Journal of Chromatography, 428 (1988) 196–202
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 4184

Note

Field-adapted method for high-performance thin-layer chromatographic detection and estimation of chloroquine in finger-stick blood

DWIGHT L. MOUNT, LESLIE C. PATCHEN and FREDERICK C. CHURCHILL*

Control Technology Branch, Division of Parasitic Diseases, Center for Infectious Diseases, Centers for Disease Control, Public Health Service, U.S. Department of Health and Human Services, Atlanta, GA 30333 (U.S.A.)

(First received December 16th, 1987; revised manuscript received February 24th, 1988)

Recent publications from several laboratories have described improved methods for determining chloroquine (Cq) and its major metabolite, desethylchloroquine (DECq) in urine in the field, information that is important for various types of malaria field studies [1]. The approaches used have included colorimetric assays [2,3] and thin-layer chromatographic (TLC) assays [1,3]. The work of Betschart and Steiger [4] permits sensitive and precise quantification of Cq and DECq in blood by using high-performance thin-layer chromatography (HPTLC) using automatic sample application and detection by a fluorescence TLC scanner in a well equipped laboratory; however, adapting this methodology to a field situation would require substantial modification and compromise [4]. More recently, we have described a field-adapted method for HPTLC separation and estimation of Cq and DECq in urine which has a detection limit of 0.25 μg/ml using a 5-ml sample [1].

In this study we describe a field-adapted HPTLC method designed to detect and estimate quantities of Cq in 100-μl samples of finger-stick blood by comparing the fluorescence intensity of the Cq and DECq spots for samples with that of standards in the presence of *n*-butylamine under ultraviolet (UV) illumination. Chromatographic conditions are chosen to focus the Cq spot to enhance detectability. The method can be used in a simple field laboratory where the only requirement in addition to easily portable equipment and supplies is a source of

0378-4347/88/$03.50

electric power. Application of the method to samples from a Cq-dosed volunteer is described, and the results are compared with a high-performance liquid chromatography (HPLC) reference method [5].

EXPERIMENTAL*

Standards

Chloroquine diphosphate was purchased from Sigma (St. Louis, MO, U.S.A.). Desethylchloroquine base was supplied by Sterling-Winthrop Research Institute (Rensselaer, NY, U.S.A.).

Reagents and materials

Spectroscopic-quality glass-distilled methyl *tert.*-butyl ether (MTBE) was from Burdick and Jackson Labs. (Muskegon, MI, U.S.A.). Sodium phosphate, tribasic dodecahydrate, and *n*-butylamine were from Aldrich (Milwaukee, WI, U.S.A.). Silica gel 60 HPTLC plates (10 cm×10 cm) were from Merck (Darmstadt, F.R.G.).

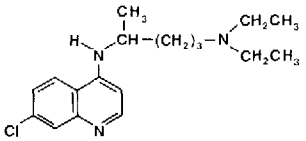
Equipment

A micro centrifuge (Haake-Buchler Instruments, Saddle Brook, NJ, U.S.A.) was used to help separate the layers after extraction. This compact centrifuge has dimensions of 18 cm×18 cm×18 cm and weighs 5.5 kg. A linear developing chamber for 10 cm×10 cm plates from Camag (Wrightsville Beach, NC, U.S.A.) was used to perform HPTLC. Transfer of sample to the plate was carried out with a 25-μl Hamilton (Reno, NV, U.S.A.) blunt-tipped syringe secured in a Hamilton PB-600-1 repeating dispenser. Plates were visualized with a Mineralight lamp, Model G-14, which provides short-wavelength UV (254 nm) illumination and is battery-operated (UVP, San Gabriel, CA, U.S.A.).

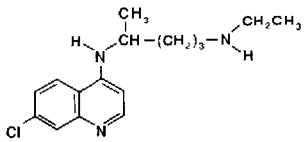
HPTLC method

A stock standard solution containing 0.60 mg/ml (expressed as base) each of Cq and DECq (Fig. 1) dissolved in 0.001 *M* hydrochloric acid was prepared. A 1:100 dilution in 0.001 *M* hydrochloric acid (6 μg/ml in Cq and DECq) was then used to provide standards at levels of 0, 60, 120, 180, 300, 450, 600 and 900 ng/ml Cq and DECq in blood. Conical polypropylene tubes (1.5-ml volume with attached caps) were used for extraction of standards and samples. The appropriate amounts of Cq and DECq were added to each standard tube, followed by a 100-μl quantity of control (blank) blood. A 100-μl amount of sample blood was added to each sample tube. MTBE (0.5 ml) and 20% aqueous trisodium phosphate dodecahydrate, pH 12.4 (0.5 ml) were added to standards and samples alike. Each

*Use of trade names and commercial sources is for identification only and does not constitute endorsement by the Public Health Service or the U.S. Department of Health and Human Services.



Chloroquine



Desethylchloroquine

Fig. 1. Structures of chloroquine and desethylchloroquine.

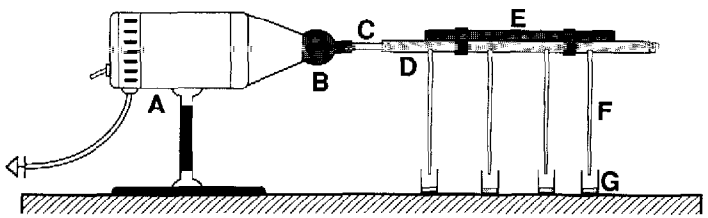


Fig. 2. Manifold for evaporation of samples. The apparatus consists of the following components: (A) cool-air blower; (B) 50-ml suction bulb, laterally bisected; (C) glass tubing; (D) rubber tubing; (E) wooden dowel for structural support; (F) Pasteur pipet tips forced into slits in rubber tubing; (G) 0 5-dram vials containing samples. A small ring-stand with clamp (not shown) is used to support the dowel and rubber tubing.

tube was capped and vortexed for 15 s and then centrifuged to help separate the layers. The organic layer for each standard and sample was then transferred by Pasteur pipet into a corresponding 0.5-dram (1.5 ml) cylindrical vial. The MTBE was evaporated by an apparatus constructed with a cool-air blower, a horizontally bisected 50-ml pipet bulb, glass and rubber tubing, and Pasteur pipet tips, which constituted a forced-air manifold evaporation system (Fig. 2). Each extract residue was then reconstituted in 25 μl of MTBE. The entire extract was applied to a 10 cm$\times$10 cm HPTLC silica gel plate (EM Science No. 5631) with a 25-μl blunt-tipped syringe mounted in a repeating dispenser. Each advance of the push-button dispenser mechanism represented 0.5 μl of standard or sample; 0.5-μl amounts were routinely applied with evaporation of solvent between incremental additions. The solvent evaporated within 1 or 2 s after each 0.5-μl incremental volume was spotted; each sample and standard extract was applied in this way. The first spot was made 10 mm from the side edge and each succeeding spot 6 mm from its predecessor. With this spacing arrangement, fourteen extracts may be applied to each end of the plate. The syringe was rinsed with MTBE between extracts.

After the samples were added, the plate was placed in the linear development chamber and eluted from the two opposite edges (to which samples had been added) to the center of the plate. The elution solvent was 4% *n*-butylamine in MTBE. The plate was removed from the tanks when elution was complete (about 10 min) and allowed to air-dry for 1 h after which it was observed under UV light. The fluorescence from the Cq spot for each sample is compared to standard spots to estimate the concentration of Cq present.

An alternative spotting scheme was also used, which aided in the estimation of higher concentration spots. For this scheme, 30 μl of MTBE were added to the dried extract. First a partial transfer of 4 μl of the sample was applied to the plate. The remainder of the sample was then applied adjacent to the partial transfer spot. All spots were spaced 4 mm apart after starting 8 mm from the side edge. With this spacing arrangement eleven samples can be applied to each side of the plate.

Volunteer study — comparison with a standard method

A volunteer (F.C.C.), who had been taking Cq chemoprophylaxis for travel to a malarious area, had blood samples drawn at intervals after a weekly dose of 500 mg of Cq diphosphate (300 mg as base). Samples were drawn into heparinized tubes at 4 h, 24 h, 3 days and 7 days subsequent to the most recent dose of the drug. These samples were analyzed by the HPTLC method, and Cq levels were estimated. These values were then compared to Cq concentrations determined by a previously published HPLC method [5].

RESULTS AND DISCUSSION

Characterization of the HPTLC method

Partitioning of 100 μl of blood between 0.5 ml of aqueous trisodium phosphate and 0.5 ml of MTBE results in the extraction of Cq and DECq into the organic phase. Evaporation of the separated MTBE phase, followed by reconstitution of the sample with a small volume of MTBE, facilitates transfer of a large fraction of each concentrated extract to the HPTLC plate. This approach contributes to the sensitivity of the method. The HPTLC conditions are designed to focus the Cq spot upon elution to give a thin, concentrated band which greatly enhances detectability. Under the elution conditions the metabolite, DECq, is not similarly focused and has a higher limit of detection. Fig. 3 shows a photograph of a representative chromatogram.

The *n*-butylamine in the mixed solvent used to elute the chromatogram enhances detectability in two ways. Both Cq and DECq fluoresce in the presence of *n*-butylamine when viewed under short-wavelength (254 nm) UV light. Further, under the elution conditions, the *n*-butylamine focuses Cq but not DECq into a narrow band, enhancing detectability. The *n*-butylamine itself does not elute with

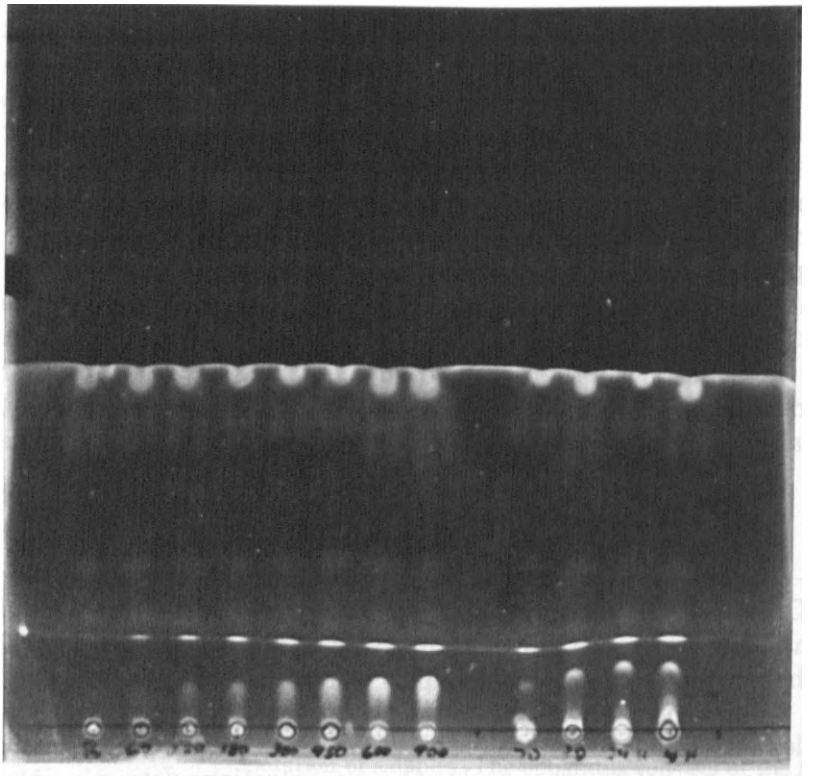


Fig. 3. Thin-layer chromatogram of extracts of standards and samples from a volunteer study. The eight standards contained 0, 60, 120, 180, 300, 450, 600 and 900 ng/ml each of Cq (R_F=0.26) and DECq (R_F=0.12) in blood. The four samples are 7-day, 3-day, 24-h and 4-h blood samples from the volunteer.

the MTBE solvent front but is retained by the silica gel and forms a second front. The Cq elutes at the *n*-butylamine front where it (Cq) is sandwiched between a region of lower elution strength in advance of the main part of the band and higher elution strength behind the main part of the band. When such conditions exist, band focusing occurs and detectability is enhanced, as has been demonstrated previously in HPLC [6]. Such elution conditions could cause loss of selectivity in the presence of compounds with elution properties similar to the analyte of interest. In the present case the selectivity of detection resulting from the fluorescent properties of Cq reduces such concerns. The contrast between the fluorescent spots and the background increases initially, while the MTBE is evaporating after removal of the plate from the elution chamber. The HPTLC plates should be read about 1 h after elution when the fluorescence of the Cq and DECq spots achieves maximum intensity. The fluorescence intensities fade over a period of a day or two as the *n*-butylamine slowly desorbs from the HPTLC plate. The detection limit for Cq by this method is 60 ng/ml for a 100-μl blood sample. The corresponding value for DECq, which is not focused by the chromatographic system, is 120 ng/ml.

TABLE I

EVALUATION OF THE HPTLC METHOD: MEASUREMENT OF Cq AND DECq IN BLOOD COMPARED WITH A REFERENCE HPLC METHOD

The blood samples were from a volunteer treated with Cq (300 mg as base) in the ninth week of a weekly chemoprophylactic regimen.

Sampling time	HPTLC (ng/ml)*		HPLC (ng/ml)	
	Cq	DECq	Cq	DECq
4 h	450-600	150-300	573	159
24 h	450-600	150-300	480	178
3 day	300-450	150	330	124
7 day	150-300	<150	226	134

*These values were estimated using the partial-spotting scheme mentioned in the text and not that shown in Fig. 3 A 150 ng/ml standard was used in the corresponding standard series.

HPTLC method compared with HPLC

The HPTLC field method for detecting and estimating Cq and DECq in blood was applied to four samples, and the results were compared with the HPLC assay [5] (Table I). The estimates from the HPTLC method were made by a chemist practiced in running the test and in reading the HPTLC plate. The HPTLC results were recorded before the HPLC analyses were run independently by a second chemist. The HPTLC values can be estimated by eye within a 150 ng/ml interval to give results consistent with the more precise results available from HPLC analysis.

For the reported HPTLC estimates, the alternative partial-spotting scheme was used. This scheme helps estimate higher concentration spots but marginally compromises detection limits, increases time for sample application, and reduces the number of samples that can be applied to each plate. Thus when applying the method, one should decide if the greater precision of estimates at higher concentrations justifies the compromises.

This HPTLC method utilizes simple and relatively light-weight equipment that can be transported easily around the world to be used in rudimentary laboratories that need only be equipped with electricity. The method, then, provides a means of monitoring concentrations of Cq and DECq in blood so that results can be available on the day of sampling if desired. The sensitivity of the method permits finger-stick blood sampling. If more precise results are required on selected samples for final interpretation of the data, finger-stick blood spots preserved on filter paper may be set aside for subsequent analysis by HPLC in an analytical laboratory [5].

ACKNOWLEDGEMENTS

This work was supported in part by the World Health Organization through a WHO Contract Technical Agreement and the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases.

REFERENCES

1 D.L. Mount, B.L. Nahlen, L.C. Patchen and F.C. Churchill, J. Chromatogr., 423 (1987) 261–269.
2 Y. Bergqvist, C. Hed, L. Funding and A. Suther, Bull. WHO, 63 (1985) 893–898.
3 D.L. Mount, L.C. Patchen, S.B. Williams and F.C. Churchill, Bull. WHO, 65 (1987) 615–623.
4 B. Betschart and S. Steiger, Acta Tropica, 43 (1986) 125–130.
5 L.C. Patchen, D.L. Mount, I.K. Schwartz and F.C. Churchill, J. Chromatogr., 278 (1983) 81–89.
6 L.B. Nilsson and D. Westerlund, Anal. Chem., 57 (1985) 1835–1840.