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High-performance liquid chromatographic method for the determination of the major quinine metabolite, 3-hydroxyquinine, in plasma and urine

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Abstract

The determination of 3-hydroxyquinine in urine and plasma samples is described. Extraction was performed using a mixture of toluene–butanol (75:25, v/v), followed by back-extraction into the mobile phase, which consisted of 0.1 *M* phosphate buffer, acetonitrile, tetrahydrofuran and triethylamine. A reversed-phase liquid chromatography system with fluorescence detection and a CT-sil C_{18} column were used. The within-assay coefficient of variation of the method was 2% at the higher concentration values in plasma, 2.95 μ*M*, 4% at 227 n*M* and 9% at the lower limit of quantitation, 4.5 n*M*. In urine, the coefficient of variation was 11% at the lower concentration, 227 n*M* and was 3% at 56.8 μ*M*. The between-assay coefficient of variation was 4% at the low concentration (5.1 n*M*) in plasma, 2% at 276.8 n*M* and 3% at 1.97 μ*M*. In urine, the between assay coefficient of variation was 4% at 204.6 n*M*, 3% at 5.12 μ*M* and 2% at 56.8 μ*M*.

Keywords: Quinine; 3-Hydroxyquinine

1. Introduction

Quinine is an old antimalarial drug, and it is still the drug of choice for the treatment of severe and complicated malaria. Knowledge of the biotransformation of quinine is incomplete. In humans, neither the metabolic pathways nor the relative importance of different metabolites are fully known. Quinine appears to be metabolized by cytochrome P450 (CYP 3A4) [1]. The activity of this enzyme varies between individuals and probably also between ethnic groups [2].

Detailed studies of the disposition of quinine requires quantitation of both the parent drug and the main metabolite(s). 3-Hydroxyquinine has been suggested to be a major metabolite of quinine [3]. One of the major biotransformation products of quinidine (which is a diastereoisomer of quinine) in man was found to be (3*S*)-3-hydroxyquinidine [4]. By analogy, the major metabolite of quinine might be the (3*S*)-3-hydroxyquinine. (3*R*)-3-Hydroxyquinine has also been found in man in smaller amounts than (3*S*)-3-hydroxyquinine (1%) (see Fig. 1 for struc-

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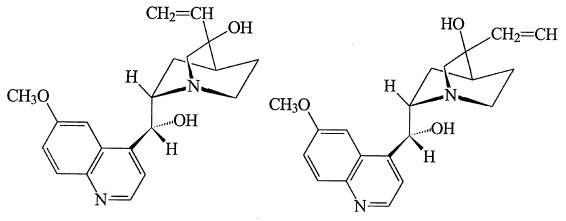

3(R)-3-hydroxyquinine 3(S)-3-hydroxyquinine

Fig. 1. Structures of the 3-hydroxyquinine diastereomers.

tures of the 3-hydroxyquinine diastereomers). 3-Hydroxyquinine, most probably the (3*S*) form, has been identified in human urine using methane chemical ionization gas chromatography–mass spectrometry [5]. It has also been found in rat urine using a high-performance liquid chromatographic method [6], and was found in human plasma [7].

One method for the determination of 3-hydroxyquinine has been established [8]. This method allows determination of concentrations down to 100 n*M*.

We intend to perform pharmacokinetic studies that require the determination of quinine and 3-hydroxyquinine for up to 96 h after dosing. At this time point, the concentration of 3-hydroxyquinine will be lower than 100 n*M*. The aim of the present work was to develop a sufficiently sensitive method for the determination of 3-hydroxyquinine in plasma and urine. The method is partly based on our previously published method for the quantitation of quinine [9].

2. Materials and methods

2.1. Chemicals

3-Hydroxyquinine hydrochloride (which is a 25:75 mixture of the two diastereomers of 3-hydroxyquinine) was obtained as a gift from Dr. Douglas J. Christie [10] at the Department of Laboratory Medicine and Pathology, Medical School, University of Minnesota, Minneapolis, Minnesota, USA. The internal standard (quinidine) was obtained from Apoteksbolaget (Stockholm, Sweden). Solvents and chemicals (HPLC or analytical grade, as required) were purchased from Merck (Darmstadt, Germany).

2.2. Instrumentation

The high-performance liquid chromatographic system consisted of a ConstaMetric 3500 pump (LDC Riviera Beach, FL, USA), a Waters 470 fluorescence detector (Millipore, Milford, MA, USA) and an LKB 2221 integrator (LKB, Bromma, Sweden).

The excitation and emission wavelengths of the detector were set at 350 and 450 nm, respectively. The emission band width was 18 nm and the electronic filter time constant was 1.5 s. The column used was a CT-sil C_{18} (100×4.6 mm I.D, 3 μm) from Chrom Tech (Hägersten, Sweden). The column was kept at room temperature. The mobile phase consisted of 460 ml of phosphate buffer (100 ml of 1 *M* phosphoric acid, 100 ml of 1 *M* di-sodiumhydrogenphosphate and 800 ml of distilled water), pH 2.2, 30 ml of acetonitrile, 20 ml of 1 *M* phosphoric acid, 10 ml of tetrahydrofuran and 8 ml of triethylamine. The pH was adjusted to 2.7 by adding phosphoric acid. The flow-rate of the mobile phase was 1 ml/min.

2.3. Samples

For the development and evaluation of the method, urine and plasma samples spiked with 3-hydroxyquinine were used. Samples from a patient treated with a single dose of quinine (500 mg) were also used.

Urine samples were diluted 100-fold before extraction, while the plasma samples were extracted directly.

2.4. Analytical procedure

To 100 μl samples (urine or plasma), 25 μl of the internal standard, quinidine (292 ng/ml in phosphate buffer) was added and, thereafter, 1 ml of 0.1 *M* NaOH was added. After vortex-mixing for 10 s and the addition of 2 ml of the extraction mixture (toluene–butanol, 75:25, v/v), the samples were shaken for 10 min and centrifuged for 10 min at 3000 *g*. The organic layer was transferred to another tube containing 100 μl of the mobile phase, shaken for 10 min, centrifuged for 10 min, and then the organic layer was discarded and 40 μl of the remainder was injected into the HPLC system.

Table 1
Extraction recovery

Sample (n=4)	Concentration	Recovery [(%)±SD]
Plasma (100 μl)	227 n*M*	84±5
Plasma (100 μl)	5.68 μ*M*	82±2
Urine (100 μl)	227 n*M*	82±3
Urine (100 μl)	5.68 μ*M*	85±3

2.5. Standard curves

Standard curves were prepared by adding known amounts of 3-hydroxyquinine to blank plasma or diluted urine. For the method validation samples, three different calibration curves were used. For plasma samples with 276.8 and 227 n*M* 3-hydroxyquinine and for urine samples with 227 n*M* and 56.68 μ*M* 3-hydroxyquinine (the latter was diluted 200-fold), concentrations of 0, 142.0, 284.0 and 426.0 n*M* were used to construct the calibration curve. For the plasma samples with a 3-hydroxyquinine concentration of 4.5 n*M*, concentrations of 0, 3.22, 6.44 and 12.88 n*M* were used to construct the calibration curve. When analysing plasma samples from a patient treated with a single dose of quinine (500 mg), three different calibration curves were used. For plasma samples collected between 0 and 24 h, a calibration curve was constructed using concentrations of 0, 1.36, 3.18, 4.54, 6.13, 7.49 and 9.08 μ*M*. For plasma samples collected at 32 and 48 h, concentrations of 0, 113.6, 227.2, 454.4, 681.6 and 908.8 n*M* were used to construct the calibration curve and for plasma samples collected at 72 and 96 h, concentrations of 0, 17.0, 34.1, 51.1, 68.2, 85.2 and 102.2 n*M* were used. These samples were extracted according to the above procedure. The resulting peak area ratios were plotted against the concentrations.

2.6. Extraction recovery

To check the extraction recovery, standard 3-hydroxyquinine was added to plasma and urine (n=4). The concentrations are given in Table 1. Samples were extracted according to the above procedure (Section 2.4), and the peak areas were compared to those of directly injected standards.

2.7. Within-assay imprecision and accuracy

Known amounts of 3-hydroxyquinine were added to plasma and urine (n=10). The concentrations are given in Table 2. The samples were extracted and analyzed according to the procedure described in Section 2.4.

2.8. Between-assay imprecision

Plasma and urine samples spiked with three different concentrations of 3-hydroxyquinine were stored at −20°C and analyzed on different occasions.

Table 2
Within- and between-assay imprecision and accuracy

Sample	Concentration added	Mean concentration found	Within-assay C.V. (%)	Between-assay C.V. (%)
Plasma (n=6)	276.8 n*M*	278.6 n*M*		2
Plasma (n=10)	227 n*M*	238.5 n*M*	4	
Plasma (n=10)	2.95 μ*M*	2.84 μ*M*	2	
Plasma (n=6)	1.97 μ*M*	1.85 μ*M*		3
Plasma (n=6)	5.1 n*M*	7.55 n*M*		4
Plasma (n=10)	4.50 n*M*	4.50 n*M*	9	
Urine (n=10)	56.80 μ*M*	48.26 μ*M*	3	
Urine (n=6)	56.80 μ*M*	53.90 μ*M*		2
Urine (n=10)	4.09 μ*M*	4.33 μ*M*	5	
Urine (n=10)	227 n*M*	225 n*M*	11	
Urine (n=6)	5.12 μ*M*	5.45 μ*M*		3
Urine (n=6)	204.6 n*M*	198.6 n*M*		4

The standard deviation and the coefficient of variation were calculated.

2.9. Interferences

Samples from patients treated with clomipramine, chloroquine, salicylate, acetaminophen, and plasma samples spiked with proguanil and imipramine, were analyzed according to the method described in Section 2.4.

2.10. Stability

The stability of the 3-hydroxyquinine in both plasma and urine samples was studied at room temperature, 4°C and −20°C.

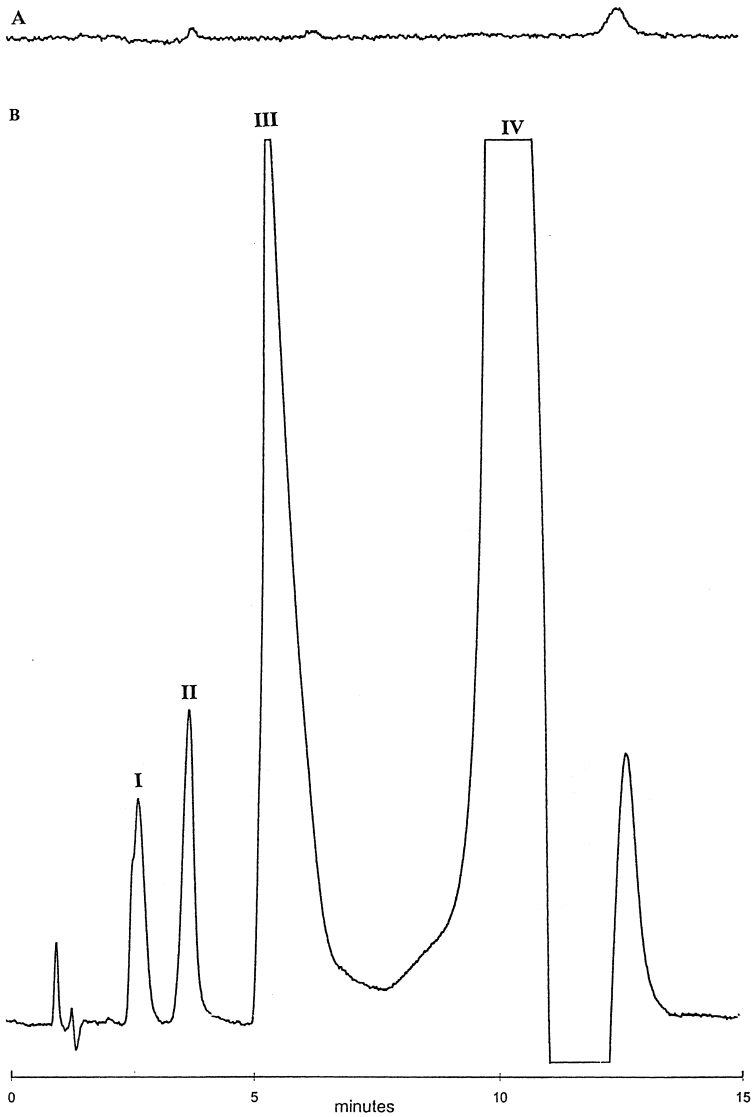

Fig. 2. Chromatograms of 3-hydroxyquinine in plasma samples. (A) Blank plasma. (B) Plasma containing 0.76 μ*M* 3-hydroxyquinine and 6.85 μ*M* quinine. (C) Patient's plasma containing 2.4 μ*M* 3-hydroxyquinine and 10.29 μ*M* quinine. Peaks I and II, 3-hydroxyquinine; retention times, 2.8 and 3.7 min; peak III, quinidine (internal standard); retention time, 6 min; peak IV, quinine; retention time, 11.6 min.

3. Results and discussion

Fig. 2 shows chromatograms of blank plasma, spiked plasma and plasma from a patient that had been treated with quinine, while Fig. 3 shows chromatograms of blank urine, spiked urine and urine from a patient. Synthetic 3-hydroxyquinine gives rise to two peaks on the chromatogram. These two peaks represent two diastereomers (Fig. 1), which was confirmed by mass spectrometry.

The ratio between the diastereomers was determined by high-performance liquid chromatography (HPLC) with UV detection and was found to be 25:75. The peak present in the chromatograms of samples from patients corresponds to the major component of the synthetic 3-hydroxyquinine. Method validation was done for the major 3-hydroxyquinine form. Since the ratio of the diastereomers in the standard substance was known, it was possible to calculate the true 3-hydroxyquinine concentration in patients' samples.

The diastereomers of 3-hydroxyquinine are well separated from each other, from the internal standard, quinidine, and from quinine. Even though quinidine is not an ideal internal standard, it is good enough for the analysis and it is readily available.

Simultaneous analysis of 3-hydroxyquinine and quinine is not possible, because of the large differ-

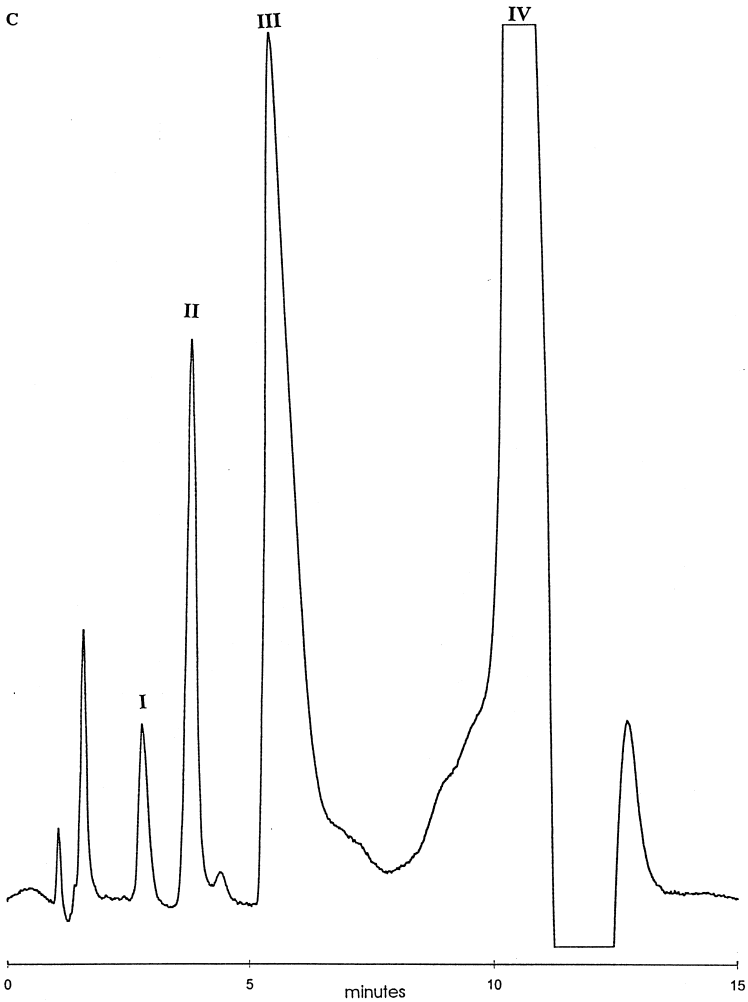

Fig. 2. (*continued*)

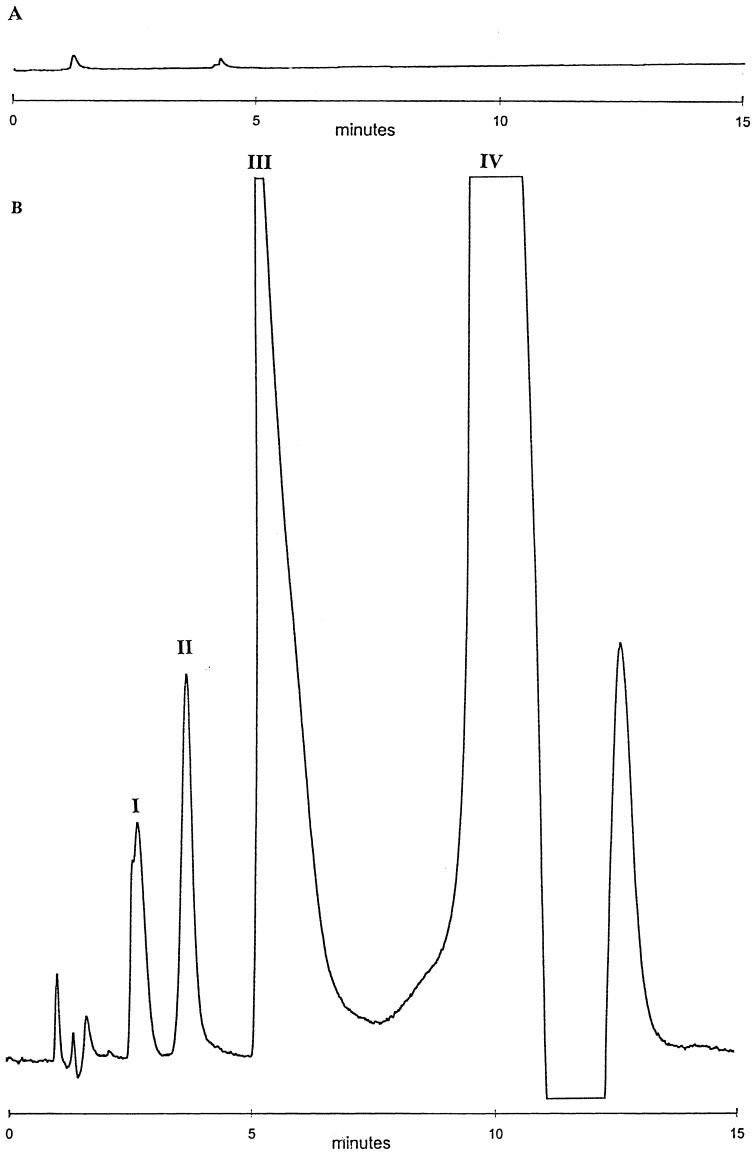

Fig. 3. Chromatograms of 3-hydroxyquinine in urine samples. (A) Blank urine. (B) Urine containing 0.76 μ*M* 3-hydroxyquinine and 6.85 μ*M* quinine. (C) Patient's urine containing 374 μ*M* 3-hydroxyquinine and 1.03 m*M* quinine, diluted 100-fold with water. Peaks I and II, 3-hydroxyquinine; retention times, 2.8 and 3.7 min; peak III, quinidine (internal standard); retention time, 6 min; peak IV, quinine; retention time, 11.6 min.

ences in concentration between them. The latter was analyzed according to our previously published method [9].

Standard curves were linear within the concentration range of interest. The correlation coefficient was more than 0.996 in all runs.

The extraction recovery is shown in Table 1. The within- and between-assay imprecision and accuracy are shown in Table 2. Pilot analysis of samples from a patient treated with a single dose of quinine (Table 3) has indicated that the concentrations chosen for evaluation of the method is relevant for the planned

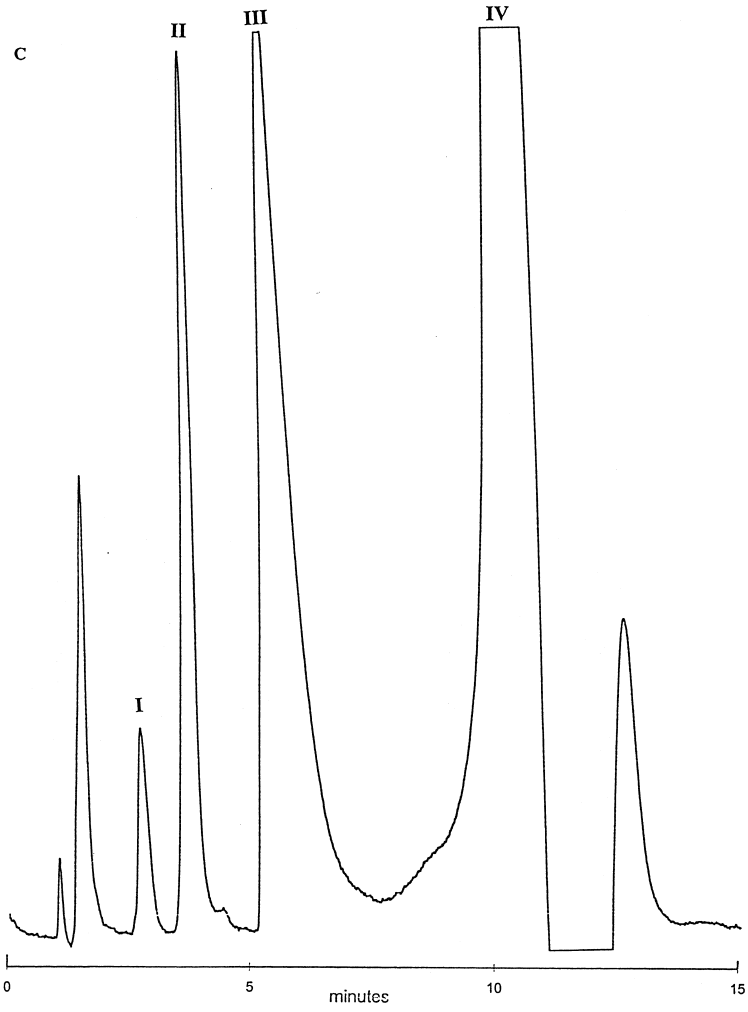

Fig. 3. (*continued*)

studies. The reproducibility is good under these concentrations.

Using 100 μl plasma samples, the lower limit of determination (coefficient of variation, C.V., 9%) was 4.5 n*M*, and it was 204.6 n*M* when 100 μl urine samples were used.

Analyses of samples from patients treated with clomipramine, chloroquine, salicylate, acetaminophen and of plasma spiked with proguanil showed that there were no interferences with 3-hydroxyquinine.

The stability of 3-hydroxyquinine in both plasma and urine was studied. At room temperature, the concentration remained unchanged for at least six days in plasma and four days in urine. At 4°C, no decrease was observed over a twenty-day period in plasma or at seventeen days in urine. At −20°C, no decrease was observed after 96 days in plasma and 92 days in urine.

The concentration of 3-hydroxyquinine in the plasma of a patient treated with a single dose of quinine (500 mg) is shown in Table 3. The method is intended for use in pharmacokinetic studies. In an on-going interaction study, it is desirable to be able to follow the concentration of quinine and 3-hydroxyquinine for up to 96 h after dosing. At this time

Table 3
Concentrations of 3-hydroxyquinine in plasma samples from a patient

Sample number	Time (h:min)	3-Hydroxyquinine concentration (μM)
1	0:0	0.00
2	0:30	2.84
3	1:00	3.31
4	1:30	3.65
5	2:00	3.31
6	2.30	3.74
7	3:00	3.18
8	4:00	3.87
9	5:00	3.30
10	8:00	3.12
11	12:00	2.36
12	24:00	1.32
13	32:00	0.52
14	48:00	0.18
15	72	0.07
16	96:00	0.04

point, the concentration of 3-hydroxyquinine will be near, or even below, the lower limit of quantitation of our method.

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