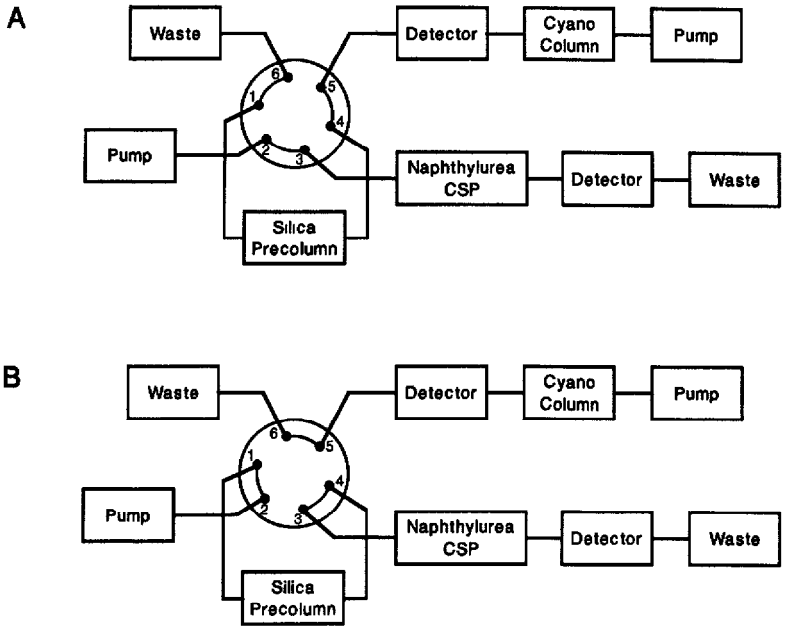


Fig. 2. Chromatographic system used in this study. See text for chromatographic conditions. (A) System configuration used to load the mefloquine on the silica precolumn. (B) System configuration used to determine total mefloquine concentrations before switching and the enantiomeric composition after switching.

The coupled achiral–chiral system used for the determination of MFQ enantiomers in plasma and whole blood differs from previously reported coupled systems [7–9] by the placement of the first detector before, rather than after, the switching valve. In the previous systems, two injections were required to quantitate the enantiomers: one to measure the total concentration and the other to determine the enantiomeric ratio. In this system, only a single injection is required. In addition, the injection loop on the switching valve was replaced by a precolumn which increased the efficiency of the transfer between achiral and chiral systems.

This paper reports the development and validation of this system and its application to a pilot pharmacokinetic study.

EXPERIMENTAL

Chemicals

Racemic MFQ was a gift from Roche (Basel, Switzerland). The (+)-MFQ and (−)-MFQ enantiomers were prepared according to Carroll and Blackwell [10]. The internal standard chloroquine (CQ) was a gift from Specia (Paris, France). Hexane, 2-propanol, acetonitrile and methanol were purchased from

Burdick and Jackson (Muskegon, MI, U.S.A.). Triethylamine was purchased from Aldrich (Milwaukee, WI, U.S.A.) and methylene chloride was purchased from Fisher Scientific (Fair Lawn, NJ, U.S.A.). All other chemicals were reagent grade and used as purchased.

Apparatus

The analytical method involved two chromatographic systems connected through a Rheodyne Model 7010 switching valve (Rainin, Woburn, MA, U.S.A.) equipped with a 10-μm silica gel guard column (30 mm$\times$2.1 mm I.D., Alltech Assoc., Deerfield, IL, U.S.A.) (Fig. 2). When the analyte was detected in the achiral system, the switching valve was rotated and the eluent flow containing the analyte diverted to the precolumn. After 75 s, the switching valve was rotated again and the analyte injected onto the chiral column. During the same time, the internal standard was detected on the achiral system.

Achiral chromatography

The achiral chromatography was performed with a Waters 600 E system controller and a Waters 484 tunable absorbance detector set at 285 nm (Waters Assoc., Milford, MA, U.S.A.), a Shimadzu C-R6A integrator (Shimadzu, Columbia, MD, U.S.A.), an Altex 210 A injection valve (Beckman, Houston, TX, U.S.A.) and a 10-μm Adsorbosphere cyano column (250 mm$\times$4.6 mm I.D., Alltech Assoc.). The separation of MFQ and the internal standard CQ from the plasma components was accomplished on the cyano column using a mobile phase composed of hexane–2-propanol–methanol (82:4:14, v/v) modified with 0.005% triethylamine. The analyses were carried out using a flow-rate of 2.0 ml/min and ambient temperature.

Chiral chromatography

The chiral chromatography was performed with a modular liquid chromatograph composed of a Beckman 116 programmable solvent delivery module pump, a Beckman 166 variable-wavelength UV detector set to 285 nm (Beckman, Houston, TX, U.S.A.) and a Shimadzu C-R6A integrator. The chiral column was a 5-μm Supelco LC-(*S*)-naphthylurea column (250 mm$\times$4.6 mm I.D., Supelco, Bellefonte, PA, U.S.A.). The stereochemical separation of (+)-MFQ and (−)-MFQ was accomplished on the (*S*)-naphthylurea column using a mobile phase composed of hexane–2-propanol–methanol (82:4:14, v/v) modified with 0.005% triethylamine. The analyses were carried out using a flow-rate of 1.0 ml/min and ambient temperature. The enantiomeric elution order was established by chromatographing the separate enantiomers.

Sample preparation

Collection of samples. A commercially available form of MFQ, Lariam® 250-mg tablets (Roche, France), was used in a pilot study. Four tablets, equivalent to 15 mg/kg MFQ base, were administered orally to a healthy male caucasian volunteer. Blood samples (10 ml) were collected in a glass vacutainer with EDTA (Aldrich, Milwaukee, WI, U.S.A.) before administration and at 2, 4, 6, 8, 10, 12, 14, 16, 24, 28, 32 h and 2, 3, 5, 7, 12, 14, 21, 30 days. The samples were separated after collection with 2 ml of whole blood transferred to one polypropylene tube while the remaining sample was centrifuged and the plasma collected and transferred to a separate polypropylene tube. The samples were stored at −20°C.

Extraction procedure. MFQ was extracted from whole blood and plasma according to Franssen et al. [11]. To 500 μl whole blood or plasma were added 250 μl of the methanol solution of the internal standard CQ (1.5 μg/ml) and 1.0 ml of acetonitrile. The mixture was vortex-mixed (30 s), centrifuged (2000 *g*, 5 min) and the supernatant transferred to a polypropylene tube. Tris buffer (1.5 ml of a 25 m*M* hydroxymethylaminoethane solution, adjusted to pH 8.2 with hydrochloric acid) was added to the supernatant followed by methylene chloride (6 ml). The resulting mixture was gently shaken for 15 min, centrifuged (2000 *g*, 10 min), and the methylene chloride phase was transferred to a polypropylene tube and evaporated under nitrogen. The remaining solid was dissolved in the mobile phase and analyzed.

The percentage recovery and the reproducibility of this method were investigated using drug-free serum samples spiked with 0.1 and 1.6 μg/ml racemic MFQ with $n=5$ at each level.

Standard curves

A standard curve for total MFQ [i.e. (+)-MFQ and (−)-MFQ] plasma concentrations was prepared by the addition of 0.1, 0.2, 0.4, 0.8, 1.6 μg/ml to drug-free serum. The standard curve was constructed by plotting the MFQ/CQ peak-height ratios versus the known MFQ concentrations. The study was carried out on the achiral chromatographic system.

RESULTS AND DISCUSSION

Achiral chromatography

The results from the chromatography of MFQ and CQ on the achiral chromatographic system are presented in Fig. 3. Under the chromatographic conditions used in this study, the chromatographic retentions, expressed as the capacity factors (k') of MFQ and CQ were 7.6 and 12, respectively. The MFQ and CQ were resolved from interfering compounds from the serum matrix (Fig. 3A and B) and from each other, selectivity factor $\alpha=1.58$.

The standard curve for the MFQ was linear over the range investigated and

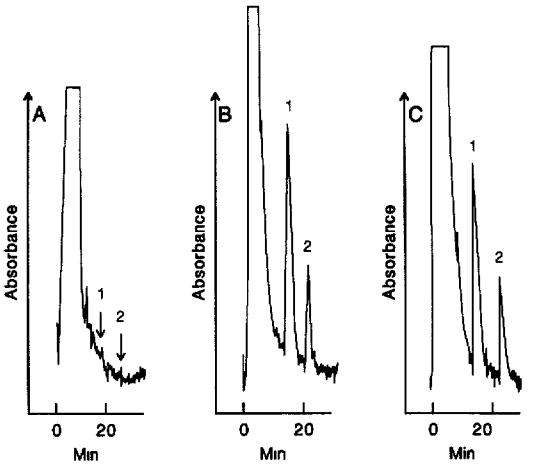


Fig. 3. Representative chromatograms of plasma samples on the achiral system. (A) Blank plasma; (B) plasma spiked with 1 μg/ml racemic MFQ; (C) plasma sample 72 h after the administration of 1000 mg of racemic MFQ to a healthy volunteer. See text for chromatographic conditions. Peaks: 1 = MFQ; 2 = CQ, internal standard.

the equation describing the curve was $y=0.43x+0.04$ with a correlation coefficient of 0.9993. The intra-day reproducibility coefficient of variation (C.V.) of the assay was 2.98% for the high standard (1.6 μg/ml) and 6.67% for the low standard (0.10 μg/ml), where $n=5$. The inter-day reproducibility ($n=5$ for three days) C.V. was 7.1% for 1.6 μg/ml and 10.7% for 0.10 μg/ml. The limit of detection was 0.05 μg/ml and the recovery was 85% at low concentrations (0.10 μg/ml, $n=5$) and 90% at high concentrations (1.6 μg/ml, $n=5$).

Chiral chromatography

Representative chromatograms of the enantiomers of MFQ on the chiral stationary phase are presented in Fig. 4. The k' values for (+)-MFQ and (−)-MFQ were 7.9 and 12.0, respectively; the observed stereoselectivity factor (α_s) was 1.45 and the stereochemical resolution factor (R_s) was 1.45.

Since a baseline resolution of the MFQ enantiomers was not obtained under the chromatographic conditions used in this study, the (+)-MFQ/(−)-MFQ peak-height ratio of racemic MFQ was investigated over the expected plasma concentration range 0.1–1.0 μg/ml. The peak height versus MFQ concentration curves for (+)- and (−)-MFQ were linear over the range investigated and the equations describing the curves were $y=14.9x-0.18$ with a correlation coefficient of 0.9984 and $y=13.4x-0.03$ with a correlation coefficient of 0.9996, respectively. The (+)-MFQ/(−)-MFQ ratio was 1.1 over this range. Since the ratio of the peak heights was not unity, the observed ratio of 1.1 was used

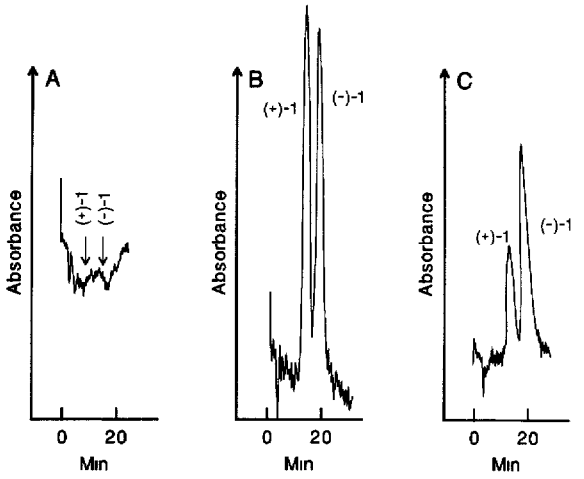


Fig. 4. Representative chromatograms of plasma samples on the chiral system. (A) Blank plasma; (B) plasma spiked with 1 μg/ml racemic MFQ; (C) plasma sample 72 h after the administration of 1000 mg of racemic MFQ to a healthy volunteer. See text for chromatographic conditions. Peaks: (+)-1 = (+)-MFQ; (−)-1 = (−)-MFQ.

as a correction factor in the calculation of the actual percentage composition of each enantiomer.

Pharmacokinetic pilot study

The serum and whole blood levels of (+)- and (−)-MFQ were determined over a 21-day period following the administration of 1000 mg (15 mg/kg) of MFQ to a healthy male caucasian volunteer. The chromatograms from a plasma sample collected 72 h after dosing are presented in Figs. 3C and 4C.

The total MFQ concentration ([MFQ]) was determined on the achiral section of the coupled chromatographic system and the percentages of (+)-MFQ [%(+)-MFQ] and (−)-MFQ [%(−)-MFQ] were determined on the chiral section. The both determinations were carried out in a single experiment. The total amounts of each enantiomer were calculated using the following equations:

$$\text{total (+)-MFQ} = [\text{MFQ}] \times \%(+)\text{-MFQ}$$
$$\text{total (−)-MFQ} = [\text{MFQ}] \times \%(-)\text{-MFQ}$$

The plasma concentration versus time curves for the enantiomers of MFQ are presented in Fig. 5. At each experimental point, the plasma concentration of (−)-MFQ was greater than that of the (+)-enantiomer. The (−)-MFQ/(+)-MFQ plasma concentration ratio varied from 1.7 at 2 h to 11.5 at 504 h. The initial results indicate that both the absorption and elimination of the drug are stereospecific.

The whole blood concentration versus time curves for the enantiomers of

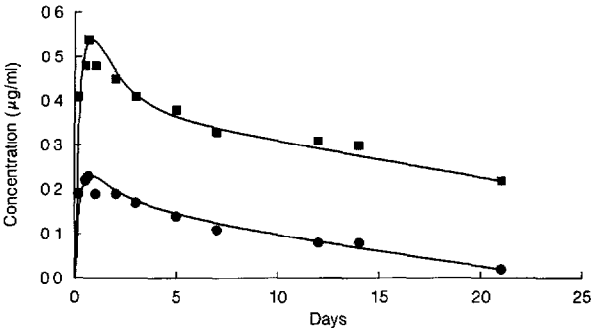


Fig. 5. Plasma concentration versus time curves for the enantiomers of MFQ after the administration of 1000 mg racemic MFQ to a healthy volunteer. (●) (+)-MFQ; (■) (−)-MFQ.

MFQ also differed, but the magnitude of the difference between the two enantiomers was not as great. The (−)-MFQ/(+)-MFQ whole blood concentration ratio varied from 1.5 at 2 h to 3 at 504 h. The results of a complete pharmacokinetic study will be reported elsewhere.

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