*Journal of Chromatography*, 615 (1993) 297–302
*Biomedical Applications*
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 6801

# High-performance liquid chromatographic method for the simultaneous determination of mefloquine and its carboxylic metabolite in 100-μl capillary blood samples dried on paper

Yngve Bergqvist*, Jelena Al Kabbani and Birgitta Krysén

*Department of Clinical Chemistry, Falun Central Hospital, S-791 82 Falun (Sweden)*

Ingela Berggren Palme and Lars Rombo

*Department of Infectious Diseases, Karolinska Institute, S-141 86 Stockholm (Sweden)*

(First received November 9th, 1992; revised manuscript received February 19th, 1993)

ABSTRACT

A reversed-phase high-performance liquid chromatographic method is described for the analysis of mefloquine and its carboxylic metabolite in 100-μl capillary blood spots dried on chromatographic paper. Each spot was cut into small pieces, and mefloquine and its metabolite were eluted with an ammonia–water solution (10:90, v/v). The compounds were extracted simultaneously after alkalization at pH 9.5 using tetrabutylammonium as ion-pairing agent and then separated on a $C_{18}$ column with ultraviolet detection at 227 nm. The recovery of the drugs from spiked blood applied to paper and dried was 70–80%, and the inter-assay precision at 1.0–5.0 μmol/l (therapeutic range) was less than 10%. The correlation between extractions from venous whole blood and capillary blood applied to chromatographic paper was more than 0.94. The analytes were stable in dried blood spots for at least fifty days at −20°C. The decrease of concentration was less than 10%, when the paper was stored at 37°C for fifty days. The assay is reliable and easy to use for therapeutic monitoring of mefloquine with a lower limit of determination of 0.3–0.5 μmol/l.

## INTRODUCTION

*Plasmodium falciparum* resistance to antimalarials is a severe problem all over the world. Mefloquine (MQ) can be used for prophylaxis and treatment of malaria in areas where chloroquine resistance exists [1].

The use of MQ for malaria treatment or as a prophylactic makes the determination of its blood concentrations necessary to insure adequate absorption or to verify patient compliance. Determination of the concentrations of MQ and its main metabolite (MMQ) is also useful for evaluation of patients with adverse reactions. For studies in malaria-endemic areas, simple and reliable methods of sample collection and storage are needed [2]. Children have an aversion to painful venepunctures, but finger pricking is well tolerated [3]. Furthermore collection of capillary samples can be performed by health workers or patients themselves, requiring minimal training.

Determination of antimalarial drugs in capillary blood samples dried on filter paper has been described for chloroquine [4,5], quinine [6], pyrimethamine and sulfadoxine [7,8], and mefloquine [9,10].

* Corresponding author.

0378-4347/93/$06.00 

Many analytical methods for measuring MQ and its metabolite MMQ have been developed in the past five years. High-performance liquid chromatographic (HPLC) techniques are commonly used [11–13] with a limit of determination of 0.1 μmol/l. However, no methods have been developed so far for capillary sampling and simultaneous determination of MQ and MMQ from blood dried on filter paper.

## EXPERIMENTAL

### *Chromatography*

The isocratic chromatographic system consisted of a Model 2150 HPLC pump (LKB-Pharmacia, Uppsala, Sweden). The sample was injected by use of a Waters WISP 710B autoinjector (Milford, MA, USA). Separations were carried out on a Beckman Ultrashere IP, $C_{18}$ reversed-phase column (5 μm; 25 cm × 4.6 mm I.D.) (Irvine, CA, USA) with a Kratos Spectroflow 757 detector (Ramsey, NJ, USA). The chromatograms were evaluated with a Model SP 4270 integrator (Spectra-Physics, San Jose, CA, USA).

The mobile phase consisted of 137 ml of acetonitrile, 173 ml of 0.1 mol/l phosphate buffer and 15 ml of 1 mol/l sodium perchlorate. N,N-Dimethyloctylamine (DMOA) was added at a concentration of 7 mmol/l. The final apparent pH of the mobile phase was adjusted to 4.0, with 5 mmol/l sodium hydroxide. The flow-rate was 1.4 ml/min and the eluent was monitored at 227 nm.

### *Chemicals and standards*

MQ and the acid metabolite of mefloquine [2,8-bis(trifluoromethyl)quinoline-4-carboxylic acid, MMQ] were supplied by Roche (Skarholmen, Sweden) and WR 184,806 [2,8-bis(trifluoromethyl)-4-[1-hydroxy-3-(N-*tert.*-butylamino)propyl]quinoline phosphate, internal standard] from the Division of Experimental Therapeutics, Walter Reed Army Institute of Research (Washington, DC, USA). HPLC-grade acetonitrile, methyl *tert.*-butyl ether (MTBE), tetrabutylammonium hydroxide (TBAH) and DMOA were obtained from E. Merck (Darmstadt, Germany).

Stock standard solutions of MQ and MMQ (100 μmol/l) were prepared separately in water. Working standards were prepared by spiking whole blood from a blood donor with stock standard solutions covering the concentration range 0–6 μmol/l and then adding 100 μl of the spiked blood to the chromatographic paper.

### *Chromatographic paper*

Whatman 31 ET Chroma chromatographic papers (Whatman, Maidstone, UK) were used.

### *Source of blood samples*

Venous and capillary blood samples were obtained from travellers taking MQ as prophylaxis.

### *Calibration*

Samples (100 μl) of whole blood spiked with MQ and MMQ at 1.25, 2.5 and 5.0 μmol/l were added to the papers. When the spots had been dried at room temperature, they were stored in a plastic bag at −20°C until analysis.

### *Determination of MQ and MMQ from blood spots dried on chromatographic paper*

Papers with 100-μl dried blood spots (patient samples or blood standards) were cut into small pieces and transferred to polypropylene tubes. A 2.0-ml volume of ammonia–water (10:90, v/v) was added to each tube. After incubation for 30–60 min at room temperature, the tubes were put into an ultrasonic bath for 30 min at 37°C. The extraction from the blood spot was performed in a polypropylene tube to which 5.0 ml of MTBE, 2.0 ml of phosphate buffer (0.5 mol/l, pH 9.0) and 2.0 ml of TBAH (0.06 mol/l) with 20 μmol/l internal standard were added. Each tube was then shaken for 30 min and centrifuged at 1000 *g* for 5 min. The upper organic layer was transferred to a conical polypropylene tube and evaporated to dryness at 80°C. The residue was reconstituted in 150 μl of mobile phase, and 125 μl were injected into the HPLC system.

### *Intra- and inter-assay precision*

Four pools of whole blood samples containing 0.25, 0.5, 1.0 and 3.0 μmol/l each of MQ and

MMQ were prepared by adding appropriate volumes of standard solutions to drug-free whole blood. Of each pool, 100 μl were added to separate papers. After drying at room temperature, the papers were stored in a plastic bag at −20°C until analyzed. Three replicate samples for each concentration were analyzed on three separate days. Concentrations were determined using a calibration graph prepared on the day of analysis. From the obtained data, intra- and inter-assay precisions were calculated.

*Recovery*

The recovery (extraction yield) was determined by spiking whole blood with MQ and MMQ at concentrations of 1.25, 2.5 and 5.0 μmol/l. A 100-μl volume was added to the paper and dried. The peak heights of MQ and MMQ determined from paper were compared with peak heights obtained by direct injection of MQ and MMQ standards dissolved in mobile phase.

*Stability*

Blood spots spiked at 2.5 μmol/l were stored at −20°C, 23°C and 37°C in plastic bags and analyzed at different time intervals. The stability of MQ and MMQ dissolved in the spots was determined and compared with the stability after direct injection of MQ and MMQ standard in mobile phase. All of the specimens were assayed in triplicate.

## RESULTS AND DISCUSSION

*System performance*

The HPLC chromatogram illustrated in Fig. 1B was obtained following an injection of a solution of standard compounds in mobile phase. MMQ, MQ and internal standard are well separated with retention times of 6.1, 7.8 and 14.6 min, respectively. No interference of endogenous substances or other commonly used antimalarial drugs were seen. The use of DMOA as modifier in the mobile phase resulted in satisfactory chromatography for MMQ, with sharp peaks and little tailing at pH 4.0.

*Recovery, precision and accuracy*

The recovery was 70–80% over the concentration range studied (Table I). The calibration of the paper method should be performed from a spiked paper spot. The precision values obtained (Table II) show that the paper method has a precision similar to that for the determination of the analytes from whole blood and plasma [13].

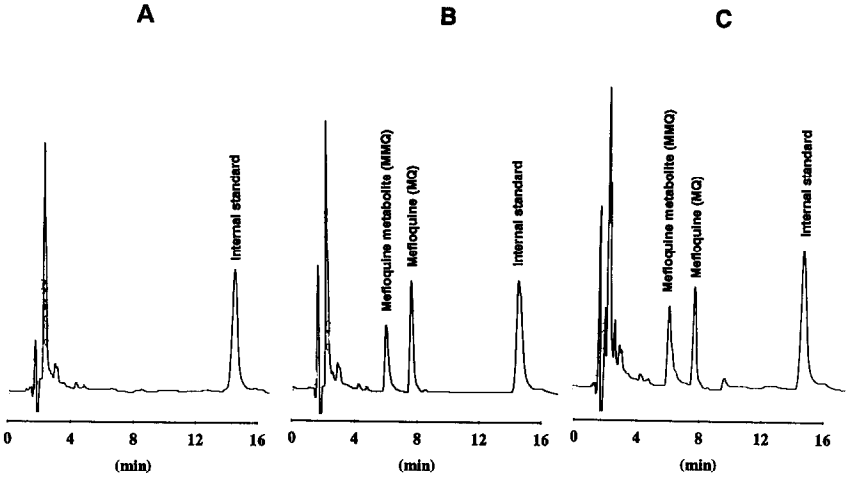


Fig. 1. (A) Chromatogram of drug-free human capillary blood spotted on chromatography paper. (B) Chromatogram obtained following injection of MQ (2.5 μmol/l) and MMQ (2.5 μmol/l) in the mobile phase. (C) Chromatogram of blood from a patient who received 250 mg of MQ as oral dose, followed by capillary sampling onto paper 12 h after dosing and analyzed by the present method. Concentrations: MQ, 1.9 μmol/l; MMQ, 2.5 μmol/l. Retention times: MMQ, 6.1 min; MQ, 7.8 min; internal standard, 14.6 min.

TABLE I

RECOVERY OF MQ AND MMQ FROM 100 μl OF WHOLE BLOOD DRIED ON PAPER ($n$ = 7)

| Sample added (μmol/l) | Recovery (mean ± S.D.) (%) |
|---|---|
| *MQ* | |
| 0.50 | 75.3 ± 3.8 |
| 1.25 | 78.4 ± 10.6 |
| 2.5 | 82.6 ± 10.9 |
| 5.0 | 80.9 ± 8.5 |
| *MMQ* | |
| 0.50 | 77.5 ± 3.5 |
| 1.25 | 72.8 ± 4.7 |
| 2.5 | 72.2 ± 5.2 |
| 5.0 | 72.3 ± 6.7 |

*Limit of determination*

The limit of determination of the analytes was defined as the minimum concentration with an inter-assay variation of less than 15%. With this definition, and using 100 μl of blood applied and dried on paper, the limits were estimated to be 0.5 and 0.25 μmol/l for MQ and MMQ, respectively.

*Correlation data*

A comparison of capillary sampling on paper with venepuncture sampling is presented in Fig. 2. There is good agreement between the different sampling techniques and the analytical methods.

*Stability*

The concentration of MQ decreased approximately 10% during storage at 37°C for fifty days. MMQ seems to be more stable as the decrease was only about 5% at room temperature for fifty days. No decrease in concentration was found during storage at −20°C, as illustrated in Fig. 3.

The clinically accepted decrease of MQ and MMQ concentrations during transport of sam-

TABLE II

DETERMINATION OF ACCURACY AND INTRA- AND INTER-ASSAY PRECISION OF MQ AND MMQ FROM DRIED BLOOD SPOT USING THE PAPER SAMPLING METHOD

| Sample | Spiked concentration (μmol/l) | Determined concentration (μmol/l) | Number of replicates/ assays | C.V. (%) |
|---|---|---|---|---|
| *Intra-assay* | | | | |
| MQ | 0.25 | 0.27 | 15 | 15 |
| | 0.50 | 0.41 | 20 | 9.2 |
| | 1.00 | 0.92 | 10 | 10 |
| | 5.00 | 4.60 | 10 | 2.5 |
| Patient sample | 2.10 | | 12 | 3.9 |
| MMQ | 0.25 | 0.25 | 15 | 8.1 |
| | 0.50 | 0.51 | 20 | 7.9 |
| | 1.00 | 0.98 | 10 | 4.8 |
| | 5.00 | 5.00 | 10 | 2.9 |
| Patient sample | 0.49 | | 12 | 9.7 |
| *Inter-assay* | | | | |
| MQ | 0.25 | 0.25 | 5 | 20 |
| | 0.50 | 0.41 | 4 | 11 |
| | 1.00 | 0.93 | 3 | 10 |
| | 5.00 | 4.60 | 3 | 4.9 |
| MMQ | 0.25 | 0.25 | 5 | 10 |
| | 0.50 | 0.51 | 4 | 8.4 |
| | 1.00 | 0.96 | 3 | 8.6 |
| | 5.00 | 4.91 | 3 | 5.0 |

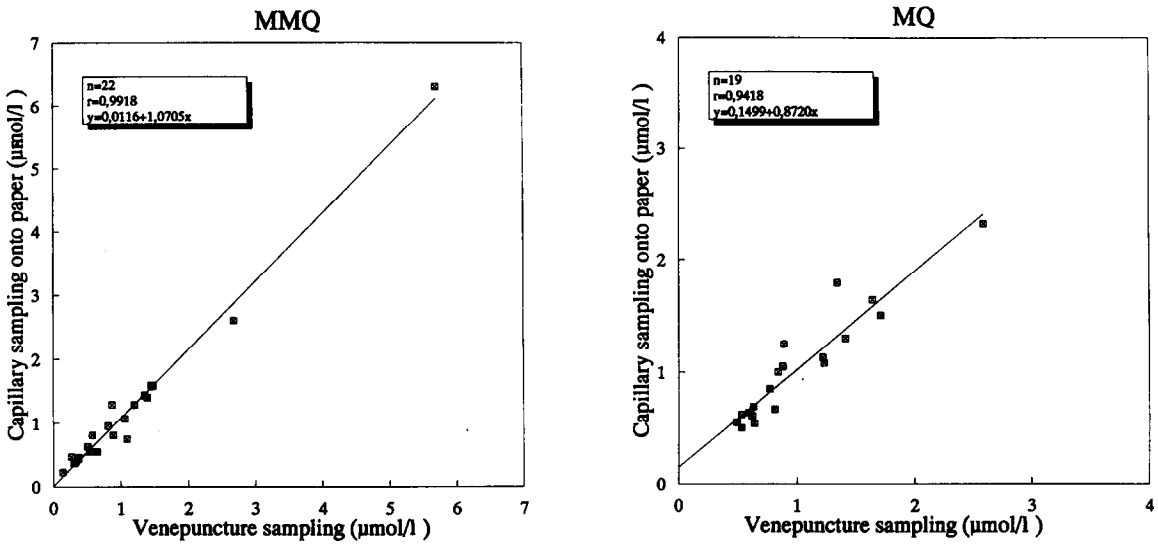


Fig. 2. Comparison of results for MQ and MMQ in blood obtained by venepuncture ($x$) and capillary sampling ($y$).

ples will vary somewhat with the application. For most clinical purposes an average loss of less than 10% is probably acceptable. In this criterion, for the stability of the analytes, the precision of the method is included.

## CONCLUSION

The present method utilises ion-pair extraction, as described in ref. 13, for the simultaneous extraction of MQ and MMQ from blood and plasma. This assay offers advantages over the previously developed filter paper methods [9] for the determination of MQ, as its metabolite (MMQ) is determined simultaneously. The method was validated from venous and capillary blood samples from volunteers, and the concentration of MQ and MMQ was analysed by different methods. The capillary sampling for analysis of MQ and MMQ made it feasible to mail blood samples at ambient temperature to the laboratory for analysis.

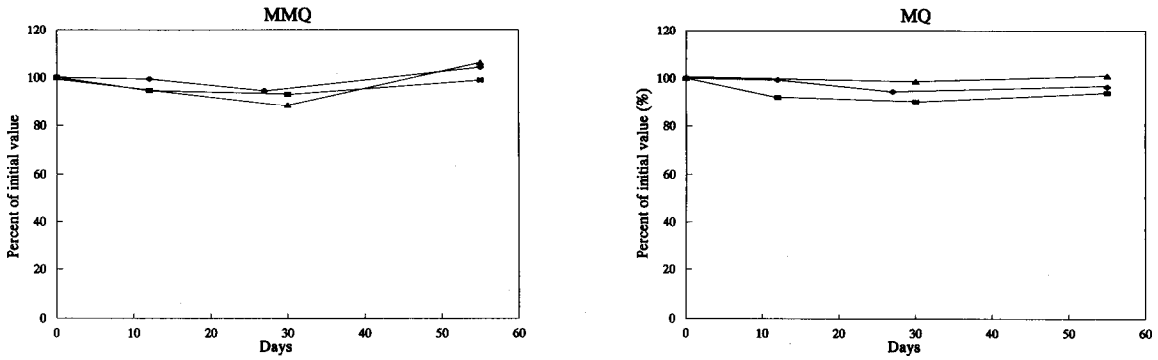


Fig. 3. Effects of storage temperature (■, 37°C; ◆, 23°C; ▲, −20°C) and duration on the stability of MQ and MMQ at 2.5 µmol/l (initial concentration) in dried blood spotted on paper. Each point is the average of three determinations, and the results are expressed as percentage of the initial value of MQ and MMQ over various time periods.

ACKNOWLEDGEMENTS

This work was supported by grants from F. Hoffman-La Roche (Basel, Switzerland) and the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR).

REFERENCES

1 C. M. Kihamia and H. S. Hill, *Lancet*, i (1982) 43.
2 E. K. Mberu, S. A. Ward, P. A. Winstanley and W. M. Watkins, *J. Chromatogr.*, 570 (1991) 180–184.
3 U. Hellgren, C. M. Kihamia, Y. Bergqvist, M. Lebbad, Z. Premji and L. Rombo, *Trans. R. Soc. Trop. Med. Hyg.*, 84 (1990) 469–473.
4 L. C. Patchen, D. L. Mount, I. K. Schwartz and F. C. Churchill, *J. Chromatogr.*, 278 (1983) 81–89.
5 B. Lindström, Ö. Ericsson, G. Alvan, L.Rombo, L. Ekman, M. Rais and F. Sjöqvist, *Ther. Drug Monit.*, 7 (1985) 207–210.
6 U. Hellgren, T. Villén and Ö. Ericsson, *J. Chromatogr.*, 528 (1990) 221–227.
7 P. A. Winstanley, W. M. Watkins, C. R. J. C. Newton, C. Nevill, E. K. Mberu, P. A. Warn, C. M. Waruiru, I. N. Mwangi, D. A. Warrell and K. Marsh, *Br. J. Clin. Pharmacol.*, 33 (1992) 143–148.
8 Y. Bergqvist, E. Hjelm and L. Rombo, *Ther. Drug Monit.*, 9 (1987) 203–207.
9 Y. Bergqvist, F. C. Churchill and D. L. Mount, *J. Chromatogr.*, 428 (1988) 281–290.
10 D. L. Mount, F. C. Churchill and Y. Bergqvist, *J. Chromatogr.*, 564 (1991) 181–193.
11 I. M. Kapetanovic, J. D. DiGiovanni, J. Bartosevich, V. Melendez, J. von Bredow and M. Heiffer, *J. Chromatogr.*, 277 (1983) 209–215.
12 P. J. Arnold and O. V. Stetten, *J. Chromatogr.*, 353 (1986) 193–200.
13 Y. Bergqvist, U. Hellgren and F. C. Churchill, *J. Chromatogr.*, 432 (1988) 253–263.