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Simultaneous determination of quinine and four metabolites in plasma and urine by high-performance liquid chromatography

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Abstract

The determination of quinine, (3*S*)-3-hydroxyquinine, 2′-quininone and (10*R*)- and (10*S*)-10,11-dihydroxydihydroquinine in plasma and urine samples is described. This is the first time the *R* and *S* configurations have been correctly assigned to the two metabolites of 10,11-dihydroxyquinine. One hundred microliter-plasma samples were protein precipitated with 200 μl cold methanol. Urine samples were 10–100× diluted and then directly injected into the HPLC. A reversed-phase liquid chromatography system with fluorescence detection and a Zorbax Eclipse XDB phenyl column and gradient elution was used. The within and between assay coefficients of variation of the method for quinine and its metabolites in plasma and urine was less than 13%. The lower limit of quantitation was in the range of 0.024–0.081 μ*M*.

Keywords: Quinine; Hydroxyquinines; Quininone

1. Introduction

Although several quinine metabolites have been isolated and characterized [1–3], neither the quantitative importance of these metabolites for the elimination of quinine nor the metabolic pathways have been fully elucidated. It has been shown that the formation of a major metabolite of quinine, (3*S*)-3-hydroxyquinine, is catalyzed by cytochrome P450 3A4 both in vitro [4] and in vivo [5]. It is interesting to investigate the isoenzyme system(s), which is involved in the metabolism of quinine to the other metabolites to be able to predict drug drug, or drug food interactions, which may be of clinical significance.

Even though (3*S*)-3-hydroxyquinine is a major metabolite of quinine, the percentage of dose excreted as (3*S*)-3-hydroxyquinine in the urine is only 14% [5]. This may indicate further metabolism of (3*S*)-3-hydroxyquinine to minor metabolites, excretion in bile or simply that other metabolic pathways are important.

In order to study quinine metabolism one should be able to quantify both the parent compound and the metabolites. There are no published methods for the simultaneous quantitation of quinine and its metabo-

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lites. We developed this high-performance liquid chromatographic method for simultaneous determination of quinine and four of its metabolites to be used in metabolic and pharmacokinetic studies.

2. Materials and methods

2.1. Chemicals

2′-quininone hydrochloride and 3-hydroxyquinine hydrochloride, which is a 25:75 mixture of the two diastereomers of 3-hydroxyquinine [6], was obtained as a gift from Dr Douglas J. Christie [7] at the Department of Laboratory Medicine and Pathology, Medical School, University of Minnesota, Minneapolis, Minnesota, USA. (10*R*)- and (10*S*)-10,11-Dihydroxydihydroquinine were synthesized using the Sharpless catalytic asymmetric dihydroxylation. (10*R*)-10,11-Dihydroxydihydroquinine was prepared by adding quinine to a solution of K_2OsO_4, dihydroquinine 2,5-diphenyl-4, 6-pyrimidinyl diether and potassium ferrocyanide. The reaction mixture was stirred for 4 days, which yielded the *R* diasteromer as the sole product. By using dihydroquinine 2,5-diphenyl-4, 6-pyrimidinyl diether as the chiral ligand a mixture of the two diasteromers *R* and *S* were obtained. The pure (10*S*)-10,11-dihydroxydihydroquinine was obtained by fractional crystallization. Further details of the synthesis can be found in [8]. Quinine sulphate was obtained from Apoteket AB (Stockholm, Sweden). Solvents and chemicals (HPLC grade) were purchased from Merck (Darmstadt, Germany). Standard solutions of quinine and metabolites were prepared by dissolving the compounds in 0.1 *M* phosphate buffer pH 4.3.

2.2. Instrumentation

The high-performance liquid chromatographic system consisted of a Rheos 4000 gradient pump (Flux Instruments AB, Karlskoga, Sweden), a Gilson 231 XL injector (Pretech Instruments, Stockholm, Sweden) a Waters 474 fluorescence detector (Millipore, Milford, MA, USA) and a computer with Chemstation software for data registration and calibration (Agilent Technologies, USA).

The excitation and emission wavelengths of the detector were set at 350 and 450 nm, respectively. The emission bandwidth was 18 nm. The column was a Zorbax Eclipse XDB phenyl 150×4.6 mm I.D. 5 μm (Chrom Tech, Hagersten, Sweden). The column was kept at room temperature. Gradient elution of the analytes was performed using two different mobile phases as shown in Table 1. The A mobile phase 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. The B mobile phase consisted of 24% acetonitrile in 0.1 *M* acetate buffer pH 4.2. 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 quinine, 3-hydroxyquinine, 2′-quininone, (10*R*)- and (10*S*)-10,11-dihydroxydihydroquinine were used. Samples from a patient treated with a single dose of quinine (500 mg) were also used.

Urine samples were diluted 10–100 times before injection, while the plasma samples were protein precipitated directly.

2.4. Analytical procedure

To 100 μ1 aliquots of the plasma 200 μ1 of cold methanol was added. The samples were then vortex mixed for 10 s and centrifuged for 10 min at 3000 *g*. The supernatant was transferred to the injection vials and 10–30 μl was injected into the HPLC. Urine

Table 1
Elution gradient

Time (min)	A (%)	B (%)
0	100	0
2	80	20
4	80	20
6	60	40
8	40	60
10	20	80
12	0	100
18	0	100
22	100	0

Table 2
Recovery in plasma

Analyte ($n=6$)	Conc. μM	Recovery (%)	C.V. (%)	Conc. μM	Recovery (%)	C.V. (%)	Conc. μM	Recovery (%)	C.V. (%)
Quinine	1.17	104	11.5	0.674	99	13.1	0.063	105	3.8
(3*S*)-3-Hydroxyquinine	0.802	90	6.7	0.308	90	10	0.058	90	11.1
2′-Quininone	1.21	92	7.6	0.421	89	6.7	0.088	106	3.8
(10*R*)-10,11-Dihydroxyquinine	0.817	108	2.8	0.435	95	7.4	0.065	85	4.7
(10*S*)-10,11-Dihydroxyquinine	1.15	89	5.6	0.307	95	4.2	0.058	85	11.8

samples were diluted 10–100 times and then directly injected into the HPLC.

2.5. Standard curves

Standard curves were prepared by adding known amounts of quinine and its metabolites to blank plasma or diluted urine. For the method validation three different calibration curves were used. A blank sample and four to five calibration points were used in all calibration curves. The concentration ranges of the standard curves for quinine, (3S)-3-hydroxyquinine, 2′-quininone, (10*R*)-10,11-dihydroxyquinine, (10*S*)-10,11-dihydroxyquinine were 0.022–1.4, 0.041–1.3, 0.043–0.98, 0.027–1.8 and 0.024–1.2 μM, respectively. When analyzing plasma samples from a patient treated with a single oral dose of 500 mg quinine, two different calibration curves were used. The concentration ranges of the standard curves for quinine, (3*S*)-3-hydroxyquinine, 2′-quininone, (10*R*)-10,11-dihydroxyquinine, (10*S*)-10,11-dihydroxyquinine were 1.1–11.1, 0.41–4.0, 0.043–1.2, 0.034–2.1 and 0.024–1.5 μM, respectively. The resulting peak areas were plotted against the concentrations.

2.6. Recovery

To document the recovery, standard quinine and metabolites were added to plasma and urine ($n=6$). The plasma concentrations are given in Table 2.

Samples were worked up according to the above procedure, and the peak areas were compared to those of directly injected standards.

2.7. Within assay imprecision and accuracy

Known amounts of quinine and metabolites were added to plasma and urine ($n=6$). The concentrations are given in Tables 3 and 4, respectively. The samples were worked up and analyzed according to the above procedure.

2.8. Between assay imprecision

Plasma and urine samples spiked with quinine and metabolites to obtain three different concentrations, were stored at −20°C and analyzed on different occasions. The concentrations are given in Tables 5 and 6, respectively. Standard deviation and coefficient of variation were calculated.

Table 3
Within assay imprecision and accuracy in plasma

Analyte ($n=6$)	Nominal conc. (μM)	Found conc. (μM)	C.V. (%)	Nominal conc. (μM)	Found conc. (μM)	C.V. (%)	Nominal conc. (μM)	Found conc. (μM)	C.V. (%)
Quinine	0.780	0.806	3.7	0.389	0.381	7.9	38.9	39.6	1.2
(3*S*)-3-Hydroxyquinine	1.000	1.030	2.0	0.359	0.390	7.3	61.6	63.7	4.2
2′-Quininone	0.752	0.730	3.4	0.362	0.367	3.1	65.3	60.6	7.4
(10*R*)-10,11-Dihydroxyquinine	1.000	1.020	1.7	0.404	0.420	5.4	40.4	42.5	2.0
(10*S*)-10,11-Dihydroxyquinine	0.930	0.961	1.6	0.381	0.349	8.4	24.3	24.6	2.3

Table 4
Within assay imprecision and accuracy in Urine

Analyte (n=6)	Nominal conc. (μM)	Found conc. (μM)	C.V. (%)	Nominal conc. (μM)	Found conc. (μM)	C.V. (%)	Nominal conc. (μM)	Found conc. (μM)	C.V. (%)
Quinine	0.780	0.805	1.3	0.389	0.358	5.1	38.1	40.8	8.9
(3*S*)-3-Hydroxyquinine	1.000	0.937	0.6	0.359	0.347	12	61.6	57.4	4.1
2′-Quininone	0.752	0.739	1.2	0.362	0.390	6.7	65.3	73.8	4.5
(10*R*)-10,11-dihydroxyquinine	1.000	1.030	1.2	0.404	0.420	1.7	80.8	81.9	2.3
(10*S*)-10,11-dihydroxyquinine	0.930	0.887	0.5	0.381	0.382	10.4	28.5	27.3	5.1

Table 5
Between assay imprecision and accuracy in plasma

Analyte (n=6)	Nominal conc. (μM)	Found conc. (μM)	C.V. (%)	Nominal conc. (μM)	Found conc. (μM)	C.V. (%)	Nominal conc. (μM)	Found conc. (μM)	C.V. (%)
Quinine	0.780	0.796	1.7	0.389	0.380	6.0	0.039	0.042	5.3
(3*S*)-3-Hydroxyquinine	1.000	1.000	0.8	0.359	0.348	6.6	0.062	0.062	5.5
2′-Quininone	0.750	0.749	2.8	0.362	0.361	1.7	0.065	0.071	10.9
(10*R*)-10,11-Dihydroxyquinine	1.000	1.030	3.2	0.404	0.408	6.9	0.040	0.045	6.6
(10*S*)-10,11-Dihydroxyquinine	0.930	0.922	1.9	0.381	0.377	4.0	0.038	0.036	8.5

2.9. Interference

Interference of chloroquine, amodiaquine, desethylamodiaquine, mefloquine, salicylate and paracetamol with the analytes of our method was studied by adding the different compounds to blank plasma and analyzing the samples according to the method.

2.10. Stability

Stability of quinine and metabolites in plasma samples was studied at room temperature, 4°C and −20°C, respectively.

3. Results and discussion

Fig. 1 shows chromatograms of blank plasma, spiked plasma, and plasma from a patient treated with a single 500 mg dose of quinine, while Fig. 2 shows chromatograms of blank urine, spiked urine, and patient urine. Peaks of quinine and metabolites are well separated from each other.

The 3-hydroxyquinine, which is found in human plasma and urine, is considered to be the (3*S*)-3-hydroxyquinine by analogy to quinidine [9]. Quinine and the metabolites differ considerably in their polarity and therefore a gradient system had to be

Table 6
Between assay imprecision and accuracy in urine

Analyte (n=6)	Nominal conc. (μM)	Found conc. (μM)	C.V. (%)	Nominal conc. (μM)	Found conc. (μM)	C.V. (%)	Nominal conc. (μM)	Found conc. (μM)	C.V. (%)
Quinine	0.780	0.791	3.4	0.389	0.372	1.7	0.038	0.037	3.9
(3*S*)-3-Hydroxyquinine	1.000	0.973	1.7	0.359	0.337	5.1	0.062	0.071	12.5
2′-Quininone	0.750	0.802	1.5	0.362	0.369	1.4	0.065	0.073	8.8
(10*R*)-10,11-Dihydroxyquinine	1.000	1.04	1.8	0.404	0.410	0.8	0.081	0.084	1.1
(10*S*)-10,11-Dihydroxyquinine	0.930	0.895	2.0	0.381	0.361	9.0	0.029	0.028	9.4

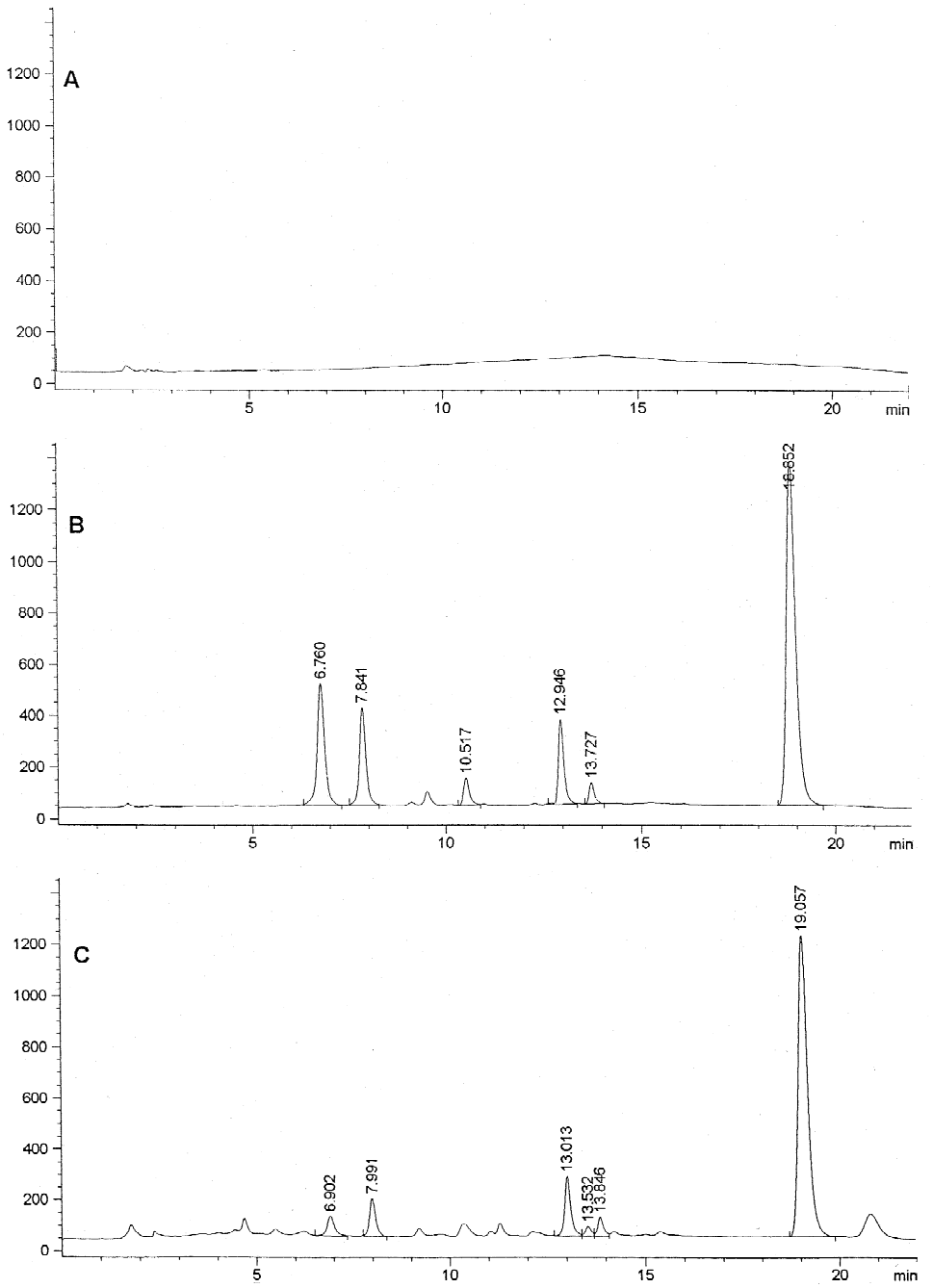

Fig. 1. Chromatograms of quinine and metabolites in plasma samples. (A) Blank plasma. (B) Plasma spiked with 6.7 μ*M* of quinine, 2.0 μ*M* of (3*S*)-3-hydroxyquinine, 0.770 μ*M* of 2′-quininone, 1.3 μ*M* of (10*R*)-10,11-dihydroxydihydroquinine and 0.952 μ*M* of (10*S*)-10,11-dihydroxydihydroquinine. (C) Patient plasma containing 5.0 μ*M* of quinine, 0.748 μ*M* of (3*S*)-3-hydroxyquinine 0.162 μ*M* of 2′-quininone, 0.290 μ*M* of (10*R*)-10,11-dihydroxydihydroquinine and 0.098 μ*M* of (10*S*)-10,11-dihydroxydihydroquinine. (10*S*)-10,11-dihydroxyquinine at 6.7 min; (10*R*)10,11-dihydroxyquinine at 7.8 min; (3*S*)-3-hydroxyquinine at 12.9 min; 2′-quininone at 13.7 min; and quinine at 18.8 min.

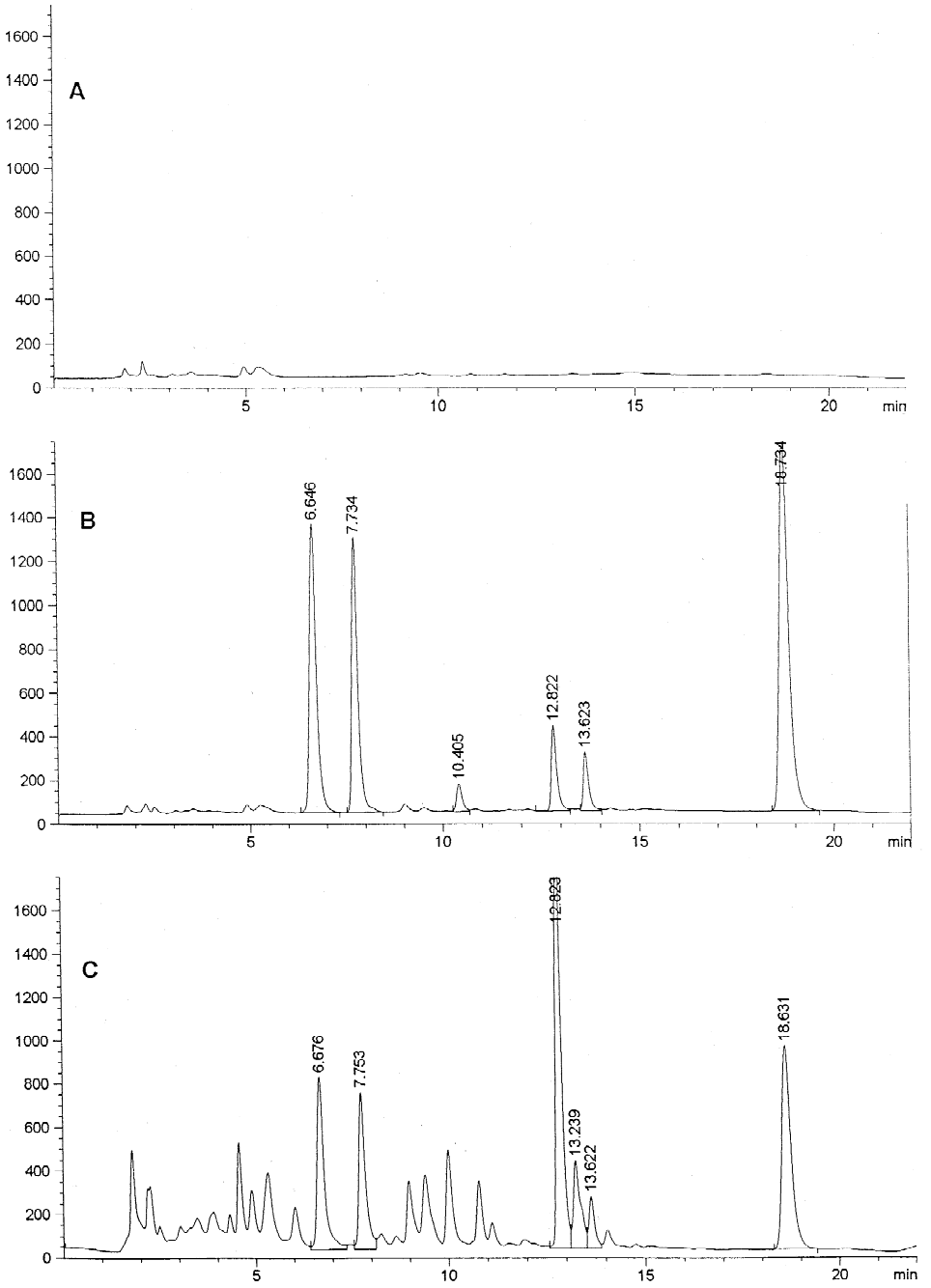

Fig. 2. Chromatograms of quinine and metabolites in urine samples. (A) Blank urine. (B) Urine spiked with 1.1 μ*M* of quinine, 0.821 μ*M* of (3*S*)-3-hydroxyquinine, 0.616 μ*M* of 2′-quininone, 1.1 μ*M* of (10*R*)-10,11-dihydroxydihydroquinine and 0.762 μ*M* of (10*S*)-10,11-dihydroxydihydroquinine. (C) Patient urine (10×diluted) containing 31.7 μ*M* of quinine, 41.2 μ*M* of (3*S*)-3-hydroxyquinine 1.8 μ*M* of 2′-quininone, 8.3 μ*M* of (10*R*)-10,11-dihydroxydihydroquinine and 5.2 μ*M* of (10*S*)-10,11-dihydroxydihydroquinine. (10*S*)-10,11-dihydroxyquinine, at 6.6 min; (10*R*)-10,11-dihydroxyquinine at 7.7 min; (3*S*)-3-hydroxyquinine at 12.8 min; 2′-quininone at 13.6 min; and quinine at 18.7 min.

used. Despite this the chromatography time is 22 min and the total run time including equilibration is 22 min. We do not consider this to be a major drawback, not even when many samples have to be run. The work up procedure is simple and rapid and the chromatographic system is stable and presents no problems when using automatic injection. Samples of 10 μl were used for routine analysis and injection of up to 30 μl caused no peak distortion.

Since the work up procedure is simple, protein precipitation for plasma and dilution of urine, no internal standard was considered.

The standard curves were linear within the concentration range of interest. The correlation coefficient has been more than 0.99 in all runs. Different standard curves were used for different concentration ranges. For analysis of plasma samples collected after 48 h, a standard curve with lower concentration of quinine than the lowest one we used should be included for more precise determination of the analyte.

The recovery in plasma is shown in Table 2. The recovery in urine was in the range of 89–117%. The within assay imprecision in plasma and urine are shown in Tables 3 and 4, respectively. The between assay imprecision in plasma and urine are shown in Table 5 and 6, respectively.

Fig. 3 shows plasma concentrations of quinine and its metabolites from a pilot analysis of samples from a patient treated with a single 500 mg dose of quinine hydrochloride. The results showed that the concentrations chosen for the evaluation of the method are relevant for the planned studies. The reproducibility is good at these concentrations.

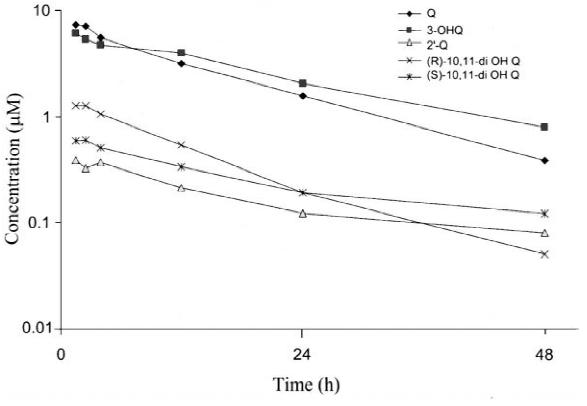

Fig. 3. Plasma concentrations of quinine and metabolites in a subject after a 500 mg dose of quinine hydrochloride.

The lowest concentration that was used for evaluation was chosen to indicate the lower limit of quantitation for each compound. Using 100 μ1 plasma samples the lower limits of quantitation for quinine, (3*S*)-3-hydroxyquinine, 2′-quinone, (10*R*)- and (10*S*)-10,11-dihydroxydihydroquinine were 0.039, 0.062, 0.065, 0.040 and 0.024 μ*M*, respectively (coefficients of variation, C.V.%, were less than 12%). When 100 μl urine samples were used the lower limits for quantitation of quinine, (3*S*)-3-hydroxyquinine, 2′-quininone, (10*R*)- and (10*S*)-10,11-dihydroxydihydroquinine were 0.038, 0.061, 0.065, 0.081, and 0.029 μ*M*, respectively (coefficients of variation, C.V.%, were less than 13%).

Chloroquine, salicylate, paracetamol, amodiaquine, desethylamodiaquine, mefloquine, acetaminophen, and proguanil showed no interference with quinine or its metabolites.

The stability of quinine, (3*S*)-3-hydroxyquinine, 2′-quininone, (10*R*)- and (10*S*)-10,11-dihydroxydihydroquinine in plasma was studied. At room temperature and at 4°C the concentrations were found to be unchanged for at least 7 days for all analytes except for 2′-quininone which was stable for 4 days. At −20°C no decrease in concentration was seen after 79 days for any of the compounds.

The concentrations of quinine, (3*S*)-3-hydroxyquinine, 2′-quininone, (10*R*)- and (10*S*)-10,11-dihydroxydihydroquinine in plasma of a patient treated with a single dose of quinine (500 mg) is shown in Fig. 3. The method is intended for use in pharmacokinetic studies and to investigate the role of 2′-quininone and (10*R*)- and (10*S*)-10,11-dihydroxydihydroquinine in quinine metabolism. The lower limits of quantitation indicate that it will be possible to determine the concentrations of quinine and its metabolites for at least 48 h after a single 500 mg dose. It may be possible to determine lower concentrations of quinine and metabolites by increasing the sensitivity settings of the detector, if needed.

3-Hydroxyquinine has always been referred to as the major quinine metabolite [10]. A comparison of the concentrations of (3*S*)-3-hydroxyquinine and the other metabolites in the plasma samples from the

patient in Fig. 3, confirms that it is indeed a major metabolite in this patient.

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