DETERMINATION OF MEFLOQUINE IN BIOLOGICAL FLUIDS USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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INTRODUCTION

Mefloquine (MQ) is the most successful antimalarial drug for chloroquine (CQ)-resistant and CQ-sensitive falciparum malaria. Despite the extensive use of the drug in the field and in clinical trials, there has been no simple and rapid method that would be suitable for routine analysis of all biological fluids. Methods which have been reported for the determination of the unchanged drug in human plasma, blood and urine (Grindel *et al.*, 1977; Mendenhall *et al.*, 1979; Nakagawa *et al.*, 1979; Schwartz 1980; Kapetanovic *et al.*, 1983; Heizmann *et al.*, 1984; Riviere *et al.*, 1985; Dadgar *et al.*, 1985), all have had drawbacks. Several of them require a large sample size (Grindel *et al.*, 1977; Mendenhall *et al.*, 1979; Nakagawa *et al.*, 1979), some do not use an internal standard, with a consequent loss of sensitivity (Schwartz 1980; Mendenhall *et al.*, 1979), and some of the methods require derivatization (Mendenhall *et al.*, 1979; Nakagawa *et al.*, 1979; Dadgar *et al.*, 1985). Of the HPLC methods, one requires a large sample size (5 ml) (Grindel *et al.*, 1977). The method of Kapetanovic *et al.*, (1983) is more sensitive but requires a 3-step extraction procedure and more recently, a method using a simple extraction procedure has been shown to be sensitive but its application is limited to only plasma samples (Riviere *et al.*, 1985). Since it has been observed that the quinoline-derived antimalarials accumulate in red blood cells during infection (Fitch 1969; Fitch *et al.*, 1979), knowledge of the concentration-time profile of the antimalarial drug in both plasma and blood may be of importance in optimizing therapy. Therefore, it was necessary to develop an assay that would allow analysis of MQ in whole blood.

MATERIALS AND METHODS

Chemical used

MQ was provided by Hoffmann La Roche (Basle, Switzerland). Internal standard, WR 184806, was provided by the Walter Reed Army Medical Research Centre, Washington D.C., U.S.A.

HPLC grade acetonitrile and dichloromethane were purchased from Fischer Scientific, U.S.A.

Glycine, dichlorodimethylsilane and HPLC grade methanol were obtained from BDH Limited Poole, U.K.

Sodium chloride, sodium hydroxide and toluene were supplied by E. Merck, Darmstadt, F.R. Germany.

The ion-pairing reagent, octanesulphonic acid, was supplied by Fisons.

Instrument used

Waters solvent delivery system Model 501, a Waters model 481 variable wavelength U.V. detector, Waters Automated Gradient Controller model 680, Waters model 740 Data module single-channel recorder/integrator, Rheodyne model 7125, Waters guard column and Waters C18 reverse phase column were used.

Chromatographic conditions

The system was operated at room temperature in isocratic mode with the mobile phase of 67% methanol and 33% water containing 0.01 M octanesulphonic acid solution (adjusted to pH 3.4 with 0.15 M phosphoric acid). Flow rate was 2 ml/min which gave a back pressure of 2,800–3,000 psi. The wavelength of the U.V. detector was 222 nm. The chart speed was set at 2 mm/min with the attenuation of 16.

Extraction procedure

The extraction was carried out in 15 ml capacity glass tubes with screw cap, pretreated with dichlorodimethylsilane in toluene (5%, v/v) in order to minimise adsorption. To 1 ml of whole blood containing 400 ng of internal standard (WR 184806) was added acetonitrile (2 ml). Each tube was vortexed for 30 seconds, followed by centrifugation at 1,000 *g* for 10 min. The acetonitrile layer was then transferred into 15 ml screw cap tubes, mixed with 2 ml of glycine buffer (pH 9.2) and vortexed for 30 seconds. The resultant mixture was extracted with 6 ml of dichloromethane by mechanical tumbling for 10 min followed by centrifugation at 1,000 *g* for 10 min. After discarding the top layer, the dichloromethane phase was evaporated to dryness at 37 ° C.

The residue was dissolved in 100 µl of methanol, and 20 µl was injected onto the chromatograph.

Standard curve

Standard curves were prepared by adding known quantities of MQ ie. 100 ng to 1,250 ng to a fixed concentration of internal standard (400 ng) in drug free whole blood. Samples were analysed as described above. The peak-height ratio of mefloquine to the internal standard was measured and the calibration curve obtained from linear regression of the peak-height ratio against concentration of mefloquine added.

Precision and accuracy study

Spiked samples with different concentrations of MQ and fixed concentrations of internal standard (400 ng) were treated as unknown to evaluate precision and accuracy of the method. The samples were analysed as described above. The peak-height ratios of MQ to internal standard were calculated and concentrations were derived from the standard curve.

RESULTS AND DISCUSSION

The apparent maximum absorbance for MQ is 222 nm (Kapetanovic *et al.*, 1983). The extraction procedure produced a simple and relatively clean sample which allowed for sufficient sensitivity to be achieved at this wavelength to detect both MQ and internal standard.

The extraction solvents, acetonitrile and dichloromethane, yielded optimum recovery

of MQ with minimal extraction of endogenous compounds. No interference with MQ or internal standard was observed from either pyrimethamine or sulfadoxine on extraction of whole blood MQ from volunteers receiving mefloquine-sulfadoxine-pyrimethamine combination (Fig. 1). Chromatograms of an extracted whole blood sample spiked with 500 ng of MQ and 400 ng of internal standard and extracted whole blood sample obtained after a multiple prophylactic dose of MQ (125 mg weekly) are shown in Fig. 2. The retention times of internal standard and MQ were 4.2 min and 5.4 min respectively.

Calibration curves for MQ in whole blood showed linearity with correlation coefficients of 0.9999 (Fig. 3). The limitation of detection using a 1 ml sample was 50 ng/ml where the signal to noise ratio was 4:1.

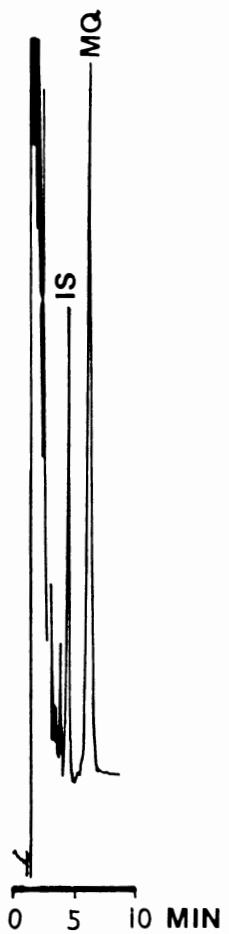

Fig. 1–Chromatogram of extracted whole blood sample obtained after oral dose of Fansimef (mefloquine-sulfadoxine-pyrimethamine combination).

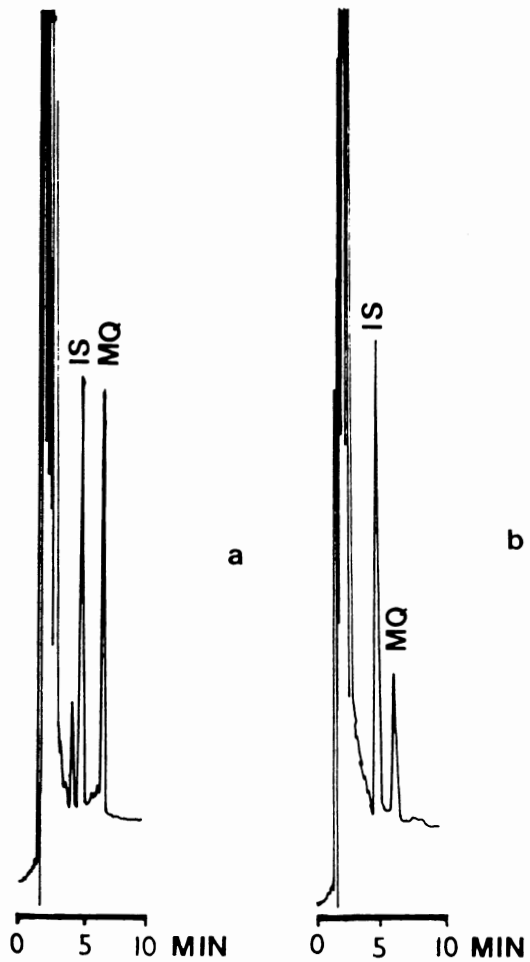

Fig. 2–(a) Chromatogram of whole blood spiked with 500 ng mefloquine and 400 ng internal standard.
(b) Chromatogram of extracted whole blood sample obtained after multiple oral dose of mefloquine.

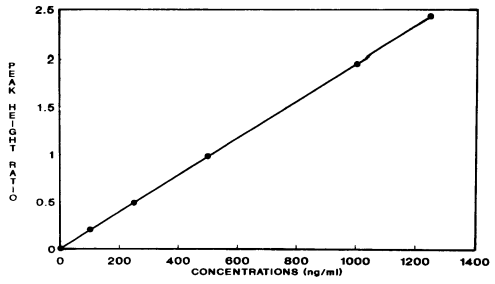

Fig. 3–Standard curve of mefloquine in human whole blood. Each point is a mean of three experiments.

Recovery of MQ varied from 61% to 81% in the range of 250 ng/ml to 1,250 ng/ml (Table 1). Due to the very similar behavior of the internal standard during extraction, changes in recovery are of minor importance.

To evaluate the precision and accuracy of the method, spiked whole blood were used. The results are summarized in Table 2, showing good accuracy and precision (intra-assay coefficient of variation ranged between 1.8% and 5% at the concentration of 100 ng/ml to 1,250 ng/ml; inter-assay coefficient of variation were less than 6% at 1,000 ng/ml and less than 10% at 100 ng/ml).

Table 1

Recovery of MQ extracted from whole blood

MQ added (ng)	% recovery	±	SD	N
250	61.0		4.2	5
500	70.9		3.2	5
1,000	80.8		3.1	5

Table 2

Precision and accuracy data for mefloquine analysis in whole blood.

Amount MQ added (ng)	Amount measured (ng, mean ± SD)	CV%	N
100	106.8 ± 4.1	3.8	5
250	257.7 ± 4.6	1.8	5
500	500.5 ± 9.5	1.9	5
1,000	1,010.3 ± 25.9	2.6	5
1,250	1,254.9 ± 63.1	5.0	5

The described method was successfully applied in a study of the pharmacokinetics of MQ in whole blood from volunteer subjects receiving mefloquine 250 mg or 125 mg weekly for prophylaxis (Karbwang *et al.*, 1988).

The assay employs a rapid and simple two-step extraction which requires a small sample volume. The low limit of detection of MQ and the short retention time make the method suitable for routine analysis of MQ. The advantage of this assay over earlier methods are that the sample treatment is rapid and simple but sensitivity and selectivity are retained. In addition, this method allows for analysis of MQ in whole blood as well as in plasma. As MQ accumulates in the red cell (Fitch *et al.*, 1979; San George *et al.*, 1984), it may be important to measure whole blood MQ in order to optimize therapy.

SUMMARY

A simple, specific and sensitive High Performance Liquid Chromatography (HPLC) method for determination of whole blood of mefloquine has been developed. WR 184806 was used as internal standard, using a two step extraction procedure followed

by revers phase HPLC. Acetonitrile and dichloromethane were used as extraction solvents. Octanesulphonic acid was used as an ionpairing reagent. Detection of extracted mefloquine and internal standard was achieved at 222 nm.

Calibration curves for mefloquine in whole blood showed linearity with correlation coefficients of 0.9999. The limitation of detection using a 1 ml sample was 50 ng/ml.

Recovery of mefloquine varied from 61% to 81%. Due to the very similar behavior of the internal standard during extraction, changes in recovery are of minor importance.

Good accuracy and precision were obtained (intra-assay coefficient of variation ranged between 1.8% and 5%; inter-assay coefficient of variation were less than 10% at 100 ng/ml and less than 6% at 1,000 ng/ml).

The assay employs a rapid and simple two-step extraction which requires a small sample volume. The low limit of detection of mefloqine and the short retention time make the method suitable for routine analysis of mefloquine.

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