Review Article

Bioanalytical Methods for Adducing Pharmacokinetic Profiles of Antimalarial Drugs Used in Africa: A Review of Progress, Pitfalls and Ways Forward

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Antimalarial drugs have been used over the years to control the impact of malaria infection. They sometimes suffer from inefficacy, suboptimal dose administration, and/or parasite resistance. Monitoring antimalarial drug concentrations and their metabolites in biological matrices becomes imperative to elucidate any case of inefficacy, inadequate drug concentration, suboptimal dose, or drug-resistant parasites. The present study conducted a critical assessment and comparison of trends and bioanalytical methods used for estimating the pharmacokinetic profiles of antimalarial drugs used in Africa between 1985 and 2021. Findings indicated random procedural inadequacies, inconsistencies, poor compliance to standards and protocols and poor documentation approaches, all of which were capable of resulting in false or misleading interpretations of results. Suggestions on how to improve method performance, why and how to select a particular method for a specific matrix, results reporting and other important standard procedural ethics on the subject are highlighted. Africans and Africa as a continent must step up and intensify research efforts that capture and accommodate African peculiarities and economic well-being. This review presents the current state of the bioanalytical approaches to antimalarial drugs and calls for an adjustment to tackle antimalarial drug treatment failure *ab initio*.

Key words: Antimalarial drugs, bioanalytical methods, malaria, method validation, pharmacokinetics

Several reports on malaria studies since the discovery of the disease to date indicate the need for new drugs, even though significant progress is recorded in different ways. This is due to the recurrent parasite resistance to available drugs[1,2]. Resistance is primarily seen in *Plasmodium falciparum* (*P. falciparum*), which is described as the most virulent of the malaria parasites in humans and a major contributor to severe and fatal cases[3,4], especially in Africa. *Plasmodium vivax*, the most geographically widespread of the *Plasmodium* species, produces less severe symptoms[5]. *Plasmodium malariae* infections produces not only typical malaria symptoms but also can persist in the blood for possibly decades without ever producing symptoms[6-8]. *Plasmodium malariae*, *Plasmodium ovale curtisi* and *Plasmodium ovale wallikeri* are understudied[9,10]. They elicit a similar magnitude of illness comparable to malaria caused by *Plasmodium vivax*[5]. Southeast Asia is affected by the zoonotic infection associated with *Plasmodium knowlesi*[5], while Africa mostly suffers from *P. falciparum*. More than 120 *Plasmodium* species infects mammals, birds and reptiles, among them only six are known to infect humans[5], but four dominate and are more pronounced, with *P. falciparum* being the most studied. Interactions between malaria parasites and human hosts have been captured by Aminake *et al.*[11], Josling *et al.*[12], and the center for disease control and prevention[13], among others.

With 54 countries and over 1.3 billion people, Africa is the second-largest continent on the globe and presently has the highest malaria burden.

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Several studies have implicated its multiple demographic, geographical, climatic, economic and lifestyle peculiarities among others, for the high malaria incidences[5,4,14-21]. The focus of these studies on the African continent is thus imperative and timely and it calls for continuous efforts until the fight against malaria, an avoidable cause of most mortality and morbidity in Africa[22-26]. There is hope (if efforts are sustainably intensified) for the total eradication of this neglected tropical disease since efforts are yielding significant results. Some of the high-burden communities and some communities previously affected have reported zero malaria cases[27] in recent times. Malaria deaths reduced from about 400 000 to 260 000 from 2010 to 2018, with the largest reduction being in Nigeria (identified as one of the 10 highest burden countries in Africa), from almost 153 000 deaths to 95 000 deaths from 2010 to 2018[28].

Past efforts have utilized the optimisation of therapy with existing agents[22,29-38], the development of analogs of existing agents[39-44], the application of natural product extracts and or their isolates[45-47], repurposed compounds active against other diseases[38,48-50], drug resistance reversers[51,52], and the use of compounds active against new targets[41-53] as approaches to effective malaria treatments and control in one part. In another approach, Indoor Residual Spraying (IRS) [18,54-56], the use of Long-Lasting Insecticide-treated Nets (LLIN), larval source management, along with entomological monitoring, advocacy, social mobilisation and information education are used to control malaria. Unfortunately, all approaches now appear slowed down, complicated or neglected with the surge in the novel coronavirus, which shares similar symptoms with malaria[57,58], leading the World Health Organisation (WHO)[59] to jointly address endemic malaria and the Coronavirus Disease 2019 (COVID-19) pandemic to prevent a predicted rise in malaria deaths (769 000 people in sub-Saharan Africa in the year 2020 alone). Individuals and other organizations are also working hard in this regard in line with the third United Nations Sustainable Development Goal (SDG)[60].

When a patient is co-infected with malaria and COVID-19 or any other infectious disease with similar symptoms, there is a high chance of an incomplete diagnosis, which increases the risk of mortality. A confirmatory laboratory test for both (all suspected) conditions becomes unavoidable to provide the best treatment options. Antimalarial drug performance in such cases is easily altered, presenting another cause for its pharmacokinetics profiling[61]. In most cases, studies of pharmacokinetic parameters often have inadequate power to define and compare the optimal dosage due to differences in assay[62], analytical methods (the focus of this study), and conditions during the investigation and clinical protocols such as eligibility criteria, standardization of diet, age, sex, genotypic and phenotypic factors, exercise, geographical factors, presence of other drugs, prevailing (diagnosed and undiagnosed) disease conditions, comparison groups, the source and quality of the drug and the use of different biological matrices[62]. Some of these factors can be addressed by method standardisation[63,64] and the introduction of quality control and quality assurance systems.

The present effort discussed how and why the selection or choice of a particular bioanalytical method and biological matrix was made. For unbiased comparison of similar or different antimalarial agents, the same matrix (where applicable) with similar experimental conditions must be used for drug concentrations in different studies or different patients. Monitoring the antimalarial drug concentrations and their metabolites (for antimalarial pharmacokinetic studies) in biological matrices remains a foundational or grass-roots solution to the identified malaria treatment challenges. Thus, it becomes imperative and unavoidable to elucidate any case of drug-resistant parasites or therapeutic failure due to inadequate drug concentration, drug interactions or efficacy. In this review, updates, trends and facts on bioanalytical methods adopted in determining the pharmacokinetic parameters of antimalarial drugs used in Africa between 1985-2021 have been discussed. A comparative assessment of the adopted methods and procedural loopholes or deficiencies is also highlighted, with a bias for the methods adopted in the antimalarial pharmacokinetic study in humans.

METHODOLOGY

We considered articles indexed in Google, Google Scholar, PubMed, PubChem and SciFinder before December 2021. The search terms included but were not limited to malarial incidences in Africa,

bioanalytical sample preparation, antimalarial drugs, malaria, antimalarial drugs used in Africa, method validation, bioanalytical methods in pharmacokinetics, pharmacokinetics and analytical methods for pharmacokinetic parameters of a specific antimalarial drug. Over 6191 articles were generated and closely related ones totaling 168 were evaluated and reviewed for this contribution. We focus on the bioanalytical methods adopted in the human antimalarial pharmacokinetic study with a bias for Africa.

Antimalarial drugs and drug combinations used in Africa:

Artesunate and artemether are the most widely used (oral) artemisinin derivatives[65,66], and their most adopted combinations in Africa for the treatment of uncomplicated *P. falciparum* malaria are artemether-lumefantrine and amodiaquine-artesunate with excellent efficacies[67-71]. Other combination therapies commonly used in Africa are sulphadoxine-pyrimethamine and dihydroartemisinin-piperaquine. Piperaquine was discovered by Chinese scientists as a suitable compound for combination with an artemisinin derivative[72]. Both artesunate and artemether are rapidly converted to the active metabolite dihydroartemisinin by Cytochrome P450 (CYP) enzymes[67], with slight variations in their activities and dihydroartemisinin as the most active of them[67]. Suputtamongkol *et al.*[66] confirmed that the antimalarial activities of oral artesunate compared to artemether were greater with better bioavailability, despite the 29 % lower dose (in molar terms) used for artesunate during their comparative study. Artesunate is the water-soluble sodium hemisuccinyl ester, while artemether is the lipid-soluble methyl ether of dihydroartemisinin[66,73,74]. Amodiaquine is converted *via* CYP enzymes to the active metabolite desethylamodiaquine[75,76] and shares some properties with its structurally similar counterpart, quinine[77]. Quinine belongs to the *cinchona* alkaloids[78], which are widely present in the *Cinchona* genus of the family *Rubiaceae*. *Cinchona* alkaloids have also been reported in the *cinchona*-related genera *Remijia pedunculata*[79] and *Ligustrum vulgare* L.[80]. Other members of this group include quinidine, cinchonidine and cinchonine as the major members. Quinine and quinidine are diastereoisomers, as are cinchonidine and cinchonine, both pairs at C-3, C-8 and C-9 and are often called pseudo-enantiomers[81-83]. Several attempts to classify antimalarial drugs have been observed in the literature in recent times[84-86].

Quinine, chloroquine, amodiaquine, piperaquine, primaquine, pyrimethamine, artesunate, artemether, doxycycline, clindamycin, lumefantrine, dihydroartemisinin and sulfadoxine are the most widely adopted antimalarials (as single or combination therapy) by the African countries. Of all these, the artemisinin-based combinations that use artemisinin derivatives (short-acting) in combination with one or more complementary compounds (long-acting and possessing different mechanisms of action) are reported to be the most effective for *P. falciparum* in present times[24,74,87-89]. Most antimalarial drugs are nitrogen-containing compounds with aromatic rings and sometimes with halogens, with the exception of the sesquiterpene lactone group (fig. 1), which represents the most effective of the antimalarials with less report on parasite resistance and a unique mode of action. Thomas *et al.*[90], among others has concisely documented the influence of the removal, introduction or presence of substituent groups in bioactive molecules; these could be synthetically taken advantage of and also guide our basic understanding during the analytical procedures for the best choice of method (fig. 1).

Bioanalytical methods for the pharmacokinetic study of antimalarial drugs:

Different approaches have been adopted in the past decades to detect, determine, optimise, and validate the quantification of different antimalarial drugs and metabolites in pharmaceutical and biological samples, as applicable. These include colorimetric field methods[91,92], refractometry[93], spectrophotometric methods[94,95], capillary zone electrophoresis[96], fluorimetric methods[97,98], immunoanalytical methods[91], electrochemical methods[88,99], High-Performance Thin-Layer Chromatography (HPTLC)[100,101], Gas Chromatography (GC), and High-Performance Liquid Chromatography (HPLC)[73,91,92,102,103]. Even though these methods are in some cases simple and affordable, with the innovation in the sophistication of instruments, the improvement of skills and the technological advancements in science over the years, they have been conveniently replaced by newer, more specific, sensitive, selective and faster techniques or their improved versions. Here, recent trends in the methods adopted over the past

35 y for bioanalytical investigations are presented.

Bioanalytical sample collection and handling for the pharmacokinetic study:

Owing to the uniqueness of each biological matrix in composition and complexity, sample collection and preparation should be considered a very sensitive aspect that must be done with utmost care and by a certified expert with ethical approvals. The different properties of the analytes and matrices often influence the best method to adopt in the collection and subsequent analysis. Methods or precaution adopted must guarantee the retention of the sample content, integrity and concentration from the time of collection, storage and analysis with strong correlation or proof for valid surrogates with its *in vivo* concentration[104]. While it is very important to clearly state the source (place of collection) of the analyte, many analytes readily decompose before investigations involving chromatographic separations (during the preparation of the sample solutions, extraction, clean-up, phase transfer or during storage of prepared vials). There are also possibilities of oxidation or reduction, acid or alkali degradation, photodegradation, thermal degradation or any other procedural influences on the sample. Under these circumstances, method development should investigate the integrity of the analyte. Based on the duration taken for the accuracy test, it should be mentioned that how long a sample (before and after extraction) can be stored before the final analysis[105]. Autosampler stability, repeated freeze-thaw cycles, benchtop stability, reinjection stability, wet extract stability and long-term stability approaches to determine the stability of analytes in human plasma samples or processed samples have been reported by Maddela *et al.*[106] during their validation of a method for the analysis of artesunate and amodiaquine before the application of their in-house developed Liquid Chromatography with tandem Mass Spectrometric (LC-MS/MS) method.

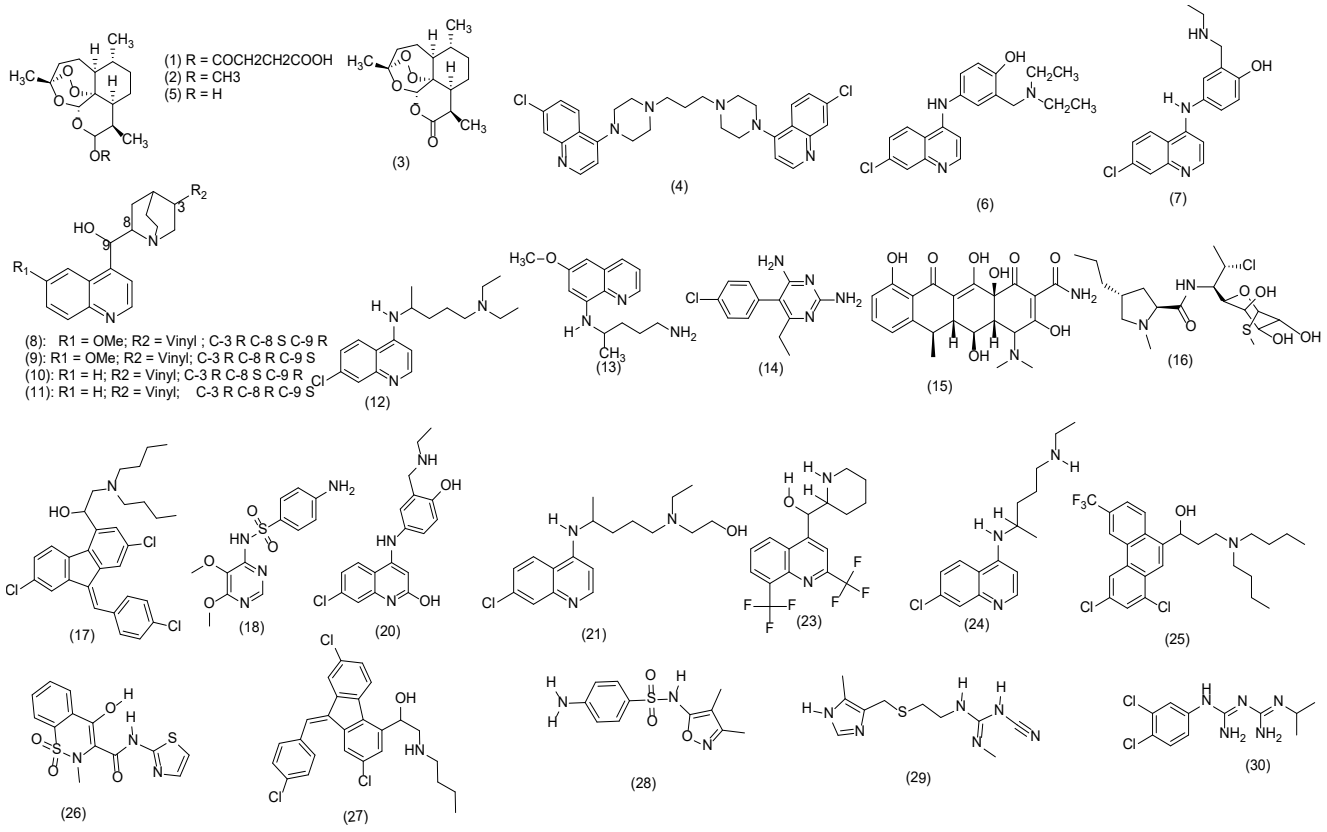

Fig. 1: Structures of some antimalarial drugs and metabolites; (1): Artesunate; (2): Artemether; (3): Artemisinin; (4): Piperaquine; (5): Dihydroartemisinin; (6): Amodiaquine; (7): Desethylamodiaquine; (8): Quinine; (9): Quinidine; (10): Cinchonidine; (11): Cinchonine; (12): Chloroquine; (13): Primaquine; (14): Pyrimethamine; (15): Doxycycline; (16): Clindamycin; (17): Lumefantrine; (18): Sulfadoxine; (19): Bisethylamodiaquine; (20): 2-hydroxydesethylamodiaquine; (21): Hydroxychloroquine; (22): Desethylamodiaquine dihydrochloride; (23): Mefloquine; (24): Desethylchloroquine; (25): Halofantrine; (26): Meloxicam; (27): Desbutyl-lumefantrine; (28): Sulisoxazole; (29): Cimetidine and (30): Chlorproguanil

Extraction or preparation of the antimalarial drug from a biological matrix:

Sample preparation in bioanalytical chemistry could be challenging, especially when it is compound[107] or matrix dependent. It is therefore not out of context to explore and confirm the most appropriate and cost-effective approach to adopt for each analyte in the pharmacokinetic study. Bioanalysis becomes more successful when sample pretreatment, treatment and handling are well-defined. This is due to the complexity of biological matrices, unlike direct pharmaceuticals with controlled composition. To date, the three most reported methods of extraction of analytes in the pharmacokinetic study of antimalarial agents from biological matrices are Solid-Phase Extraction (SPE)[67,106,108-114], Protein Precipitation (PP)[115-120] and Liquid-Liquid Extraction (LLE) [102,119,121,122], though other bioanalytical methods of extraction exit, such as Micro Extraction by Packed Sorbent (MEPS), Salting-out Liquid-Liquid Extraction (SALLE), Stir Bar Sorptive Extraction (SBSE), Restricted Access Material (RAM), Molecularly Imprinted Polymers (MIP), Liquid-Liquid Micro Extraction (LLME), and Solid-Phase Micro Extraction (SPME)[123]. Details on bioanalytical methods have been elaborated in study by Navakova *et al.*[123]. For the simultaneous determination of amodiaquine and artesunate using LC-MS/MS in human plasma, PP can lead to ion suppression despite the sensitivity of LC-MS/MS since this method suffers from unprecipitated plasma components such as lipids, phospholipids and fatty acids[106], among others. Lindegardh *et al.*[124] highlighted major pitfalls in the bioanalytical investigation of artesunate and dihydroartemisinin in human plasma by PP and recommended SPE for their investigation before liquid chromatography. When treatment with PP is done before a chromatographic run, there is an increased tendency for the adsorption of protein on the stationary phase, leading to a loss of column efficiency, an increase in backpressure and subsequently inaccurate quantification.

SPE, compared to PP and LLE procedures (Table 1) has been associated with high recovery, ease of operation, a better quality of extracts, effective sample preparation requiring less organic solvent and a greater possibility of automation[106]. With SPE, amodiaquine and artesunate were found free of significant matrix effects. The reduced efficiency, higher volume of solvent required and its environmental implications when considered in most cases may have deterred most researchers from using LLE, coupled with the fact that in many instances, different batches of the extractions may be required. Often, SPE, PP and LLE have been used in chloroquine analysis[125]. A lot of methods have reported the use of LLE and SPE (Table 2) for quinine and other quinolone derivatives, even though PP can be more suitable for lipophilic compounds. While the "best method" of extraction for a specific antimalarial should be used, it is also important to be conscious of other procedural expectations such as handling, exposure to light, container compatibility, interaction with biological fluids pH and temperature[64] as critical factors that can lead to false estimation (i.e., positive or negative error) of the drug concentration or its degradation products. All in all, a simple method with fewer analytical steps would reduce the tendency to introduce systematic errors (Table 1).

Methods for adducing pharmacokinetic data of an antimalarial drug:

After sample collection and preparation, they are analyzed for the concentration of the analyte (antimalarial) present using different methods. Method choice is mostly influenced by the nature of the matrix, cost, safety, sensitivity, selectivity, robustness, duration, level of precision, and accuracy anticipated from such an analytical procedure. The structure (fig. 1) and chemical properties of the analytes are also important factors to consider. Since the mid-1980s, most analytical methods for biological matrices for currently used antimalarial drugs in Africa have used both normal and reversed-phase chromatography[64,67,102,106,119,120,122,126]. In several studies today, Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) methods are still very relevant and widely reported[121,122,127-129] most times due to the affordability of water, which is the main component of the mobile phase, in place of expensive HPLC grade organic solvents used for normal phase separations, especially in a resource-poor setting, which dominates in Africa. A wide variety of validated LC methods have been used in the past three and a half decades for the determination of antimalarial drug concentration in biological fluids. Recent observations indicate that the assays are becoming more sensitive, allowing for better characterization of the pharmacokinetic

profiles of these drugs and granting sufficient consideration to other parameters highlighted in this review. Despite reservations about HPLC, it has the ability to generate signals free from interference[130] and is the preferred technique for the determination of antimalarials in biological matrices (Table 2). It is also superior to GC for polar and non-volatile drugs[64,91,131]. However, GC has advantages for certain antimalarial drugs that are non-polar and volatile, but also for polar analytes that can be derivatized[64,91].

Apart from technological advancement in instrumentation or the availability of expertise, the procedure and validation of instrument performance and method are critical. Khuda *et al.*[121] reported an isocratic RP-HPLC with Ultraviolet (UV) detection and applied it for adducing the pharmacokinetic profile of lumefantrine. This method had significantly lower sensitivity relative to other reports in the literature[118,132]. However, there was also no mention of the validation guidelines used in developing the method. This made the revalidation and applicability of the method outside the originating laboratory difficult and reduced the confidence and adaptability of the method for official purposes. The works of Choemang *et al.*[128], Mwesigwa *et al.*[67], Mount *et al.*[102], Chen *et al.*[115] and Arun *et al.*[133] also suffer from this procedural oversight. Some methods reported in the literature were done without the inclusion of internal standards as prescribed by many regulatory guidelines[83,134]. The recommendation that must be encouraged as standard practice is that bioanalytical methods should adopt the use of internal standards, possibly one per analyte[62]. Although the Food and Drug Administration guidelines[135] prescribe conditions under which a method should be partially, fully, or cross-validated, it is important to specify whether a method has been validated or just meets a selected set of analytical performance characteristics. Additionally, methods that have been applied for pharmacokinetic profiling of malaria drugs are rarely indicated to have been validated in many research publications. It is important to encourage researchers to implement regulatory, academic and industrial research-specific guidelines. This is especially true in method development to facilitate simplified cross-validation, promote confidence in the quality of research and ease the adoption of academic research findings in industry and regulatory settings.

Hodel *et al.*[117] developed for the first time a broad-range (LC-MS/MS) assay for the simultaneous quantitation of 14 antimalarial drugs currently in use in Africa and their metabolites in human plasma. Similar efforts have been reported by Taylor *et al.*[136] for 11 antimalarial agents in one assay. Artemisinin derivatives (alongside their slow-acting counterpart) in combination or single therapy seem to have confined most reports and dominated most LC methods[73,137-139] of antimalarial drugs in human biological fluid investigations. Mount *et al.*[102] compared the sensitivity of HPLC-ED and HPLC-UV (at 340 nm) for the determination of amodiaquine and its metabolites and found HPLC-ED to be a better option. Amodiaquine, desethylamodiaquine, bisethylamodiaquine and 2-hydroxydesethylamodiaquine had Limit of Detection (LOD) of 15, 10, 20, and 100 ng/ml, respectively, compared to 1 ng/ml for amodiaquine, desethylamodiaquine and bisethylamodiaquine[102]. LOD for 2-hydroxydesethylamodiaquine was 3 ng/ml using HPLC-ED. Both LC-MS/MS and IP-LC-MS/MS had good sensitivities and were the best options for the analysis of amodiaquine (Table 2) in biological matrices. Dry Blood Spot (DBS) innovation offers the advantage of handling dried whole blood in a much safer way than liquid blood[122], especially in resource-poor facilities, as it is in most cases in Africa. HPLC-ED is comparably affordable and sensitive for pharmacokinetic profiling studies of drugs[110], while LC-MS/MS is the most sensitive of them all for antimalarial bioanalysis. Table 2 summarises analytical methods for adducing pharmacokinetic data of antimalarial drugs (and some metabolites) used in Africa, from which we selected examples for our discussion[140-152].

In this work, a variety of LC methods used for the separation of antimalarial drugs in biological matrices were uncovered. From literature reports, LC-MS/MS gave the most sensitive results with low LOD, Lower Limits of Quantification (LLOQ), shorter run times, and consequently much higher throughputs. GC is best suited for the separation of volatile compounds, while HPLC is suited for the separation of non-volatile compounds. This made it slightly difficult to critically compare the superiority of one of these separation methods over the other since they both function very effectively under different conditions.

TABLE 1: COMPARISON OF TRADITIONAL SAMPLE PREPARATION METHODS[62,106,107,123,125]

Methods of extraction	Advantages	Disadvantages
LLE	Ease of automation	A large sample volume is used
	Relatively selective	Toxic organic solvent/environmental hazard
	Good recovery for most analytes	High solvent consumption
	Low cost	Takes a longer time to complete.
	It takes approximately 15-25 min	Incompatibility with hydrophilic samples
	Produces cleaner samples compared to PP	Emulsion formation
	Conveniently applied to LC-UV and LC-MS	Relatively expensive and difficult to automate
PP	Cost-effective	Contamination from an unprecipitated plasma component (protein residue)
	Relatively faster	Poor selectivity
	It takes less than or around 10 min	May increase the possibility of backpressure on the HPLC system
	It generates the least organic waste	Solvent consumption is high
	Can be used in aqueous and organic sample preparation	A highly selective analyte detector is required
		This is Less applied with LC-UV methods due to possible contaminations but more applied with LC-MS/MS due to the sensitivity of the latter
SPE	High recovery	Time consuming and cost-ineffective
	Better sensitivity	Poor reproducibility
	Relatively selective	Relatively high solvent consumption
	More efficient	
	Produces contaminant-free samples	
	Effective pre-concentration	
	Better solvent economy	
	It takes about 15 to 25 min	
	Operation-friendly with high promise for automation	

TABLE 2: SELECTED ANALYTICAL DETAILS OF ANTIMALARIAL DRUGS (AND SOME METABOLITES) COMMONLY USED IN AFRICA

Antimalarial drug	Internal standard	Extraction method	Biological matrix	Analytical method (s)	LLOQ (ng/ml)	RGV	References
Amodiaquine	Hydroxychloroquine	PP	Plasmas	LC-MS/MS	1	NS	[119,120]
	Hydroxychloroquine	PP	blood	IP-LC–MS/MS	0.15	NS	[115]
	Isobutyl analog of desethylamodiaquine dihydrochloride	LLE	Whole blood	HPLC-ED/ HPLC-UV	1/15	NS	[102]
	Quinidine base	LLE	Whole blood	RP-HPLC-UV	5	NS	[122]
	Amodiaquine (6) d10 (isotope-labeled)	SPE	Plasmas	LC-MS/MS	0.31	US FDA and EMA	[106]

Desethyl amodiaquine	Hydroxychloroquine	LLE	Plasmas	LC-MS/MS	2	NS	[119]
	Isobutyl analog of desethylamodiaquine dihydrochloride			HPLC-ED/ HPLC-UV	1/10	NS	[102]
	Quinidine base	LLE	Whole blood	RP-HPLC-UV	10	NS	[122]
	Hydroxychloroquine		blood	IP-LC–MS/MS	1.50	NS	[115]
Bisdesethyl amodiaquine	Isobutyl analog of desethylamodiaquine dihydrochloride	LLE	Whole blood	HPLC-ED/ HPLC-UV	1/20	NS	[102]
2-Hydroxydesethyl amodiaquine	Isobutyl analog of desethylamodiaquine dihydrochloride	LLE	Whole blood	HPLC-ED/ HPLC-UV	3/100	NS	[102]
Artesunate	Stable isotope-labeled artesunate	SPE	Plasma	LC-MS/MS	1.2	NS	[67]
	Artesunate d4 (isotope-labeled)		Plasma	LC-MS/MS	3.11	US FDA and EMA	[106]
Artesunate	Artemether	BTD	NA	HPLC-UV	0.0523	ICH	[126]
Artemether		SPE	Plasma	LC-MS/MS	1.43	NS	[67]
	Artemisinin	SPE	Plasma	GC–MS–SIM	*0.1	NS	[140]
	Artemisinin	LLE	Plasma	HPLC-MS/MS	5	US FDA	[141]
	mefloquine	PP	Plasma	HPLC–ESI-MS/ MS	10	US FDA	[118]
	Artesunate		Plasma	HPLC–ESI-MS/ MS	10	US FDA	[142]
Dihydroartemisinin	Stable isotope-labeled dihydroartemisinin	SPE	Plasma	LC-MS/MS	2.0	NS	[67]
Dihydroartemisinin	Mefloquine	PP	Plasma	HPLC–ESI-MS/ MS	10	US FDA	[118]
Artemisinin	Artesunate	SPE	Plasma	LC-MS/MS	1.03	US FDA	[143]
Chloroquine	Quinine	SPE	Whole blood and plasma	HPLC-DAD	NS	US-FDA	[144]
	Chloroquine-D4-diphosphate salt	SPE	DBS/plasma/ whole blood	IP-HPLC-UV	1.82/1.41/2.56	US-FDA	[145]
Desethyl chloroquine	Desethylchloroquine-D4	SPE	DBS/plasma/ whole blood	IP-HPLC-UV	2.95/1.41/3.36	US-FDA	[145]
Lumefantrine (benflumetol)	Hexyl analog of desbutyl-lumefantrine		Plasma	LC-MS/MS	25	NS	[67]
	Halofantrine	SPE	Plasma	LC-MS/MS	2	US FDA	[132]
	Mefloquine	PP	Plasma	HPLC–ESI-MS/ MS	5	US FDA	[118]
	Artesunate		Plasma	HPLC–ESI-MS/ MS	10	US FDA	[142]
	Meloxicam	LLE	Plasma	RP-HPLC–UV	18	NS	[121]
	(9Z)-2,7-Dichloro-9-[(4-chlorophenyl) methylene]-α-[(hexylamino)methyl]-9H-fluorene-4-methanol	PP	Plasma	HPLC-UV	25	US FDA / ICH	[139]
Desbutyl-lumefantrine	Mefloquine	PP	Plasma	HPLC–ESI-MS/ MS	5	US FDA	[118]

Mefloquine	Chlorpromazine hydrochloride	SPE	Plasma	RP-HPLC-UV	50	*US FDA	[146]
Piperaquine	Mefloquine	LLE	Plasma	RP-HPLC-UV	10	NS	[128]
	Deuterated piperaquine	SPE	Plasma	LC-MS/MS	1.5	US-FDA	[147]
Primaquine	Primaquine diphosphate	LLE	Plasma	HPLC-DAD	-	EMA/ US FDA	[148]
Sulphadoxine	Sulisoxazole	-	Whole blood	RP-HPLC-UV	*120	NS	[127]
Doxycycline	Cimetidine	PP	Plasma (rat)	HPLC-MS/MS	5	FDA	[149]
Quinine	Primaquine	-	Plasma, saliva, urine	RP-IP-HPLC-UV	*10	NRG	[150]
	Cinchonine	LLE	Plasma, whole blood, and erythrocytes	RP-HPLC-Fluorescence	*40	NRG	[82]
	Chlorproguanil	LLE /SPE	Plasma and whole blood on filter paper	RP-HPLC-UV/ Fluorescence	-	NRG	[151]
	Cimetidine	PP	Plasma (rat)	HPLC-MS/MS	5	FDA	[149]
	Hydroxychloroquine	SPE	Plasma, erythrocytes, and urine	HPLC-Fluorescence	*23	NRG	[152]

Note: PP: Protein Precipitation, SPE: Solid-Phase Extraction, LLE: Liquid-Liquid Extraction, BTD: Bulk and Tablet Dosage form, FDCT: Fixed-Dose Combination Tablets, NA: Not Applicable, NS: Not Stated, RGV: Regulatory Guidelines on Validation followed, NRG: Non-Regulatory Guidelines, *n: Limit of detection, *US FDA: Guideline not necessarily followed but the result was compared

For the HPLC methods, the type, quality, and handling (including degassing) of the solvent, column type, and choice of mobile phase used are very important to guarantee reproducible results, enhanced sensitivity, stable pump operation, better peak resolution, minimal troubleshooting, a better baseline, and enhanced column condition during each run. In the selection of the method of analysis of choice, the Absorption, Distribution, Metabolism, and Excretion (ADME) properties of the drug are very important because most drugs could be sequestered in higher concentration in one body fluid than the other. For example, the preferential distribution of chloroquine and amodiaquine to the Red Blood Cells (RBC)[62,64,153] reduces their concentrations in any other biological matrix. This underscores the need for a careful selection of a matrix based on the required analytical outcomes. It is recommended that a matrix with the highest concentration of the drug be carefully selected, except where the study is intended for comparison in a specified matrix. In case, a restriction to a particular matrix exists, with a lower level of drug concentration, a very sensitive and applicable method becomes imperative. It is advisable to strike a balance between the choice of method to adopt, the cost-effectiveness and the quality of the analytical outcome, especially in the resource-poor setting in Africa.

Detector devices/internal standard for antimalarial pharmacokinetic study:

Due to lower sensitivity, selectivity and structural issues, UV detection is not adequate for artemether quantitation in biological matrices[133]. When mass spectrometric detection is used in a bioanalytical method, a stable isotope-labeled (with the highest isotope purity and no possibility of isotope exchange reaction) internal standard is recommended for use whenever possible[154]. The recommendation further provides for the use of Stable Isotopically Labeled (SIL) internal standards, which should compensate for matrix effects, but this is not always the case[124,155,156]. This demands careful thought during analytical processes involving SIL internal standards. Little *et al.*[157] identified glycerophosphocholines as a known cause of matrix ionisation effects in LC-MS/MS during the investigation of biological samples and suggested how it could be detected and monitored. Other phospholipids are generally abundant in different biological membranes, but glycerophosphocholines constitute a major component in plasma[158-160]. UV or fluorescent detectors should not be used for artemisinin, its analog and metabolites in biological fluids since they lack UV absorbent

or fluorescent chromophores (fig. 1). Instead, the MS/MS and ED are strongly recommended for artemisinin, its analogs and metabolites. UV and fluorescent detectors have been applied for quinine, piperaquine, amodiaquine, mefloquine and chloroquine (Table 2). MS/MS is the most widely used detector due to its high sensitivity and the ability to simultaneously quantify different analytes in one run with the flexibility of using software to resolve co-eluting peaks. Table 3 gives a comparative assessment of the different detector devices (Table 3).

Bioanalytical method validation/revalidation for antimalarial drugs:

Bioanalytical method validation has been practiced over the years (especially since the 1940s), but it gained better documentation and recognition in the early 1970s and different guidelines have been developed since then. Several analytical methods for the analysis of antimalarial drugs were developed before this time and a few afterward, but they rarely specified the validation guidelines that were employed during the development process[161-164]. Consequently, only a few validation parameters can be cited in published papers written during that period. Analytical method validation is a very important component that ensures the reproducibility and higher quality of results and guarantees the reliability of a method to determine an analyte concentration in a specific biological matrix, such as blood, serum, plasma, urine, or saliva. Important validation parameters that should be presented depending on the matrix and what interferences may be expected and the guidelines followed are selectivity, specificity, sensitivity, linearity, precision (repeatability, intermediate precision, and reproducibility), accuracy, recovery, dilution integrity, stability, LOD and Limit of Quantification (LOQ), Lower Limits of Detection (LLOD) and LLOQ, system suitability, matrix effect, carryover test, run size evaluation, robustness and method ruggedness. For any pharmaceutical industry or regulated laboratory or standard research to conform to and be in compliance with the requirements of Good Clinical Practice (GCP), Good Laboratory Practices (GLP), current Good Manufacturing Practices (cGMP), or the International Organisation for Standardisation (ISO), as the case may be, a validation Standard Operating Procedure (SOP) or policy should be properly documented and archived (to support the verification and qualification process)[165]. Guidelines provided by United States Pharmacopeia (USP), International Council for Harmonisation (ICH)[166], FDA, European Medicines Agency (EMA) and several other organisations[167] provide frameworks for the validation of bioanalytical methods, though with distinct idiosyncrasies. Whichever is adopted based on choice and type of research, due reference should be captured in the final report.

TABLE 3: COMPARISON OF THE MOST FREQUENTLY USED DETECTOR DEVICES[62,133]

Detector devices	Advantages	Disadvantages
UV	Robust	Primarily for analytes with compatible structures (chromophores)
	Cheap	High sample volume is required
	Easy to operate	Interferences from solvents
	Sensitive to the compatible analyte (with high molar absorptivity)	UV-transparent solvents are required
	Can be used for identification and confirmation of purity of analyte	Quantification is restricted to analytes with chromophores
	Can be used to detect compatible unknown metabolites	
Fluorescence	More sensitive than UV	Only for analytes with compatible structure
	Fixed and variable wavelengths can be used	Background interference from other fluorescent substances
	Fluorescent drugs can be detected with high sensitivity	Quenching or matrix effect
MS/MS	High sensitivity	Very expensive

ED	High selectivity	Requires high technical know-how
	High specificity	High maintenance cost
	Used for identification and quantification	Requires a highly controlled environment.
	Small sample volume is required	Prone to matrix effect
	Can be used for identification and quantification of analytes	Large volume of sample is required compared to MS/MS for LC coupled system
	Better sensitivity than UV	Prone to metal leakage from tubing
	Can be conducted in the oxidative and reductive modes	Expensive

According to ICH[166], full validation for chromatographic methods should include selectivity, specificity (if necessary), matrix effect, calibration curve (response function), range (LLOQ to the Upper Limit Of Quantification (ULOQ)), accuracy, precision, carry-over dilution integrity, stability, and reinjection reproducibility. Observations from most academic research involving antimalarial drug studies indicated that partial method validations have been common practice with omissions of the validation guidelines. In cases where a validated method was adopted, partial validation and clear reference to or documentation of the adopted method are strongly recommended. Although there were improvements in adherence and adoption of guidelines in the mid-2000s, compliance was less than 45 % in the present survey, which was not satisfactory. It has been suggested that academic research findings should be reliable enough for adoption by any organisation or agency[64], further raising the need for standardisation and full validation of bioanalytical research methods. The statistical, operational and economic considerations of a validation process or method define the quality of such processes or methods[167]. After all the mathematical and statistical manipulations to build confidence and quality, the operational flexibility for even non-experts with weak technical know-how would promote the developed or adopted method. Adding to the merits of method selection are cost-effectiveness, the safety of materials needed, the ease of online adoption, etc., which define the economic component of the method[167].

Ongas *et al.*[118] demonstrated the selectivity of their method by analysing 6 independent blanks (plasma) from different sources. Each blank sample was tested for interference using the proposed extraction procedure and chromatographic/mass spectrometric conditions and compared to those containing their analyte at LLOQ by the Guidance for Industry-bioanalytical method validation recommended by FDA[168]. A similar approach was adopted by Maddela *et al.*[106] in the determination of amodiaquine and artesunate in human plasma using the ICH guidelines. According to the ICH guidelines, a minimum of 9 measurements over a minimum of 3 concentration levels should be used to assess accuracy over the range of the method. In the same study, Ongas *et al.*[118] assessed the linearity of the calibration curves for artemether, lumefantrine and their metabolites by assaying standard plasma samples at nine concentrations in the range of 5-1500 ng/ml for artemether/dihydroartemisinin and 5-5000 ng/ml for lumefantrine/desbutyl-lumefantrine on 3 consecutive d using the ICH guidelines. According to the ICH, linearity should be established using a minimum of 5 concentrations. The USP, ICH and FDA prescribed different validation parameters as possible requirements. The choice of guidelines to follow should be guided by the requirements of the outcomes. Preferably, a standardised guideline for all regulatory bodies would rapidly aid the advancement of and promote excellence in this area of bioanalysis.

CONCLUSION

The analytical methods used in adducing pharmacokinetic parameters must be treated as important as the pharmacokinetic parameters if we are to clearly and accurately differentiate between treatment failure due to drug inefficacy, suboptimal dose, or parasite resistance to the drug, or possibly avoid all of these from the early stages of the drugs development. The unification of bioanalytical guidelines for antimalarial drugs to synchronise regulatory, academic, and industry requirements

for improved, applicable, reproducible, and reliable analytical outcomes within and outside the originating laboratory would accelerate the fight to end malaria treatment failures. This review provides updates, trends, and gaps in the various approaches. A comparative assessment of the different methods is also highlighted to redirect research options to the most appropriate choice of method and approach. If possibly all procedural lapses are addressed, the chances of finding a permanent solution to the treatment failure of an antimalarial agent will become clearer and more achievable. The present contribution strongly appeals to researchers to redirect efforts toward making their contribution count from the onset of any bioanalytical investigation. Even though Africa is the most malaria-affected continent, most of the research findings originate outside Africa. This is cause for concern, and there is a need for African researchers to develop methods and adjust research that should capture and accommodate the African genotype, phenotype, and geographical and economic well-being.

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Conflicts of Interest

The authors declare no conflict of interest.

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