A High-Performance Liquid Chromatographic Method for the Quantitative Enantioselective Analysis of Mefloquine Stereoisomers

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A rapid quantitative, enantioselective HPLC method for the analysis of the four stereoisomers, (+) and (−) erythro and (+) and (−) threo forms, of mefloquine has been developed using a Chiralpak AD analytical column containing amylose tris-3,5-dimethylphenyl carbonate coated on silica gel and hexane/ethanol/diethylamine (96:4:0.1, v/v%) as the mobile phase. This method made it possible to quantitate small amounts of threo form in the presence of the erythro form of mefloquine, the form which is used as the active ingredient in commercial mefloquine tablets. Tablets from three sources were studied to estimate their optical purity, and it was found that tablets from one source contain 0.27 w/w% of the (−)-threo and 0.25 w/w% of the (+)-threo form, tablets from the second source contain 0.056 and 0.042 w/w% (−)- and (+)-threo, respectively, and tablets from the third source contain 0.052 w/w% (+)-threo, with the remainder erythro.

KEY WORDS: mefloquine; high-performance liquid chromatography; enantiomer separation; optical purity; determination in tablets.

INTRODUCTION

Mefloquine hydrochloride (Fig. 1) is a synthetic 4-quinoline methanol compound effective against chloroquine- and quinine-resistant strains of *Plasmodium falciparum*. F. I. Carroll and J. T. Blackwell synthesized four optical isomers (Fig. 2) of the compound, which chemically is α-[2,8-bis(trifluoromethyl)-4-quinolyl]-α-(2-piperidyl)-methanol hydrochloride (1). The agent is administered orally as the erythro form, that is, a racemic mixture of the (+)-(11*R*,2′*S*) and (−)-(11*S*,2′*R*) forms.

Gimenez *et al.* have reported on the resolution of two of the enantiomers of erythro mefloquine on an (*S*)-naphthylurea chiral stationary phase using a hexane–2-propanol–methanol (82:4:14, v/v) mobile phase. The stereoselectivity factor (α) was 1.63 (2). They have also employed a coupled achiral–chiral system, with chloroquine as internal standard to separate the two enantiomers in plasma and whole blood. The system they used consisted of a cyano-bonded phase and a (*S*)-naphthylurea chiral stationary phase connected by a switching valve equipped with a silica precolumn. In a pilot pharmacokinetic study performed on a single subject, the authors found that the plasma concentration of (−)-mefloquine was greater than that of the (+)-enantiomer. The (−)-mefloquine/(+)-mefloquine plasma concentration ratio varied from 1.7 at 2 hr to 11.5 at 504 hr. They also reported that both the absorption and the elimination of the drug are stereospecific (2). In an earlier *in vitro* study, Ngiam and Go demonstrated that (−)-mefloquine is a more potent inhibitor of acetylcholinesterase and butyrylcholinesterase than (+)-mefloquine. However, no reports have appeared concerning the therapeutic usefulness or toxicity of threo mefloquine. Therefore, it seems reasonable to expect that compendial standards developed for this drug product will include a measurement of enantiomeric purity, since some stereoisomers could potentially exhibit toxic effects.

We have developed a rapid, quantitative, enantioselective HPLC method for the analysis of the four stereoisomers of mefloquine.

MATERIALS AND METHODS

Reagents and Chemicals

Erythro and threo racemates and four stereoisomers of mefloquine hydrochloride were characterized products supplied by the Walter Reed Army Institute of Research. One lot of tablets (Lot E598) was obtained from the same source and had been manufactured by a generic firm. These tablets are referred to hereafter as WR tablets. Lariam (mefloquine hydrochloride; Roche) tablets (Lot 0014) were purchased from a local wholesaler. Mephaquin (mefloquine hydrochloride; Mepha) tablets (Lot 91565) were generously supplied by Mepha Ltd., Aesch-Basle, Switzerland. Hexane, ethanol, and methanol were HPLC grade; diethylamine and concentrated ammonia solution were reagent and GR grade, respectively.

Chromatographic Method

The HPLC system used consisted of a solvent delivery pump (Shimadzu LC-6A), an injection valve (Rheodyne 7161) fitted with a 20-μl loop, a variable-wavelength UV-VIS detector (Shimadzu SPD-6AV), and an integrator (Shimadzu CR-601). The detector wavelength was set at 285 nm, and the sensitivity range was 0.005–0.04 AUFS. The mobile phase consisted of hexane/ethanol/diethylamine (96:4.0:0.1%, v/v) and was filtered through an 0.50-μm filter before use. The flow rate was set at 1.0 ml/min. The HPLC column used was a Chiralpak AD analytical column containing amylose tris-3,5-dimethylphenyl carbamate coated on silica gel with a particle size of 10 μm (250 × 4.6 mm; Daicel Chemical Industries). Analyses were performed at room temperature.

Identification of Four Stereoisomers by HPLC

Ten milligrams of the hydrochloride salt of each isomer was dissolved in 10 ml of water, and 0.5 ml of ammonia solution was slowly added. The free bases obtained were filtered off, washed with water, and dried in a vacuum desiccator for 2 days. These free bases of four isomers were

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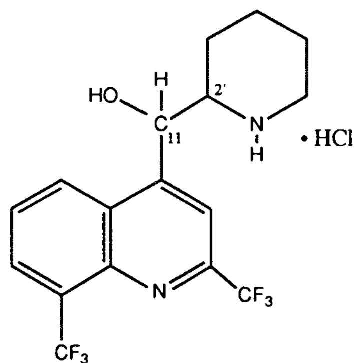


Fig. 1. Mefloquine hydrochloride.

Fig. 3. Chromatogram illustrating the separation of a mixture of racemic (±)-threo and racemic (±)-erythro mefloquine. Column: Chiralpak AD (250 × 4.6 mm). Mobile phase: hexane–ethanol–diethylamine (96:4:0.1). Flow rate: 1.0 ml/min. Retention times: (+)-(11*R*/2′*R*)-threo, 5.72 min; (−)-(11*S*/2′*S*)-threo, 6.29 min; (−)-(11*S*/2′*R*)-erythro, 6.95 min; (+)-(11*R*/2′*S*)-erythro, 11.67 min.

dissolved in the HPLC mobile phase and injected onto the HPLC to determine the enantiomeric elution order.

Preparation of Standard Curve

One hundred milligrams of the erythro and threo racemates of mefloquine hydrochloride was dissolved in 100 ml of water, and 5 ml of ammonia solution was added. These free bases of erythro and threo racemates were filtered off, washed with 20 ml of water, and dried in a vacuum desiccator for 2 days. Stock solutions of these erythro and threo racemates were prepared by dissolving them in the HPLC mobile phase (250 and 50 μg/ml, respectively) and dilutions were performed to obtain a series of solutions with concentrations ranging from 0.25 to 2.5 μg/ml of the threo and 5 to 50 μg/ml of the erythro form. These standard solutions were injected onto the HPLC and the standard curves for each individual isomer were obtained.

Sample Preparation for Determining the Optical Purity of Mefloquine in Commercial Tablets

Ten tablets of mefloquine hydrochloride were weighed and finely ground. Then 0.1, 0.5, or 1.0 mg of the hydrochloride salt of the threo form was added to the ground tablets, equivalent to 100 mg of the erythro form of mefloquine hydrochloride, and these mixed samples were sonicated with 50 ml of methanol for 5 min. After filtration, the filtrate was evaporated to dryness in a rotary evaporator and the residue was stored in a vacuum desiccator for 2 days. The residue was dissolved in 100 ml of water, the solution was filtered, and 5 ml of ammonia solution was added to obtain tablet free base. The precipitate was filtered, dried, washed with 20 ml of water, and dried in a vacuum desiccator for 2 days. The dried samples were used to determine the optical purity of the mefloquine hydrochloride in tablets obtained from three sources. The HPLC method described above was used for the analyses.

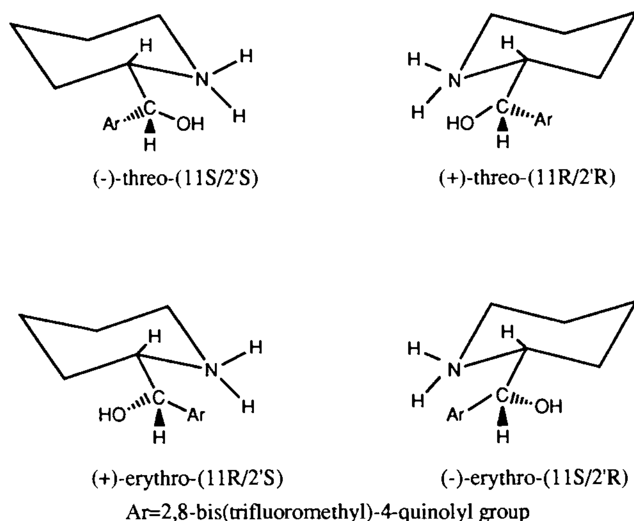


Fig. 2. Absolute configurations of four stereoisomers of mefloquine.

RESULTS AND DISCUSSION

Chiral Separation of the Four Mefloquine Isomers

In order to achieve optimum direct separation of the mefloquine stereoisomers, different concentrations of 2-propanol or of ethanol in hexane were used as the mobile phase. Since mefloquine is a secondary amine, the addition of 0.1%

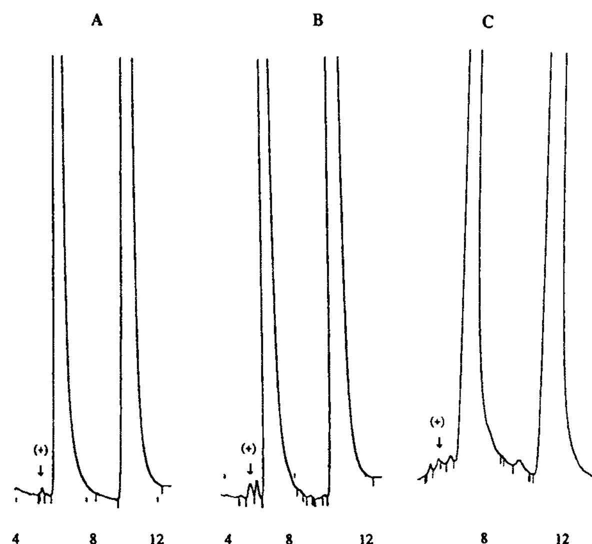
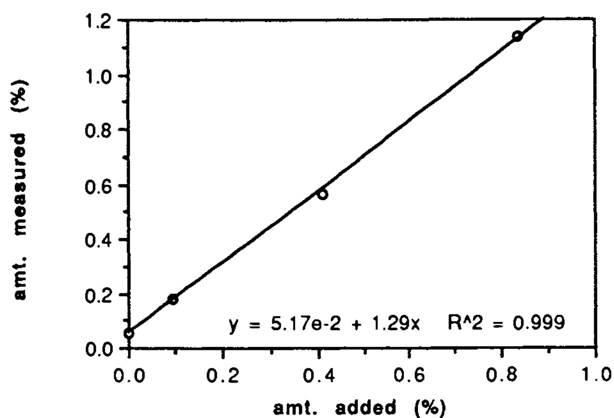
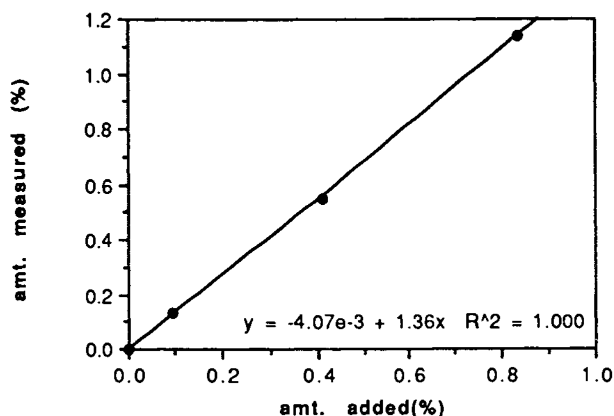


Fig. 4. Chromatograms of the samples obtained from tablets of racemic (±)-erythro mefloquine. Conditions were the same as in Fig. 3. (A) Lariam tablets. Retention times: 5.40, 6.48, and 10.39 min. (B) WR tablets: retention times—5.46, 5.84, 6.49, and 10.40 min. Mephaquin tablets: retention times—5.72, 6.40, 7.10, and 11.83 min.

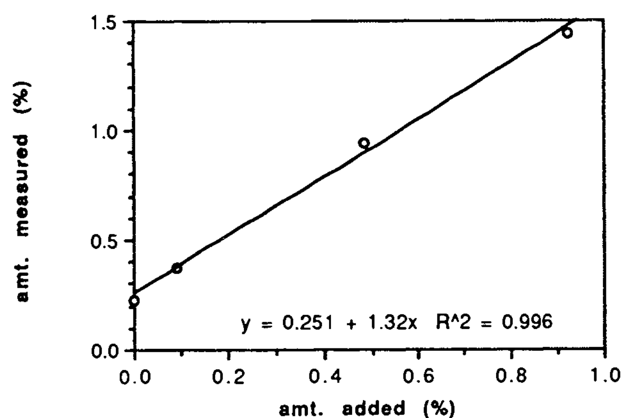


(a)

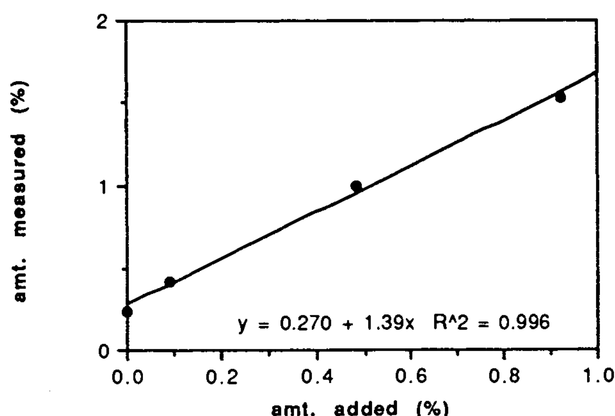


(b)

Fig. 5. Relationship between percentage added and percentage measured of mefloquine for Lariam tablets: (a) (+)-threo; (b) (−)-threo mefloquine.



(a)



(b)

Fig. 6. Relationship between percentage added and percentage measured of mefloquine for WR tablets: (a) (+)-threo; (b) (−)-threo mefloquine.

of diethylamine was found to improve the resolution of the threo form and suppress the tailing of the elution peaks. A representative chromatogram illustrating baseline enantiomeric separation of the mixture of racemic (±)-threo and racemic (±)-erythro mefloquine is shown in Fig. 3. In the case of the threo form the peak that eluted first was identified as (+)-(11*R*/2′*R*)-threo, and the second peak that eluted was identified as (−)-(11*S*/2′*S*)-threo. Similarly for the erythro form, the peak that eluted first was (−)-(11*S*/2′*R*)-erythro and the second peak was (+)-(11*R*/2′*S*)-erythro.

As shown in Fig. 3, the four stereoisomers of mefloquine can be completely separated using the above-mentioned HPLC method.

Quantitation

Mean peak heights for each enantiomer from duplicate injections of solutions of mefloquine with six concentrations ranging from 0.25 to 2.5 μg/ml for threo and 5 to 50 μg/ml for erythro were plotted against the corresponding concentrations. Good linear relationships were obtained for each enantiomer of mefloquine. The regression equations obtained at different sensitivities were as follows.

$y = 370x - 3.73$, $R^2 = 0.997$, $n = 6$, for (+)-threo at 0.005 AUFS.

$y = 343x - 3.26$, $R^2 = 0.997$, $n = 6$, for (−)-threo at 0.005 AUFS.

$y = 23.7x - 8.17$, $R^2 = 1.000$, $n = 6$, for (+)-erythro at 0.04 AUFS.

$y = 37.3x - 12.8$, $R^2 = 1.000$, $n = 6$, for (−)-erythro at 0.04 AUFS.

It is evident that it is possible to detect and quantitate small amounts of the threo form in the presence of the predominant erythro form.

Optical Purity Determination of Commercial Tablets

Commercial mefloquine hydrochloride tablets from

three sources were studied to confirm optical purity. Figure 4 shows chromatograms of the active ingredient obtained by extraction from the tablets. Figures 5 and 6 show the relationship between added amounts of the standard threo form and measured amounts of the threo form in the active ingredient extracted from two tablet lots. Figure 4 shows that small amounts of the (+)- and (−)-threo forms were observed in the WR tablets, while it was difficult to detect peaks corresponding to the threo form in Lariam tablets. In order to determine the exact amounts of the threo form in tablets, known amounts of the threo form were added to ground material obtained from the three lots of commercial tablets. Good linear relationships between percentage added and percentage measured for threo mefloquine are observed in all cases, as shown in Figs. 5 and 6. Therefore, the impurity level of threo mefloquine in the tablets containing erythro mefloquine can be determined from the intercept of the regression equation for each enantiomer. It was found that the Lariam tablets contain 0.00 w/w% of (−)-threo and 0.052 w/w% of (+)-threo, while the WR tablets contain 0.270 w/w% of (−)-threo and 0.251 w/w% of (+)-threo mefloquine.

A similar plot (not shown) was obtained for the Mephaquin tablets, with the relationships between percentage added and percentage measured of mefloquine as follows.

(+)-Threo: $Y = 0.0417 + 1.31x$, $R^2 = 1.000$.
(−)-Threo: $Y = 0.0562 + 1.24x$, $R^2 = 0.999$.

Thus, these tablets contain 0.042 w/w% of (+)-threo and 0.056 w/w% of (−)-threo mefloquine.

CONCLUSIONS

The HPLC method described here is capable of exhibiting baseline resolution of all four isomers of mefloquine. Furthermore, this method has the advantage of being rapid and direct since it requires no derivatization and can be used for optical purity determination of the individual enantiomers in formulations as well as in pure materials. It can also provide a reliable and less tedious alternative to the chemical isolation of mefloquine enantiomers because of the availability of the Chiralpak AD preparative column.

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