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Short communication

High-performance liquid chromatographic method for the determination of quinine and 3-hydroxyquinine in blood samples dried on filter paper

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Abstract

A simple high-performance liquid chromatographic method for the simultaneous analysis of quinine and 3-hydroxyquinine in blood samples dried on filter paper is described. Sample preparation involves liquid–liquid extraction with toluene–butanol 75:25 (v/v) followed by evaporation. A reversed-phase liquid chromatography system with fluorescence detection was used. The limit of determination was 10 n*M* for both quinine and 3-hydroxyquinine and the recovery varied between 78 and 109%. The within- and between-assay coefficients of variation varied between 2–5% and 4–10%, respectively. No loss of either analyte occurred after storage for 2 months at room temperature or at 37 °C. This method for sampling has advantages that make it of great value for clinical and pharmacokinetic studies especially in remote regions where storage and transportation is problematic.

Keywords: Quinine; 3-Hydroxyquinine

1. Introduction

Quinine is an old antimalarial drug that has been used for over 350 years [1]. Since the rapid development of chloroquine-resistant *Plasmodium falciparum*, quinine has become the drug of choice for treatment of severe and complicated malaria [1]. Determination of the concentration of quinine in different body fluids makes it possible to monitor adverse effects and to study the pharmacokinetics of the drug.

Studies in Africa are often located in rural areas remote from the laboratory where the analysis is to be performed. Therefore storage and transportation of the samples is a problem. Some high-performance liquid chromatography (HPLC) methods for determination of quinine in blood samples dried on filter paper have been developed [2–6]. Such methods are of great value for field studies since filter paper samples are easy to handle and transport. Heat treatment of the samples has also been reported to reduce the viability of the human immunodeficiency

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virus (HIV), thus reducing the risk of infection for laboratory personnel [7,8].

Since we will perform clinical studies to investigate the metabolism of quinine we need to measure the concentrations of both the parent drug and the major metabolite 3-hydroxyquinine. There are no methods available for the simultaneous analysis of quinine and 3-hydroxyquinine in blood samples dried on filter paper.

The aim of this study was to develop a simple HPLC method for the analysis of quinine and 3-hydroxyquinine in blood samples dried on filter paper.

2. Experimental

2.1. Chemicals

Quinine sulfate and the internal standard quinidine were obtained from Apoteket AB (Stockholm, Sweden). The 3-hydroxyquinine hydrochloride, which is a 25:75 mixture of the two diastereomers of 3-hydroxyquinine, was obtained from Dr Douglas J Christie [9] at the Department of Laboratory Medicine and Pathology, Medical School, University of Minnesota, Minneapolis, MN, USA. Solvents and chemicals (HPLC or analytical grade, as required) were purchased from Merck (Darmstadt, Germany).

2.2. Instrumentation

The high-performance liquid chromatography system consisted of a Rheos 4000 gradient pump (Crelab Instruments, Uppsala, Sweden), a Gilson 231 XL injector (Pretech Instruments, Stockholm, Sweden), a Waters 474 scanning fluorescence detector (Milford, MA, USA) and a computer with Chemstation software for data registration and analysis (Agilent Technologies, Kista, Sweden).

The excitation and emission wavelengths of the detector were set at 350 and 450 nm, respectively. The column was a Zorbax Eclipse XDB phenyl 150×4.6 mm, I.D. 5 μm (Chrom Tech, Hägersten, Sweden). The column was kept at room temperature. Gradient elution of the analytes was performed using two mobile phases as shown in Table 1. Mobile phase A consisted of 8% acetonitrile in 0.1 *M* acetate buffer (880 ml acetic acid 0.2 *M*, 120 ml ammonium acetate 0.2 *M* and 1000 ml distilled water), pH 3.9. Mobile phase B consisted of 24% acetonitrile in 0.1 *M* acetate buffer, pH 4.25. The flow-rate of the mobile phase was 1.0 ml/min.

Table 1
Elution gradient

Time (min)	A (%)	B (%)
0	30	70
6	20	80
8	10	90
15	30	70

2.3. Samples

Samples for the development and evaluation of the method were prepared by adding known amounts of quinine and 3-hydroxyquinine to blank whole blood. Spiked blood (100 μl) was applied to filter paper (Whatman 3 MM Chr, Whatman International Ltd, Maidstone, UK) and dried at room temperature. Samples were also obtained from healthy volunteers taking a single dose of 250 mg quinine hydrochloride. Capillary blood samples were taken in 100 μl heparinized precision capillaries (Drummond Scientific Company, Broomall, PA, USA). Two hundred microliters of venous blood from the same volunteers was dried on filter paper for comparison of the concentrations of quinine and 3-hydroxyquinine between capillary and venous blood. The dried samples were stored in plastic folders at room temperature until analysis.

2.4. Analytical procedure

The filter paper samples were cut into pieces and placed in 4.5 ml polypropylene tubes. One milliliter of NaOH 0.1 *M* and 100 μl of the internal standard quinidine was added then the tubes were vortexed for 10 s. After the addition of 2 ml of the extraction mixture (toluene–butanol, 75:25 v/v) the samples were extracted for 10 min using a rotating mixer then centrifuged for 10 min at 3500 *g*. The organic layer was transferred to a new tube and evaporated to dryness under vacuum for 45 min. The residue was reconstituted in 100 μl of 10% acetonitrile in acetate

buffer (pH 3.97) then 5–70 μl was injected into the HPLC system.

2.5. Standard curves

Standard curves were prepared by adding known amounts of quinine and 3-hydroxyquinine to blank whole blood which was applied to filter paper. The samples were extracted according to the above procedure. The peak height ratios were plotted versus the concentration in standard curves. For method validation, three different concentrations of quinine and 3-hydroxyquinine were used; 1 μ*M*, 300 n*M* and 10 n*M*.

2.6. Extraction recovery

To document the extraction recovery, quinine and 3-hydroxyquinine were added to blank whole blood and 100 μl was applied to filter paper ($n=10$). The concentrations are given in Table 2. Samples were extracted according to the above procedure and the peak areas were compared to those of directly injected standards.

2.7. Within-assay precision and accuracy

Known amounts of quinine and 3-hydroxyquinine were added to blank whole blood and 100 μl was applied to filter paper ($n=10$). The concentrations are given in Table 3. Samples were extracted and analyzed as described above.

2.8. Between-assay precision and accuracy

Known amounts of quinine and 3-hydroxyquinine were added to blank whole blood and 100 μl was applied to filter paper. Double samples were analyzed on different occasions. The concentrations are given in Table 4. Samples were extracted and analyzed as described above.

2.9. Interference

Solutions of chloroquine, amodiaquine, pyrimethamine, mefloquine, acetylsalicylic acid, paracetamol, prometazine, sulfamethoxazole and ampicillin were injected in the HPLC system to study if these interfered with our samples.

Table 2
Extraction recovery

Analyte ($n=10$)	Concentration (μ*M*)	Recovery (%)	CV (%)
Quinine	1.0	103	8
	0.3	79	6
	0.01	87	5
3-Hydroxyquinine	1.0	109	8
	0.3	96	6
	0.01	104	7

2.10. Stability

The stability of the filter paper samples was studied at both room temperature (20 °C) and in an incubator set at 37 °C. Samples were analyzed over a period of 2 months. The stability of the samples after heat treatment at 56 °C for 1 h was also investigated.

2.11. Samples from healthy subjects

Capillary blood samples (100 μl) drawn 16 h after intake of a single oral dose of 250 mg quinine hydrochloride from 10 healthy Swedish subjects were dried on filter paper, extracted and analyzed as described above. Venous blood samples (200 μl) were taken from six subjects to compare the concentrations of quinine and 3-hydroxyquinine in venous vs. capillary blood. The venous blood samples were dried on filter paper and analyzed as described above.

3. Results and discussion

Chromatograms of blank whole blood, spiked whole blood and capillary whole blood from a volunteer taking a single 250 mg oral dose of quinine hydrochloride are shown in Fig. 1. Even though the two analytes, quinine and 3-hydroxyquinine, differ in polarity, the gradient elution made it possible to have an analysis time of merely 15 min. Quinine is well separated from both the internal standard quinidine

Table 3
Within-assay precision and accuracy

Analyte ($n=10$)	Nominal concentration (μM)	Mean concentration found (μM)	Accuracy (%)	CV (%)
Quinine	1.0	1.128	+13	3
	0.3	0.298	−1	3
	0.01	0.0105	+5	2
3-Hydroxyquinine	1.0	1.110	+11	4
	0.3	0.289	−4	5
	0.01	0.0109	+9	5

and 3-hydroxyquinine. There were no endogenous compounds interfering.

Analysis of solutions of chloroquine, amodiaquine, pyrimethamine, mefloquine, acetylsalicylic acid, paracetamol, prometazine, sulfamethoxazole and ampicillin showed that none of these drugs interfered with any of the analytes that was studied. However, samples of patients treated with quinidine cannot be analyzed since this substance is used as an internal standard.

The limit of detection (defined as the concentration injected that gives at least a signal-to-noise ratio of 3:1) was 2.5 n*M*. The limit of determination was taken as the lowest concentration used for evaluation of the method, which was 10 n*M* for both quinine and 3-hydroxyquinine.

Several extraction solutions were tested. Since quinine and the metabolite differ in polarity an extraction mixture containing both a polar and a non-polar organic solute was required. Liquid–liquid extraction with toluene–butanol 75:25 followed by evaporation gave the best extraction recovery for both quinine and its metabolite. Table 2 shows the recovery for spiked samples, which was in the range of 78 to 109%. The within- and between-assay precision and accuracy are shown in Tables 3 and 4. Typical values of the coefficient of variation (CV) for the within assay variability was 2 to 5%. Inter-assay CV was in the range of 4 to 10%.

During the study a large number of standard curves were run. The most frequently used ranges for the standard curves were 0.2–2.0 μM and 5–20 n*M*. All were linear and the correlation coefficients were all above 0.995 in the higher concentration range and above 0.991 in the lower.

Hellgren et al. have previously showed that quinine in filter paper samples is stable for 3 months at 30 °C [2]. We here wanted to examine the stability of 3-hydroxyquinine in filter paper samples. The stability of the analytes was studied at both room temperature (20 °C) and tropical temperature (37 °C) over a period of 2 months. The concentration of quinine and 3-hydroxyquinine did not change after storage in either temperature. The samples also resisted heat treatment at 56 °C for 1 h. This treatment is considered to efficiently inactivate HIV [7,8] and make the method safer for the personnel working with the samples.

Table 4
Between-assay precision and accuracy

Analyte ($n=8$)	Added concentration (μM)	Mean concentration found (μM)	Accuracy (%)	CV (%)
Quinine	1.0	1.089	+9	7
	0.3	0.314	+5	6
	0.01	0.01		10
3-Hydroxyquinine	1.0	1.064	+6	8
	0.3	0.297	−1	4
	0.01	0.0106	+6	8

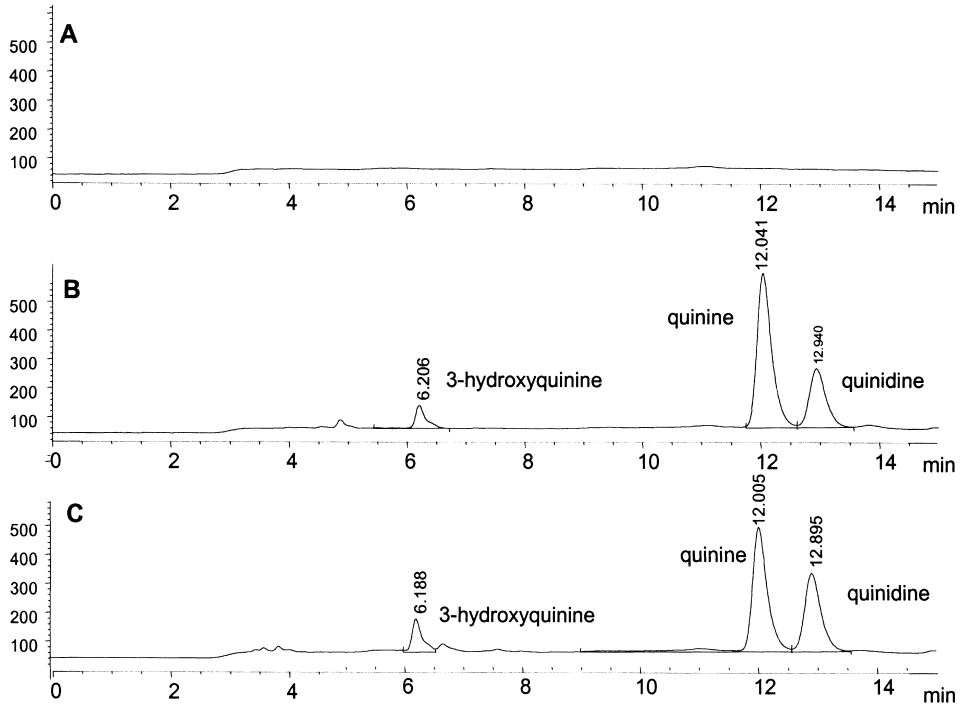

Fig. 1. Chromatograms of quinine and 3-hydroxyquinine in filter paper samples. (A) Blank whole blood. (B) Spiked whole blood containing 3.0 μ*M* of quinine, 500 n*M* of 3-hydroxyquinine and 500 n*M* of quinidine. (C) Capillary sample containing 1.87 μ*M* of quinine, 280 n*M* of 3-hydroxyquinine and 500 n*M* of quinidine. 3-Hydroxyquinine at 6.2 min; quinine at 12 min; and quinidine at 12.9 min.

The concentrations in capillary samples from 10 volunteers that took a single oral dose of 250 mg quinine hydrochloride are shown in Table 5. Venous samples were drawn from six of these individuals.

Table 5
Capillary and venous blood samples

Subject (n=10)	Concentration of quinine (μ*M*)		Concentration of 3-hydroxyquinine (μ*M*)	
	Capillary	Venous	Capillary	Venous
1	1.155	n.d.	0.132	n.d.
2	1.058	n.d.	0.213	n.d.
3	0.402	0.563	0.115	0.069
4	1.706	1.598	0.390	0.307
5	0.627	n.d.	0.112	n.d.
6	0.214	0.226	0.083	0.083
7	2.811	2.207	0.304	0.212
8	2.066	n.d.	0.308	n.d.
9	0.727	0.511	0.121	0.110
10	1.943	1.904	0.236	0.199

n.d.=not determined.

The concentrations are presented in Table 5. Hellgren et al. [2] and Dua et al. [4] have previously established that there are no differences between capillary and venous concentrations of quinine. Even though 200 μl were analyzed from the venous blood and 100 μl were analyzed from the capillary blood we think that the results could still be compared. There was no significant difference (P=0.72) in the mean concentration of quinine between capillary (1.3±1.0) and venous (1.3±0.78) blood, which is in agreement with the previously published results. On the other hand there was a significant difference (P=0.03) in the mean 3-hydroxyquinine concentrations determined in capillary (0.208±0.12) and venous (0.163±0.0.09) blood. This needs to be further evaluated and confirmed by using the same volume in capillary and venous blood samples and by including a larger number of samples.

In conclusion, this method for analyzing quinine and its main metabolite 3-hydroxyquinine is simple and reliable. The recovery and limit of determination

presented make this method suitable for clinical and pharmacokinetic studies. Among the advantages of filter paper samples over regular blood sampling are the ease of storage and transportation. Capillary sampling from finger pricking is also much more accepted and cheaper than venous sampling. This method of sampling is becoming more popular, especially in children, when small samples are preferred. All of this, in combination with the reduced risk of HIV infection when handling the samples, results in an excellent method for field studies.

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