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Sensitive fluorescence HPLC assay for AQ-13, a candidate aminoquinoline antimalarial, that also detects chloroquine and *N*-dealkylated metabolites☆

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Abstract

A sensitive, specific and reproducible fluorescence high performance liquid chromatography (HPLC) assay has been developed for the separate or simultaneous measurement of AQ-13 (a candidate 4-aminoquinoline antimalarial), chloroquine (CQ), and their metabolites in whole blood. After liquid–solid extraction using commercially available extraction cartridges, these two aminoquinolines (AQs) and their metabolites were separated on C18 (Xterra RP18) columns using a mobile phase containing 60% borate buffer (20 mM, pH 9.0) and 40% acetonitrile with isocratic elution at a flow-rate of 1.0 ml/min. The assay uses a biologically inactive 8-chloro-4-aminoquinoline (AQ-18) as its internal standard (IS). There is a linear relationship between the concentrations of these AQs and the peak area ratio (ratio between the peak area of the AQ or metabolite and the peak area of the IS) on the chromatogram. Linear calibration curves with correlation coefficients ≥0.997 ($r^2 \geq 0.995$, $p < 0.001$) were obtained for AQ-13, CQ and their *N*-dealkylated metabolites. Reproducibility of the assay was excellent with coefficients of variation (CVs) ≤3.8% for AQ-13 and its metabolites, and ≤2.5% for CQ and its metabolites. The sensitivity of the assay is 5 nM using 1.0 ml of blood and a 20 μl injection volume, and can be increased by using 5.0 ml of blood with an injection volume of 40 μl.

Keywords: Chloroquine; Aminoquinoline; Fluorescence HPLC; AQ-13; Malaria

1. Introduction

The worldwide morbidity and mortality of malaria are overwhelming, with 3 million deaths and between 500 million and 4 billion cases each year [1–3]. In addition, aminoquinoline (AQ) resistance is the single most important factor in the worldwide resurgence of malaria due to *Plasmodium falciparum*. The well-known antimalarial drug chloroquine (CQ) has been used widely for more than 40 years, and is exceptionally safe for children and pregnant women. However, its value has been compromised by the widespread emergence of CQ-resistant *P. falciparum* in Southeast Asia, South America, and sub-Saharan African [4–5]. For this reason, it would be extremely helpful to develop AQs as safe as CQ that are also active against CQ-resistant *P. falciparum*.

To address this challenge, we have performed a series of studies, examining mechanisms of AQ action and resistance, and the activity of AQ analogues against CQ-susceptible and CQ-resistant parasites [6–7]. Recent studies from our laboratory have shown that AQs with short (2–3 carbon) and long (10–12 carbon) diaminoalkane side chains are active against CQ-resistant and multi-resistant strains of *P. falciparum* [8]. One of these AQs (AQ-13, with a linear propyl side chain, *N*′-[7-chloroquinolin-4-yl]-*N*,*N*-diethylpropane-1, 3-diamine, Fig. 1) is now finishing Phase 1 Human safety and toxicity studies (which have shown that it is as safe as CQ), and is scheduled to begin Phase 2 Efficacy studies shortly (Abstracts for the 53rd Annual Meeting of the American Society of Tropical Medicine and Hygiene at www.astmh.org and http://www.som.tulane.edu/gcrc/Bios/DKrogstad.htm). In order to define the pharmacokinetics of this candidate AQ antimalarial

Abbreviations: AQ, 4-aminoquinoline; AQ-13, 4-aminoquinoline with a linear propyl side chain; AUC, area under the curve; BDCQ, bi-desethyl CQ (di *N*-dealkylated CQ); CQ, chloroquine, 4-AQ with an isopentyl side chain; CV, coefficient of variation (standard deviation divided by the mean); HPLC, high performance liquid chromatography; IS, internal standard; LC–MS, liquid chromatography–mass spectrometry; MDCQ, mono-desethyl CQ (mono *N*-dealkylated CQ); NMR, nuclear magnetic resonance; UV, ultraviolet

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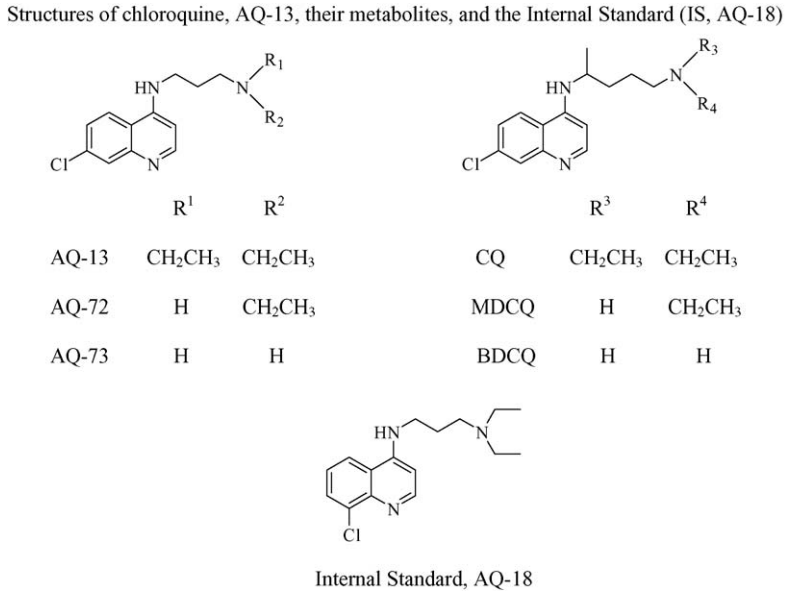

Fig. 1. Structures of chloroquine, AQ-13, their metabolites, and the Internal Standard (IS, AQ-18). All compounds tested were 4-aminoquinolines (AQs). With the exception of AQ-18, the internal standard (IS) in which the Cl is at position 8, all the AQs tested were 4-amino-7-chloroquinolines.

during the Phase 1 and 2 Studies in humans and to compare its pharmacokinetics to those of CQ, it was necessary to develop a sensitive and specific assay for AQ-13 and its mono- and di-*N*-dealkylated metabolites (AQ-72 and AQ-73) that would also detect CQ and its metabolites (mono-desethylchloroquine, MDCQ and di-desethylchloroquine, BDCQ).

The analytical method of choice for the measurement of antimalarial drug and metabolite levels in blood is high performance liquid chromatography (HPLC) [9,10]. Several HPLC methods have been reported for the determination of CQ levels in blood. These include normal- and reverse-phase chromatography after liquid-liquid extraction, using either ultraviolet (UV) or fluorescence detection. Fluorescence detection has been particularly valuable because of its greater sensitivity [11]. The columns used most frequently have been reverse phase C18 columns [12–14]. Mobile phases used have included methanol–phosphate buffer–perchloric acid (0.1 M, pH 3; 250:747.5:2.5, v/v/v) [12], acetonitrile–phosphate buffer (10 mM, pH 3.5; 69:31, v/v) [13], acetonitrile–phosphate buffer (pH 6.5; 20:80, v/v) [14], and methanol–water (70:30, v/v) with 0.1% triethylamine (0.1%, v/v) [15]. These HPLC methods have been used to estimate the concentrations of CQ and other AQs in plasma [16–22], serum [23], blood cells [16–18,24], human biological fluids [18,25], urine [18,19,21,24,26–28], saliva [21] and whole blood [23,27].

The purpose of this study was to develop a sensitive, specific and reproducible fluorescence HPLC assay for the measurement of AQ-13 and its *N*-dealkylated metabolites (AQ-72 and AQ-73) in whole blood that would also be applicable to the detection of CQ and its metabolites (monodesethylchloroquine, MDCQ and di-desethylchloroquine, BDCQ). Such an assay would reduce the cost and complexity of analysis (one assay method for all specimens), and would simultaneously provide an independent external check on the double-blind administration of CQ and AQ-13 in Phase 1 and 2 Human Studies conducted as randomized clinical trials to evaluate the safety, toxicity and efficacy of AQ-13 in comparison to CQ.

2. Experimental methods

2.1. Reagents and standards

All chemicals were analytical reagent grade. Ammonium hydroxide and boric acid were purchased from Fisher Scientific (Pittsburgh, PA). Reagent grade water was obtained from a Milli-Q system (Millipore, Bedford, MA). The two-dimensional structures of the AQs examined in these studies are shown in Fig. 1. AQ-13, its primary mono-*N*-dealkylated metabolite, AQ-72 [*N*-(7-chloroquinolin-4-yl)-*N*′-ethylpropane-1,3-diamine], its secondary di-*N*-dealkylated metabolite, AQ-73 [N^1-(7-chloroquinolin-4-yl)-propane-1, 3-diamine], and the internal standard, AQ-18 (IS), were synthesized as reported elsewhere ([6–8,29], Liu et al., personal communication) CQ [N^4-(7-chloroquinolin-4-yl)-N^1, N^1-diethylpentane-1, 4-diamine] was purchased from Sigma–Aldrich (St. Louis, MO); MDCQ [N^4-(7-chloroquinolin-4-yl)-N^1-ethylpentane-1,4-diamine] and BDCQ [N^4-(7-chloroquinolin-4-yl)-pentane-1,4-diamine] were kindly provided by Dr. Michael Green at the Centers for Disease Control and Prevention (Atlanta, GA). The structures of these compounds were tested and confirmed with liquid chromatography–mass spectrometry (LC–MS, Shimadzu LC–MS 2010 mass spectrometer, Columbia, MD), proton nuclear magnetic resonance (^{1}H NMR) spectrometry (Bruker 500 MHz spectrometer, Rheinstetten, Germany), and elemental analysis.

2.2. Instrumentation and chromatographic conditions

The fluorescence HPLC system (Waters, Milford, MA) consisted of a 600E solvent delivery pump connected to a refriger-

ated 717 auto sampler, with UV and fluorescence detectors. The 486 UV absorbance detector was set at a wavelength of 340 nm, the 474 fluorescence detector was set at excitation and emission wavelengths of 320 and 380 nm [23]. The detectors' responses were recorded using the Millenium^{32} software, Version 4.0.

Chromatography was performed at ambient temperature using an Xterra RP18 analytical column (5 μm particle size, 250 mm × 4.6 mm I.D.) with an Xterra RP18 guard column (3.9 mm × 4.6 mm I.D.) between the injector and the analytical column. Chromatographic separations were accomplished at ambient temperature (25 °C) using a mobile phase containing 60% borate buffer (20 mM, pH 9.0) and 40% acetonitrile in the isocratic mode at a flow-rate of 1.0 ml/min. The mobile phase was prepared from a 20 mM solution of boric acid adjusted to pH 9.0 using NaOH (10 N). Solid-phase extraction of blood samples was performed using Oasis MCX 3.0 ml solid-phase extraction cartridges with a vacuum manifold (Waters). After elution from the Oasis MCX cartridges, the AQs and their metabolites were concentrated using a Thermo Savant System (Holbrook, NY) with an SPD Speed Vac, an RVT4104 refrigerated Vapor Trap, and an OFP-400 vacuum pump.

2.3. Preparation of stock solutions, standard samples and the internal standard (IS)

Stock solutions were prepared in 10 ml of MeOH at a concentration of 1.0 mM (10 μmol AQ in 10 ml MeOH = 1.0 mM). The stock solutions were then diluted 1:10 with MeOH to prepare working solutions at concentrations of 100 μM in 100% MeOH. Both the stock and working solutions were stored at −15 °C for up to 7 days. Standard samples of the AQs and their metabolites were prepared similarly in 1.0 ml volumes of MeOH at concentrations of 250, 500, 1000, and 2000 nM. A 10 μl aliquot of the 100 μM internal standard working solution was added to a 990 μl volume of each standard sample for internal standardization. Thus, the final concentration of the IS in all samples was 1.0 μM.

2.4. Extraction of AQ-13, CQ and their metabolites from whole blood

To extract AQ-13, CQ and their metabolites from blood, 1.0 ml (990 μl) aliquots of blood in buffered sodium citrate (3.2% sodium citrate tubes with a 5 ml draw, BD Biosciences, Franklin, NJ) were mixed with 10 μl of the 100 μM IS working solution and 9.0 ml of 0.1 M KH_2PO_4 in 15 ml screw-crapped tubes (430052 polypropylene conical tubes, Corning Life Sciences, Corning, NY) producing 1.0 μM final concentrations of the IS. After shaking for 15 min, the samples were centrifuged for 15 min at room temperature and 3000 × *g*. The supernatants (5.0 ml) were then aspirated with a Pasteur pipette and loaded onto Oasis MCX 3.0 ml cartridges, which had been washed first with methanol (2.0 ml) and then with water (2.0 ml). The matrix of the Oasis MCX solid-phase extraction cartridges is a modification of the poly(divinylbenzene-co-*N*-polyvinylpyrrolidone) copolymer used for the HLB sorbent with a 30 μm particle size to which sulfonic acid (HSO_3) groups have been bonded to produce a cation exchange resin that facilitates the retention of basic drugs such as AQs. After loading with supernatant, the cartridges were washed with 0.1 N HCl (4.0 ml × 1 ml), and eluted with 5% NH_4OH in MeOH (4.0 ml × 1 ml). After the eluates had been evaporated to dryness using an SPD Speed Vac (SPD131DDA, ThermoSavant, Holbrook, NY), the residues were dissolved in 200 μl of MeOH, vortex-mixed and filtered with PVDF Durapore 0.22 μm filters (Millipore, Bedford, MA). Two aliquots of each filtered sample (20 μl apiece) were then injected onto the column and run in duplicate.

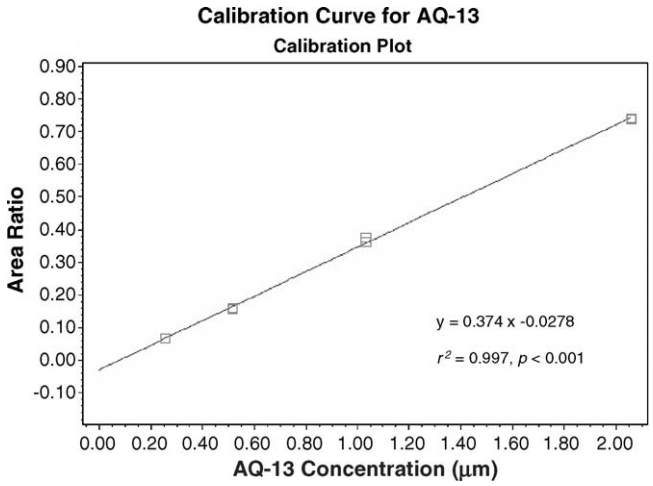

Fig. 2. Calibration curve for AQ-13. As indicated in the Results section of the text, the relationship between the peak area ratio for AQ-13 (peak area for AQ-13 divided by the peak area for the IS) and the actual AQ-13 concentration is linear and is described by a regression line of the form $y = mx + b$ ($y = 0.374x - 0.0278$, $r^2 = 0.997$, $p < 0.001$). Similar formulas (see Section 3) and chromatograms (not shown) were obtained for AQ-72, AQ-73, CQ, MDCQ and BDCQ.

3. Results

3.1. Quantitation of individual AQs and their metabolites (Fig. 2)

Quantitation was based on the peak area ratio (the ratio between the peak area of the individual AQ or metabolite and the peak area of the IS, Fig. 2). Standard curves were obtained from peak areas for a series of AQ-13 and CQ concentrations (250, 500, 1000, and 2000 nM) in relation to the peak area of the IS. Each calibration sample was prepared in whole blood and run in duplicate. The means of the peak area ratios were plotted against the concentrations of the individual AQs and metabolites. Linear regression was then performed (using the Millenium software) to estimate the linearity, slope, *y*-intercept and correlation coefficient for each AQ and metabolite. The individual regression equations each have r^2-values ≥0.995: (a) AQ-13, $y = 0.374x - 0.0278$; (b) AQ-72, $y = 0.880x - 0.0807$; (c) AQ-73, $y = 0.597x + 0.013$; (d) CQ, $y = 0.851x - 0.055$; (e) MDCQ, $y = 1.31x - 0.107$; and (f) BDCQ, $y = 0.568x - 0.0768$ (y = peak area ratio [peak area for each AQ or metabolite divided by the peak area for the IS on the same chromatogram]; x = molar concentration of the AQ or AQ metabolite being measured).

3.2. Sensitivity

Sensitivity was examined by determining the smallest change in analyte concentration that produced a detectable change in fluorescence on HPLC relative to the IS. The results of this testing (using 5 nM increments in AQ concentration from 45 to 75 nM) indicate that the sensitivity of this assay is ≤5 nM for both CQ and AQ-13 (ANOVA and Kruskal Wallis p-values <0.001 between groups provide evidence that the groups are different, the Student–Newman–Keulls test demonstrates each

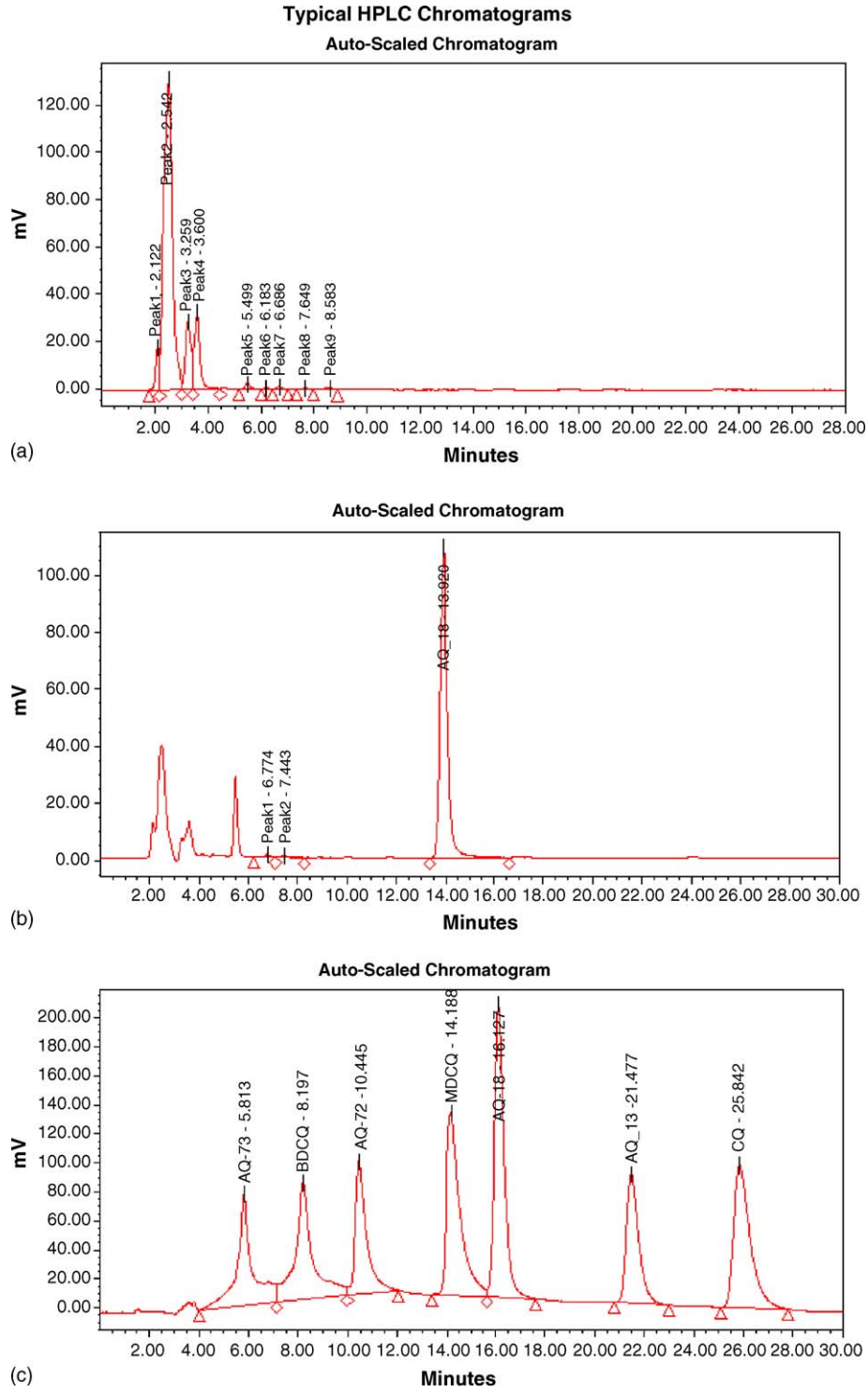

Fig. 3. Typical HPLC chromatograms: (panel a) chromatogram of a control blood specimen from a subject who had not received either AQ-13 or CQ, to which the internal standard (IS) was not added; (panel b) chromatogram of a control blood specimen, to which the internal standard (IS, 1.0 μM) was added; and (panel c) HPLC chromatogram of a control blood specimen to which AQ-13 (1.5 μM), AQ-72 (0.75 μM), AQ-73 (0.5 μM), CQ (1.5 μM), MDCQ (0.75 μM), BDCQ (0.5 μM) and the IS (1.0 μM) were added.

Table 1
Reproducibility of the fluorescence HPLC assay for AQ-13 and its metabolites, as estimated by coefficients of variation (CVs, %) between runs

Concentration (nM)	AQ-13	AQ-72	AQ-73
100	1.03	1.02	1.55
250	1.14	1.71	1.17
1000	1.59	1.60	1.64

Coefficients of variation (CVs) were estimated by dividing the standard deviation by the mean at each AQ concentration ($n = 10$ independent runs on different days).

concentration is different from all the others, $p < 0.001$). When examined for linearity, the X^2 Goodness of Fit test yielded r^2-values of 0.94 and 0.97 for AQ-13 and CQ, respectively.

3.3. Specificity (Fig. 3)

Specificity was examined by comparing the fluorescence HPLC chromatograms obtained for four groups of blood samples: (a) control blood samples to which the IS was (and was not) added (panels a and b); (b) control blood samples to which AQ-13, its two metabolites and the IS were added; (c) control blood samples to which CQ, its two metabolites and the IS were added; and (d) control blood samples to which AQ-13, CQ, their metabolites and the IS were added (panel c). As demonstrated (Fig. 3, panels a–c), the reverse phase C18 column (Xterra RP18), together with the 60% borate buffer plus 40% acetonitrile mobile phase clearly separated these seven compounds: AQ-13 and its metabolites (AQ-72, AQ-73), CQ and its two metabolites (MDCQ, BDCQ), and the IS. The elution sequence for AQ-13, its metabolites and the IS was: AQ-73, AQ-72, IS and AQ-13; the elution sequence for CQ, its metabolites and the IS was: BDCQ, MDCQ, IS and CQ. When examined simultaneously on the same chromatogram, the elution sequence for AQ-13, CQ, their metabolites and the IS was: AQ-73, BDCQ, AQ-72, MDCQ, IS, AQ-13 and CQ.

Specificity was also tested by using the same extraction procedure, mobile phase and elution conditions with two quinoline antimalarials: quinine and primaquine (an 8-aminoquinoline). Under these conditions, quinine (8.8 min) eluted after bidesethyl-CQ (7.6 min) and before AQ-72 (9.3 min). In contrast, under these conditions, no peaks were detected with primaquine (chromatogram not shown).

3.4. Analytical precision—reproducibility (Tables 1 and 2)

Analytical precision for AQ-13, CQ and their metabolites was estimated from repetitive analyses ($n = 10$) of blood samples spiked with 100, 250, or 1000 nM AQ-13, CQ or their metabolites (Tables 1 and 2). The results obtained for AQ-13 and its metabolites between runs on different days were excellent with coefficients of variation (CVs) $\leq 1.6\%$. Similar results were obtained between runs for CQ and its metabolites based on repetitive analyses ($n = 10$ analyses run on different days) of blood samples spiked with 100, 250, or 1000 nM CQ or metabolites with CVs <3% (Table 2). Results obtained for analytical precision within runs were similar, with CVs <4% for both AQ-13 and CQ. CVs were 3.80 and 3.10% for 100 and 250 nM AQ-73, respectively. Otherwise, CVs were <3% (data not shown).

Table 2
Reproducibility of the fluorescence HPLC assay for CQ and its metabolites, as estimated by coefficients of variation (CVs, %) between runs

Concentration (nM)	CQ	MDCQ	BDCQ
100	1.82	2.30	2.89
250	1.98	1.23	2.47
1000	1.23	1.62	2.72

Calculations were performed as in Table 1 ($n = 10$ independent runs on different days).

Table 3
Recovery of AQ-13 and its metabolites from human blood[a]

Concentration (nM)	AQ-13	AQ-72	AQ-73
100	93.6 ± 3.3	96.2 ± 1.2	83.3 ± 2.7
250	93.9 ± 3.0	93.2 ± 2.2	84.4 ± 2.6
1000	94.0 ± 1.6	96.2 ± 1.6	84.7 ± 1.4

[a] Data provided are the mean recovery (±SD) at each concentration. Mean recoveries were calculated as described in Section 3.4 ($n = 5$ independent runs on different days).

3.5. Analytical recovery (Tables 3 and 4)

The recoveries of AQ-13, CQ and their metabolites were estimated from their peak area ratios by extracting blood samples containing known concentrations of AQ-13, CQ and their metabolites. Those peak area ratios were then compared with the peak area ratios obtained with MeOH solutions containing the same concentrations of AQ-13, CQ and their metabolites. Based on five analyses at each concentration, the mean recoveries were then estimated for each of the AQ-13 and CQ samples (Table 3 for AQ-13 and its metabolites, Table 4 for CQ and its metabolites). With the exception of AQ-73 (di-*N*-dealkylated metabolite of AQ-13, 83% recovery), all recoveries were $\geq 93\%$.

3.6. Analyte stability

Based on repeated HPLC measurements, there was no evidence for instability of any of the analytes examined (AQ-13, AQ-72, AQ-73, CQ, MDCQ or BDCQ). In each case, the repeat measurements 3 months later were within 1–2% of the original measurements (data not shown), and were thus within the margin of error established by the CVs obtained in studies of between-run precision (Tables 1 and 2).

Table 4
Recovery of CQ and its metabolites from human blood[a]

Concentration (nM)	CQ	MDCQ	BDCQ
100	101.2 ± 1.7	95.9 ± 1.7	98.9 ± 2.5
250	101.4 ± 1.7	96.2 ± 1.5	95.6 ± 2.4
1000	102.0 ± 1.7	96.9 ± 1.2	93.5 ± 0.4

[a] Calculations were performed as in Table 3 ($n = 5$ independent runs on different days).

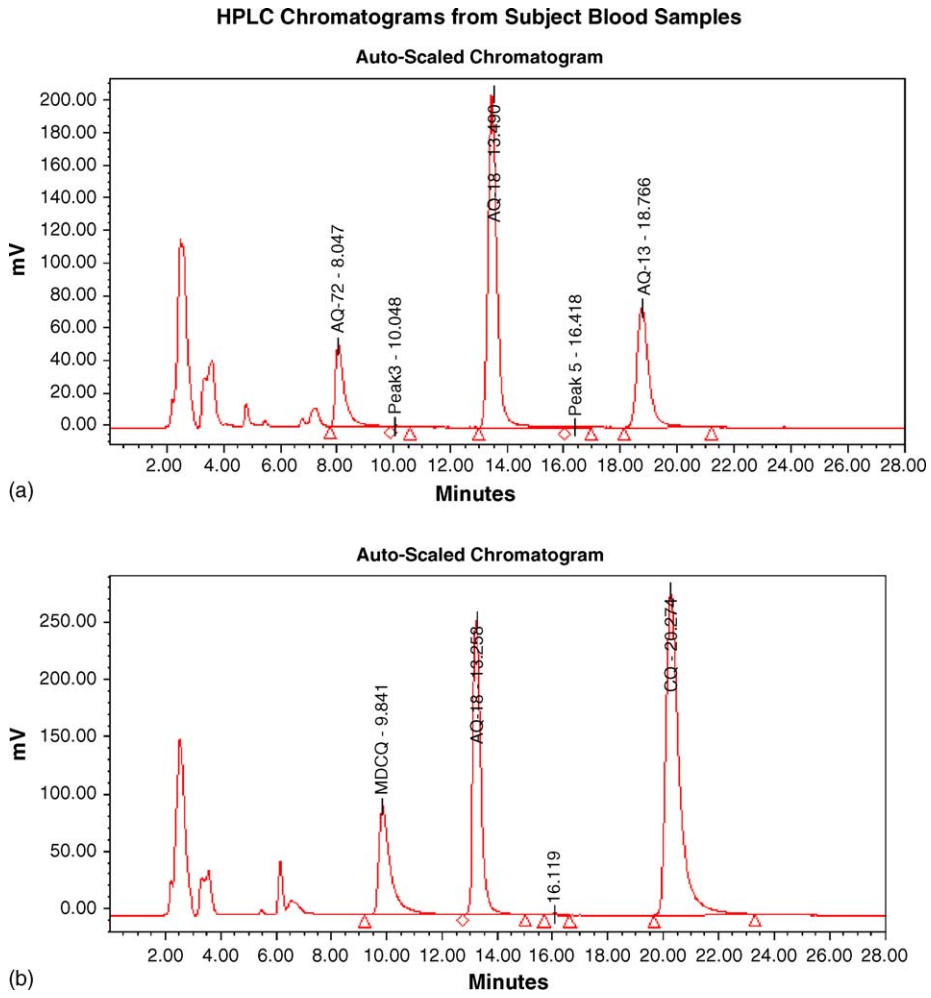

Fig. 4. (panel a) HPLC chromatogram obtained from the blood of subject who received a single oral dose of AQ-13 equivalent to 600 mg CQ base 4 h previously and (panel b) HPLC chromatogram obtained from a subject who received a single oral dose of CQ equivalent to 600 mg base 4 h previously.

3.7. Clinical applicability: pharmacokinetic studies (Figs. 4 and 5)

The clinical applicability of this assay was examined during the Phase 1 (safety and toxicity) Human Studies of AQ-13 in comparison with CQ. During those studies, this assay was used to follow blood levels of AQ-13 and CQ in human subjects who had received single oral doses of AQ-13 or CQ equivalent to 600 mg CQ base (Fig. 4, panel a: AQ-13 dose equivalent to 600 mg CQ base, panel b: CQ dose equivalent to 600 mg CQ base). Also shown are the blood concentration curves for the parent compounds (AQ-13 and CQ) and their major metabolites, AQ-72 and MDCQ, respectively, beginning with the baseline (pre-drug) blood specimen at 0 h on day 1, and concluding with the final blood sample 28 days after an AQ-13 dose equivalent to 600 mg CQ base on day 1 (Fig. 5, panel a) or a CQ dose equivalent to 600 mg CQ base on day 1 (Fig. 5, panel b). Note that the concentrations of the parent compounds (AQ-13, CQ, diamonds) are higher than those of the metabolites (AQ-72, MDCQ, circles), that neither AQ-73 nor BDCQ was identified in these blood specimens, and that AQ-72 (the major metabolite of AQ-13) and MDCQ (the major metabolite of CQ), as well as the minor metabolites of AQ-13 and CQ (AQ-73 and BDCQ) are inactive against CQ-, mefloquine and multi-resistant *P. falciparum* in vitro (Krogstad et al., unpublished observations).

4. Discussion

The HPLC assay method described here for the determination of AQ-13 and CQ in whole blood is sensitive, reproducible and specific. Its precision is excellent with CVs $\leq$3.8% for AQ-13 and its metabolites, and CVs $\leq$2.50% for CQ and its metabolites. Linearity is likewise excellent across the relevant range of therapeutic concentrations. In comparison to previously published assays for CQ and other AQs [12–28,30], the advantages of this method include its ability to measure the concentrations of AQ-13, CQ and their metabolites simultaneously (in the same blood specimen), solid-phase extraction using commercially available extraction cartridges, the choice of an appropriate IS, and the 60% borate plus 40% acetonitrile mobile phase. The IS used for these studies (AQ-18) was chosen because of its structural similarity to AQ-13 and CQ, its elution time, and its lack of antiparasite activity.

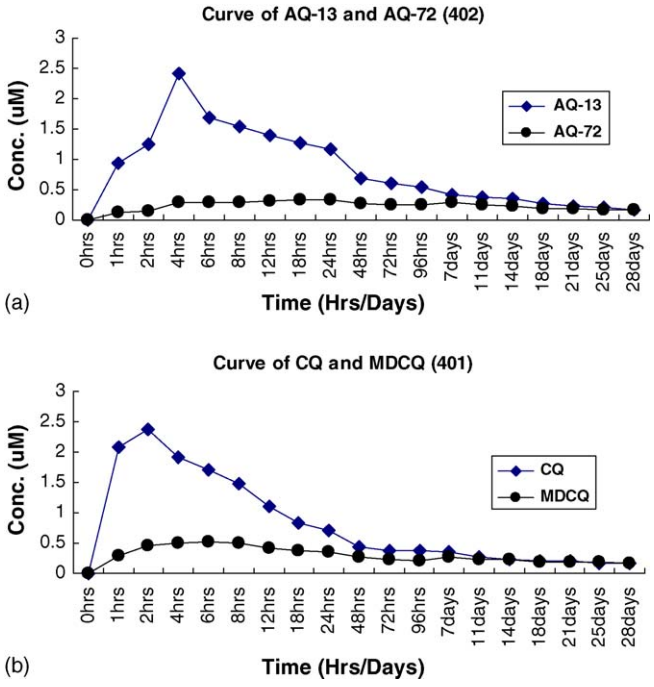

Fig. 5. (panel a) Blood concentration curve for a subject who received an oral dose of AQ-13 equivalent to 600 mg CQ base on day 1 and (panel b) blood concentration curve for a subject who received an oral dose of CQ equivalent to 600 mg CQ base on day 1.

An accurate HPLC assay for AQ-13 and CQ is necessary to compare and understand their pharmacokinetics. In addition to the Phase 1 Studies noted above, there is a need for assays such as this in the field studies of efficacy (Phase 2 and 3) to determine whether failures to clear *P. falciparum* parasitemias with AQ-13 or CQ are due to pharmacokinetic problems (inadequate absorption or excessively rapid metabolism as indicated by low drug blood levels or reduced areas under the curve, AUCs) or AQ resistance (blood levels and AUCs similar to those observed in the Phase 1 Studies in subjects with parasites that are resistant in vitro).

In conclusion, the fluorescence HPLC method described here for the measurement of AQ-13, CQ and their metabolites in whole blood is sufficiently rapid and sufficiently sensitive for pharmacokinetic studies. The small amount of blood required (1.0 ml per determination) makes this method suitable for routine analysis in clinical studies and drug monitoring.

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References

[1] S.C. Oaks Jr., V.S. Mitchell, G.W. Pearson, C.C. Carpenter (Eds.), Malaria: Obstacles and Opportunities, Institute of Medicine, Washington, DC, 1991.
[2] R.W. Snow, C.A. Guerra, A.M. Noor, H.Y. Myint, S.I. Hay, Nature 434 (2005) 214.
[3] J.G. Breman, M.S. Alilio, A. Mills, Am. J. Trop. Med. Hyg. 71 (Suppl. 2) (2004) 268.
[4] C.F. Curtis, Trop. Med. Int. Health 1 (1996) 137.
[5] D.J. Krogstad, in: P.K. Russell, C.P. Howson (Eds.), Vaccines against Malaria: Hope in a Gathering Storm, Institute of Medicine, National Academy Press, Washington, DC, 1996.
[6] D. De, F.M. Krogstad, F.B. Cogswell, D.J. Krogstad, Am. J. Trop. Med. Hyg. 55 (1996) 579.
[7] D.J. Krogstad, D. De, in: I.W. Sherman (Ed.), Malaria: Parasite Biology, Pathogenesis, and Protection, American Society for Microbiology Press, Washington, DC, 1998, p. 331.
[8] D. De, F.M. Krogstad, L.D. Byers, D.J. Krogstad, J. Med. Chem. 41 (1998) 4918.
[9] M.A. Staiger, P. Nguyen-Dinh, F.C. Churchill, J. Chromatogr. 225 (1981) 139.
[10] Y. Bergqvist, F.C. Churchill, J. Chromatogr. 434 (1988) 1.
[11] L.R. Snyder, J.J. Kirkland, J.L. Glajch (Eds.), Practical HPLC Method Development, Wiley, New York, 1997, p. 676.
[12] O. Minzi, M. Rais, J.O. Svensson, L.L. Gustafsson, O.J. Ericsson, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 783 (2003) 473.
[13] S.N. Muchoh, B.R. Ogutu, C.R. Newton, G.O. Kokwar, J. Chromatogr. B Biomed. Sci. Appl. 761 (2001) 255.
[14] A.M. Ronn, T.K. Mutabingwa, S. Kreisby, H.R. Angelo, K. Fuursted, I.C. Bygbjerg, Ther. Drug Monit. 21 (1999) 609.
[15] J. Ducharme, R. Farinotti, J. Chromatogr. B Biomed. Sci. Appl. 698 (1997) 243.
[16] V.K. Dua, N.C. Gupta, P.K. Kar, J. Nand, G. Edwards, V.P. Sharma, S.K. Subbarao, Ann. Trop. Med. Parasitol. 94 (2000) 565.
[17] V.K. Dua, P.K. Kar, N.C. Gupta, V.P. Sharma, J. Pharm. Biomed. Anal. 21 (1999) 199.
[18] J.F. Chaulet, G. Grelaud, P. Bellemin-Magninot, C. Mounier, J.L. Brazier, J. Pharm. Biomed. Anal. 12 (1994) 111.
[19] O. Walker, O.G. Ademowo, Ther. Drug Monit. 18 (1996) 92.
[20] E.A. Karim, K.E. Ibrahim, M.A. Hassabalrasoul, B.O. Saeed, R.A. Bayoumi, J. Pharm. Biomed. Anal. 10 (1992) 219.
[21] F.A. Ogunbona, C.O. Onyeji, A.A. Lawal, C.M. Chukwuani, O.O. Bolaji, J. Chromatogr. 380 (1986) 425.
[22] Y. Bergqvist, M. Frisk-Holmberg, J. Chromatogr. 221 (1980) 119.
[23] K. Croes, P.T. McCarthy, R.J. Flanagan, J. Analyt. Toxicol. 18 (1994) 255.
[24] J.F. Chaulet, Y. Robet, J.M. Prevosto, O. Soares, J.L. Brazier, J. Chromatogr. 613 (1993) 303.
[25] R.B. Taylor, R. Behrens, R.R. Moody, J. Wangboonskul, J. Chromatogr. 527 (1990) 490.
[26] R.W. Steketee, D.L. Mount, L.C. Patchen, S.B. Williams, F.C. Churchill, J.M. Roberts, D.C. Kaseje, A.D. Brandling-Bennett, Bull. World Health Org. 66 (1988) 485.
[27] P. Houze, A. de Reynies, F.J. Baud, M.F. Benatar, M. Pays, J. Chromatogr. 574 (1992) 305.
[28] N.D. Brown, B.T. Poon, J.D. Chulay, J. Chromatogr. 345 (1985) 209.
[29] D. De, L.D. Byers, D.J. Krogstad, J. Heterocyclic Chem. 34 (1997) 315.
[30] P. Volin, J. Chromatogr. B Biomed. Appl. 666 (1995) 347.