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 amyllose tris-3,5-dimethylphenyl carbonate coated on silica gel and hexane/ethanol/diethylamine (96: 4:0.1, v/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 remaining 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-quinoyl]-α-(2-piperidyl)-methanol hydrochloride (1). The agent is administered orally as the erythro form, that is, a racemic mixture of the (+)-(11R,2’S) and (−)-(11S,2’R) forms.

Gimenez et al. have reported on the resolution of two of the enantiomers of erythro mefloquine on an (S)-naphthyleurea chiral stationary phase using a hexane–2-propanol–methanol (82:4:14, v/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)-naphthyleurea 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 three 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 hereafter as WR tablets. Lariam (mefloquine hydrochloride; Roche) tablets (Lot 0014) were purchased from a local wholesaler. Mephaprin (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 amyllose 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
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 threee 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 threee 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 threee 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 three 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 three 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.

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%