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GAS CHROMATOGRAPHIC DETERMINATION OF MEFLOQUINE IN HUMAN AND DOG PLASMA USING ELECTRON-CAPTURE DETECTION

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SUMMARY

A sensitive and selective gas—liquid chromatographic method for the determination of plasma levels of mefloquine in human and dog plasma is described. The drug and internal standard were extracted from plasma at pH 9.0 into isopropyl acetate. After evaporation of the solvent, the residue was taken up in toluene and derivatised with heptafluorobutyrylimidazole. The derivative was quantified by gas—liquid chromatography on a 3% GC GE-SE30 column with electron-capture detection. The limit of detection for mefloquine in plasma was 10 ng/ml. The mean overall recovery from plasma was 102.7 ± 3.3%. The method was shown to be specific for mefloquine without any interference from endogenous compounds in plasma or from the drugs pyrimethamine and sulfadoxine (compounds often administered in combination with mefloquine).

The assay described was successfully applied to the determination of plasma levels of mefloquine in man and dog following oral and intravenous administration, respectively.

INTRODUCTION

Mefloquine, racemic *erythro*-α-(2-piperidyl)-2,8-bis(trifluoromethyl)-4-quinolinemethanol (Fig. 1), is an antimalarial drug used for the treatment and prevention of chloroquine-resistant *Falciparum malaria*. Several methods have

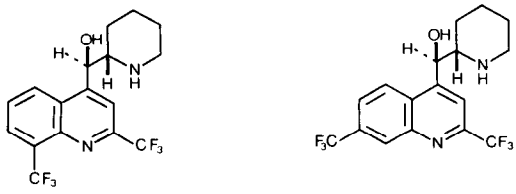

Fig. 1. Chemical structures of mefloquine and internal standard.

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been proposed for the determination of mefloquine in blood, plasma and urine [1—5]. All these methods lack sufficient sensitivity and/or suitability for routine use.

In this paper we describe a chromatographic method for the quantitation of mefloquine in plasma, without interference from either sulfadoxine or pyrimethamine. The method involves solvent extraction, derivatisation with heptafluorobutyrylimidazole and electron-capture detection.

EXPERIMENTAL

Reagents and solvents

Mefloquine and the internal standard (I.S.) [racemic erythro-α-(2-piperidyl)-2,7-bis(trifluoromethyl)-4-quinolinemethanol] were provided by Hoffmann-La Roche (Basle, Switzerland). Glycine (AnalaR), sodium chloride (GPR), sodium hydroxide (CVS), toluene (AnalaR) and ammonia solution (35% AnalaR) were purchased from BDH (Poole, U.K.). Isopropyl acetate (Puriss p.a.) was obtained from Fluka (Buchs, Switzerland). Heptafluorobutyrylimidazole (HFBI) was purchased from Pierce (Chester, U.K.) and methanol (HPLC grade) was obtained from Fisons Scientific (Leicester, U.K.). Aqueous solutions were prepared with nanopure water (deionised water obtained by reverse osmosis, Barnstead System).

Standards

Mefloquine hydrochloride (2.631 mg; equivalent to 2.4 mg mefloquine) was dissolved in 100 ml methanol to yield the stock solution (24 μg/ml).

A series of standard solutions containing 18, 12, 9, 6, 3, 1.5, 0.75, 0.375, 0.187 and 0.094 μg/ml mefloquine was prepared by serial dilution of the stock solution with methanol. Plasma standards were freshly prepared each day by spiking blank plasma with working standards at a ratio of 1:10 to yield mefloquine concentrations ranging from 9.4 to 2400 ng/ml in plasma. A stock solution of internal standard was prepared by dissolving 0.5 mg in 50 ml methanol. This solution was further diluted to give concentrations of 1 and 2 μg/ml.

All standard solutions were stored at 4°C for up to two months. Drug-free plasma was obtained from healthy volunteers, kept at 4°C and used within one week. The buffer solution was prepared by dissolving glycine (7.5 g) and sodium chloride (5.85 g) in nanopure water (1 l) and adjusting to pH 9.0 with sodium hydroxide (1 *M*).

Instrumentation

A Pye-Unicam Series 104 gas chromatograph equipped with ^{63}Ni electron-capture detector in pulse mode (period of 150 μsec) was used. The column (1.5 m × 4 mm I.D., glass) was packed with 3% GC GE-SE-30 on Gas-Chrom Q (100—120 mesh) and conditioned overnight at 280°C with nitrogen at a flow-rate of 30 ml/min.

The gas chromatographic conditions used in this assay were as follows: carrier gas: nitrogen at a flow-rate of 40 ml/min; column temperature: 180°C for 9 min, raised to 280°C for 7 min; detector temperature: 350°C.

Extraction procedure

Plasma (0.25, 0.50 or 1.0 ml depending on the expected concentration of mefloquine) spiked with mefloquine standard (25, 50 or 100 μl, respectively) and internal standard (50 μl, 1 or 2 μg/ml) was mixed with 1 ml buffer solution in a glass-stoppered tube. After the addition of isopropyl acetate (8 ml), the drugs were extracted by rotating gently for 20 min on a mechanical rotator, followed by centrifugation for 10 min at 700 *g* at 5°C. The organic phase was transferred into a glass culture tube which was placed in a water bath at 40°C and the solvent was evaporated under a gentle stream of oxygen-free nitrogen. The residue was transferred quantitatively into a small narrow-bore culture tube of 5 ml capacity using an aliquot of 200 μl and followed by a second aliquot of 100 μl isopropyl acetate. The tube was placed in a water bath at 40°C and the solvent was evaporated under nitrogen.

Samples were extracted exactly as described above for standards except in place of the mefloquine standards an equivalent amount of methanol was added.

Derivatisation procedure

Toluene (100 μl) and HFBI (30 μl) were added to the dried residue and the tube was capped immediately, vortexed and left in a water bath at 37°C for 30 min. The excess of derivatising reagent was removed by addition of water (1 ml) and vortexing for 30 sec followed by addition of toluene (100 μl) and 2% ammonia solution (1 ml). This was further vortexed for another 30 sec. The tube was capped and centrifuged for 3 min at 1000 *g*. The toluene layer was transferred into another tube and 0.5—1 μl was injected into the gas chromatograph.

Calibration and calculation

Evaluation of the assay was carried out using five-point calibration standards in the concentration range 37.5—600 ng/ml mefloquine in plasma. The calibration curves were obtained by linear regression of the peak height ratios of mefloquine/I.S. versus concentrations of mefloquine.

Because of the wide concentration range found for mefloquine in plasma samples, different calibration curves were set up, each consisting of at least four points and the amount of internal standard was accordingly adjusted. These calibration curves were then used to interpolate the concentrations of mefloquine in plasma from the measured peak height ratio of mefloquine/I.S.

RESULTS AND DISUCSSION

Stability of acyl derivatives

It is known that acyl derivatives should be stored in the reaction medium until they are required for chromatography [6—8]. Derivatives of mefloquine and internal standard exhibit satisfactory stability in the presence of water and this allowed the use of aqueous washes to remove excess derivatising reagent. However, one technical point should be observed after removing the excess of derivatising reagent. The derivatives of internal standard and mefloquine should be analysed within 8 h after removal of the excess reagent if kept at room

temperature. However, if for any reason the excess of reagent is removed but samples cannot be analysed, addition of 10 μl HFBI will prolong the storage time. Excess of derivatising reagent should again be removed before analysis.

Selectivity

The emergence of plasmodial resistance to many currently applied antimalarials is becoming a severe problem in many parts of the world, especially to chloroquine, one of the most frequently used antimalarials. A similar resistance phenomenon may be anticipated for mefloquine when given alone, but to a lesser degree when combined with other drugs [9, 10]. The use of the triple combination of mefloquine (MQ), pyrimethamine (PY) and sulfadoxine (SX) results in a marked delay of resistance development and because lower individual doses may be given, is better tolerated with fewer side-effects.

To determine whether or not pyrimethamine and sulfadoxine interfere with the determination of mefloquine the following study was carried out. Drug-free plasma samples were spiked with each of the following: (1) PY (500 ng/ml); (2) SX (500 ng/ml); (3) PY plus I.S. (200 ng/ml); (4) SX plus I.S.; and (5) PY plus SX plus I.S. plus MQ (150 ng/ml). These samples, including a drug-free sample, were taken through the entire extraction and derivatisation procedure and injected into the gas chromatograph. Under the described procedural conditions no interference with mefloquine or internal standard was observed from either pyrimethamine or sulfadoxine. Many endogenous compounds present in plasma elute later than mefloquine and the internal standard and therefore a rise in temperature was necessary to elute all these compounds before the next injection was made.

Limit of detection

Under procedural conditions the limit of detection using a 1-ml plasma sample and 0.8-μl injection was 10 ng/ml, where the signal-to-noise ratio was greater than 3:1. A calibration curve consisting of 9.38, 18.75, 37.5 and 75 ng/ml mefloquine in plasma demonstrated a correlation coefficient of 0.990 and the regression equation was $y = 0.0107x - 0.0209$.

Recovery

The overall recovery was calculated in two different ways (Table I). First by comparing the peak heights of a series of mefloquine-spiked samples after its extraction from plasma and derivatisation, with the peak heights of a series of unextracted, derivatised reference standards. Secondly, by comparing the slope (determined by linear regression analysis) of a processed (extracted, derivatised) standard curve to that of the reference (derivatised) standards. Using these methods in the concentration range 37.5—600 ng/ml the mean overall recoveries were 102.7% and 103.1%, respectively.

Precision and accuracy

The data presented in Table II show the precision and accuracy of this assay. Intra-assay precision was determined at five concentrations in quadruplicate analysis, at levels of 37.5, 75, 150, 300 and 600 ng/ml mefloquine in plasma. Inter-assay precision was determined singly at the same five concentrations in

TABLE I

RECOVERY

Method 1

Concentration (ng/ml)	Mefloquine peak height (cm) (mean of duplicates)		Recovery (%)
	Set A: derivatised authentic standards	Set B: standards extracted from plasma and derivatised	
Blank	N.D.*	N.D.	—
37.5	1.25	1.25	100.0
75	2.75	2.90	105.4
150	6.45	6.40	99.2
300	12.70	13.55	106.7
600	24.40	25.00	102.4

Mean overall recovery ± S.D. = 102.7 ± 3.3%

Method 2

Regression line for Set A: $y = 0.0411x - 0.0428$, $r = 0.999$

Regression line for Set B: $y = 0.0424x - 0.0271$, $r = 0.999$

Overall recovery $= \frac{0.0424}{0.0411} \times 100 = 103.1\%$

Mean recovery determined by Methods 1 and 2 = 102.9%.

*N.D. = Not detectable.

TABLE II

PRECISION AND REPRODUCIBILITY

Concentration added (ng/ml)	Mean (n = 4) concentration found ± S.D. (ng/ml)	C.V. (%)	Difference between added and found concentration (%)
*Intra-assay precision** *(repeatability)*			
37.5	41.6 ± 0.0	0.0	10.9
75	76.8 ± 5.0	6.5	2.4
150	139.4 ± 6.0	4.3	−7.1
300	292.7 ± 10.1	3.4	−2.4
600	607.3 ± 22.7	3.7	1.2
	Mean C.V. =	3.6%	
Inter-assay precision (reproduciblity)			
37.5	39.4 ± 4.4	11.3	5.1
75	72.6 ± 3.2	4.5	−3.2
150	136.7 ± 8.0	5.9	−8.9
300	303.6 ± 18.1	6.0	1.2
600	600.5 ± 8.8	1.5	0.1
	Mean C.V. =	5.8%	

*Linear regression line for intra-assay precision: $y = 0.0028x - 0.0174$ and correlation coefficient (r) = 0.999.

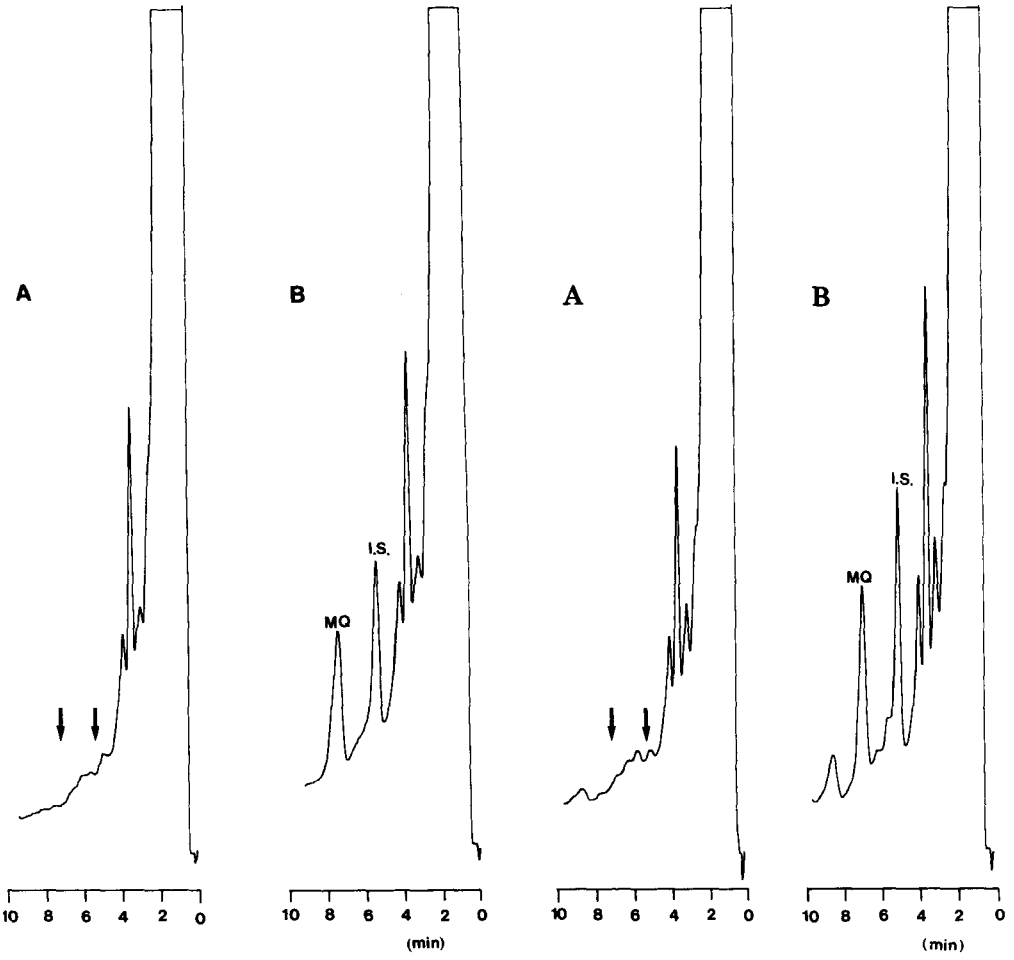

Fig. 2. Chromatograms of a human drug-free plasma (A) and a human drug-free plasma spiked with internal standard and 75 ng/ml mefloquine (B). Arrows indicate the retention times of internal standard (I.S.) and mefloquine (MQ).

Fig. 3. Chromatograms of a dog drug-free plasma (A); and a dog drug-free plasma spiked with internal standard and 75 ng/ml mefloquine (B). Arrows indicate the retention times of internal standard (I.S.) and mefloquine (MQ).

four replicate runs. The precision of the method (mean coefficient of variation, C.V.) was 3.6% and 5.8% for intra- and inter-assay, respectively. The accuracy (mean percentage differences between added and measured amounts) for the values of recovered standards, when calculated as unknowns against the linear regression line were between 0.1—10.9% over the concentration range investigated.

Separation

Figs. 2, 3 and 4 show typical chromatograms of mefloquine as determined in extracts of human and dog plasma, respectively. The mean retention times of internal standard and mefloquine were 5.2 and 7.3 min, respectively.

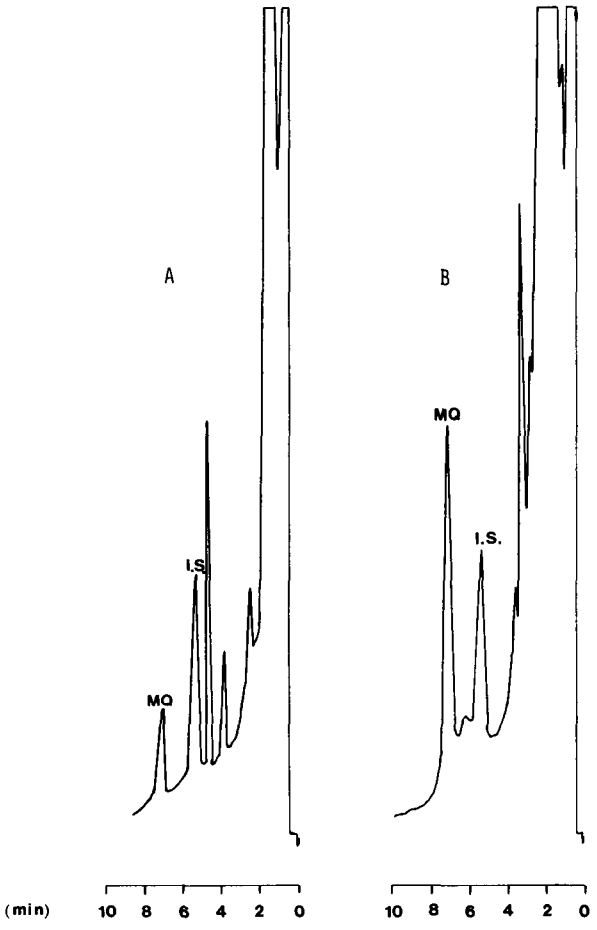

Fig. 4. Chromatograms of a human plasma sample (A) 21 days after receiving a single oral dose of a tablet containing MQ (750 mg), SX (1500 mg) and PY (75 mg); and a dog plasma sample (B) 3 days after receiving a single intravenous dose of MQ (100 mg).

Linearity

A measure of linearity is given under intra-assay precision, Table II. The correlation coefficient (r) was 0.999 and the intercept did not differ significantly from zero.

Application of the method to biological samples

The described method has been successfully applied to plasma samples from humans and dogs dosed with mefloquine. Table III presents the plasma concentrations of mefloquine in human and dog plasma, respectively. Determinations were carried out singly, while standard curves were evaluated in duplicate.

TABLE III

PLASMA CONCENTRATIONS OF MEFLOQUINE IN HUMAN AND DOG SAMPLES

The human subject received a single oral dose of a tablet containing mefloquine (750 mg), sulfadoxine (1500 mg) and pyrimethamine (75 mg). The dog received a single intravenous dose of mefloquine (100 mg).

Human plasma		Dog plasma	
Time after dose	Concentration (ng/ml)	Time after dose	Concentration (ng/ml)
2 h	620	10 min	2900
4 h	560	20 min	1720
6 h	860	40 min	1915
8 h	910	1 h	2155
10 h	570	2 h	1820
Day 1	560	3 h	1810
2	500	4 h	2260
4	470	6 h	1600
7	365	8 h	1625
14	350	12 h	1575
21	175	25 h	675
28	160	Day 2	405
35	105	3	265
42	120	4	140
49	55	6	70
56	40	7	50
63	20	8	35

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