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Note

Liquid chromatographic analysis of chloroquine and desethylchloroquine in human plasma, saliva and urine

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Chloroquine, a 4-aminoquinoline, is used in the prophylaxis and treatment of malaria and is also indicated in rheumatoid arthritis. Recent vigorous attempts to elucidate the complex pharmacokinetics of the drug have depended heavily on the development of three sensitive and specific liquid chromatographic methods for analysis of the drug and its major metabolite, desethylchloroquine, in biological fluids [1—3]. Two of these methods employed fluorescence detection [1, 2], while all three involved time-consuming extraction procedures. Another method [4], employing UV detection, was found unsuitable because it could not separate chloroquine (CQ) and desethylchloroquine (CQM).

We report here a simple, sensitive and rapid ion-pair liquid chromatographic method for the analysis of CQ and CQM in biological fluids. The method has been applied to the analysis of the two compounds in plasma, saliva and urine obtained from volunteers following administration of chloroquine.

EXPERIMENTAL

Chemicals and reagents

Chloroquine diphosphate (from ICI), desethylchloroquine base (from Sterling-Winthrop) and papaverine (from BDH) were used to prepare all the working standards. Stock solutions, containing 50 μg/ml of desethylchloroquine and papaverine, (internal standard), respectively, were prepared in 0.1 *M* hydrochloric acid, while that of chloroquine was prepared in water.

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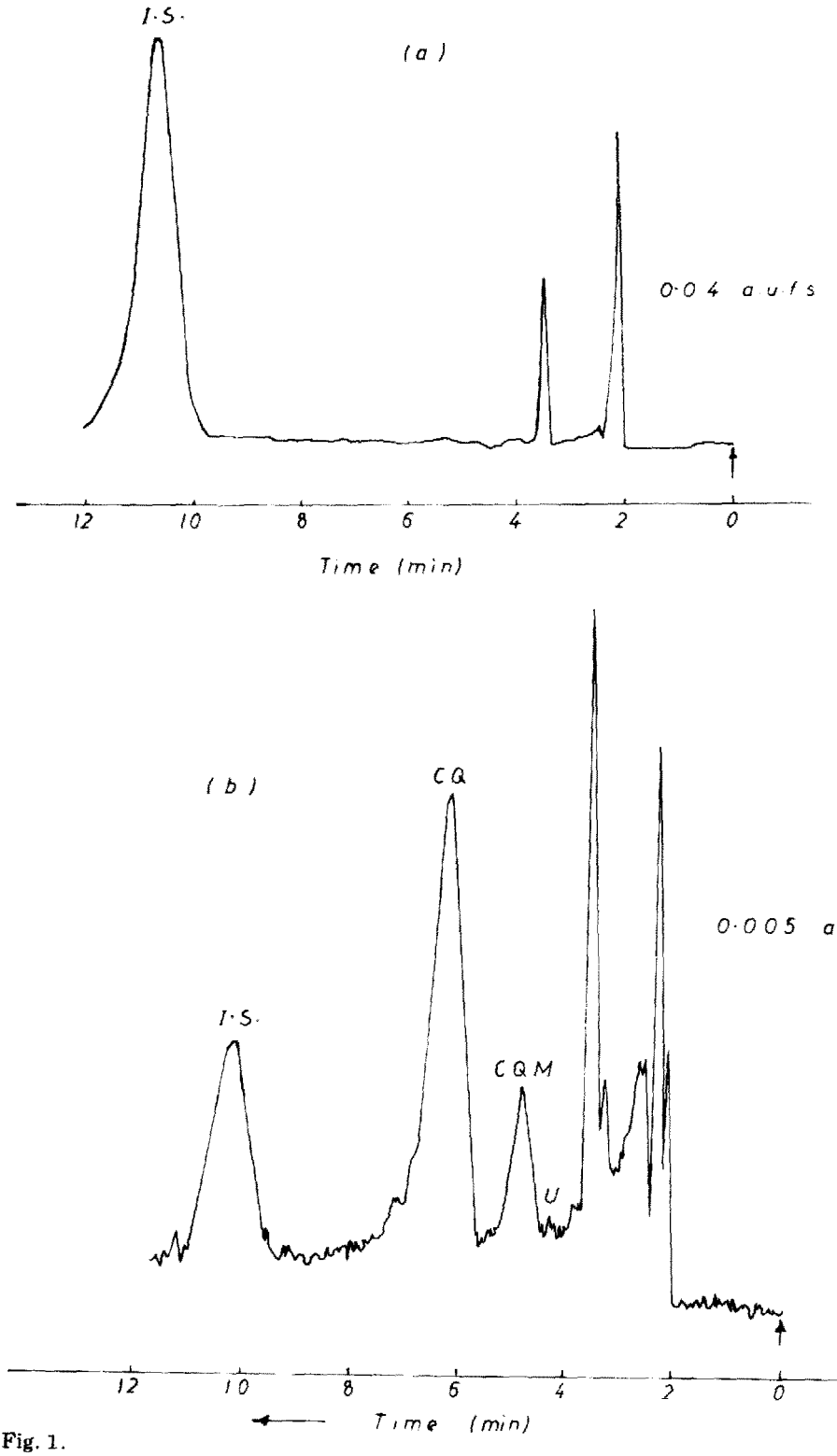
I·S·
(a)
0·04 a u f s
12
10
8
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Time (min)
(b)
CQ
I·S·
CQM
U
0·005 a·u·f s
12
10
8
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4
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0
Time (min)

Fig. 1.

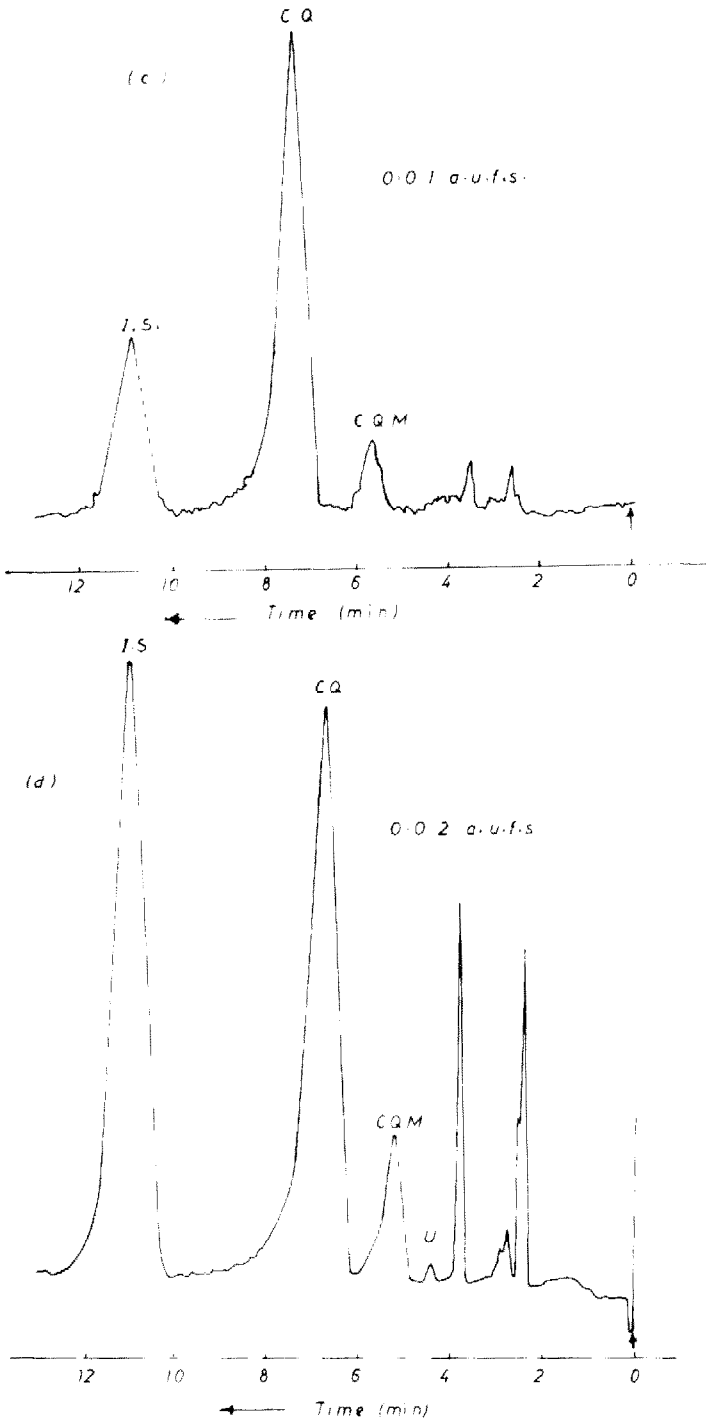

Fig. 1. High-performance liquid chromatograms of (a) an extract of blank human urine containing the internal standard (I.S.), (b) an extract of test plasma sample obtained from a volunteer 12 h after a single 600-mg oral dose of chloroquine, (c) an extract of test saliva sample obtained 24 h after drug administration, and (d) an extract of test urine sample obtained 8 h after drug administration (U = CQMM?).

Working standards were prepared from the stock standards. Methanol (analytical grade) and *n*-hexane (H & W) were glass-distilled twice, before use.

Chromatographic conditions

A Varian Model 5000 liquid chromatograph fitted with a fixed-wavelength (254 nm) UV detector was used. Injection was through a Varian manual loop valve injector fitted with a 10-μl loop. The detector output was connected to a Varian Model 9176 recorder. The analysis was carried out on MicroPak MCH-10, a 10-μm reversed-phase column (300 × 4.0 mm I.D.).

The mobile phase of methanol—0.2 *M* sodium dihydrogen phosphate (50:50) with 75 mmol/l perchloric acid was pumped through the column at a flow-rate of 1 ml/min. The pH of the mobile phase was 3.8. Analysis was carried out at ambient temperature.

Clinical samples

The study involved several volunteers and patients who received a single 600-mg oral dose of chloroquine. Blood samples were collected into heparinized tubes and centrifuged immediately to collect the plasma. Stimulated saliva samples were collected into wide-mouthed bottles. Total urine voided was also collected. The samples were collected before drug administration and at predetermined time intervals thereafter.

Plasma assay

To 1 ml of plasma placed in a 20-ml screw-capped extraction tube were added 1 ml of 2 *M* sodium hydroxide, 20 μl of internal standard solution (5 μg/ml) and 3 ml of *n*-hexane, before mixing with a whirlmixer for 1 min and then centrifuging for 10 min at 2000 *g*. The hexane layer was transferred into another tube. A 100-μl volume of the mobile phase was added, followed by mixing for 1 min and centrifuging for 10 min. The hexane layer was rejected. A 10-μl aliquot of the aqueous phase was injected into the high-performance liquid chromatograph.

Calibration curves based on peak-height ratio were prepared by spiking drug-free plasma with standard CQ and CQM solutions (5 μg/ml) to give a concentration range of 10—400 ng/ml.

Saliva assay

After thawing, 1 ml of saliva was taken through the extraction procedure described above. Calibration curves were prepared by spiking blank saliva in the concentration ranges of 50—2000 ng/ml and 10—2000 ng/ml for CQ and CQM, respectively.

Urine assay

A 1-ml volume of urine sample was diluted to 10 ml with water, and 1 ml of the diluted solution was then taken through the extraction procedure as described for the plasma sample above, but in this case 20 μl of 50 μg/ml internal standard solution was used. Calibration curves for the concentration ranges 10—4000 and 50—3000 ng/ml were prepared for CQ and CQM, respectively.

Precision and recovery studies

Replicate samples of blank plasma, urine and saliva were spiked with chloroquine and desethylchloroquine standards to give pre-determined concentrations . A 20-μl aliquot of the internal standard was added to each sample and then taken through the extraction procedure as described previously. Peak-height ratios were calculated and converted to concentrations using the calibration curves. The coefficients of variation (C.V.) of the estimated concentrations were determined and used for the assessment of precision. The absolute recovery was determined by comparing the peak-height ratios of the extracts with those obtained by direct injection of the compounds.

RESULTS AND DISCUSSION

The method reported here involved a less cumbersome extraction procedure than those previously reported. CQ was well resolved from its major metabolite, CQM, and the internal standard (I.S.), papaverine. The retention

TABLE I

PRECISION OF ANALYTICAL METHOD

Sample	Concentration (ng/ml)	C.V. (%)	*n*
Within-run			
Chloroquine:			
Plasma	50	3.2	8
	400	3.1	8
Urine	100	3.9	10
	3000	6.4	10
Saliva	50	6.0	4
	2000	2.8	4
Desethylchloroquine:			
Plasma	50	7.3	5
	400	6.3	5
Urine	100	5.8	5
	3000	3.8	5
Saliva	50	5.3	4
	2000	5.9	4
Between run			
Chloroquine:			
Plasma	50	3.2	6
	400	4.0	6
Urine	100	3.9	7
	3000	6.1	7
Saliva	50	7.5	4
	2000	3.8	4
Desethylchloroquine:			
Plasma	50	7.1	6
	400	5.3	6
Urine	100	6.3	7
	3000	6.0	7
Saliva	50	7.2	4
	2000	6.1	4

TABLE II

RECOVERY OF ANALYTICAL METHOD

Sample	Concentration (ng/ml)	Recovery (% ± S.D.)	*n*
Chloroquine:			
Plasma	200	90.0 ± 7.0	6
Urine	1000	92.0 ± 4.0	8
Saliva	2000	93.4 ± 3.0	4
Desethylchloroquine:			
Plasma	200	81.0 ± 6.0	5
Urine	1000	84.0 ± 5.0	5
Saliva	2000	84.5 ± 4.0	4

times (t_R) of CQ and CQM were 6.5 and 5.0 min, respectively, while that of the I.S. was 10.8 min. Another peak with t_R = 4.1 min was also seen in the plasma and was quite prominent in the urine samples of the volunteers and patients (Fig. 1). This peak is most probably due to the other known basic metabolite of CQ, bidesethylchloroquine (CQMM). Lack of reference sample of CQMM prevented the identification of this metabolite. The limits of detection in all the biological fluids were 10 and 5 ng/ml for CQ and CQM, respectively. Linearity of response was observed for the concentration ranges tested for the two compounds in all of the biological fluids ($r > 0.99$). We found no interference from endogenous compounds in the biological fluids or from the commonly used antimalaria drugs such as amodiaquine, proguanil, pyrimethamine and sulphadoxine and from other drugs usually co-administered with chloroquine, e.g. promethazine and chlorpheniramine. The method was found to be quite reproducible, as indicated by the low values of the coefficients of variation (Table I). The extraction efficiency was quite high and never less than 80% for both CQ and CQM in the biological fluids (Table II).

This method has been found adequate for the pharmacokinetic studies of CQ in volunteers and patients who were given a single dose (600 mg) of the drug. In one of these studies, the concentration of CQM in plasma was 63 ± 8% of CQ in four female healthy volunteers. The percentage dose excreted in the urine in 24 h in 30 other male volunteers was 14.67 ± 8.7% as CQ and 1.49 ± 0.78% as CQM. The method has also been used to demonstrate the secretion of CQ into human saliva [5].

The high-performance liquid chromatographic method described here is simple, selective and reproducible and permits simultaneous determination of CQ and its major metabolite, CQM, in plasma, saliva and urine.

REFERENCES

1 J. Bergqvist and M. Frisk-Holmberg, J. Chromatogr., 221 (1980) 119.
2 G. Alván, L. Ekman and B. Lindström, J. Chromatogr., 229 (1982) 241.
3 N.D. Brown, B.T. Poon and J.D. Chulay, J. Chromatogr., 229 (1982) 248.
4 M.A. Staiger, P. Nguyen-Dinh and F.C. Churchill, J. Chromatogr., 225 (1981) 139.
5 F.A. Ogunbona, A.A. Lawal and C.O. Onyeji, J. Pharm. Pharmacol., (1986) in press.