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ScienceDirect
Journal of Chromatography B, 850 (2007) 481–487
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Very small injected samples to study chloroquine and quinine in human serum using capillary-LC and native fluorescence

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Received 22 September 2006; accepted 18 December 2006
Available online 30 December 2006

Abstract

A comparison between HPLC with conventional fluorescence detection and capillary-LC (μHPLC) with native laser-induced fluorescence (LIF) detection was done to determine chloroquine (CQ) and quinine (Q) in human serum. HPLC experiments were run with parameters of the conventional fluorimeter set at the highest level of sensitivity. Results were compared with those obtained on μHPLC coupled to a ZETALIF (He–Cd 325 nm) detector which provided a 50-fold increase in sensitivity. In μHPLC-LIF injection volumes were 200 nL instead of 10 μL in conventional HPLC. The separation was completed within 3 min (6 min on HPLC). The limit of detection on μHPLC-LIF was 1.9 and 1.3 fmol for CQ and Q, respectively. Both experiments were validated on serum samples. The mean recovery was more than 95% for CQ and Q. The intra- and inter-day precision and accuracy were found to be within the acceptable limits (<10%).

Keywords: μHPLC; Laser-induced fluorescence detection; Chloroquine; Quinine

1. Introduction

In many cases, a combination of drugs is employed for the treatment of infectious diseases for which the causative parasite or virus has become resistant to treatment. When two or more drugs are employed, it is necessary to study the pharmacokinetic interactions of drugs in combination and the analyst should be able to quantify both the parent compounds and the metabolites simultaneously. Due to the widespread appearance of antimalarial drug-resistant *Plasmodium falciparum* (*P. falciparum*) strains [1–4], we were interested in developing a very sensitive analytical methodology for simultaneous determination of antimalarials in blood samples. In this work the pair chloroquine (CQ)–quinine (Q) was chosen; CQ is the prototype synthetic drug for which *P. falciparum* strains have become resistant but is still largely used in Africa and Q is a natural occurring alkaloid which has received renewed interest in the treatment of malaria [2,3].

Several methods have been reported for the determination of the concentration of chloroquine in human biological fluids including the use of normal [5,6] or reversed-phase HPLC using either ultra-violet (UV) [7–9] or fluorescence (FL) detection [5,6,10,11]. In addition, a flow injection fluorimetric method [12], a radioimmunoassay (RIA) [13] and an enzyme-linked immunosorbent assay (ELISA) [14] have been reported. Methods for the determination of Q in biological fluids have been reported [15,16]. Only three methods have been reported for the simultaneous determination of Q and CQ in biological fluids [17–19]; all require an extraction step using either a liquid–liquid phase extraction or a solid phase extraction. In addition, these methods require a relatively large injected volume (≥50 μL). Only one method has been reported for the determination of CQ and its metabolites without extraction [11] and another one for the Q and its metabolites [16].

HPLC and μHPLC were considered to be excellent techniques for this assay due to the resolving power of the separation. In these studies, micro-HPLC (μHPLC) was used because this technique can offer several advantages over the use of a conventional HPLC system. μHPLC provides a significant decrease in the solvent consumption (with the obvious economic

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doi:10.1016/j.jchromb.2006.12.028

consequences) and the reduction of the sample amount that is required for analysis; this can often be a limiting factor for biological samples, e.g., in pharmacokinetic studies. μHPLC offers a higher eluted peak concentration than conventional HPLC, which leads to a higher sensitivity due to the reduction of peak volume and enhanced signal-to-noise ratio of chromatographic peaks using concentration sensitive detectors [20].

Fluorescence detection was employed as it can provide excellent selectivity and sensitivity. Fluorescence involves two measuring wavelengths (excitation and emission), thereby providing enhanced selectivity relative to an absorbance measurement. The sensitivity of fluorescence is greater than absorbance, as the measurement is made with reference to a zero background (for absorbance the reference and the blank have very similar transmission at low concentration, therefore the sensitivity is limited). We should note that the fluorescence characteristics of the two compounds of interest is significantly different, CQ fluorescence shows maximum emission in alkaline pH [11] while Q shows maximum emission at pH 1 [21], and the overall protocol that we have developed optimizes the sensitivity of both compounds.

When a fluorescence detector is used with μHPLC (and capillary electrophoresis), the detection window is quite small and it is difficult to properly illuminate the window with the conventional Xe lamp normally used in fluorescence detection. To solve this problem, laser-induced fluorescence (LIF) detection is commonly used to focus on the very small volume detection cell and LIF detection provides a higher light intensity inside the cell [22].

In this paper, we describe a new simple, cost-effective and sensitive method for the analysis of CQ and Q in small injected volumes (200 nL) in μHPLC-LIF, the method will be validated on serum samples.

2. Experimental

2.1. Instrumentation

1. Direct fluorescence of analytes was measured with a FluoroMax-2 spectrofluorimeter (Jobin Yvon-Spex, Longjumeau, France).
2. Conventional HPLC/fluorescence: The system included—an automatic injector (Waters 717 plus), a degasser (Waters In-Line), a quaternary pump (Waters 600) coupled to a fluorescence detector (Waters 474). Data acquisition was performed using Waters Millenium^{32} software, Version 3.2 (Waters, St. Quentin, France).
3. Capillary LC/LIF: The μHPLC system included—a micro autosampler, a degasser (micro vacuum degasser), a binary capillary pump (Agilent 1100) delivering a flow rate (1–20 μL/min) with an electronic flow control (EFC) (Agilent; Waldbronn, Germany), a ZETALIF fluorescence detector with a 325 nm He–Cd laser (Picometrics, Toulouse, France). The power irradiating the capillary flow cell was 7.5 mW. Data acquisition was performed using Agilent Chemstation software.

2.2. Reagents and materials

Quinine sulfate salt (Q) (purity 99% by HPLC) Mw = 782.94 g/mol; Mw of the free Q base = 324.42 g/mol (weak base $pK_{a_1} = 4.2^{\circ}$; $pK_{a_2} \approx 8.3$), chloroquine diphosphate salt (CQ) (purity 97% by HPLC) Mw = 515.9 g/mol; Mw of the free CQ base = 319.9 g/mol (weak base pK_{a_1} 8.1; $pK_{a_2} = 10.2$) and pyridoxine hydrochloride salt (vitamin B6) (purity 98% by HPLC) Mw = 205.64 g/mol were obtained from Sigma, St. Quentin, France. Methanol and acetonitrile were HPLC gradient grade (Fisher, Illkirch, France). Ammonium acetate was HPLC grade (Sigma, St. Quentin, France). All aqueous solutions were prepared using high-purity water obtained from a Milli-Q® water purification system (Millipore, St. Quentin, France). A glass vacuum-filtration apparatus was employed for the filtration of the aqueous solutions, using 0.20 μm membrane filters (Millipore). Dissolution of compounds was enhanced by sonication in an ultrasonic bath (Elma®, Germany). A small vortex mixer and a centrifugation system Biofuge A (Heraeus-Sepatech, Germany) were employed for the sample pre-treatment. Serum samples were provided from "Etablissement Français du Sang" (Purpan, Toulouse, France).

2.3. Preparation of standard solutions and samples

A stock standard solution of chloroquine (0.20 mM) was prepared in water/methanol (1:1) and kept in a polypropylene tube to prevent the adsorption of CQ which occurs when it is in contact with glass [11,23]. A stock standard solution of quinine 0.13 mM was prepared in water and working standards were prepared from these stocks in the range of 20–1600 nM (20–1357 nM for μHPLC) and 13–1030 nM (13–639 nM for μHPLC) for CQ and Q, respectively, by appropriate dilution with the mobile phase. All working standards contained the vitamin B6 internal standard (IS), which was 8.5 μM for HPLC (2.4 μM for μHPLC). The 100 μL (40 μL for μHPLC) of human serum samples were spiked with 20 μL (10 μL for μHPLC) of each concentration of CQ, Q and vitamin B6 (IS). Samples were treated with 300 μL (120 μL for μHPLC) of acetonitrile to precipitate the protein and diluted with the mobile phase until the total volume was 0.5 mL (0.2 mL for μHPLC). The samples were then vortexed for 30 s and centrifuged for 10 min at 2800 × *g*. The supernatant was transferred directly to the injection vial (10 μL for HPLC, 200 nL for μHPLC). All the concentrations mentioned above corresponding to the actual concentrations in the serum samples after five-fold dilution.

2.4. Chromatographic conditions

2.4.1. HPLC/conventional fluorimeter

The chromatographic separation was performed on a Synergi^{TM} Max-RP 80 Å, C-12 (4 μm) analytical column (150 mm × 4.6 mm). The mobile phase [methanol/acetonitrile/0.5 M ammonium acetate (50:10:40, v/v/v)], pH 7.4, was delivered at a flow rate of 0.7 mL/min in an isocratic mode. The backpressure observed was 179 bar and the injected volume was 10 μL. The separation was conducted at room temperature with

retention times of 2.7 min for vitamin B6, 4.4 min for CQ and 6.8 min for Q.

Fluorescence detection was performed at 325 nm (λ_{ex}) and 380 nm (λ_{em}) with a bandwidth of 18 nm, the detector gain was fixed at 1000, the attenuation was set to 1, the electronic filter time constant was 3 s and the volume of the detection cell was 16 μL.

2.4.2. μHPLC/ZETALIF laser-induced fluorescence detector

The chromatographic separation was performed on Zorbax SB C-18 (3.5 μm) column (150 mm × 0.3 mm). The mobile phase [MeOH/0.2 M ammonium acetate (60:40, v/v)], pH 7.4, was delivered at a flow rate of 7 μL/min with a backpressure observed was 235 bar. The temperature was fixed at 35 °C; the injected volume was 200 nL.

Fluorescence detection was performed with a He–Cd 325 nm laser. The detector gain was fixed at 770 V with rise time of 1.5 and the detection cell was a capillary of 50 μm internal diameter with 150 μm bubble set exactly in the front of the detector ball lens. The LIF detector is equipped with the Picometrics ball-lens cell. The length of the capillary detection cell was 20 cm from the outlet of the column to the detector. Retention times were 1.7 min for vitamin B6, 2.4 min for CQ and 3.5 min for Q.

2.5. Standardization, sensitivity and determination of reproducibility

Standard curves were prepared by adding known amounts of Q and CQ to human serum. The response factors for each concentration were calculated from the ratio of peak area of analyte to that of the internal standard. Eight determinations were performed for each concentration level. The reproducibility of the procedure was determined by the consistency of the relationship between each concentration and the corresponding factor. Stability was examined by repeated injections on the same day or on different days.

2.6. Recovery

The recoveries of different concentrations of CQ and Q after protein precipitation were determined by comparing the response factor of precipitated CQ and Q-spiked samples with the response factor obtained from direct injections containing the same concentration of CQ and Q. Intra-day precision was determined by five analyses of spiked samples at different concentrations. Inter-day precision was determined by analysis of spiked samples on 5 different days.

3. Results and discussion

3.1. Chromatography

Fig. 1 shows representative chromatograms obtained with conventional HPLC and μHPLC systems with standard CQ and Q in spiked serum samples. The resolution factors calculated by the equation $R_s = 2(t_2 - t_1)/(w_1 + w_2)$ were greater than 2.5, it is

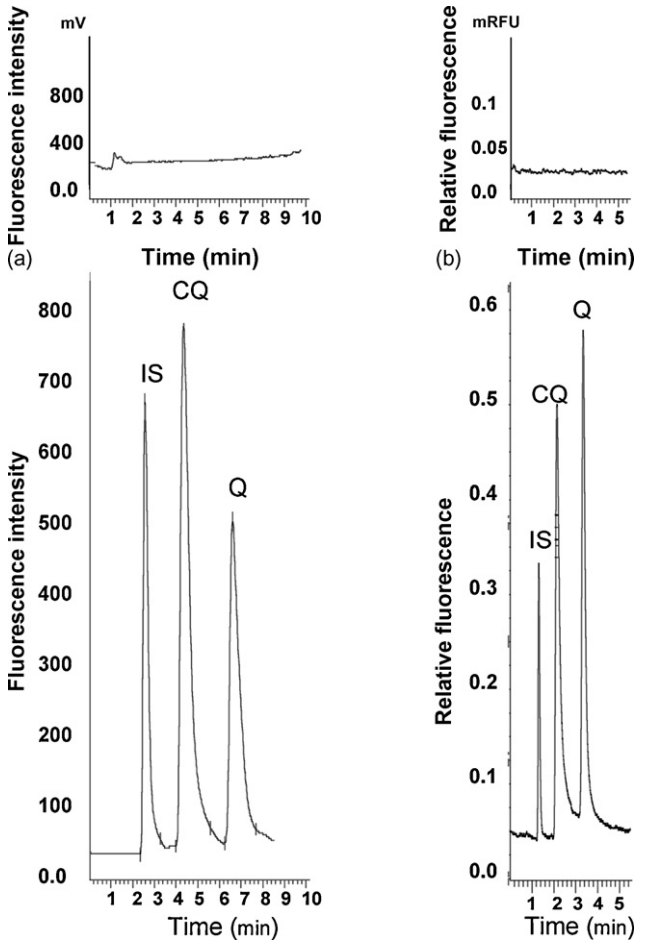

Fig. 1. Chromatograms of blank serum sample at the top and of CQ and Q in spiked serum sample at the bottom on conventional HPLC (a) and on μHPLC (b).

clear that this separation provides a good separation of the two compounds of interest. Vitamin B6 was chosen as IS because it has an appropriate retention time with suitable fluorescence properties at the working wavelengths and was found to give a sharp and a good resolved peak under the experimental conditions. However, high levels of endogenous vitamin B6 may be present in blood of some patients (e.g., nutritional supplementation, red blood cell lysis) which could prevent the use of vitamin B6 as IS. In this situation, after recording the blank chromatogram for confirmation, it could be decided to omit vitamin B6 since the simplicity of the work up procedure and the stability of the light source allow this experimental alternative. To confirm the reproducibility of this alternative method, calibration curves were done using peak areas against the corresponding concentration. Regression equations revealed correlation coefficients higher than 0.999, indicating good linearity. The within and between assay coefficient of variation was lower than 5.4%.

3.2. pH studies

Since the CQ fluorescence is extremely pH-dependent, an alkaline mobile phase was required to provide adequate

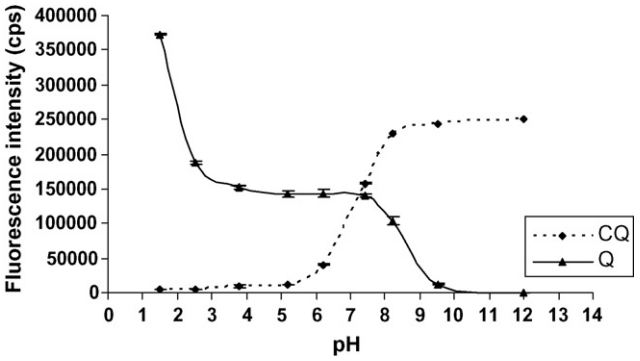

Fig. 2. Intensity of the fluorescence vs. pH at a 1 μg/mL aqueous solutions of CQ and Q, where λ_{ex} was 325 nm for both compounds, λ_{em} was 377 nm for CQ and 383 nm for Q, respectively at pH 7.4.

sensitivity [10,11]. In contrast, Q shows maximum emission at pH 1 [21]. The fluorescence intensity was studied as a function of pH and an optimal value in the range of 7.4 for CQ and Q was found. Fig. 2 shows the fluorescence intensity of Q and CQ plotted versus pH with a maximum emission for CQ at pH 12 and for Q at pH 1.5 at a concentration of CQ and Q = 1 μg/mL in aqueous solutions adjusted to the desired pH using either NaOH or H_3PO_4 ($n = 3$). When the excitation was set to 325 nm for both compounds, the emission maxima were 377 and 383 nm for CQ and Q, respectively, at pH 7.4.

3.3. Optimization of the mobile phase composition

For ionisable compounds, an increase in ionic strength can decrease solute and silica ionization. In addition, such an increase can reduce secondary interactions between analytes and the stationary phase, while increasing the salt concentration can reduce peak tailing for both bases and acids. For these reasons, we studied the effect of the ionic strength on the chromatographic behavior of the analytes. In Fig. 3 we show the effect of the ionic strength on the resolution of the two peaks and on the column efficiency. As the ionic strength increases, a decrease in the tailing of the CQ peak was observed and an increase in the resolution was found (similar results were obtained with conventional HPLC, data not shown). For HPLC studies, we used a 0.5 M salt concentration and with μHPLC we used a 0.2 M salt concentration. The salt concentration used in μHPLC was selected because it provided an optimal resolution/column efficiency ratio while the effect of the salt concentration over the mechanical parts of the μHPLC was minimized. A plot of the retention factor k' versus the inverse of the salt cation concentration (Fig. 4) provides a straight line. An extrapolation of the line to infinite cation concentration can be used to predict the contribution of ion exchange to the retention [24] and demonstrates the effect of the interaction of the acidic residual silanol groups with the analytes that contributes to the retention of the analytes. Comparable results were obtained on conventional HPLC (data not shown). The column void volume was determined using deionised water.

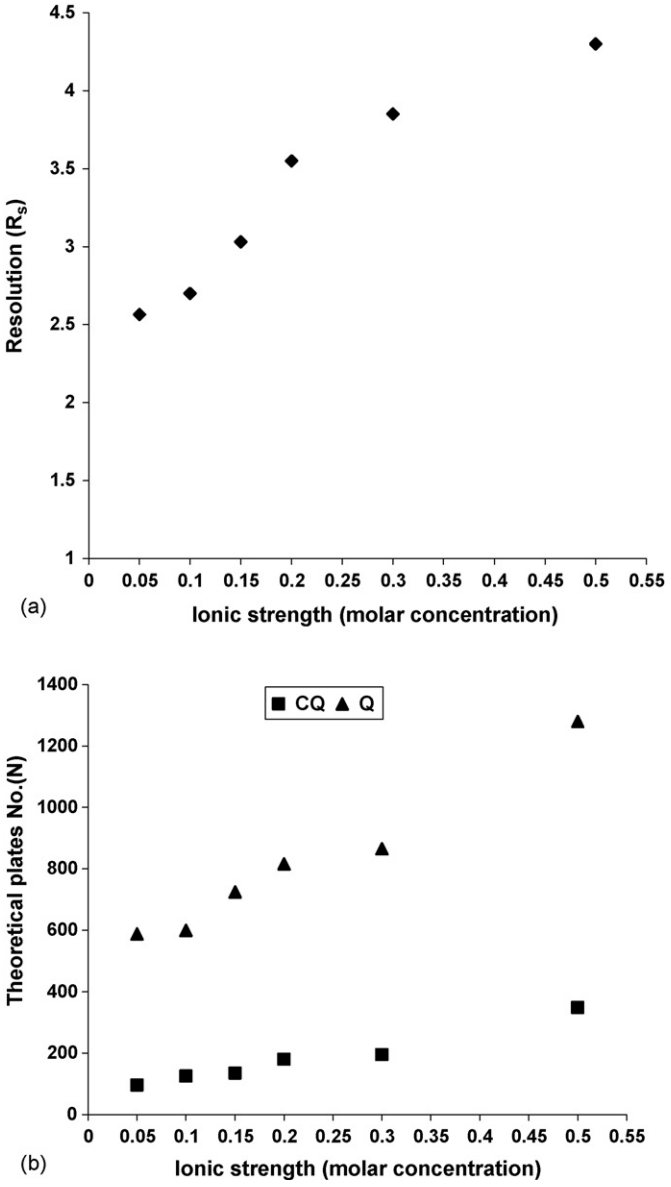

Fig. 3. Effect of ionic strength on the resolution (a) and on the column efficiency (b). [CQ (0.385 μM) and Q (0.255 μM)].

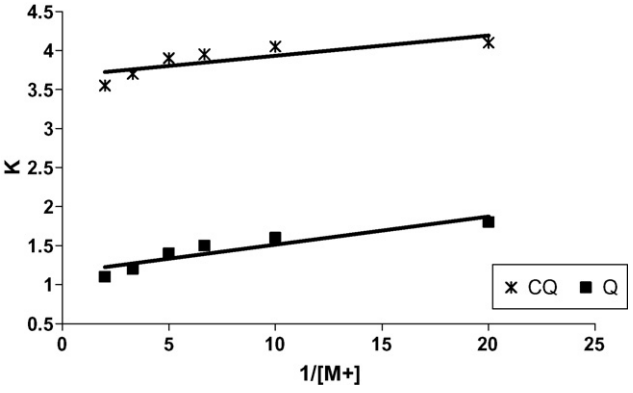

Fig. 4. Plot of the retention factor (k') against the reciprocal of the cation salt concentration.

3.4. Optimization of LIF detection

When LIF is employed with μHPLC, the velocity of the mobile phase in front of the laser beam has an effect on the sensitivity of the LIF detector. At a constant flow rate, the sensitivity depends on the internal diameter of the capillary connected to the output of the column [25]. An additional concern with LIF detection is the possibility of photodegradation; it has been demonstrated [22] that the number of molecules that are photodegraded is inversely proportional to the velocity of the fluorophore in front of the laser beam. We studied different capillaries with different internal diameters and their effect on the sensitivity and on the efficiency of the separation. Fig. 5 presents the effect of the internal diameter of the capillary on LIF intensity; when injecting a constant concentration of the analyte at a constant mobile phase composition and a flow rate. An increase in LIF intensity was observed as the diameter of the capillary was increased. We note, however, that the expected quadratic increase in the fluorescence intensity was not seen due to the photodegradation. At larger diameter (slower velocity) the rate of photodegradation of vitamin B6 is higher than for Q, as the curve is an asymptotic curve, rather than a straight line. In Fig. 6, we show that an increase in the efficiency occurs as the i.d. of the capillary decreases. Fig. 7 shows that a decrease in retention time occurs as a consequence of the decrease in dead volumes. This decrease is directly proportional to the capillary i.d. To get the lowest dead volume, a 50 μm i.d. capillary is preferable while to get the best sensitivity, a 150 μm i.d. capillary is required. A capillary of 50 μm i.d. with a bubble of 150 μm offers a good compromise between sensitivity and dead volumes was selected. The quality of separation was not affected by the bubble cell.

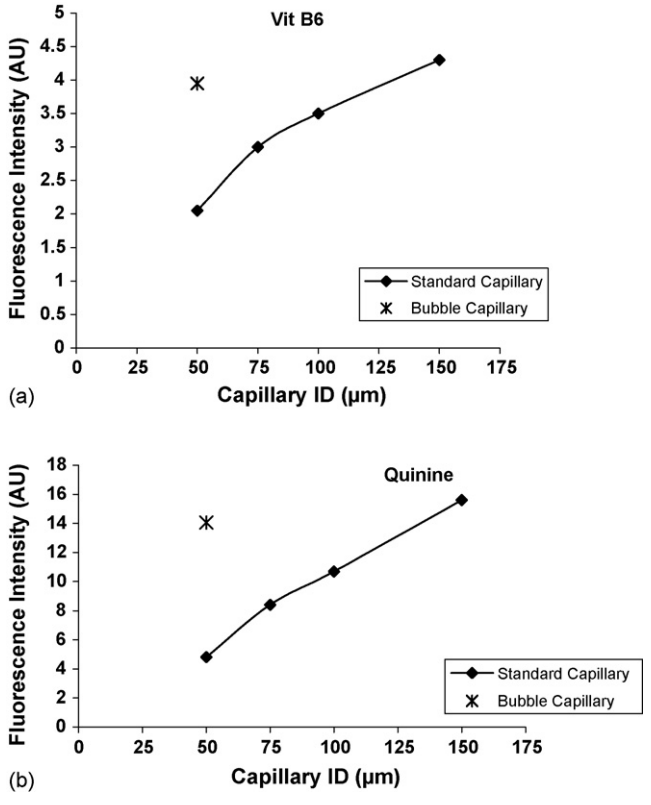

Fig. 5. Effect of the internal diameter of the detection cell on the peak height of vitamin B6 (0.83 μM) (a) and of Q (0.34 μM) (b).

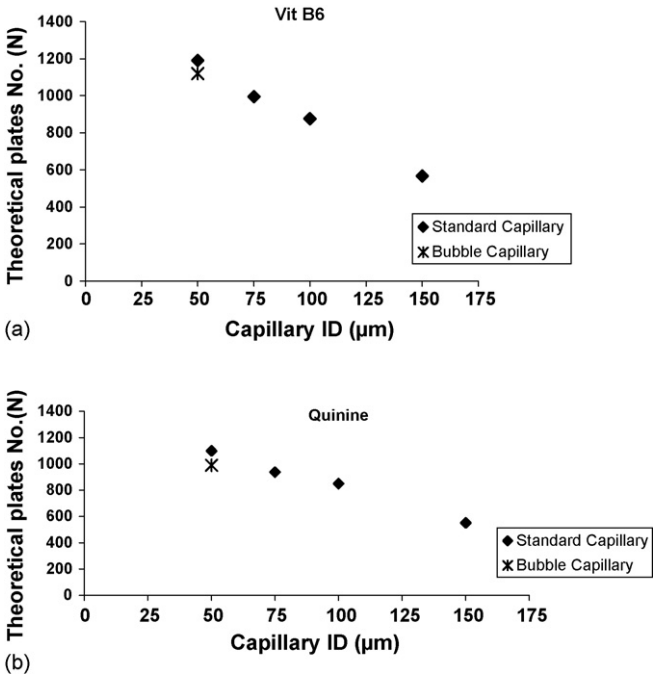

Fig. 6. Effect of the internal diameter of the detection cell on the number of theoretical plates (*N*) (a) vitamin B6 (0.83 μM) and (b) Q (0.34 μM) using Agilent-SB C-18 (3.5 μm, 0.5 mm × 150 mm); flow rate was 20 μL/min, mobile phase was MeOH/0.2 M ammonium acetate (60:40, v/v), temperature = 35 °C, 100 nL injected volume.

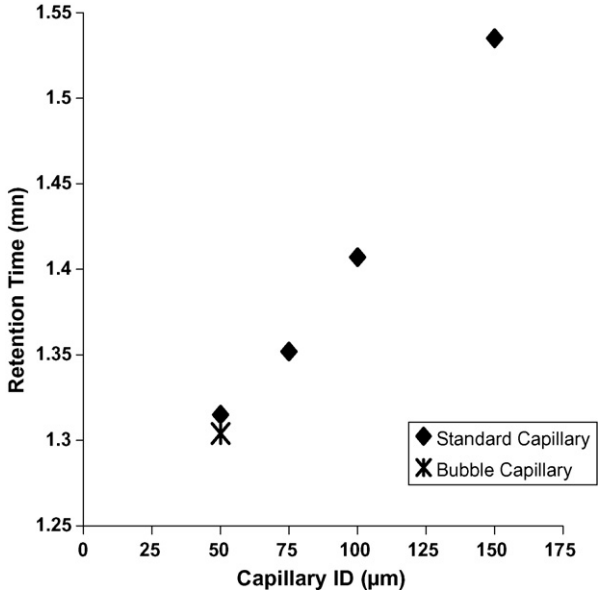

Fig. 7. Effect of the internal diameter of the detection cell on the retention time of vitamin B6 (0.83 μM) using Agilent-SB C-18 (3.5 μm, 0.5 mm × 150 mm); flow rate was 20 μL/min, mobile phase was MeOH/0.2 M ammonium acetate, temperature = 35 °C, 100 nL injected volume.

3.5. Linearity and sensitivity

Calibration curves with seven points were constructed by injecting a series of standards mixtures covering the tested concentration range. The method was linear up to 1030 nM for Q (639 nM on μHPLC) and up to 1600 nM for CQ (1357 nM on μHPLC). Equations were obtained by least-squares linear regression analysis of the peak area ratio of analyte/internal standard versus analyte concentration. The regression equations on HPLC were $y = (3.4588 \pm 0.0965)x - (0.0331 \pm 0.0403)$ and $y = (3.8195 \pm 0.1248)x - (0.1066 \pm 0.0759)$ for the CQ and Q, respectively. The regression equations on μHPLC were $(y = (2.3389 \pm 0.0833)x - (0.0267 \pm 0.0204)$ and $y = (4.4277 \pm 0.0930)x + (0.0537 \pm 0.0063)$ for the CQ and Q, respectively. The correlation coefficients were higher than 0.996.

The LOD was defined as the compound concentration that produced a signal-to-noise ratio greater than three, while the limit of quantification of the assay was evaluated as the concentration equal to or greater than 10 times the value of the signal-to-noise ratio. LOD and LOQ values for each compound in standards based upon these criteria are shown in Table 1. The lowest concentration used for evaluation was chosen to indicate the lower limit of quantification for each compound. The LOD and the LOQ in serum samples were five-fold greater than those in standards due to the dilution of the serum samples during the analysis. The LOD in the standards were 9.7 and 6.4 nM for the CQ and Q, respectively which corresponds to an on-column detection limit of 95 and 65 fmol on HPLC for the CQ and Q, respectively and 1.9 and 1.3 fmol on μHPLC for the CQ and Q, respectively. The LOD in serum were 48.5 and 32 nM for the CQ and Q, respectively and the LOQ in serum were 97 and 64 nM for the CQ and Q, respectively (coefficient of variation, CV%, were less than 10%). To our best knowledge the obtained LOD in μHPLC are the lowest reported in the literature.

3.6. Precision, accuracy and recovery

Table 1 summarizes the results of the determination of reproducibility regarding accuracy, within-day and day-to-day precision assays and the recovery in the spiked serum samples, injecting in μHPLC-LIF only 200 nL sample volume. The intra-assay precision of the method based on within-day repeatability was performed by replicate injections ($n = 5$) of three standard solutions covering different concentration levels: low, medium and high, where peak areas were measured in comparison to the peak area of the internal standard. Statistical evaluation provided the relative standard deviations (R.S.D.) at different values. The inter-assay precision (day-to-day variation) of the method was established using the same concentration range as above. A triplicate determination of each concentration was conducted during routine operation of the system over a period of 5 different days. The measured concentrations had R.S.D. values <8.4%. Accuracy data were expressed as recovery of added analyte to spiked serum samples.

All analytes were found to be stable for at least 24 h in the automatic sampler. This was confirmed by replicate injections of standards. After 4 months stock solutions of CQ and Q (stored refrigerated and protected from light) did not show any degradation (peak areas ≈100% of the peak areas of freshly prepared solutions).

In conclusion, we have demonstrated that using very small injected samples (200 nL), the sensitivity using laser-induced fluorescence is much better than that reported either with UV or conventional fluorescence; the work up procedure is simple

Table 1
Intra- and inter-day reproducibility data for CQ and Q in the serum on HPLC and on μHPLC

Theoretical concentration (nM)	Intra-day ($n = 5$)		Inter-day ($n = 5$)		The recovery (%)
	Measured concentration average (nM ± S.D.)	CV (%)	Measured concentration average (nM ± S.D.)	CV (%)	
CQ					
400[a]	397 ± 15	3.8	414 ± 24	5.8	103.5
800[a]	785 ± 22	2.8	800 ± 20	2.5	100.0
1200[a]	1259 ± 37	3.0	1265 ± 23	1.8	105.5
1600[a]	1622 ± 29	1.8	1635 ± 39	2.4	101.8
97[b]	95 ± 4.5	4.7	94 ± 5.3	5.6	97.5
388[b]	393 ± 15	3.8	381 ± 21	5.5	98.2
775[b]	792 ± 32	4.0	809 ± 35	4.3	104.2
1164[b]	1169 ± 22	1.9	1188 ± 31	2.6	102.1
Q					
260[a]	250 ± 7	2.8	260 ± 9	3.5	98.7
520[a]	480 ± 16	3.3	490 ± 11	2.2	94.3
780[a]	770 ± 24	3.1	770 ± 3	0.4	99.4
1030[a]	1030 ± 8	0.8	1030 ± 10	1.0	99.5
64[b]	62.5 ± 1.8	2.9	61.5 ± 2.5	4.1	96.9
128[b]	123 ± 8	6.8	120 ± 9	7.5	93.7
255[b]	251 ± 15	5.9	245 ± 13	5.3	96.1
551[b]	549 ± 13	2.4	541 ± 9	1.7	98.1

[a] On HPLC.
[b] On μHPLC.

and rapid, with deprotenization of serum samples and direct injection, without prior liquid-liquid phase extraction or solid phase extraction. The recovery in serum is more than 95% for CQ and Q. It is clear that μHPLC/LIF is an appropriate technique to separate and detect very low quantities (fmol) of drugs such as CQ and Q.

This work reports the first separation of CQ and Q on μHPLC-LIF, the shortest time of analysis within 3 min and the lowest LOD (<2 fmol). The usefulness of a bubble capillary to increase the sensitivity in μHPLC is also reported. The very sensitive μHPLC-LIF method provides the basis for further development to study pharmacokinetics and mechanisms of action of new antimalarial compounds in single parasite which require very low injected volumes (below than 500 nL) and are difficult to do using conventional HPLC-fluorescence detection.

Acknowledgements

We thank Agilent Technologies for their gracious Cap LC loan and for their technical support and Peter Froehlich for his careful review and editing of this manuscript. H.I. thanks the Egyptian Government for fellowship. F.N. thanks European Commission for support (RTD-Project 6th FP, Contract No. 018602, READ-UP).

References

[1] M. Foley, L. Tilley, Pharmacol. Ther. 79 (1998) 55.
[2] H. Tinto, J.B. Ouedraogo, B. Traoré, S.O. Coulibaly, T.R. Guiguemde, Bull. Soc. Pathol. Exot. 94 (2001) 188.
[3] J. May, C.G. Meyer, Trends Parasite 19 (2003) 432.
[4] N.J. White, P.L. Olliaro, Parasite Today 12 (1996) 399.
[5] B. Traore, E. Lazaro, F. Gay, Trop. Med. Int. Health 2 (10) (1997) 929.
[6] V.K. Dua, P.K. Kar, N.C. Gupta, V.P. Sharma, J. Pharm. Biomed. Anal. 21 (1999) 199.
[7] M. Estadieu, A. Durand, A. Viala, P.P. Rop, M. Fornaris, J. Quicke, J. Anal. Toxicol. 13 (2) (1989) 89.
[8] O.M.S. Minzi, M. Rais, J.O. Svensson, L.L. Gustafsson, O. Ericsson, J. Chromatogr. B 783 (2003) 473.
[9] O. Walker, O.G. Ademowo, Ther. Drug Monit. 18 (1996) 92.
[10] J. Ducharme, R. Farinotti, J. Chromatogr. B 698 (1997) 243.
[11] Y. Bergqvist, M. Frisk-Holmberg, J. Chromatogr. 221 (1980) 119.
[12] J. Amador-Hernandez, J.M. Fernandez-Romero, M.D. Luque de castro, Fresenius J. Anal. Chem. 369 (2001) 438.
[13] C. Freier, G. Alberici, P. Turk, F. Baud, C. Bohuon, Clin. Chem. 32 (1986) 1742.
[14] A.M.C. Witte, H.H. klever, B.J. Brabin, T.A. Eggelte, H.J. Van der Kayy, M.P. Alpers, Trans. R. Soc. Trop. Med. Hyg. 84 (1990) 521.
[15] R.A. Mirghani, Ö. Ericsson, L.L. Gustafsson, J. Chromatogr. B 708 (1998) 209.
[16] R.A. Mirghani, Ö. Ericsson, J. Cook, P. Yu, L.L. Gustafsson, J. Chromatogr. B 754 (2001) 57.
[17] V.F. Samanidou, E.N. Evaggelopoulou, I.N. Papadoyannis, J. Pharm. Biomed. Anal. 38 (1) (2005) 21.
[18] K. Croes, P.T. McCarthy, R.J. Flanagan, J. Anal. Toxicol. 18 (1994) 255.
[19] J.F. Chaulet, Y. Robert, J.M. Prevosto, O. Soares, J.L. Brazier, J. Chromatogr. 613 (1993) 303.
[20] J.P.C. Vissers, H.A. Claessens, C.A. Cramers, J. Chromatogr. A 779 (1997) 1.
[21] B.B. Brodie, S. Udenfriend, W. Dill, G. Downing, J. Biol. Chem. 168 (1947) 311.
[22] C. Bayle, V. Poinsot, C. Fournier-Noël, F. Couderc, in: U. Pyell (Ed.), Electrokinetic Chromatography, John Wiley & Sons, 2006, p. 263.
[23] T.G. Geary, M.A. Akood, J.B. Jensen, Am. J. Trop. Med. Hyg. 32 (1983) 19.
[24] D.V. McCalley, J. Chromatogr. A 902 (2000) 311.
[25] N. Siméon, R. Myers, C. Bayle, M. Nertz, J.K. Stewart, F. Couderc, J. Chromatogr. A 913 (2001) 253.