Journal of Chromatography, 311 (1984) 411—417
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 2271

Note

Determination of the antimalarial mefloquine in human plasma by gas chromatography with electron-capture detection

P. HEIZMANN* and R. GESCHKE

Biological and Pharmaceutical Research Department, F. Hoffmann-La Roche & Co., Basel (Switzerland)

(First received April 17th, 1984; revised manuscript received July 16th, 1984)

Mefloquine, DL-*erythro*-α-(2-piperidyl)-2,8-bis(trifluoromethyl)-4-quinoline-methanol (Ro 21-5998) (I, Fig. 1), is a new antimalarial drug for the treatment of drug-resistant falciparum malaria [1]. Pharmacokinetic studies in humans showed a long biological half-life of the parent drug [2, 3]. The main plasma and urine metabolite is 2,8-bis(trifluoromethyl)-4-quinoline carboxylic acid (II, Fig. 1) [4].

Several methods have been reported for the determination of the unchanged drug in human plasma including thin-layer chromatography (TLC) [5], high-

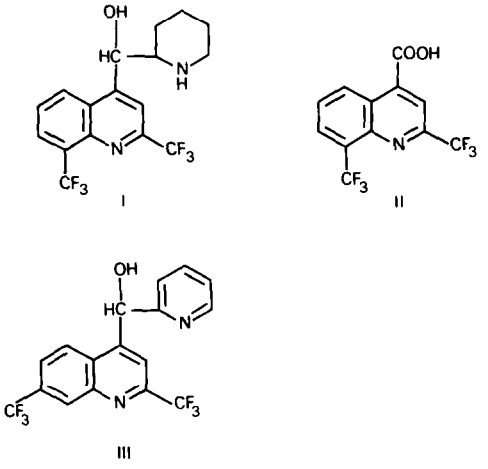

Fig. 1. Structural formulae of mefloquine (I), its metabolite (II) and of the internal standard (III).

0378-4347/84/$03.00

performance liquid chromatography (HPLC) [6] and gas chromatography—mass spectrometry (GC—MS) [7]. All of these methods have some inherent drawbacks. The sensitivity of both the TLC method [5] and the HPLC method [6] is limited and not sufficient for pharmacokinetic studies. Recently a more sensitive HPLC method has been published [8]. However, a three-step extraction procedure was required. GC—MS, on the other hand, is very sensitive but not available in every routine analytical laboratory. Due to the presence of two trifluoromethyl groups in the molecule, gas—liquid chromatography (GLC) with electron-capture detection (ECD) was expected to be more sensitive than TLC or HPLC. A GLC—ECD method for use in animal studies has been reported [9]. The present paper describes a simple and sensitive GLC—ECD method for the determination of mefloquine in human plasma, using packed columns. As the metabolite (II) is pharmacologically inactive, its measurement was not attempted by the present method.

EXPERIMENTAL

Reagents

Dichloromethane p.a. (freshly distilled), methanol p.a., and acetonitrile (LiChrosolv) were all from E. Merck, F.R.G.), as well as tris(hydroxymethyl)-aminomethane (Tris) and 0.1 *M* hydrochloric acid.

Tris—HCl buffer, 0.2 *M*, pH 8, was prepared by mixing equal volumes of a solution of Tris (12.5 g Tris in 500 ml of water) and of 0.1 *M* hydrochloric acid and adjusting the pH to 8.0.

N-Trimethylsilylimidazole (TSIM) was from Pierce Eurochemie.

Internal standard

The structurally closely related compound DL-α-(2-pyridyl)-2,7-bis(trifluoromethyl)-4-quinolinemethanol (Ro 12-9744) (III, Fig. 1) was used as internal standard.

Chromatographic system

The following instruments were used: gas chromatograph Varian 2700 with a 8.5-μCi ^{63}Ni electron-capture detector (Varian, Darmstadt, F.R.G.), modified for pulse mode with a Pye electron-capture amplifier (Philips, Kassel, F.R.G.); autosampler Hewlett-Packard 7660 A (Hewlett-Packard, Böblingen, F.R.G.); integrator SP 4100 (Spectra-Physics, Darmstadt, F.R.G.). A packed column, 150 cm × 4 mm filled with 3% SP-2250 on 80—100 mesh Supelcoport (Art. No. 1-1767, Supelco, Crans, Switzerland) was used. The chromatographic conditions were as follows: flow-rate 50 ml/min (argone—methane), oven 205°C, injector 270°C, detector 320°C. The high detector temperature was chosen to keep the foil clean of contaminations. The chromatographic response itself was independent of the electron-capture detector temperature.

Under these conditions, the following retention times were obtained: internal standard 4.6 min, mefloquine 6.4 min.

Teflon®-faced septa were used in the injector. For the autosampler, 250-μl glass micro-vials (Perkin-Elmer, Ueberlingen, F.R.G.), capped with aluminium foil, prepared in the laboratory, were used.

Plasma standards and internal standardization

Mefloquine base (10 mg) was weighed into a 10-ml flask and dissolved in methanol.

Plasma standards (calibration standards) at the following concentrations were prepared: 2000, 1500, 1000, 500, 250, 125, 62.5, 31.25, and 15.6 ng/ml. The 2000 ng/ml standard was prepared by adding 100 μl of the mefloquine solution to 50 ml of drug-free human plasma. The other standards were then prepared by stepwise dilutions with drug-free plasma. These calibration standards were stored deep-frozen (−18°C) in small portions until needed for analysis.

Internal standard (10 mg) was weighed into a 10-ml flask and dissolved in methanol. After appropriate dilution with water, this aqueous solution was used for internal standardization.

Sample preparation

Plasma samples and calibration standards were thawed at room temperature and then briefly mixed on a Vortex mixer. Aliquots of 0.25 ml of plasma (concentration range 15.6—125 ng/ml) or 0.1 ml of plasma (concentration range 62.5—2000 ng/ml) were transferred into a conical glass tube (15 ml). An equal volume of pH 8 Tris buffer, 100 μl of the aqueous solution of the internal standard and 6 ml of dichloromethane were added and the tube was closed by a glass stopper. The plasma was then extracted on a rotary extractor (10 min, 30 rpm). After centrifugation (5 min, 1000 *g*), the aqueous phase was carefully aspirated and discarded; 5 ml of the organic phase were transferred into a conical glass tube (7 ml volume) and evaporated to dryness under a gentle stream of nitrogen; 100 μl of a 10% solution of TSIM in acetonitrile were then added to the dry residue. The glass tube was stoppered and, after mixing on a Vortex mixer, the solution was maintained at room temperature for 15 min. After this, 400 μl of acetonitrile (concentration range 15.6—125 ng/ml) or 1000 μl of acetonitrile (concentration range 62.5—2000 ng/ml) were added. Samples with concentrations > 2000 ng/ml needed further dilution. The mixture was then transferred to the 250-μl micro-vials by means of a glass pipette and 1 μl was injected for analysis.

Calibration

Four or five calibration standards, covering the anticipated concentration range (15.6—125 or 62.5—2000 ng/ml), were processed as described above and analysed alongside the unknown samples. Peak height ratios of unchanged drug to the internal standard were measured and the calibration was obtained from linear regression of the peak height ratio against concentration. This line was then used to calculate the concentration of the unchanged drug in the unknown samples.

RESULTS

Trimethylsilylation

The GC—MS spectrum of the mefloquine TMS derivative was identical to that previously published [4], and confirmed the formation of the O-silyl

derivative. A 100-μl volume of a 10% solution of TSIM in acetonitrile was sufficient for complete reaction. This was established as follows: the dried residue of 300 ng of mefloquine base (corresponding to a plasma concentration of 3000 ng/ml in this procedure) was derivatized with the 10% TSIM solution; a second 300-ng sample was reacted with 100% TSIM. The same peak height was obtained from both reactions. A silylation time of 15 min was sufficient for complete derivatization. For reaction times of more than 15 min, no measurable change in the peak intensity was observed.

Recovery

Spiked plasma samples of various concentrations were prepared and extracted as described above. Solutions of mefloquine in dichloromethane were added to evaporated extracts of drug-free plasma to give samples in the same concentration range as the spiked plasma. Following evaporation and silylation, injection of these samples provided the "100%" values. The recovery was calculated by comparing the peak heights from these two experiments. The recovery varied between 97% and 107% in the range 20—2000 ng/ml (Table I).

TABLE I

RECOVERY AND INTRA-ASSAY PRECISION

$n = 4$.

Concentration (ng/ml)	Recovery (%) (mean ± S.D.)	Precision (C.V., %)
20	97 ± 2.5	1.2
200	107 ± 3.4	2.7
2000	105 ± 3.6	2.5

Linearity

A linear correlation between peak height ratio and concentration of mefloquine in plasma was found in the range 15.6—125 ng/ml (0.25 ml of plasma, final dilution to 500 μl) and in the range 62.5—2000 ng/ml (0.1 ml of plasma, final dilution to 1100 μl). Plasma samples with higher concentrations needed further dilution in the final step in order to ensure that the chromatographic response fell within the linear range of the detector used ($2 \cdot 10^2$, from 10^{-12} ng to $2 \cdot 10^{-10}$ ng of mefloquine).

TABLE II

INTER-ASSAY PRECISION AND ACCURACY

Added (ng/ml)	Found (ng/ml)	C.V. (%)	Deviation between added and found (%)	Replicates (n)
7.8	7.8	6.0	±0.0	5
62.5	62.2	3.9	—0.5	8
250	249	2.3	—0.4	6
500	501	3.2	+0.2	6
1000	993	2.8	—0.7	6

Precision

Intra-assay precision was calculated from spiked samples which were analysed as replicates during one working day. The mean coefficient of variation was ± 2.1% in the range 20—2000 ng/ml (Table I).

Inter-assay precision was calculated from spiked plasma samples (quality control samples), which were analysed as unknowns on different days using a new calibration each day. The mean coefficient of variation was 3.1% in the range 62.5—1000 ng/ml and 6.0% at the limit of detection (Table II).

Detection limit

Using 0.25 ml of plasma and injecting 1 μl from 500 μl of the final mixture, the limit of detection was 5 ng/ml at a signal-to-noise ratio of 5:1. This corresponds to an injected amount of $2.5 \cdot 10^{-12}$ g of mefloquine. Injecting the pure compound as its TMS derivative, the minimum detectable amount was $0.7 \cdot 10^{-12}$ g. Pharmacokinetic studies undertaken up to now, however, showed that the above detection limit was more than adequate.

Selectivity

The method is selective for mefloquine. No interference from the main metabolite of mefloquine, or as a result of the simultaneous administration of

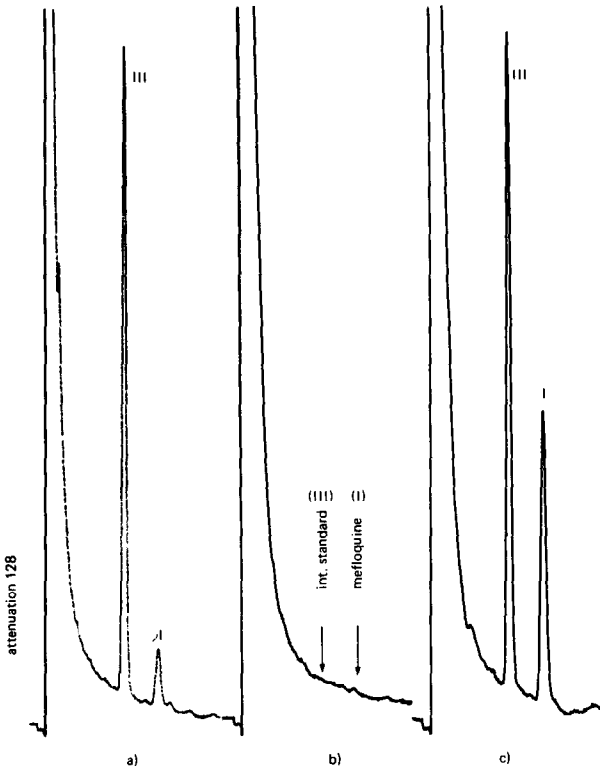

Fig. 2. Chromatograms of plasma extracts (0.25 ml of plasma extracted; injected volume 1 from 500 μl): (a) calibration standard, 15.6 ng/ml; (b) volunteer's pre-dose plasma; (c) volunteer's plasma, eight weeks after oral administration of 750 mg of mefloquine. The peak corresponds to 75 ng/ml mefloquine.

Fansidar® (an antimalarial containing pyrimethamine and sulfadoxine) was observed.

Stability in plasma and in solution

Stability of the drug in biological fluids was described by Schwartz and Ranalder [7]. According to these authors the drug is stable at room temperature for three days and at −20°C for two months. Under the conditions we used, the TMS derivative was found to be stable in the final solution for at least 24 h at room temperature.

Application of the method to biological samples

Plasma from volunteers and patients receiving mefloquine per os has been analysed by this method. Fig. 2 shows chromatograms of plasma extracts from a volunteer after oral administration of mefloquine (750 mg).

Plasma levels of the parent drug are presented in Fig. 3. In this case a terminal elimination half-life of 16 days was calculated.

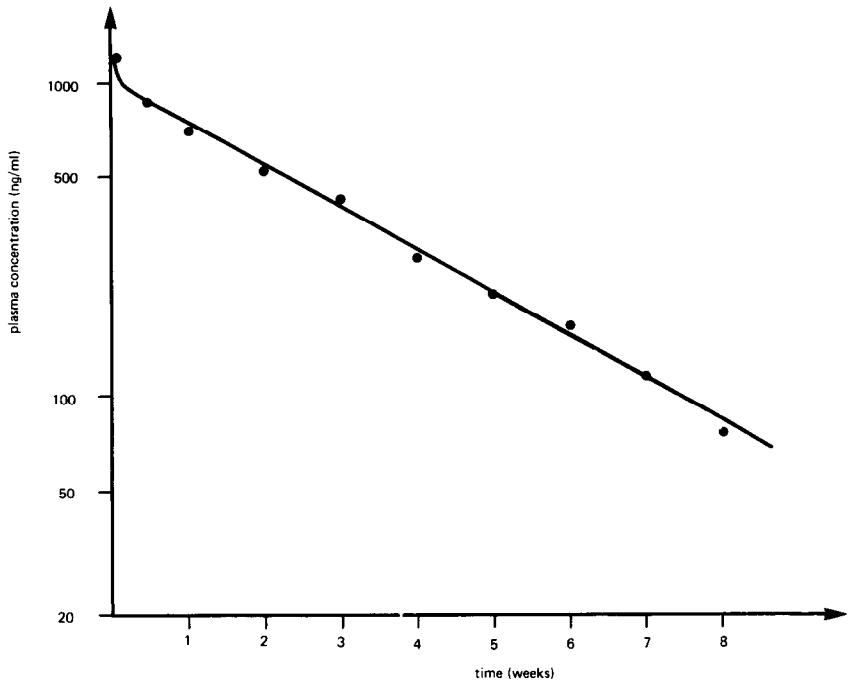

Fig. 3. Plasma levels of mefloquine after oral administration of 750 mg of mefloquine. Terminal half-life, $t_{1/2\beta}$, = 16 days.

ACKNOWLEDGEMENTS

We thank Dr. Dell for helpful discussions and for revising the manuscript, and Mr. Oesterhelt for having carried out the GC—MS spectra.

REFERENCES

1 G.M. Trenholme, R.L. Williams, R.E. Desjardins, H. Trischer, P.E. Carson, K.H. Rieckmann and C.J. Canfield, Science, 190 (1975) 792.
2 R.E. Desjardins, C.L. Pamplin, J. von Bredow, K.G. Barry and C.J. Canfield, Clin. Pharmacol. Ther., 26 (1979) 372.
3 D.E. Schwartz, B. Weber, D. Richard-Lenoble and M. Gentilini, Acta Trop., 37 (1980) 238.
4 R. Jauch, E. Griesser and G. Oesterhelt, Arzneim.-Forsch., 30 (1980) 60.
5 D.E. Schwartz, in A. Frigerio and M. McCamish (Editors), Recent Developments in Chromatography and Electrophoresis, Vol. 10, Elsevier, Amsterdam, 1980, p. 69.
6 J.M. Grindel, P.F. Tilton and R.D. Shaffer, J. Pharm. Sci., 66 (1977) 834.
7 D.E. Schwartz and U.B. Ranalder, Biomed. Mass Spectrom., 8 (1981) 589.
8 I.M. Kapetanovic, J.D. Di Giovanni, J. Bartosevich, V. Melendez, J. von Bredow and M. Heiffer, J. Chromatogr., 277 (1983) 209.
9 T. Nakagawa, T. Higuchi, J.L. Haslam, R.D. Shaffer and W.D. Mendenhall, J. Pharm. Sci., 68 (1979) 718.