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REVIEW

DETECTION AND DETERMINATION OF ANTIMALARIAL DRUGS AND THEIR METABOLITES IN BODY FLUIDS

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1. INTRODUCTION

This review of methods for the detection and determination of antimalarial drugs and metabolites in body fluids is selective rather than exhaustive. The determination of antimalarial drugs should be used in several circumstances. Recent drug history is essential for correct interpretation of alleged resistance of malaria parasites to the drug in vitro or in vivo. This information is difficult to obtain in the field, because drugs are sometimes used indiscriminately, and contents of tablets are not always known. For example, in some African communities chloroquine is present in the blood of many persons who deny having taken it [1]. Patterns of drug use have an impact on the incidence and spread of drug-resistant strains of *Plasmodium falciparum*.

High-performance liquid chromatography (HPLC) is by far the preferred technique for the assay of antimalarials and in most instances is superior to gas chromatography (GC) for this purpose; however, the latter has advantages for certain antimalarials when a non-polar, volatile derivative amenable to selective, sensitive GC determination may be conveniently formed. Colorimetric and thin-layer chromatographic methods find application for field-adapted assays. Immunoanalytical methods are beginning to find application in antimalarial drug assays, but will not be considered in this review.

Simple tests to determine drugs in urine have been extensively used both to exclude participants with recent drug intake from in vitro and in vivo tests and to ensure drug absorption or compliance during in vivo tests. Until recently, urine determinations have been semiquantitative at best. In addition, urine concentrations of drugs do not directly reflect plasma concentrations because the diuresis at voiding varies. Therefore, when quantitative information on drug concentration is needed (during malaria treatment and before in vitro tests), whole blood assays are indicated.

The analytical method requirements differ depending on the concentration range of interest. In therapeutic drug monitoring, the determinations are usually performed with higher drug concentrations than in pharmacokinetic studies, where extreme sensitivity is needed if the terminal phase of the elimination curve

also must be accurately determined. The use of sensitive chromatographic methods has already caused re-evaluation of previous information on the pharmacokinetic properties of older antimalarial drugs. Chromatographic methods are also selective in that concentrations of metabolites are determined separately. This selectivity is essential whenever the biological activities of the metabolite and of the parent compound differ.

1.1. Abbreviations

The following abbreviations are used: BTB = bromthymol blue; C.V. = coefficient of variation; GC = gas chromatography; HPLC = high-performance liquid chromatography; HPTLC = high-performance thin-layer chromatography; TLC = thin-layer chromatography; CQ = chloroquine; CQM = deethylchloroquine; Q = quinine; AQ = amodiaquine; AQM_1 = deethylamodiaquine; AQM_2 = bideethylamodiaquine; AQM_3 = 2-hydroxydeethylamodiaquine; MQ =

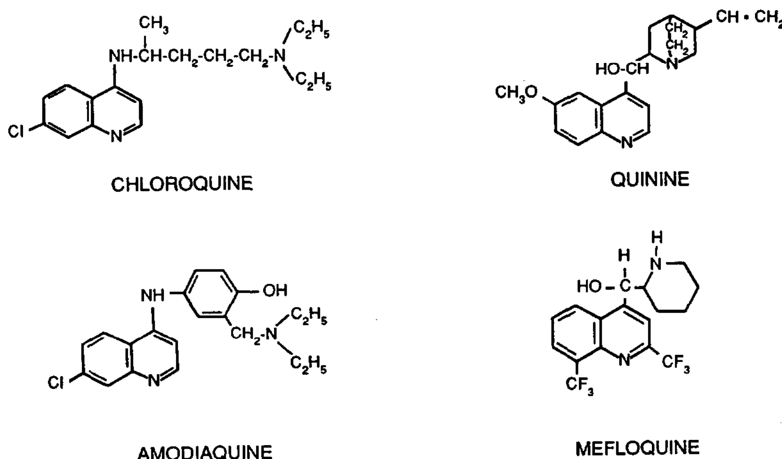

Fig. 1. Structures of compounds.

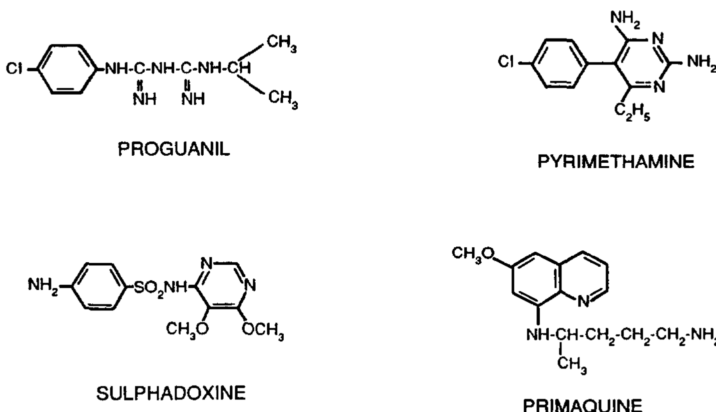

Fig. 2. Structures of compounds.

mefloquine; MMQ=mefloquine, carboxylic acid metabolite; PO=proguanil; PYR=pyrimethamine; SU=sulphadoxine; PQ=primaquine.

1.2. Structures of compounds

The structures of chloroquine, quinine, amodiaquine and mefloquine are shown in Fig. 1 and those of proguanil, pyrimethamine, sulphadoxine and primaquine in Fig. 2.

2. ANALYTICAL CONSIDERATIONS

If biological specimens are improperly sampled and handled, results may be invalidated even when the most sophisticated techniques are used. For example, some antimalarials, including chloroquine (CQ) and amodiaquine (AQ), are degraded by exposure to light, so blood samples must be protected from sunlight. Even the sampling tubes can affect the results. This is true of gel barrier sampling tubes used to determine some antiepileptic drugs [2].

Adsorptive losses of drugs and metabolites must be considered whenever the drug is in contact with glass surfaces, especially in the nanomole per litre concentration range [3–6]. The risk of adsorption is most pronounced for amines (most antimalarials are amines). Adding blood greatly decreases CQ adsorption [3]. CQ concentrations decreased by up to 40% in glass containers, but adding human serum at concentrations of 5–50% inhibited the binding of CQ to glass [4,5]. However, Yahya et al. [6] showed that CQ is bound only to soda glass and not to borosilicate glass.

For CQ and deethylchloroquine (CQM), the result depends greatly on the type of sample (serum, plasma or whole blood) used (Table 1).

The different concentration ratios are due to the binding of CQ to thrombocytes and granulocytes to such an extent that the plasma concentration is only about 15% of that in whole blood [5]. Therefore, failure to remove these blood

TABLE 1

CONCENTRATION RELATIONSHIPS OF CQ AND CQM IN DIFFERENT BIOLOGICAL MEDIA

All samples were taken after at least two to three months of treatment of rheumatoid disease (160–250 mg of CQ base per day) [7].

Biological media	CQ			CQM		
	Mean ratio	r^*	n	Mean ratio	r^*	n
Serum/plasma	2.1	0.87	13	4.8	0.75	13
Whole blood/serum	3.3	0.69	13	1.8	0.87	13
Whole blood/plasma	7.0	0.66	13	8.5	0.63	13

* Correlation coefficient between concentrations obtained in two biological media.

cells from plasma during centrifugation will lead to erroneously high plasma concentration values [5,8]. Strictly standardized handling of plasma samples is necessary to obtain the true plasma concentration, but this is rarely possible in areas with limited technical facilities.

The ratio of sulphadoxine (SU) between whole blood and plasma was about 0.56 [9]. This is due to the fact that about 90% of SU is bound to albumin, which is found in the plasma fraction. The rest is distributed in plasma (ca. 6%) and erythrocytes (ca. 4%) [10].

Finger-puncture capillary serum quinine (Q) concentrations average 63% of venous serum Q concentrations [11]. This is contrary to results obtained on comparing capillary and venous whole blood concentrations for CQ and CQM in studies on sampling on filter paper, where Patchen et al. [12] and Lindström et al. [13] found no difference. It has also been reported that the concentration of quinidine was higher in serum than in plasma [14]. A confirmatory study, by a high-performance liquid chromatographic (HPLC) method, of the difference in concentration in serum and plasma showed that the concentration in serum is on average 38% higher [15].

Methods using samples collected in the field are very desirable. Such methods using dried blood spots have been successfully developed to detect inborn errors of metabolism and to monitor concentrations of glucose and some antimalarial drugs and are now also used to determine CQ [12,13], SU [9] and mefloquine (MQ) [16]. Capillary blood sampling with a lancet puncture technique provides whole blood sample volumes of 50–200 μl. Finger-puncture sampling can be performed by semi-skilled field workers after minimal training, with little discomfort or risk to the participant. Capillary blood sampling is also more readily accepted (low sample volume) than venous blood sampling by people in developing countries. In addition, a technique with dried blood on filter papers reduces the need to provide facilities for separating and storing blood samples in the field. However, all contamination on the filter paper must be avoided. In developing countries, antimalarial tablets are often handled by the person who draws blood samples and there is therefore an appreciable risk of contaminating the filter paper [17].

2.1. Extraction of the antimalarial drug from a biological matrix

Extraction studies describing in detail the distribution properties of the antimalarial drug are not common. Extraction studies should be performed to determine the best extraction conditions with regard to solvent, phase volume ratio and extraction type (base or acid versus ion-pair extraction) to provide high recovery and yet avoid interference from endogenous compounds.

Schill et al. [18] described an approach for extraction studies of different drugs that would be most valuable in designing bioanalytical methods for antimalarial drugs. Extraction conditions for antimalarials have been studied for CQ and metabolites [19] and for pyrimethamine (PYR) and SU [20] and recently for MQ and its metabolite [21].

Difficulties arise when different antimalarials are used in a combination drug. For example, Fansidar is composed of PYR and SU. PYR is a base and SU is an

acid, and the concentration ratio between these drugs in plasma is high. MQ is also a base and its metabolite is an acid. Ordinary base- or acid-extraction procedures cannot be used for the simultaneous extraction of the acids and bases before the chromatographic determination.

With the increased demand for analyses and the development of automation and data processing, sample preparation has now become the limiting step in analysing antimalarial drugs. Liquid–solid extraction in small disposable columns as an alternative to solvent extraction has been used [22]. Protein precipitation before injection on to the HPLC column has been exemplified for Q [11], CQ [23] and MQ [24]. On-line column switching procedures, which enable drug concentrations in biological fluids to be assayed by direct injection of plasma samples, have recently been reported for MQ and its carboxylic acid metabolite [25].

2.2. *Preparation of calibration graphs*

An important aspect of quantitative methodologies that is not considered is the method for preparing standard solutions. The use of aqueous solutions rather than blank plasma for antimalarial standards should be avoided; they may give incorrect results because aqueous standards may give low recoveries compared with plasma standards. This has been demonstrated for CQ determination [5]. The recovery from aqueous solutions is only 70–80%, but addition of plasma to 50% improves the recovery to 90%. Even though the recovery of CQ from aqueous solutions in glass tubes is low, adding blood to the glass tubes increases the recovery [3].

2.3. *Validation*

Methods of determining antimalarials must be repeatedly controlled and validated. This should be accomplished by analysing control samples with known concentrations together with patients' samples and by calculating inaccuracy and imprecision, as measures of analytical quality in daily work. Precise measurements depend largely on the proper use of good laboratory practices. These include the consistent use of standard operating procedures and the establishment of and adherence to carefully designed protocols for specific analytical processes. Good laboratory practice also includes the consistent use of qualified and closely supervised personnel, reliable and well maintained equipment and appropriate calibrations and standards.

Every analytical system contains sources of inaccuracy and imprecision that have variable components, so a strategy to minimize errors is needed. More information on bioanalytical aspects of method validation for determination of drug concentrations in pharmacokinetic and therapeutic drug monitoring is given in refs. 26 and 27.

Metabolites of the drug must not interfere with the method. Samples taken from patients on medication are preferred for interference studies in comparison

to blank body fluid samples, to which the synthesized metabolite is added in vitro. Conditions for sampling, transport and storage must also be stated.

Standardization of analytical methods used for antimalarial drugs is essential, as analytical results must be comparable and independent of the laboratory where the analyses were performed.

3. CHLOROQUINE

CQ binds to blood cells [5], and all blood cells must be removed from plasma with a high centrifugal force if plasma concentrations are to be determined [5,8]. Hence whole blood may be preferable to plasma for the determination of CQ. There is also the advantage that concentrations in whole blood are higher than those in the corresponding plasma, so that analysis of the former yields more precise and accurate values at low whole blood concentrations. Plasma concentrations do not directly reflect how much of the drug (CQ and CQM) is bound to blood cells [5].

Methods for determining CQ and CQM in whole blood should have a limit of determination of 50 nmol/l or less; methods for determining CQ and its metabolites in urine should have a limit of determination of 5 μmol/l or less.

3.1. Field assays in urine and whole blood

Simple tests for determining CQ in urine have been used to document both drug absorption and compliance failures. Three qualitative urine tests have been widely used, the Haskins [28], the Wilson–Edeson [29] and the Dill–Glazko tests [30]. All urine samples after a single CQ dose of 5 mg/kg body weight were positive for at least ten days with the Haskins test and for at least three days with the Wilson–Edeson test. The Dill–Glazko test was only positive on the first day after a dose of 10 mg/kg body weight [31]; this test is unsuitable for field use.

Several colorimetric tests have been developed recently for quantitating CQ and metabolites in the field [32,33]. The bromthymol blue (BTB) test quantifies CQ and metabolites based on ion-pair extraction into dichloromethane to give a yellow colour proportional to CQ+metabolite concentration up to 400 μmol/l [32]. A single CQ dose of 5 mg/kg yielded detectable levels in urine for at least eight days. Analysis with the BTB test of urine samples from patients taking 150 mg of CQ base per day compared with the sum of the urine concentrations of CQ and CQM determined by HPLC gave a correlation coefficient of 0.95. The limit of determination was about 10 μmol/l using 1 ml of urine and the coefficient of variation (C.V.) was about 5–10% for the range 10–400 μmol/l. The method codetermines the main metabolites of CQ and the other antimalarial agents Q and proguanil (PO), if present.

The Haskins test has been modified to permit the quantitation of CQ and its metabolites in the field using a hand-held, battery-operated filter photometer [33]. Two modifications were developed, one optimized for sensitivity and the other for simplicity. The latter method (Haskins MMII) shows absorbance values with a hand-held, battery-operated filter photometer to be linearly related to

CQ and metabolites in urine at levels up to 100 μmol/l. The limit of determination is 3 μmol/l using 2 ml of urine. A single dose of chloroquine diphosphate (5 mg/kg as base) permits the quantitation of CQ and its metabolites fourteen days after dosing. Field application of the Haskins MMII modification has been reported [34]. A related thin-layer chromatographic (TLC) method [33] permits the confirmation of the presence of CQ and CQM in urine and allows a semiquantitative determination of the concentration of CQ and CQM by visual comparison of the intensities of the sample spots with those of standard spots.

The urine tests based on ion-pair extraction of CQ (Dill–Glazko, Haskins, modified Haskins and BTB tests) are affected by pH variations in the urine sample and should be buffered. Both the BTB test and the Haskins MMII test are validated by HPLC determinations of CQ and CQM in urine and, additionally, correlate significantly ($r=0.80$) with CQ concentration in whole blood determined by HPLC [32,33].

A simple colorimetric test for phenylcyclidine in urine [35] has been shown to provide values for CQ in urine samples tested in Nigeria that correlate well with Haskins MMII results (Mount, Nahlen, Patchen and Churchill, unpublished results). This method is as easy to run as the Dill–Glazko test but is much more sensitive (2–5 μmol/l) and may replace the latter in routine field testing for CQ in urine.

A high-performance thin-layer chromatographic (HPTLC) method was developed for the determination of CQ and CQM in plasma, whole blood and urine with a limit of determination of 10 nmol/l in a 1-ml sample [36]; the feasibility of adapting HPTLC to CQ assay in the field was discussed [36]. Mount et al. [37] developed a field-adapted HPTLC method that requires no electricity and allows the semiquantitative determination of CQ and CQM with a detection limit of 1 μmol/l in 5 ml of urine. The method has been applied to a survey of drug use practices in Esmeraldes Province, Ecuador [37]. More recently, HPTLC methodology has been developed that permits the determination of CQ in 100-μl amounts of finger puncture capillary blood with a detection limit of 200 nmol/l [38]. The assay uses easily portable supplies and equipment and requires only a building with electricity for its application.

3.2. Non-chromatographic laboratory methods

The first analytical method for CQ was a fluorescence method developed by Brodie et al. [39], but the most cited method is a modification by McChesney and co-workers [40,41]. The limit of determination of these non-chromatographic methods is 30 nmol/l. They are non-selective, as the extraction step does not separate CQ from its main metabolites, which have the same fluorescence characteristics as the parent compound. A comparison [42] between these non-chromatographic methods and a specific HPLC method [43] showed a good correlation with the sum of CQ and CQM for serum, plasma and whole blood.

3.3. *Liquid chromatography*

HPLC methods for the simultaneous determination of CQ and its main metabolite CQM in plasma, whole blood and urine were reported by Bergqvist and Frisk-Holmberg [23]. The limit of determination using 1-ml samples was 10 nmol/l for CQ and CQM with UV detection and 0.5 nmol/l for both analytes with fluorescence detection. The within-day C.V. was 4% at 50 nmol/l for CQ and CQM and the between-day C.V. was 7% at 200 nmol/l for CQ and CQM. Additional validation of the method was provided by comparison with a capillary GC method [44]. A systematic extraction study in combination with the chromatographic behaviour of CQ and CQM was reported [19]. Alván et al. [43] utilized diethylamine in the mobile phase for the normal-phase HPLC quantification of CQ and CQM. By this system and with fluorescence detection the post-column pH adjustment required in ref. 23 could be avoided. In aqueous media CQ shows pH-dependent fluorescence with a maximum near pH 9.1–9.5. The C.V. for the method [43] is similar to that for the method reported in ref. 23, but the limit of determination is higher (2–3 nmol/l). CQ is used as a racemate. An HPLC method for separating the two enantiomers of CQ and those of CQM was presented in a study of the disposition of the enantiomers in humans [45].

3.4. *Determination after sampling on filter paper*

Patchen et al. [12] developed an HPLC method with fluorescence detection for determining of the concentration of CQ and CQM in a small sample of capillary blood collected on filter paper from a finger puncture. The limit of determination was 16 nmol/l for CQ and CQM in 100-μl samples and the C.V. was less than 5% for both CQ and CQM. No loss of CQ and CQM occurred from filter paper-collected blood spots stored over a twelve-week period at room temperature. Another filter paper HPLC method by Lindström et al. [13] was reported. The C.V. of this method, using 75 μl of capillary whole blood eluted from filter paper at 40 nmol/l, was 5% for CQ and 15% for CQM. In both studies the concentrations of CQ and CQM in venous whole blood and in capillary blood samples dried on filter paper correlated very well.

4. QUININE

A method for the assay of Q in plasma during medication should be sensitive enough to cover the therapeutic range of 5–30 μmol/l.

4.1. *Non-chromatographic methods*

There has been a long tradition of monitoring plasma levels of quinidine. Non-chromatographic methods (which quantitate Q and quinidine equally) have been available since 1943 [46]. The most commonly used extraction procedure is the double extraction method of Cramer and Isaksson [47] with fluorescence measurements.

A comparison between the non-chromatographic method [47] and HPLC methods [11] after oral administration of Q to children showed that the non-chromatographic values were on average 37% higher than those obtained by HPLC. This difference is due to the contribution by metabolites of Q in serum when the non-chromatographic method was used.

4.2. Liquid chromatography

Several HPLC methods for determining Q and quinidine have been published during the last ten years. A selective, simple and accurate HPLC method for the assay of Q with fluorescence detection was described by Edstein et al. [11]. The plasma sample (100 μl) is mixed with acetonitrile to precipitate the proteins and a protein-free aliquot of 10 μl is injected into the HPLC system. The limit of determination using 100-μl samples was 100 nmol/l. The within-day and between-day C.V. were 5 and 7%, respectively, at 15 μmol/l. No interferences were found from the common antimalarial drugs. Most of the recent studies of Q disposition have used the non-chromatographic method [48], but in the future HPLC methods should be used since accurate separation and quantitation of metabolites are required. Mihaly et al. [49] recently published an HPLC method that allows both Q and its diastereoisomer quinidine to be determined simultaneously. The limit of determination was 30 nmol/l in 1000 μl of plasma with a within-day C.V. of about 10%.

5. AMODIAQUINE

5.1. Non-chromatographic methods

AQ does not exhibit analytically useful fluorescence. Trenholme et al. [50] found that fluorescence results from heating AQ in an alkaline borate solution. In this method the sample of biological fluid is made alkaline and partitioned with 1,2-dichloroethane, in turn partitioned with 0.1 mol/l hydrochloric acid. Borate buffer is added to the separated acid phase and the solution is heated in boiling water to produce the fluorescence. Churchill et al. [51] showed that the fluorescent compound produced in this procedure is 7-chloro-4-aminoquinoline. Further, as AQ acts as a prodrug [51], most of the fluorescence seen in whole blood and urine samples from orally dosed persons in the assay of Trenholme et al. [50] is produced by hydrolysis of deethylamodiaquine (AQM_1), which also contributes most antimalarial activity [51–55]. The limit of determination is about 0.5 μmol/l with a C.V. of 7.5% at this level and the most appropriate standard for use with the method of Trenholme et al. is deethylamodiaquine [52,53].

5.2. Liquid chromatography

Several methods for assaying AQ and its metabolites have recently been published. The most sensitive and complete assay of AQ and its three known metabolites used HPLC with oxidative electrochemical detection and has a limit of

determination of 3 nmol/l for AQ, AQM_1 and AQM_2 (bideethylamodiaquine) and 10 nmol/l for AQM_3 (2-hydroxydeethylamodiaquine) in whole blood [53]. The methods of Pussard et al. [55] and Winstanley et al. [56] permit the sensitive determination of AQ and AQM_1 using HPLC with UV spectrophotometric detection and suffice for most purposes. The method of Pussard et al. [55] quantifies CQ, CQM, AQ and AQM_1 simultaneously in body fluids with a limit of determination of 10 nmol/l for each of the analytes using 1000 μl of sample with a between-day C.V. of $<8\%$. This method illustrates the simultaneous determination of two commonly used antimalarial drugs and their metabolites.

6. MEFLOQUINE

Mefloquine is a relatively new antimalarial drug effective against chloroquine and multidrug-resistant strains of *Plasmodium falciparum* [57,58]. The main metabolite of MQ is a carboxylic acid derivative (MMQ). Mimica et al. [59] reported steady-state plasma concentrations of MQ and MMQ in five volunteers who received 250 mg of MQ weekly for 21 weeks. Mean plasma concentrations ranged between 1.5 and 2.9 μmol/l for MQ and between 4.8 and 18.0 μmol/l for MMQ. After six months of weekly administration, the elimination half-life of MQ from plasma was found to range from 17 to 35 days [60,61].

6.1. Gas chromatography

Several gas chromatographic (GC) methods [62–65] for the determination of MQ in biological samples, using silyl [62–64], perfluoroacyl [65] or silyl and perfluoroacyl [63] derivatization procedures and either electron-capture or mass spectrometric detection, have been reported. The detection limits for two of the methods described [63,64] are between 2.6 and 26 nmol/l. Carbonic dichloride (phosgene) has been shown recently to react readily with MQ to form a stable cyclic carbamate structure that eliminates the polar hydroxy and secondary aliphatic amine functional groups in MQ [66]. A practical application of the phosgene derivatization reaction was used in a new method [67] to produce derivatives of both MQ and an analogue internal standard, which have excellent GC properties and are sensitive to electron-capture detection. By this method MQ concentrations down to 25 nmol/l are determined in 100-μl plasma or whole blood samples with a within-day C.V. of 12% at the 25 nmol/l level.

6.2. Liquid chromatography

HPLC methods [68,69] for determining MQ have been described. The current methods for the simultaneous assay of MQ and MMQ are TLC [60] and HPLC involving direct injection of plasma with precolumn enrichment and column switching techniques [25]. The C.V.'s in plasma at 2–10 μmol/l levels for MQ and MMQ were about 2 and 4%, respectively, with a limit of determination of 25 nmol/l for both substances using 500-μl volumes of plasma or whole blood. In a recently published HPLC method by Bergqvist et al. [24], an ion-pair extraction

is used to permit the simultaneous determination of MQ and MMQ down to 100 nmol/l in 500-μl plasma and whole blood samples using an analogue of MQ as internal standard. The within-day C.V. was 5–6% for MQ and MMQ within the range 1–6 μmol/l in plasma or whole blood; no interference from other antimalarial drugs was seen [21]. This method compared favourably with a GC method [67] for MQ assay in both plasma and whole blood.

6.3. Determination after sampling on filter paper

The first practical application for determining MQ in capillary blood collected on filter paper was published recently [67]. The limit of determination of MQ in 100-μl blood samples collected on filter paper was 100 nmol/l with a C.V. of 12% and the within-day C.V. was 2–3% for the range 0.5–3.0 μmol/l. The concentration of MQ in blood spots on filter paper appears to be stable for at least seven to ten days at room temperature (20–25°C), but at lower temperature (4°C) the stability is at least twenty days. There was close agreement between the results obtained from blood samples collected by venipuncture and corresponding capillary blood samples collected by finger puncture [67].

7. PROGUANIL

Little information has been published concerning the concentrations in blood of PO and its active metabolite cycloguanil resulting from 100 and 200 mg per day regimens. Bygbjerg et al. [70] measured concentrations of PO, cycloguanil and *p*-chlorophenyl biguanide in plasma from five volunteers administered 200 mg of Paludrine daily for fourteen days. The trough concentrations (predose in the morning) of PO and cycloguanil were about 200 and 100 nmol/l, respectively. The active metabolite cycloguanil comprised about 30% of the total plasma drug concentration.

7.1. Liquid chromatography

Several selective and sensitive HPLC methods [22, 70–73] for the simultaneous determination of PO and its metabolite cycloguanil in body fluids have been developed recently. One such method [22] exploits solid-phase extraction in small disposable columns, which has the advantage of rapid and simple sample throughput. To achieve the HPLC separation of PO, cycloguanil and 4-chlorophenyl biguanide in a small-bore column packed with 3-μm particles, an ion-pair system was used. The precision at the 400 nmol/l level was estimated to be about 7% for PO and 10–16% for the metabolites. The detection limit was reported to be 2–4 nmol/l for the compounds, although no data on precision at this level were given. Urine samples can also be assayed by this method.

8. PYRIMETHAMINE

PYR is mostly used in combination with other antimalarial drugs, as in PYR–SU or PYR–SU–MQ preparations. The level of PYR determined in plasma is in the range 200–600 nmol/l.

8.1. Non-chromatographic methods

One report is available on the determination of PYR by non-chromatographic methods. PYR has a weak native fluorescence, but a fluorescent derivative may be used for PYR determination. Idowu and Dada [74] described a simple, sensitive and selective method for quantifying PYR in plasma based on its reaction with chloroacetaldehyde. No interference from SU, MQ and Q were reported. The recovery of PYR from plasma was 94% and the limit of determination using 1 ml of plasma was 35 nmol/l.

8.2. Liquid chromatography

Three HPLC methods for the simultaneous determination of PYR and SU in plasma have been published [20,75,76]. The method of Bergqvist and Eriksson [20] is the most sensitive, has a simple extraction procedure and permits the simultaneous determination of SU. The within-day C.V. of this method was 5–10% for PYR within the range 50–1000 nmol/l in plasma. No interference from other antimalarial drugs was reported.

9. SULPHADOXINE

9.1. Urine test

A modification of the Bratton–Marshall technique [77] for a urine test for SU was presented [78], which can be carried out in a simple field laboratory. The method could be used down to a SU concentration of 16 μmol/l in urine. The test gives positive results for arylamines and for other drugs that carry an aromatic primary amino group or are metabolized to such compounds.

9.2. Liquid chromatography

Bergqvist and Eriksson [20] reported a simple and selective HPLC method for the simultaneous determination of SU and PYR in plasma. No interference from other antimalarial drugs was observed. The acetylsulphadoxine metabolite could be separated and quantified. The limit of determination was 5 μmol/l using 1-ml samples with a C.V. of 3–6% in the concentration range 5–1000 μmol/l. Whole blood, plasma and urine can be assayed by this method. Two other methods have been reported for the selective and sensitive quantification of SU, the N^4-acetyl metabolite of SU, and PYR in body fluids [75,76], as indicated above.

9.3. Determination after sampling on filter paper

An HPLC method for determining SU in whole blood obtained by finger puncture and dried on filter paper has been published [9]. The technique was validated by comparing the SU concentration in simultaneously collected capillary blood, dried on filter paper, and conventional venous whole blood samples ($r = 0.99$). Using 100 µl of capillary blood the method has a limit of determination of 25 µmol/l, with a precision of 3–5% in the concentration range 50–400 µmol/l. SU was stable on the dried filter papers for at least fifteen weeks at 37°C.

10. PRIMAQUINE

Ward et al. [79] reported that administration of 15 mg of PQ daily for fourteen days resulted in plasma concentrations ranging from 135 to 413 nmol/l for PQ and from 2040 to 4967 nmol/l for the carboxylic acid metabolite of PQ.

10.1. Urine test

A colorimetric method of screening urine samples in field work for PQ metabolites has been reported [80]. The extraction of PQ metabolites in urine is performed in a C_{18} extraction cartridge. The metabolites are then rendered visible by reaction with a commercially available diazonium salt. The limit of determination using a 5-ml sample of urine is 310 nmol/l and the method is selective for acidic or neutral metabolites of PQ [80]. This method seems simple, but more studies with samples of human urine after therapeutic doses are needed, as most of the results were obtained from analyses of rat urine.

10.2. Liquid chromatography

Several HPLC methods have been reported for determining PQ in biological fluids. Ward et al. [81] developed a rapid and simple HPLC method for use in pharmacokinetic studies. The carboxylated main metabolites of PQ did not interfere, nor did CQ or PYR. The method could be used in the concentration range 4–770 nmol/l, with within-day and between-day C.V. for spiked plasma of 8.7 and 5% at 97 nmol/l and 4.2 and 2.7% at 385 nmol/l, respectively. No data were reported on the assay of whole blood by this method.

Mihaly et al. [82] reported an HPLC method for the simultaneous determination of the carboxylic acid and N-acetyl derivatives of PQ in plasma and urine. The method has a limit of determination of 274 nmol/l for carboxy-PQ and 32 nmol/l for N-acetyl-PQ. The within-day and between-day C.V. for the carboxy-PQ were 2.9 and 5.0%, respectively. There was no interference reported from PQ, nor from the commonly used antimalarials CQ and PYR.

11. CONCLUSIONS

Reference methods that have a chromatographic step to ensure sufficient selectivity must be available for the determination of each antimalarial drug and its active metabolites. Inclusion of a sensitive and selective means of detection, such as electron-capture or mass spectrometric detection for GC and electrochemical or fluorescence detection for HPLC, further enhances method selectivity. It is highly desirable that the various steps of reference and routine methods be carefully investigated. Studies to optimize sample preparation steps, such as extraction and separation, should be methodically undertaken. Conditions chosen for the various steps should be characterized, including the determination of absolute recoveries and values for the separation parameters. The overall method performance in terms of imprecision and inaccuracy should be evaluated statistically and validated by comparison with existing methods. Good routines for laboratory quality control and adherance to good laboratory practices in the day-to-day analysis of samples should be followed. Final interpretation of validation data from the evaluation of analytical methods should consider pre-instrumental variations from the collection of biological samples, sample transport and storage stability before the introduction of the sample into the analytical systems. Variations in these steps can have significant effects on the results.

Recently there has been considerable interest in developing field methods for the assay of antimalarial drugs. Much of this work has centred on CQ and its metabolites, and there is need for such methods for other antimalarials. Recently developed field-adapted procedures have included colorimetric [32,33] and TLC [37,38] methods. Field adapted methods for assay of CQ and metabolites in blood and urine have been reviewed [83]. The methods for the analysis of filter paper-adsorbed, finger puncture blood [9,12,13,16] may be characterized as field-interfaced, as they facilitate the acquisition and transport of blood samples from the field to the laboratory. Laboratory reference methods validate these procedures and will also validate other approaches, such as the various immunoanalytical procedures that can be applied to assays of antimalarial drugs and their metabolites.

With the advent of additional multiple drug regimens for chemoprophylaxis (CQ+PO) or chemotherapy (MQ–SU–PYR) of malaria, there is need for methods that can quantify several drugs and their active metabolites simultaneously.

12. SUMMARY

This review of methods for determining antimalarial drugs in biological fluids has focused on the various analytical techniques for the assay of chloroquine, quinine, amodiaquine, mefloquine, proguanil, pyrimethamine, sulphadoxine, primaquine and some of their metabolites. The methods for determining antimalarials and their metabolites in biological samples have changed rapidly during the last eight to ten years with the increased use of chromatographic techniques. Chloroquine is still the most used antimalarial drug, and various methods of different complexity exist for the determination of chloroquine and its metabolites

in biological fluids. The pharmacokinetics of chloroquine and other antimalarials have been updated using these new methods.

The various analytical techniques have been discussed, from simple colorimetric methods of intermediate selectivity and sensitivity to highly sophisticated, selective and sensitive chromatographic methods applied in a modern analytical laboratory. Knowledge concerning the method for a particular study is determined by the type of application and the facilities, equipment and personnel available. Often is it useful to apply various methods when conducting a clinical study in malaria-endemic areas. Field-adapted methods for the analysis of urine samples can be applied at the study site for screening, and corresponding blood samples can be preserved for subsequent analysis in the laboratory. Selecting samples for laboratory analysis is based on clinical, parasitological and field-assay data. The wide array of methods available for chloroquine permit carefully tailored approaches to acquire the necessary analytical information in clinical field studies concerning the use of this drug. The development of additional field-adapted and field-interfaced methods for other commonly used antimalarials will provide similar flexibility in field studies of these drugs.

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