DETERMINATION OF QUININE AND QUINIDINE IN BIOLOGICAL FLUIDS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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INTRODUCTION

The cinchona alkaloids, quinine and quinidine have been successful antimalarial drugs for centuries. They are still the only two available parenteral treatments for severe chloroquine-resistant falciparum malaria, and are therefore widely used throughout Southeast Asia. Quinine (usually combined with tetracycline) also remains the oral treatment of choice in much of this region. As parenteral quinine or quinidine is given to severely ill patients in whom clearance and distributions are usually markedly abnormal, drug monitoring by measurement of plasma or serum concentrations may become necessary (concerning the toxicity).

Fluorescence techniques (initially after protein precipitation, and subsequently after benzene or toluene extraction) have been used to measure quinine and quinidine in biological fluids for the past forty years (Spinks and Tottey 1948). However, the technique lacks selectivity and sensitivity (Edstein *et al.*, 1983). High Performance Liquid Chromatography (HPLC) has been shown to be a more sensitive and selective assay technique than extraction fluorescence technique (Edstein *et al.*, 1983). There are several HPLC methods which enable to measure either quinine or quinidine but not both (Edstein *et al.*, 1983; Ochs *et al.*, 1980). However, recently, Mihaly *et al.* (1987) described HPLC method that is able to quantitate quinine and/or quinidine, either separately or simultaneously but requires large plasma sample size (1 ml), and so this is not suitable for routine drug monitoring in children. The present paper describes a rapid, sensitive, selective method requiring a plasma sample size of only 250 µl.

MATERIALS AND METHODS

Chemical

Ammonium formate was purchased from Fluka (Switzerland). Chloroform, sodium hydroxide and phosphoric acid were obtained from MERCK. HPLC grade acetonitrile and methanol were supplied by J.T. Baker (U.K.) Quinine sulphate and quinidine sulphate were purchased from Sigma (St. Louis, Mo, U.S.A.).

Chromatography

This method was developed on a HPLC, system consisting of a 501 Waters delivery pump, a model 680 automated gradient controller, a Rheodyne valve injection system. This was coupled to a model FP 210 spectrofluorometer (Jasco, spectroscopic Co. LTD.), at emission frequencies of 425 nm and excitation of 340 nm. The separation was carried out on a revers-phase plastic column (Rad-Pak uBondapak C18, 10-µm particles, 100 mm × 8 mm I.D., Waters Assoc.) and was housed in a Z-module (Waters Assoc.). The output from the detector was connected to a Waters integrator model 740 recorder. The mobile phase consisted of 6.5% acetonitrile and 93.5% water containing 0.05 M NH_4COOH buffered to pH 2.0 with H_3PO_4 and the flow rate was 4 ml/min.

Extraction procedure

The extraction was carried out in 10 ml capacity glass tubes. To a 250 µl of plasma containing internal standard, quinine (1,000 ng) or quinidine (500 ng), was added 1 M NaOH (1ml). Each tube was vortexed for 5 seconds. The mixture was extracted with 5 ml chloroform by mechanical tumbling for 30 mins, followed by centrifugation at 1,000 g for 30 mins. The chloroform phase was evaporated to dryness at 37 ° C. The residue was dissolved in 100 µl of methanol and 20 µl was injected onto the chromatograph.

Standard curves

Standard curves were prepared by adding known amount of quinine or quindine to a fixed concentration of internal standard (1,000 ng) in drug-free plasma. Samples were analysed as described above and the peak area ratios of drug to internal standard was plotted against the corresponding weight ratio.

Precision and accuracy study

Six spiked samples of 250 ng, 1,000 ng and 1,500 ng of either quinine or quinidine were treated as unknowns to evaluate the accuracy and precision of the method. Peak area ratios were calculated and concentrations were obtained from the standard curve. The coefficient of variation was calculated using the equation:

$$\text{C.V.} = \frac{\text{S.D.}}{\text{mean value}} \times 100$$

Recovery study

Comparison of the peak area ratios (of quinine or quinidine and internal standard) between direct injection of quinine (or quinidine) and those obtained from extracted spiked plasma (of quinine or quinidine with the same amount with those direct injection). The recovery was calculated as following:

$$\% \text{ recovery} = \frac{\text{peak area ratio from extracted sample}}{\text{peak area ratio from direct injection}} \times 100$$

RESULTS AND DISCUSSION

The extraction procedure produced a simple and clean sample. The retention times of quinidine and quinine were 9 min and 11 min, respectively. Chromatogram of extracted plasma sample of quinine (quinidine was used as internal standard) is shown in Fig. 1.

Calibration curves for quinine and quinidine in plasma showed linearity with correlation coefficients of 0.9999 and 0.9997, respectively (Fig. 2, 3). The limitation of detection using a 250 µl of plasma was 4 ng/ml where signal to noise ratio was 4:1. Recovery of quinine and quinidine varied

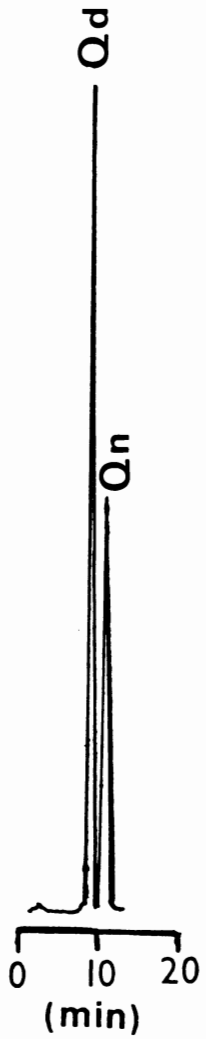

Fig. 1–Chromatogram of extracted plasma sample obtained after oral dose of quinine.

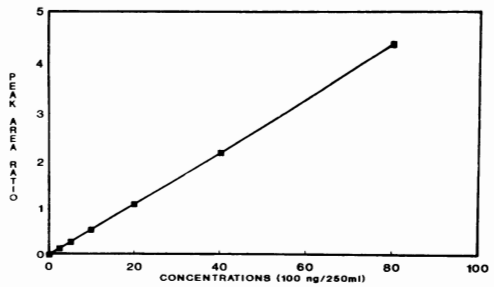

Fig. 2–Standard curve of quinine in plasma.

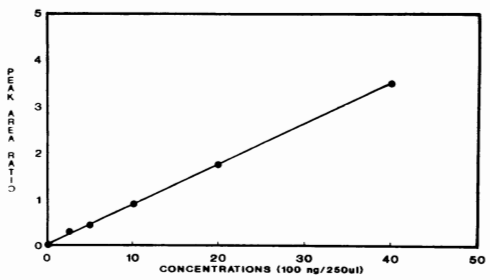

Fig. 3–Standard curve of quinidine in plasma.

from 62% to 92% (mean of 76% and 81% for quinine and quinidine, respectively) in the range of 500 ng to 3,000 ng. Due to the very similar behavior of quinine and quinidine during extraction, changes in recovery are of minor importance.

To evaluate the precision and accuracy of the method, spiked plasma was used. The results are summarized in Table 1, 2, showing good accuracy and precision, both quinine and quinidine. Inter- and intra-assay coefficient of variation was less than 6.8% for quinine and 3.7% for quinidine.

The assay provided a rapid and simple one-step extraction which requires a small sample volume. The low limit of detection of quinine and quinidine with the short retention times make the method suitable for routine analysis of quinine and quinidine.

The method has been used in the analysis of quinine and quinidine in healthy volunteers receiving quinine or quinidine intrave-

Table 1

Precision and accuracy data for quinine analysis in plasma.

Amount quinine added (ng)	Amount measured (ng, mean ± SD)	CV%	N
250	254.3 ± 17.4	6.8	6
4,000	4,006.7 ± 13.0	0.3	6
8,000	8,120.7 ± 99.7	1.2	6

Table 2

Precision and accuracy data for quinidine analysis in plasma.

Amount quinidine added (ng)	Amount measured (ng, mean ± SD)	CV%	N
500	504.1 ± 9.0	1.8	6
1,500	1,501.2 ± 40.4	2.7	6
3,000	3,079.3 ± 115.7	3.7	6

nously (Fig. 4). The method is now being used to assay samples from field studies with satisfactory results.

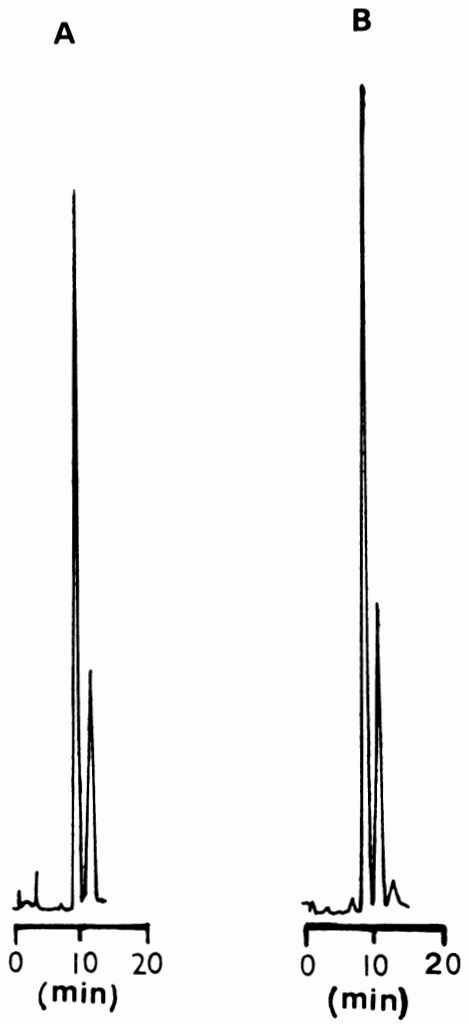

Fig. 4—Chromatograms of extracted plasma sample of (a) quinine (b) quinidine

SUMMARY

A simple, specific, rapid and sensitive High Performance Liquid Chromatography (HPLC) method has been developed to measure plasma level of quinine and quinidine.

The drugs were extracted successively from plasma at basic pH with chloroform and quantified on a revers-phase Z-module C18 HPLC column with fluorescence detector (excitation 340 nm, emission 425 nm). The isocratic mobile phase used was the mixture of 0.05 M ammonium formate and acetonitrile (93.5:6.5, v/v), adjusted pH to 2.0 with ortho-phosphoric acid.

The limits of quantitation for these compounds were as low as 4 ng/ml of plasma, using a 0.25 ml specimen. Calibration curves were linear (R squared = 0.9994) in the range 0–7,000 ng/ml. An interassay repro-

ducibility was 6.8%, 0.3% and 1.2% at the concentrations of 250 ng, 4,000 ng and 8,000 ng of quinine, respectively. Inter-assay coefficient of variation of quinidine was 1.8%, 2.7% and 3.7% at the concentrations of 250 ng, 1,500 ng and 3,000 ng, respectively. Recovery of quinine and quinidine were 76% and 81%, respectively.

The method has been used in the analysis of quinine and quinidine in healthy volunteers receiving quinine or quinidine intravenously. The method is now being used to assay samples from field studies with satisfactory results.

ACKNOWLEDGEMENT

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