Journal of Chromatography, 616 (1993) 151–154
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 6831

Short Communication

Column liquid chromatographic analysis of quinine in human plasma, saliva and urine

C. P. Babalola and O. O. Bolaji

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife (Nigeria)

P. A. F. Dixon

Department of Pharmacology, Faculty of Pharmacy, Obafemi Awolowo University, Ile-ife (Nigeria)

F. A. Ogunbona*

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife (Nigeria)

(First received November 10th, 1992; revised manuscript received March 9th, 1993)

ABSTRACT

A new simple, selective and reproducible high-perfiormance liquid chromatographic method for the determination of quinine in plasma, saliva and urine is described. The ion-pair method was carried out on a reversed-phase C_{18} column, using perchlorate ion as the counter ion and ultraviolet detection at 254 nm. Quinine was well resolved from its major metabolite, 3-hydroxyquinine, and the internal standard, primaquine. The limit of detection was 10 ng/ml and the recovery was greater than 90% from the three biological fluids.

INTRODUCTION

Quinine, a quinolinemethanol, is widely used for the treatment of chloroquine-resistant strains of *Plasmodium falciparum* and for the management of patients presenting with severe or complicated malaria. This renewed interest in the use of quinine in malaria chemotherapy makes it necessary to elucidate fully the pharmacokinetics of the drug in humans, a situation that requires a highly sensitive, accurate and specific method for the analysis of the drug in biological fluids.

There have been reports [1–5] on the high-performance liquid chromatographic (HPLC) analysis of quinine in biological fluids. Most of these are either of low sensitivity [1,3,5] or do not employ any internal standard [1,3]. Some other methods [2,4] that are highly sensitive and specific give a poor recovery of quinine.

This paper describes a simple and selective HPLC method for determining quinine in plasma, whole blood, urine and saliva.

---

* Corresponding author.

0378-4347/93/$06.00

EXPERIMENTAL

Chemicals and reagents

Quinine sulphate (BDH, Poole, UK) and primaquine diphosphate (BDH) were used to prepare the working standards, and stock solutions containing 100 μg/ml quinine and primaquine were prepared in 0.1 *M* HCl. Primaquine was used as the internal standard. The required working standards were prepared from the stock solutions. Methanol was redistilled before used. Acetonitrile (HPLC grade), potassium dihydrogenphosphate, perchloric acid (H & W, Essex, UK) and diethyl ether (Koch-Light, Haverhill, UK) were used as supplied.

Chromatographic conditions

A Waters Model 501 liquid chromatograph fitted with a fixed UV detector, a Waters Model 441 (254 nm), was used for the analysis. The column was a reversed-phase C_{18} 10-μm Bondapak column (300 mm × 3.9 mm I.D.) (Waters). The detector output was connected to a Perkin Elmer Model 56 recorder. A mobile phase of 0.02 *M* potassium dihydrogenphosphate–methanol–acetonitrile (75:15:10) containing 74 m*M* perchloric acid was pumped through the column at a flow-rate of 1.2 ml/min. The pH of the mobile phase was 2.8, and the chromatogram was run at ambient temperature.

Assay

To 1 ml of plasma placed in a 10-ml tapered extraction tube were added 20 ml of internal standard solution (100 μg/ml), 0.2 ml of perchloric acid mixed for 5 s, 1 ml of 5 *M* NaOH and 4 ml of diethyl ether. After mixing for 1 min followed by centrifuging for 10 min at 3000 *g* the ether layer was transferred to another tube, and 100 ml of 0.1 *M* HCl were added. The tube contents were mixed for 1 min and centrifuged for 5 min. A 10-ml aliquot of the aqueous layer was injected into the HPLC column.

For the saliva assay, 1 ml of saliva was taken through the procedure described above for plasma. For the urine assay, 1 ml of urine was diluted to 10 ml with water and 1 ml of the diluted urine was taken through the same procedure.

Calibration curves

Calibration curves based on peak-height ratio were prepared by spiking drug-free plasma, saliva and urine with standard quinine solutions (5 and 50 μg/ml) to give a concentration range of 0.01–3.0 μg/ml. The solutions were taken through the above procedure.

Precision, recovery and selectivity studies

Replicate samples of blank plasma, saliva and urine were spiked with quinine standard solution to give pre-determined concentrations. To each sample were added 20 ml of internal standard solution (100 μg/ml) before taking it through the analytical procedure previously described. The precision of the method was assessed by computing the peak-height ratios and the coefficients of variation (C.V.) of the concentrations employed.

The absolute recovery was determined by comparing the peak heights of extracted quinine standards with those obtained by direct injection of known amounts of quinine. The selectivity study was carried out by injecting some other antimalarials (amodiaquine, chloroquine, proguanil, pyrimethamine and sulphadoxine) and other drugs such as promethazine, chlorpheniramine and paracetamol commonly co-administered with antimalarials, and recording their retention times.

RESULTS AND DISCUSSION

Quinine was well resolved from its major metabolite, 3-hydroxyquinine [6], and the internal standard, primaquine. The retention times, of quinine, 3-hydroxyquinine and primaquine were 7.8, 3.4 and 10.8 min, respectively (Fig.1). There was no interference from endogenous compounds in the biological fluids, or from commonly used antimalarials, or from drugs commonly co-administered with antimalarials, such as promethazine, chlorpheniramine, chloroquine, amodiaquine, pyrimethamine, proguanil and paracetamol. The limit of detection of quinine was 10 ng/ml in all biological fluids tested.

The calibration curves were linear with a correlation coefficient (*r*) greater than 0.99 for the concentration ranges tested in all the biological fluids

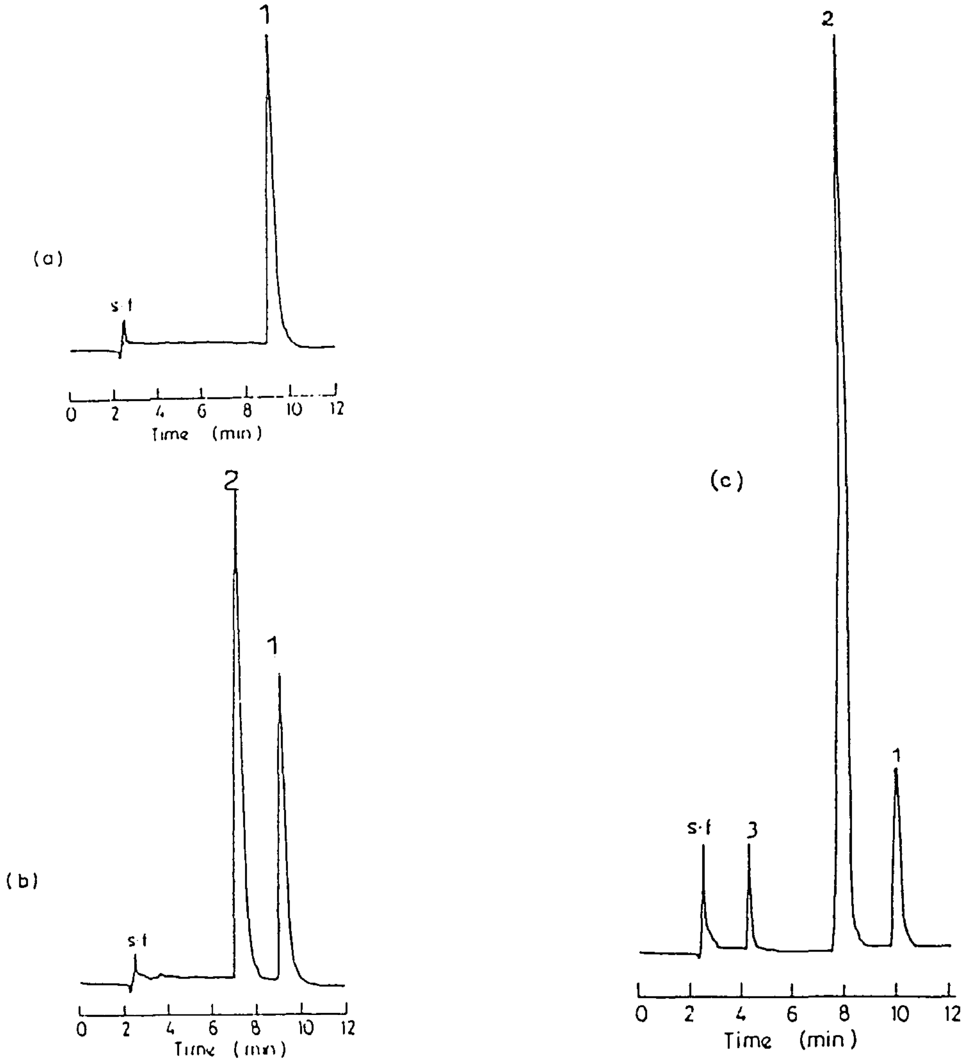

Fig. 1. HPLC of (a) extracted blank plasma containing the internal standard (2.5 μg/ml), (b) extracted spiked plasma containing quinine (1.5 μg/ml) and the internal standard and (c) extract of test plasma obtained from a volunteer 2 h after a single 600-mg oral dose of quinine. Peaks: 1 = internal standard; 2 = quinine; 3 = 3-hydroxyquinine; s.f. = solvent front.

examined. This method has been found to be quite reproducible giving low C.V. (Table I).The extraction efficiency was quite high, with the recovery being greater than 90% in all the biological fluids.

This method has been used to study the pharmacokinetics of quinine in healthy volunteers and patients. In one study in some volunteers given a single dose of 600 mg of the drug, C_{max} was found to be 8.0 ± 4.0 μg/ml, $t_{1/2}$ was 14.0 h, and the percentage dose excreted unchanged in 24 h was 5.55 ± 1.14. The saliva-to-plasma concentration ratio was 0.2 ± 0.02. The method has also been used to study the pharmacokinetics of quinine in healthy volunteers and kwashiorkor children [7]. It has been found to be simple, selective and reproducible, and less cumbersome than methods previously described for the measure-

TABLE I

PRECISION AND RECOVERY OF ANALYTICAL METHOD

Precision	Concentration (μg/ml)	C.V. (%)	Recovery (mean ± S.D.) (%)	*n*
Plasma				
Within-run	0.4	1.45		6
	1.2	1.65		6
Between-run	0.4	3.31		6
	1.2	5.55		6
Recovery	0.4		98.7 ± 3.2	4
	1.5		96.0 ± 7.5	5
Saliva				
Within-run	0.5	1.42		4
	1.5	2.23		4
Between-run	0.5	5.29		4
	1.5	4.60		4
Recovery	0.5		91.2 ± 4.7	4
	1.5		97.8 ± 3.6	5
Urine				
Within-run	0.5	1.25		4
	1.5	3.62		5
Between-run	0.5	2.11		4
	1.5	4.60		5
Recovery	0.5		94.7 ± 5.1	6
	1.5		97.4 ± 4.3	6

ment of nanogram amounts of the drug in plasma, saliva and urine.

REFERENCES

1 M. Edstein, J. Stace and F. Shann, *J. Chromatogr.*, 278 (1983) 445.

2 J. Karbwang, K. NaBargchang, P. Molunto and D. Bunnag, *S.E. Asian J. Trop. Med. Publ. Health*, 20 (1989) 65.

3 U. Hellgren, T. Villen and O. Ericsson, *J. Chromatogr.*, 528 (1990) 221.

4 A. R. Zoest, S. Wanwimolruk and C. T. Hung, *J. Liq. Chromatogr.*, 13 (1990) 3481.

5 E. K. Mberu, S. A. Ward, P. A. Winstanley and W. M. Watkins, *J. Chromatogr.*, 570 (1991) 180.

6 O. O. Bolaji, C. P. Babalola and P. A. F. Dixon, *Xenobiotica*, 21 (1991) 447.

7 L. A. Salaḳo, A. Sowunmi and F. O. Akinbami, *Br. J. Clin. Pharmacol.*, 27 (1989) 1.