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ANALYSIS OF THE ANTIMALARIAL, MEFLOQUINE, IN BLOOD AND PLASMA USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

An analytical method is described for the quantitation of mefloquine, a new antimalarial agent, in plasma and blood. A structurally similar quinolinemethanol compound, WR 184,806, is used as the internal standard. The method employs a three-step extraction procedure followed by reversed-phase high-performance liquid chromatography, and octanesulfonate is used as an ion-pairing reagent. Detection is achieved at 222 nm. The entire procedure is relatively simple and requires only 1 ml of sample. Good accuracy and precision are obtained over the wide concentration range tested.

INTRODUCTION

Mefloquine [(*R**,*S**)-(±)-α-2-piperidinyl-2,8-bis(trifluoromethyl)-4-quinolinemethanol] hydrochloride is a recently developed antimalarial agent. It is effective as a single oral dose against multidrug-resistant malaria in man [1, 2]. It also provides suppressive prophylaxis against *Plasmodium vivax* and *P. falciparum* infections in human volunteers [3, 4]. Several methods have been published on the analysis of mefloquine in blood and/or plasma, including thin-layer chromatographic (TLC) [5], high-performance liquid chromatographic (HPLC) [6], ion-selective electrode [7], gas—liquid chromatographic (GLC) [8], and selected-ion monitoring (SIM) [9] methods. All of these methods have some inherent drawbacks. Several of them require a large sample size (5 ml) [6—8]. Certain methods used a relatively uncommon technique such as

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an ion-selective electrode to detect hydrophobic cations in aqueous solution [7], or relatively sophisticated and expensive instrumentation such as gas chromatography—mass spectrometry [9]. Some of the methods require derivatization [7—9] and some do not use internal standard [5, 7]. The TLC technique, in addition to lacking the resolving power of HPLC [10], is further hampered by its sensitivity to changes in ambient conditions, a limited concentration range and by plate-to-plate differences [5]. Furthermore, in the reported methods [5—9], the evaporation step is carried out in ethyl acetate, isopropyl acetate or ether. It has been our experience that evaporation in these solvents can cause major decomposition of mefloquine even when solvents of the highest commercial grade or redistilled solvents are used.

Therefore, a relatively simple, yet sensitive and selective, method was developed for the analysis of mefloquine in blood and plasma and is currently being employed in pharmacokinetic studies.

EXPERIMENTAL

Chemicals

Mefloquine hydrochloride [WR 142,490 HCl, (*R**,*S**)-(±)-α-2-piperidinyl-2, 8-bis(trifluoromethyl-4-quinolinemethanol) hydrochloride] and WR 184,806 H_3PO_4 {[DL-2,8-bis(trifluoromethyl)-4-[1-hydroxy-3-(N-*tert.*-butylamino)-propyl] quinoline phosphate} were obtained on contract from Ash Stevens (Detroit, MI, U.S.A.), and Starks Associates (Buffalo, NY, U.S.A.), respectively. ^{14}C-Labeled mefloquine ([^{14}C] methanol, 57.8 mCi/mmole) was obtained on contract from Research Triangle Institute (Research Triangle Park, NC, U.S.A.). Quinine, primaquine, chloroquine, sulfadoxine, pyrimethamine, WR 180,409, WR 194,965, WR 171,669, WR 177,602 and WR 160,972 were obtained from Walter Reed stock. Solvents were purchased from Burdick & Jackson Labs. (Muskegon, MI, U.S.A.). Commercially available ion-pairing reagent, low UV PIC B8, was purchased from Waters Assoc. (Milford, MA, U.S.A.). Other chemicals were of the best commercially available grade.

Extraction of mefloquine

To disposable glass centrifuge tubes with Teflon-lined screw caps, were added 1 ml of blood or plasma and 1 ml of deionized water. Contents were mixed by vortexing and appropriate amounts of mefloquine and WR 184,806 (the internal standard) were added. Contents of the tubes were mixed again by vortexing and 1 ml of 0.5 *M* dibasic sodium phosphate was added and samples were vortexed well to ensure homogeneity. All extractions were carried out by slow mixing for 15 min using a Roto-Torque rotator (Cole-Parmer Instrument Co., Chicago, IL, U.S.A.) and centrifugation for 10 min at about 2500 *g* and 4°C in a Sorvall refrigerated RC-3B centrifuge (Dupont, Newtown, CT, U.S.A.). Blood or plasma samples were extracted with 5 ml of ethyl acetate–hexane (3:2, v/v). The organic phase was extracted with 2 ml of methanol plus 3 ml of 0.5 *M* monobasic ammonium phosphate. To the methanolic aqueous phase were added 2 ml of 0.2 *M* perchloric acid and the resultant mixture was extracted with 6 ml of dichloromethane. The dichloromethane phase was transferred to clean tubes and evaporated to dryness under nitrogen at room

temperature. The residue was redissolved in 100 μl of mobile phase just prior to analysis.

High-performance liquid chromatographic analysis

The chromatographic system consisted of a Model 6000A pump (Waters Assoc.), a Model U6K injector (Waters Assoc.), a Model LC-85 variable-wavelength UV detector (Perkin-Elmer, Norwalk, CT, U.S.A.), a Model 385 strip chart recorder (Linear Instruments, Irvine, CA, U.S.A.) and a 30 cm × 3.9 mm (I.D.) 10-μm particle size μBondapak C_{18} column (Waters Assoc.). The column was at ambient temperature. The mobile phase consisted of 0.005 *M* low UV PIC B8 in methanol–water (70:30, v/v) and the flow-rate was 1.0 ml/min. Injections were made using 10–50 μl of the sample and the UV signal was monitored at 222 nm and 0.01 or 0.02 a.u.f.s.

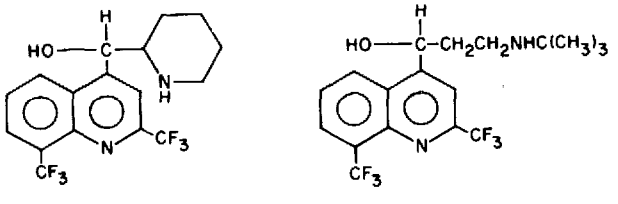
Quantitation

For each analysis, a standard curve was generated by adding known, varying amounts of mefloquine to outdated blank human blood or plasma (Walter Reed Army Medical Center Blood Bank, Washington, DC, U.S.A.) prior to extraction. Known and constant amounts (50 or 250 ng) of WR 184,806 (the internal standard) were also added to all samples prior to extraction. Standards (10–150 ng or 25–1500 ng) were selected to encompass the range of experimental values. Spiked samples were treated as unknowns to evaluate the accuracy and precision of the method. Quantitation was achieved using the peak height ratio of mefloquine to WR 184,806.

RESULTS AND DISCUSSION

The quinoline-derived antimalarials are used in the treatment of erythrocytic forms of the disease and it has been observed that the infection causes greater accumulation of these drugs in the red blood cells [11, 12]. The time-dependent concentration profile of antimalarials in both plasma and blood (and therefore also in red blood cells if hematocrit is known) may be of importance in understanding their action and optimizing the therapy. Therefore, it was deemed necessary to develop an assay which would allow analysis of mefloquine in both of these biological fluids.

In addition, quantitation of relatively low levels of mefloquine required combination of sensitive detection and relatively clean samples. Sufficient sensitivity was achieved at 222 nm, the apparent maximum absorbance for



I II

Fig. 1. Chemical structures of mefloquine (I) and WR 184,806 (II) (the internal standard)

mefloquine. A three-step extraction procedure was developed to provide clean samples compatible with high-sensitivity detection at 222 nm. Mefloquine and the internal standard are weak bases as can be seen from their chemical structures (Fig. 1) and the extraction procedure utilized this characteristic. These compounds were first extracted from biological matrix into ethyl acetate—hexane from which they were then extracted into methanolic acidic buffer. Perchloric acid was added to the methanolic buffer extract and the compounds were finally extracted as ion pairs into dichloromethane. The overall extraction efficiency was determined using 214 ng of [^{14}C]mefloquine and ranged between 60% and 70% for plasma and blood. Chromatographic separation was achieved by reversed-phase HPLC in the presence of octanesulfonate ion-pairing reagent. Representative chromatograms of extracts from blank plasma, spiked plasma and patient plasma and blank and spiked blood depicted in Figs. 2 and 3 demonstrate that sensitivity and clean sample criteria were met.

In order to cover a large (10—1500 ng/ml or ng/g) concentration range without sacrificing accuracy or precision, it was advisable to use two standard curves. The lower end standard curve was designed for 10—150 ng/ml or ng/g concentration range and the higher end standard curve for 25—1500 ng/ml or ng/g. Each standard curve consisted of at least eight points covering the anticipated assay range. Routinely, excellent linearity and a negligible Y-intercept were found. Using a least-squares linear regression analysis, representative equations of the line and the regression coefficients (r^2) for lower end and higher end standard curve were: $Y = 0.0132X + 0.0051$, $r^2 = 0.991$ and $Y = 0.00242X - 0.00531$, $r^2 = 0.999$, respectively.

The validation of the method was performed by using spiked plasma and blood. The results are summarized in Tables I—III and show good accuracy and precision in both plasma and blood.

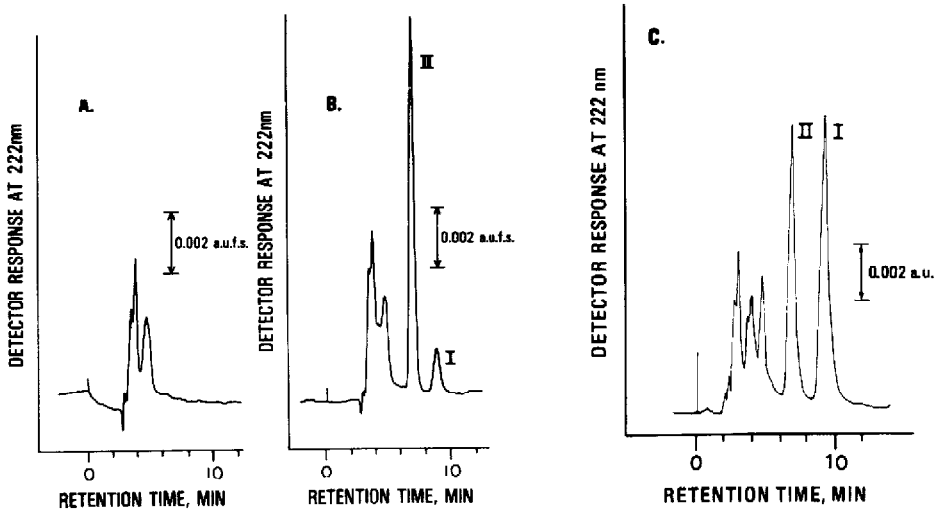


Fig. 2. High-performance liquid chromatogram of extracts from (A) blank human plasma, (B) human plasma spiked with 250 ng of WR 184,806 (II) and 50 ng of mefloquine (I), and (C) patient sample spiked with 250 ng of WR 184,806 (II) and containing 486 ng of mefloquine (I). In all instances, 25 μl of a total 100 μl were injected. For additional details, see the Experimental section.

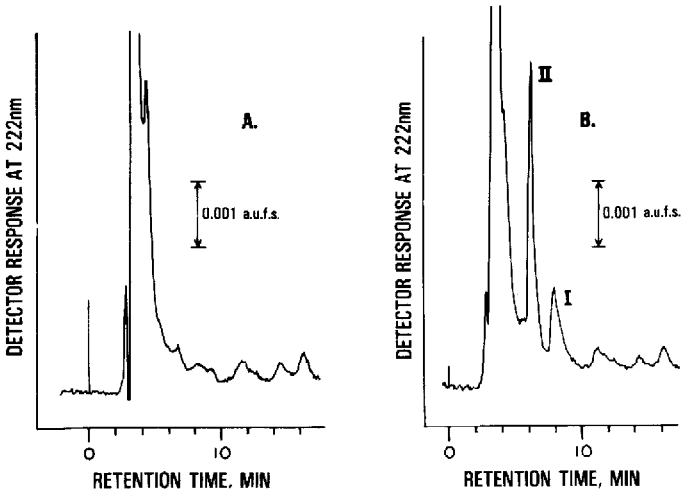


Fig. 3. High-performance liquid chromatogram of extracts from (A) blank human blood and (B) human blood spiked with 50 ng of WR 184,806 (II) and 15 ng of mefloquine (I). In both instances, 50 μl of a total 100 μl were injected. For additional details, see the Experimental section.

TABLE I

PRECISION AND ACCURACY DATA FOR MEFLOQUINE ANALYSIS IN PLASMA

Data represent a compilation of three separate experiments performed by the same investigator. Spiked unknowns were bracketed by a standard curve ranging from 25 to 1500 ng of mefloquine. For additional details, see the Experimental section.

Amount added (ng)	Amount measured (ng, mean ± S.D.)	C.V. (%)	*n*
50.0	49.2 ± 4.12	8.4	12
100.0	99.9 ± 2.16	2.2	4
300.0	303.2 ± 4.02	1.3	8
1400.0	1413.9 ± 19.5	1.4	9

TABLE II

PRECISION AND ACCURACY DATA FOR ANALYSIS OF HIGHER CONCENTRATIONS OF MEFLOQUINE IN BLOOD

Data represent a compilation of two separate experiments performed by the same investigator. Spiked unknowns were bracketed by a standard curve ranging from 25 to 1500 ng of mefloquine. For additional details, see the Experimental section.

Amount added (ng)	Amount measured (ng, mean ± S.D.)	C.V. (%)	*n*
50.0	49.6 ± 2.84	5.7	8
100.0	98.0 ± 5.73	5.8	8
1400.0	1442.5 ± 41.3	2.9	6

TABLE III

PRECISION AND ACCURACY DATA FOR ANALYSIS OF LOWER CONCENTRATIONS OF MEFLOQUINE IN BLOOD

Data represent a compilation of three separate experiments performed by the same investigator. Spiked unknowns were bracketed by a standard curve ranging from 10 to 150 ng of mefloquine. For additional details, see the Experimental section.

Amount added (ng)	Amount measured (ng, mean ± S.D.)	C.V. (%)	*n*
15.0	14.6 ± 1.92	13.2	12
30.0	29.4 ± 1.96	6.7	12
50.0	49.8 ± 1.49	3.0	12
90.0	92.9 ± 4.21	4.5	9

TABLE IV

RETENTION TIMES FOR MEFLOQUINE AND RELATED COMPOUNDS

Retention times were obtained under the HPLC conditions used for mefloquine analysis. For additional details, see the Experimental section. The elution time of the solvent front was 3.0 min.

Compound	Retention time (min)
Mefloquine	8.8
WR 184,806	6.8
WR 160,972	4.0
WR 177,602	7.1
Pyrimethamine	4.7
Sulfadoxine	3.4
Quinine	5.4
Primaquine	5.7
Chloroquine	5.9
WR 180,409	14.5
WR 194,965	18.8
WR 171,669	63.0

In order to check for possible interferences, chromatographic retention times for related compounds were also examined under these conditions and the data are presented in Table IV. Pyrimethamine, sulfadoxine, quinine, primaquine and chloroquine are other commonly used drugs for the treatment of malaria while WR 180,409 [DL-*threo*-α-(2-piperidyl)-2-trifluoromethyl-6-(4-trifluoromethylphenyl)-4-pyridinemethanol], WR 194,965 [(4-(*tert.*-butyl)-2-(*tert.*-butylaminomethyl)-6-(4-chlorophenyl)phenol], and WR 171,669 {halofantrine; [1-(1,3-dichloro-6-trifluoromethyl-9-phenanthryl)-3-di(*n*-butyl)aminopropanol]} are in investigational stages. WR 160,972 (2,8-trifluoromethylquinoline-4-carboxylic acid) is the major reported metabolite of mefloquine [13, 14] and WR 177,602 is a *threo* diastereoisomer of mefloquine. Based on the retention times, none of the compounds tested would be expected to interfere with mefloquine.

In separate additional experiments (data not shown), [^{14}C]mefloquine (542 ng) was added to fresh canine blood and samples were maintained for seven days at various temperatures. Using the analytical method described herein, essentially the same peak height ratio (mefloquine/WR 184,806) was found for freshly spiked samples and spiked samples which were kept for seven days at temperatures ranging from −20°C to +37°C. Furthermore, on the basis of chromatographic radioactivity data, no decomposition of mefloquine was observed during extraction or seven-day incubations.

In summary, the analytical method described here offers the sensitivity, selectivity and reliability necessary for accurate and precise determination of mefloquine in only 1 ml of blood or plasma. The method is relatively simple and is suitable for pharmacokinetic studies where it is being currently employed. Furthermore, the procedure is also easily adaptable for analysis of other quinoline antimalarials.

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