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Note

High-performance liquid chromatographic determination of quinine in plasma, whole blood and samples dried on filter paper

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As chloroquine-resistant *Plasmodium falciparum* now is widespread, the classical antimalarial drug quinine has received renewed interest and remains the drug of choice for severe and complicated malaria [1,2]. However, since decreased sensitivity to quinine has also been reported, careful monitoring of possible resistance is required [1]. This can be done either by laborious in vitro methods or by relating drug concentrations achieved during treatment to in vivo parasitological response [3]. Such studies have been difficult to perform in the field since existing methods often require that large volumes of blood be obtained by venepuncture and that centrifugation and freezing facilities be available.

Methods for determining quinine levels in biological fluids are commonly based on the fluorescence of the native compound. Early methods used protein precipitation or extraction and direct measurement of the fluorescence [4,5]. However, these methods co-determine quinine metabolites and thus are not suitable for pharmacokinetic and other studies [6]. Edstein et al. [6] and Rauch et al. [7] have described reversed-phase high-performance liquid chromatographic (HPLC) methods, and Salvadori et al. [8] a normal-phase HPLC method using quinidine as internal standard. More recently, Mihaly et al. [9] have reported an HPLC method with a minimum detectable concentration as low as 10 ng/ml (31 nM) using 1-ml plasma samples. Gas chromatographic [10] and gas chromatographic–mass spectrometric [11] methods for the

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quantification of quinine also have been reported. Recently, a specific and sensitive enzyme-linked immunosorbent assay that can be used for blood dried on filter paper has been described [12].

Quinidine is a diastereomer of quinine. There are several HPLC methods reported for determination of quinidine (see refs. 13–15 and references cited therein). Although not evaluated for this application, these quinidine methods can also be used for quinine determination with or without minor modifications.

The aim of the present study was to develop a simple HPLC method that can be used to determine quinine concentrations both in conventional blood or plasma samples and in samples dried on filter paper. This sampling technique is especially suitable for field use.

EXPERIMENTAL

Instrumentation and chemicals

The HPLC equipment used consisted of a Constametric III pump, (LDC, Riviera Beach, FL, U.S.A.), a Rheodyne 7125 injector (Berkeley, CA, U.S.A.) with a 200-μl loop and an RF-530 fluorescence detector (Shimadzu, Kyoto, Japan). The detector was operated with the excitation and emission wavelengths set at 330 and 365 nm, respectively. The column was a Supelcosil LC-Si (15 cm $\times$ 4.6 mm I.D.) with 3-μm particles (Supelco, Bellefonte, PA, U.S.A.). The mobile phase was acetonitrile–methanol–ammonia (400:175:1, v/v), and the flow-rate was 1.0 ml/min.

Quinine hydrochloride was obtained from the Department of Pharmacy at Huddinge Hospital. All chemicals were of analytical grade.

The filter paper used was Munktell No. 5 (Kebo, Spånga, Sweden).

Samples

To evaluate the method, plasma or venous blood spiked with different concentrations of quinine was used. Samples were also obtained from a volunteer given 900 mg of quinine hydrochloride orally. Capillary blood and venous blood obtained in 5-ml heparinized vacuum tubes (Vacutainer) were drawn simultaneously on several occasions during 48 h after administration. Plasma was obtained by centrifugation at 1000 *g* for 10 min. The plasma and venous whole blood samples from the volunteer were stored at $-20°C$ until analysis. Capillary blood (100 μl) was filled into heparinized precision capillaries and applied to the filter paper. The filter papers were stored in plastic folders at room temperature for two to eight weeks unless stated otherwise.

Analytical procedure

The dried blood spots, cut into small pieces, or aliquots (25–100 μl) of whole blood or plasma were mixed with 1.0 ml of 0.1 *M* sodium hydroxide and extracted with 100 μl of chloroform on a rotating mixer for 10 min. After centrif-

ugation at 1000 *g* for 10 min, 20 μl, measured with a syringe, of the organic layer were injected into the column. Concentrations were calculated by comparing the heights of the quinine peaks in the samples with those in a standard curve, prepared by adding quinine to plasma, venous blood or venous blood dried on filter paper (1–50 μ*M*). All samples were analysed in duplicate.

Recovery

The recovery was estimated by spiking samples, extracting them, and comparing the peak heights with those of directly injected standards dissolved in the mobile phase.

Interferences

Serum samples were spiked with therapeutic concentrations of antimalarial drugs. Authentic samples were obtained from patients on malaria prophylaxis and from patients whose samples were sent to our laboratory for determination of drug concentrations.

RESULTS

Chromatograms obtained from extracts of dried samples of drug-free blood, blood spiked with 1 μ*M* quinine and of capillary blood 24 h after quinine intake are shown in Fig. 1. No peaks other than that of quinine were observed in any of the chromatograms.

Tables I and II show the accuracy, reproducibility and recovery for spiked 100-μl samples. When 20-μl aliquots were injected, the limit of determination (coefficient of variation, C.V. $<10\%$) was 1 μ*M*. The corresponding limit of detection (three times the baseline noise) was 0.2–0.3 μ*M*. During the study a large number of calibration curves were run. All were linear and the correlation coefficients were always above 0.990.

Analysis of serum spiked with the antimalarial drugs sulfadoxine, pyrimethamine, proguanil, chloroproguanil, mefloquine, chloroquine and primaquine showed that these drugs did not interfere with the quinine determination. In order to evaluate possible interferences from metabolites, serum samples from patients treated with chloroquine, paracetamol, salicylic acid and imipramine, as well as serum from persons undergoing malaria prophylaxis with mefloquine or sulfadoxine/pyrimethamine, were analysed. None of them gave rise to interfering peaks. The method has also been used in a field study in Guinea Bissau, where eighteen children were given quinine (5 mg/kg body weight) twice daily for five days. A total of 100 capillary whole blood samples were taken, and the blood was applied to filter paper. Interferences from other substances or metabolites were not detected in any of the samples. It should be noted that the chromatographic system we used cannot separate quinine from its diastereomer quinidine.

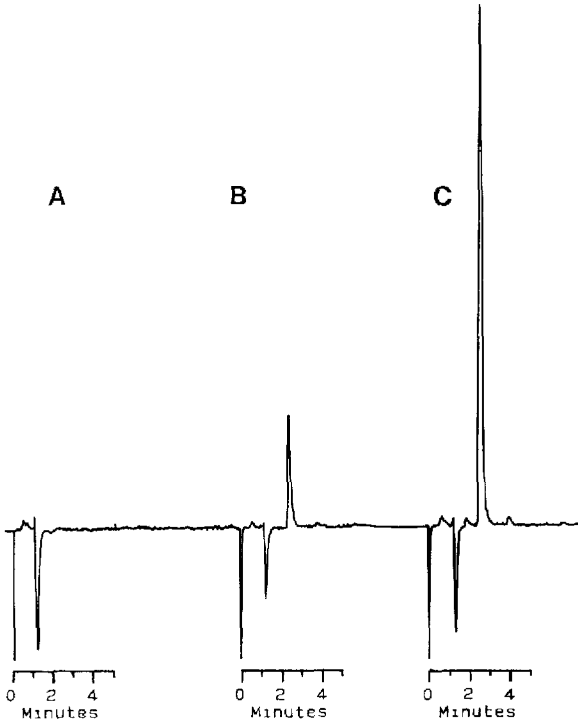

Fig. 1 Chromatograms of extracts of dried samples of (A) drug-free blood, (B) blood spiked with 1 μM quinine and (C) capillary blood 24 h after quinine intake. The retention time for quinine is 3 min.

TABLE I

ACCURACY AND COEFFICIENT OF VARIATION FOR 100-μl SAMPLES SPIKED WITH QUININE

Accuracy is deviation from spiked value.

Concentration (μM)	Plasma ($n=10$)		Whole blood ($n=10$)		Whole blood filter paper ($n=10$)	
	Accuracy (%)	C.V. (%)	Accuracy (%)	C.V. (%)	Accuracy (%)	C.V. (%)
25	+8.1	5.6	+4.8	5.0	−8.9	4.6
1	+4.4	9.2	+10	4.9	+12	3.8

Fig. 2 shows plasma, venous and capillary whole blood concentrations in a subject after quinine intake. There were no apparent differences between venous and capillary blood concentrations in this person, but the concentrations

TABLE II

EXTRACTION RECOVERY OF QUININE FROM 100-μl SAMPLES

Concentration (μM)	Extraction recovery (%)		
	Plasma (n=4)	Whole blood (n=4)	Whole blood filter paper (n=4)
25	92	74	69
2.5	92	78	77

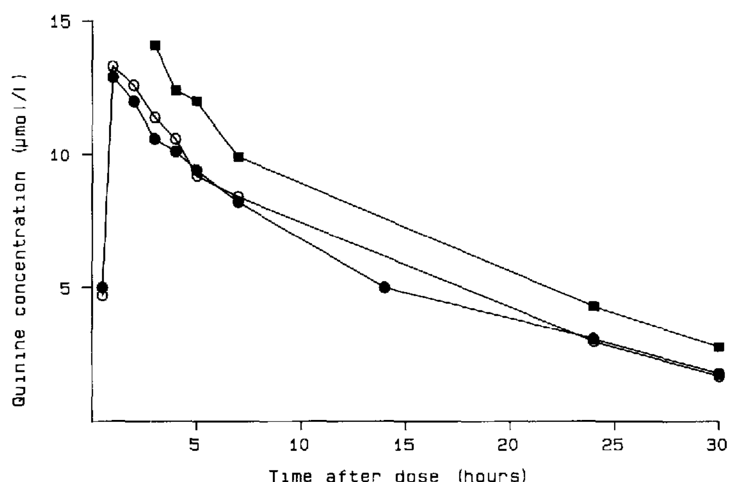

Fig. 2. Plasma (■), venous (●) and capillary whole blood (○) concentrations after oral intake of 900 mg of quinine hydrochloride.

in venous plasma were higher than those in whole blood. Concentrations in capillary plasma were also essentially the same as those in venous plasma.

Storage of the filter papers in an incubator at 30°C for three months or at 54–56°C for 5 h did not change the concentration measured.

DISCUSSION

This simple method is the first HPLC assay that has been developed for determination of quinine concentrations in small blood samples dried on filter paper. It is equally well suited to the analysis of venous blood and plasma samples.

The extraction recovery from blood dried on filter paper was essentially the same as that from blood not dried on filter paper (Table II), and thus the latter might be used for preparation of standard curves even when concentrations in filter paper samples are determined.

The limit of determination with this method is similar to that reported by Edstein et al. [6], but higher than those reported by Salvadori et al. [8], who used post-column acidification with sulphuric acid, and Mihaly et al. [9], who used larger sample volumes and an evaporation step before reconstitution and injection. Also the method of Rauch et al. [7] is more sensitive than ours. However, the quinine concentration could be determined in the volunteer even 30 h after a single treatment dose. This sensitivity is sufficient for normal monitoring of drug concentrations during antimalarial therapy as therapeutic plasma concentrations are in the range 25–45 μM [16]. If higher sensitivity is required, e.g. for pharmacokinetic studies, both sample and injection volumes can be increased. On the other hand, it is sometimes necessary to use smaller volumes, even at the expense of sensitivity. We have, for example, determined quinine in samples from guinea-pigs by using 25-μl aliquots [17].

The chromatographic system described here is not capable of separating quinidine from quinine, but this is a minor disadvantage as patients with malaria are normally not treated with both drugs at the same time. Except for this interference with quinidine, field application and analyses of serum from persons using common antimalarials did not show any signs of interference indicating that the method is specific when used in connection with antimalarial studies.

Inactivation of HIV virus in dried samples is considered to be complete after incubation for 5 h at 54–56 °C and at normal room temperature for a week [18]. Heat inactivation of the filter paper samples did not affect the quinine determination. The filter paper samples were also stable for at least three months at tropical temperatures; this fact, together with the high level of acceptance of repeated fingerpricking among the population, makes this sampling method very suitable for field studies [3].

In this study, we did not find any differences between capillary and venous whole blood concentrations contrary to an earlier report [6].

To elucidate whether there is a real difference between capillary and venous concentrations, further studies in healthy volunteers and in malaria patients are needed.

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