Contribution of Mass Spectrometry to the Study of Antimalarial Agents

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1. Introduction

Mass spectrometry (MS) has become a powerful analytical tool for qualitative and quantitative applications, providing information about the structure and purity of compounds, and also about the chemical composition of complex samples.

The most recent applications of mass spectrometry are oriented towards biochemical applications such as proteome, metabolome and drug discovery. During the last decade, mass spectrometry has progressed rapidly and an evolution has been observed in the type of applications, software and equipments. Atmospheric pressure ionization sources are now used, an analyser based on a new concept (the orbitrap) was recently developed, existing ones were modified, and new hybrid instruments were developed using combinations of different analysers, depending on applications. One of the major trends was the transition to high resolution/accurate mass analysis, made routine by new MS instruments. The use of separation techniques as gas chromatography (GC), liquid chromatography (LC) and capillary electrophoresis (CE) coupled with mass spectrometry and tandem mass spectrometry, expanded the interest in this methodology.

In this chapter are presented general aspects related with characteristics of mass spectrometry equipments. The contribution of this technique to new discoveries concerning one of the major infectious diseases in man, malaria, is also discussed.

2. Basics of mass spectrometry

Mass spectrometry is a technique used to analyse from small inorganic molecules to biological macromolecules and relies on the formation of gas-phase ions (positively or negatively

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charged) that are isolated based on their mass-to–charge ratio (m/z). In order to achieve this state, the sample must be volatilized and this may become a problem to biological samples, as biomolecules have usually high molecular mass and high polarity, factors that limit their volatility.

All mass spectrometers share common components as an ionization source, a mass analyser and a detector (Fig. 1). As there are available equipments with different specifications, even from the same supplier, it is necessary to choose carefully the most adequate equipment for each type of application.

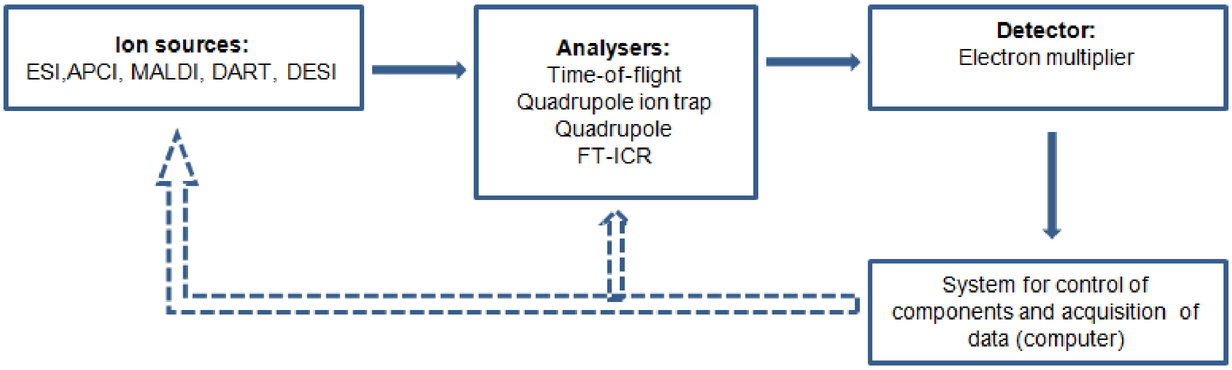

Figure 1. Basic components in a mass spectrometer. ESI, electrospray ionization, APCI, atmospheric-pressure chemical ionization; MALDI, Matrix-Assisted Laser Desorption ionization; DESI: Desorption Electrospray Ionization ; DART: Direct Analysis in Real Time; FT-ICR: Fourier transform ion cyclotron resonance (adapted from Glish & Vachet, 2003)

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Different ionization techniques may be used in mass spectrometry equipments, depending on the need of molecule disruption for the induction of ion formation. These techniques may perform strong and soft ionization processes. Soft ionization methods, like fast atomic bombardment (FAB), liquid secondary ion mass spectrometry (LSIMS), matrix-assisted laser desorption ionization (MALDI) and electrospray ionization (ESI) allow the detection of molecular ions and are more suitable for the analysis of biomolecules and non-purified analytes. The ions are generated by protonation, deprotonation or formation of adducts. In table 1 are summarized some of the main characteristics of the most used ionization methods.

The use of FAB is useful to assign the molecular ion peaks. The sample is dissolved in a suitable liquid matrix, with low vapour pressure (e.g. diethanolamine, triethanolamine, glycerol, thioglycerol or 3-nitrobenzyl alcohol), inserted into the mass spectrometer and bombarded with high energy argon or xenon atoms, providing efficient means to analyze polar, ionic, thermally labile, energetically labile, and high molecular mass compounds (El-Aneed et al., 2009).

Ionization method	Analytes	Sample introduction	Mass range	Type of ionization
FAB	Organometallic compounds	Direct injection, LC	<5000 Da	Soft
MALDI	Biomolecules	Sample co-crystallized with a matrix	500-500 000 Da	Very soft. Generates mainly single charged ions
ESI	Organic and inorganic compounds	Direct injection, LC	Large range	Softer than MALDI. Generates multiple charged ions
DESI	Small non-polar and large polar molecules (peptides and proteins)	Analysis of a surface	Large range	Generates single or multiple charged molecular ions from small or large analytes
DART	Low molecular mass compounds	Analysis of a surface	Less broad than DESI	Simple mass spectra (molecular ion)

Table 1. Characteristics of the most used ionization sources. FAB: Fast Atomic Bombardment; MALDI: Matrix Assisted Laser Desorption Ionization; ESI: Electrospray Ionization; DESI: Desorption Electrospray Ionization ; DART: Direct Analysis in Real Time

In MALDI, ions are produced by pulsed-laser irradiation (e.g. nitrogen lasers) of the sample co-crystallized with an organic matrix (e.g. gentisic, sinapic or ferulic acid) and operating in the vacuum or more recently, at atmospheric pressure. MALDI ionization uses a low amount of sample but low molecular mass molecules (below 500 Da) are difficult to analyse, due to strong interferences of the organic matrix ions.

The development of other soft ionization techniques has become crucial to the analysis of biomolecules dissolved in a mixture of water and a volatile organic solvent (e.g. methanol, acetonitrile). Techniques as ESI and MALDI make MS methodologies versatile as both techniques accomplish the conversion into gas-phase ions of non-volatile and thermally labile large molecules, allowing the study of biological compounds. Both techniques produce protonated peptide and protein ions, deprotonated deoxyribonucleic acids (DNA) and ribonucleic acids (RNA). Some reviews have been published on the covalent and non-convalent interactions between drug molecules with DNA and RNA, protein and enzyme targets for drug action and toxicity (Feng, 2004). When using ESI, proteins are ionized as they have several sites of protonation or deprotonation, and this multiple charging enables mass spectrometers with limited *m*/*z* ranges to analyse higher molecular mass molecules. However, ion suppression may occur when solutions contain high concentrations of salt, or when the target analytes are present in low concentration in matrices with high content of other analytes. Strategies based on the type of analyte, ionization reaction, ionization efficiency, analyte solution composition and pH, have been described for producing positive or negative ion modes when operating with an ESI source (Feng, 2004). APCI, is less susceptible to matrix interferences from salts, and is used for monitoring weakly polar compounds. However, labile compounds can be thermally decomposed, and due to its high sensitivity, the solvents used with this technique must have higher purity.

DART and DESI are well established open-air ionization techniques, as no sample preparation is required, making these techniques suitable for screening a large number of samples (Fernández et al., 2006). The DART ion source produces a heated stream of protonated reactant ions and the analytes in the sample are ionized, producing protonated molecules $[M+H]^+$ or deprotonated molecules $[M-H]^-$ in the open air of the laboratory environment, making possible the analysis of organic compounds directly, in real time, without time-consuming analytical protocols and destruction of the sample. The method may detect concentrations of analytes as low as femtomole (Arnaud, 2007). Due to these characteristics, DART has become an ionization method useful for rapid screening of pharmaceutical products. In DESI analysis, a high-speed charged liquid spray is directed to the sample (Takats et al., 2005). The DESI spray dissolves the material from the sample and the charged droplets are sampled downstream by a mass spectrometer. Desolvation and evaporation from these droplets creates ions that will generate a mass spectrum of the sample components.

Following the ionization process, the selected ions are extracted, accelerated, and analyzed. A mass analyser is characterized by its mass range limit, analysis speed, transmission, mass accuracy and resolution, expressed as full width at half maximum (FWHM). The most used analysers and their characteristics are summarized in Table 2.

Quadrupoles are widely used mass analysers, where ions are separated according to the stability of their trajectories in the oscillating electric fields applied between the four parallel rods. The QTOF, a hybrid quadrupole time of flight mass spectrometer, is a high-resolution mass spectrometer with MS/MS capability, and has been often used in drug studies (Nyunt et al., 2005). FT-ICR is also a high resolution and high mass accuracy analyser that enables the study of the binding of ligands (drugs) to RNA targets (Hofstadler et al., 1999; Masselon et al., 2000). The Orbitrap mass analyzer employs electrostatic trapping and it bears similarities to FT-ICR as both belong to the same Fourier Transform MS (FTMS) family of instruments. Orbitrap mass spectrometry is expected to provide maximum resolving powers of 100,000–200,000. A modified Orbitrap instrument has shown that this technology is capable of a resolution of 1,000,000 for $m/z < 300$–400, which makes it compatible to be used with chromatographic separation techniques (Denisov et al., 2012).

Tandem mass spectrometry (MS^n) development was crucial for the structural analysis of compounds. In tandem experiments, a molecular ion is selectively isolated and fragmented in a controlled environment. With this type of analysers, it is possible to perform different types of experiments (e.g. parent scan, daughter scan, neutral loss) and data obtained, will allow to identify or quantify the analytes, even in complex matrices (e.g. natural product extracts and biological fluids). Multiple Reaction Monitoring (MRM), has become an important tool, used for quantification purposes, allowing an increment of methods specificity and sensitivity.

Finally the signal obtained in the detector will produce a mass spectrum, the x-coordinate represents m/z values and the y-axis indicates total ion counts.

	Quadrupole	**Ion Trap**	**Time-of-flight**	**Time-of-flight reflectron**	**Magnetic sector**	**Fourrier Transform ion cyclotron resonance**	**Fourrier Transform Orbitrap**
Symbol	Q	IT	TOF	TOF	B	FT-ICR	FT-OT
Principle of separation	*m/z* (trajectory stability)	*m/z* (resonance frequency)	Velocity (flight time)	Velocity (flight time)	Momentum	m/z (resonance frequency)	m/z (resonance frequency)
Mass limit (Th)	4 000	6 000	>1 000 000	4 000	20 000	30 000	50 000
Resolution FWHM (m/z 1000)	2 000	4 000	5 000	20 000	100 000	500 000	100 000
Accuracy (ppm)	100	100	200	10	<10	<5	<5
Ion sampling	continuous	pulsed	pulsed	pulsed	continuous	pulsed	pulsed
Pressure (Torr)	10^{-5}	10^{-3}	10^{-6}	10^{-6}	10^{-6}	10^{-10}	10^{-10}
Tandem mass spectrometry	MS/MS Fragments Precursors Neutral loss Low-energy collision	MS^n Fragments Low-energy collision	-------	MS/MS Fragments Low-energy collision	MS/MS Fragments Precursors Neutral loss High-energy collision	MS^n Fragments Low-energy collision	-------

Table 2. Comparison of mass analysers (adapted from Hoffmann & Stroobant, 2002)

Liquid chromatography coupled to mass spectrometry (Triple Quadrupole TQ, QTOF, Linear ionTrap and Linear QTRAP analyzers) is today a well established methodology used due to its high sensitivity, speed, selectivity, versatility and ease of automation. Recently the advantages of using mass spectrometry in comprehensive liquid chromatography (LC X LC system) have been discussed and different applications have been described for pharmaceutical compounds (Donato et al., 2012).

3. An overview of malaria

Malaria is caused by *Plasmodium* parasites, which are transmitted through the bite of an infected female *Anopheles* mosquito, and remains one of the major infectious disease in man.

Five species from the genus *Plasmodium* namely: *P. falciparum*, *P. vivax P. ovale*, *P. malariae*, and *P. knowlesi* cause infection in humans. Of these, *P. falciparum* and *P. vivax* account for more than 95% of malaria cases in the world, with *P. falciparum*, being responsible for most of the deaths caused by malaria every year. The species of human malaria differ in the periodicity of their life cycle, as well as in the outcomes of the disease. Generally clinical manifestations can include fever, chills, prostration and anemia. Severe disease can include delirium, metabolic acidosis, cerebral malaria and multi-organ system failure, coma and death may ensue. (Kantele & Jokiranta, 2011)

The World Health Organization (WHO) estimated 225 million cases of malaria and about 800,000 deaths worldwide in 2010 (WHO, 2010). Due to the rapid evolution and spread of multi-resistant parasites to the current antimalarial drugs, both, chemotherapy and prophylaxis are at risk of impairment. Malaria is most prevalent in developing countries of tropical areas such as sub-Saharan Africa, East Asia and South America (Rodrigues et al., 2010; Eisenstein, 2012).

3.1. Life cycle of the malaria parasite

The malaria parasite exhibits a complex life cycle (Fig. 2) involving an insect vector (mosquito) and a vertebrate host (human). It includes an asexual cycle in humans, encompassing an asymptomatic liver-stage, a symptomatic blood-stage and a sexual cycle in a mosquito.

The liver or hepatic stage (A) is initiated when sporozoites injected through the bite of a mosquito travel to the liver and infect hepatocytes, where a clinically silent asexual multiplication takes place, generating thousands of merozoites. The release of merozoites into the bloodstream (B) marks the beginning of the erythrocytic stage of infection (C), during which parasites infect red blood cells, undergo repeated asexual replication cycles, and give rise to clinical illness. Some merozoites differentiate into gametocytes (D) that can be taken up by the mosquito during a posterior blood meal. Within the mosquito, gametocytes undergo a sexual development to form sporozoites that migrate to the salivary glands and can infect another host trough another bite (E).

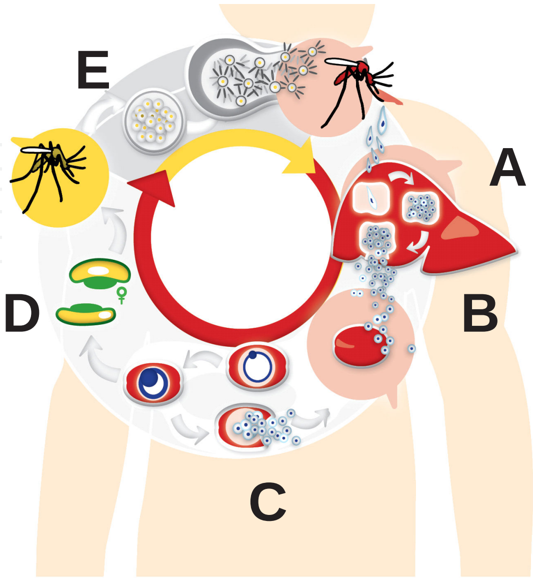


Figure 2. *Plasmodium* life cycle

3.2. Agents with antimalarial activity

The public health problem of malaria has been addressed by different approaches (Biot et al., 2012):

- use of insecticides to control the mosquito vector, but mosquitoes are developing resistance to insecticides;
- vaccines, but in spite of all the efforts there is yet not available a vaccine that effectively targets the parasite;
- chemotherapy to control malaria has relied mainly on a restricted number of chemically related drugs belonging to either the quinoline or the antifolate groups.

Increasing resistance of *P. falciparum* to the commonly used drugs is recognized as one of the major problems in eradication of the disease. The severe malaria situation underscores the continuing need of research for new classes of antimalarial agents with new mechanisms of action or re-utilization of the existing drugs with new types of therapies. The existing drug armamentarium is insufficient to answer the call for malaria eradication. The first line of treatment for malaria currently relies on a single class, the artemisinins. To overcome this problem scientists are exploring many approaches, targeting different stages of the parasite life cycle, to find agents that will prevent, cure or eliminate malaria. (Hobbs, C. & Duffy, P., 2011)

Many antimalarial agents contain a 4-aminoquinoline, 8-aminoquinoline or quinolone methanol scaffolds (Rosenthal, 2001). Chloroquine and amodiaquine are 4-aminoquinolines used to treat and prevent malaria, while primaquine is the single 8-aminoquinoline clinically approved to treat relapsing malaria caused by *P. vivax*. Mefloquine (Fig. 3) is a quinoline methanol antimalarial structurally similar to quinine, the first pure substance used to treat malaria and extracted from the bark of the cinchona tree. Other relevant classes of antimalarial agents include the antifolates (e.g. pyrimethamine and proguanyl), phenanthrene methanols (e.g. halofantrine), and naphthoquinones (e.g. atovaquone). More recently, artemisinin (Fig. 3), a sesquiterpene lactone isolated from the *Artemisia annua* chinese herb, and its analogues were a major breakthrough in malaria chemotherapy because they produce a very rapid therapeutic response, particularly against multidrug-resistant *Plasmodium falciparum* malaria.

With exception of primaquine, (Vale et al., 2009) most available antimalarials are active against the blood stage of the disease. However to achieve the eradication goal, new compounds with new modes of action are needed to block parasite transmission and eliminate the asymptomatic and latent hepatic forms. (Rodrigues et al., 2012) A strategy used to address this major goal is to combine two chemotypes - each one targeting a specific stage of the parasite's life cycle in a single chemical entity, to develop effective hybrid antimalarials capable of killing both the blood and liver-stage parasites with identical efficacy. (Capela et al., 2011)

4. Study of antimalarials agents by mass spectrometry

Structural and stability information is fundamental for any drug study, including antimalarials. In fig. 3 are presented currently available antimalarial drugs. Due to the rapid emergence and spread of resistant parasites to well-established antimalarial drugs, there is an urgent need for novel drugs. Studies performed on new antimalarial compounds using mass spectrometry are scarce but are useful for the elucidation of structures, also for prediction of compound stability and properties, isomer characterization, and detection of counterfeit products. Furthermore, studies using this technique coupled to chromatographic methods have been conducted for the evaluation of pharmacokinetics, metabolite identification, and detection of impurities.

4.1. Structural elucidation

One of the main applications of mass spectrometry is the structural elucidation of molecules. Based on the molecular ion peaks and their fragmentation patterns, the structure of compounds can then be proposed.

Among the different equipments that can be used for these purposes, FAB ion sources are frequently described. Applications can be found in the study of the oxidation products of primaquine, 5,5-di-[6-methoxy-8-(4-amino-1-butyl amino)] quinoline (PI), 6-methoxy-5,8-di-[4-amino-1-methyl butyl amino] quinoline (PII) and 5,5-di-[7-hydroxy-6-methoxy-8(4-amino-1-methyl butylamino)] quinoline (PIII) (Fig. 4) (Sinha & Dua 2004). The mass spectrum of PI with molecular formula $C_{30}H_{40}N_6O_2$ presents the molecular ion at *m/z* 517 confirming the molecular mass of the compound, and a fragment at *m/z* 500 is detected due to the presence of a terminal amino group at position 4′.

An LC-MS/MS method was developed for the analysis of bulaquine (BQ) 3-[1-[4-[(6-methoxy-8-quinolinylamino] pentylamino] ethylidene]dihydro-2 (3H)-furanone (Fig. 5) and its metabolite primaquine in monkey plasma. Protonated species at *m/z* 370 and 260 were detected for bulaquine and primaquine, respectively. MS/MS conditions were optimized generating product ions through fragmentation of the molecular ions. Based on the fragmentation spectra obtained from $[M+H]^+$ under the established analytical conditions, a fragmentation pattern was presented for these compounds. This type of study is important to establish analytical conditions for the quantification of drugs and their metabolites in biological fluids. (Nitin et al., 2003)

An ESI-Ion trap mass spectrometer was used to perform MS^n analyses, in the study of imidazolidin-4-one peptidomimetic derivatives of primaquine. (Vale et al., 2008a)

4.2. Pharmacokinetic studies

Pharmacokinetic (PK) studies provide a mathematical basis to assess the time course of drug in the body. It enables to quantify absorption, distribution, metabolism and excretion of the drug and their metabolites. The primary requirement to undertake a PK study, is to have an

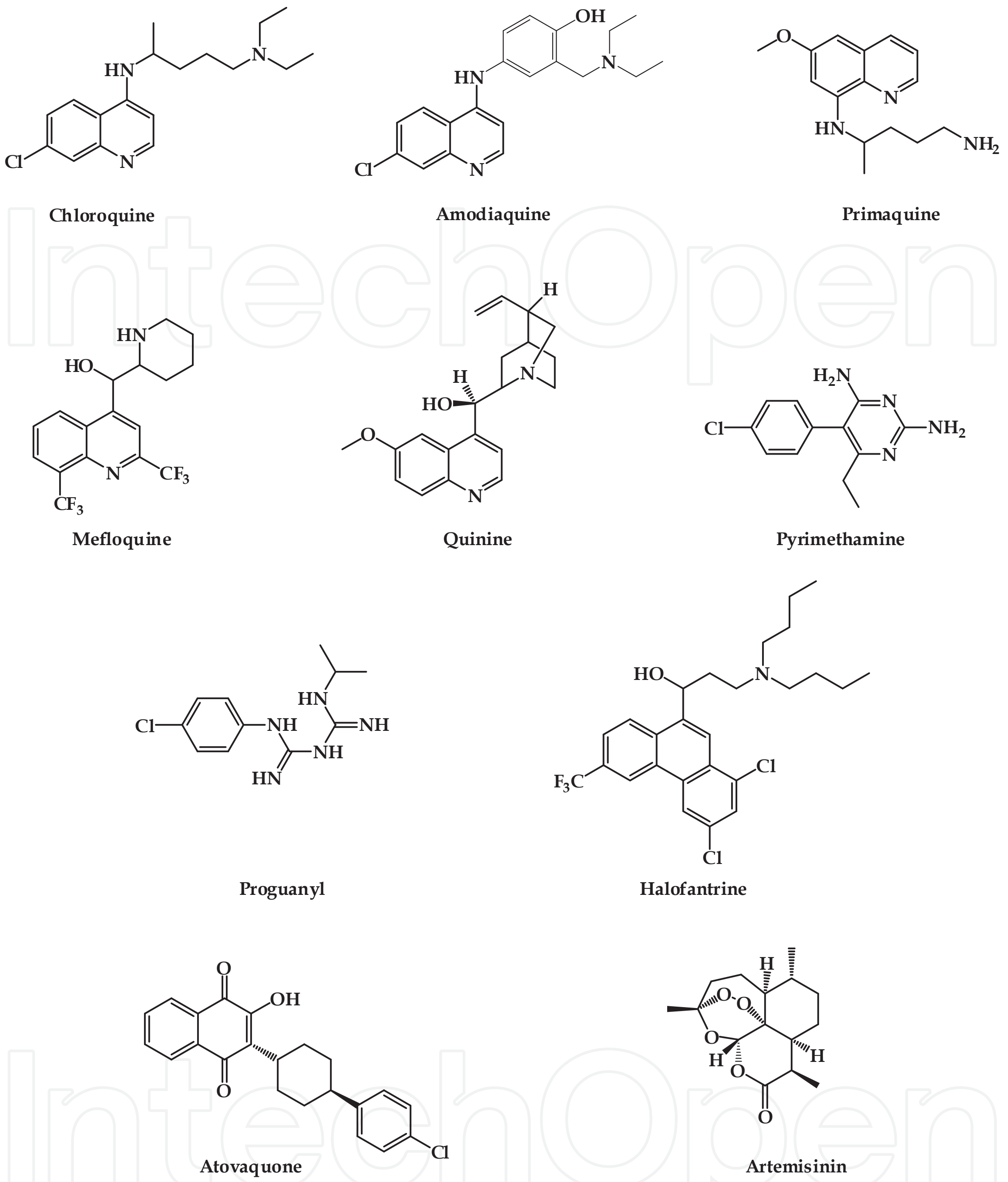
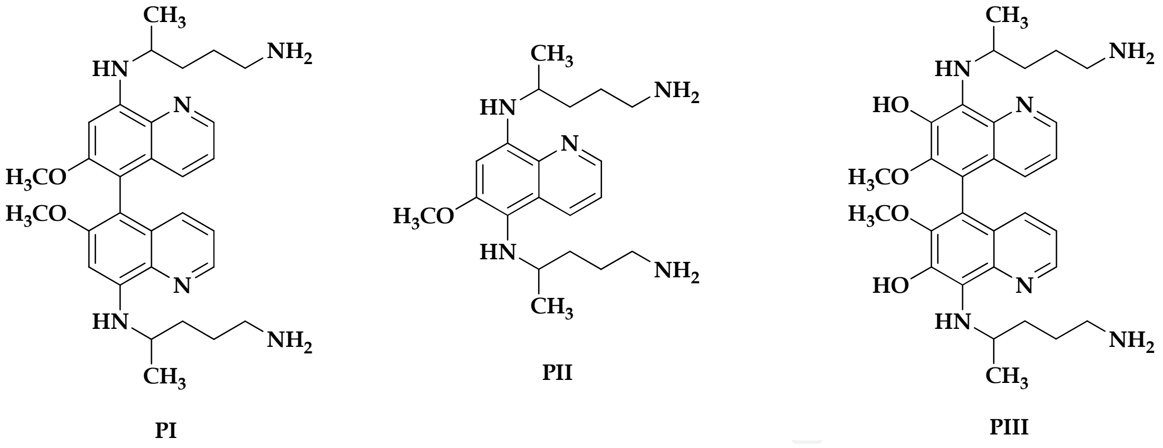


Figure 3. Currently available antimalarial drugs

analytical method which is reliable, reproducible, sensitive, selective, and if possible, compatible with high-throughput pharmacokinetic approaches.

Drug efficacy requires adequate drug concentration at the site of action. Monitoring drugs and their metabolites in biological samples (*in vivo* studies), is fundamental in order to control the intake of the antimalarials by the infected populations. Mass spectrometry has been successfully used for this purposes, coupled with liquid chromatography, and analytical methods



PI PII PIII

Figure 4. The oxidative antimalarial primaquine analogous (PI, PII and PIII)

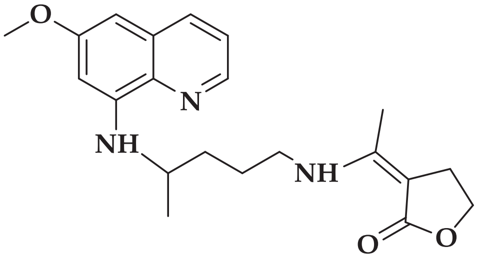


Figure 5. Bulaquine

have been optimized and validated for quantification of different drugs and metabolites in biological fluids.

A LC-MS/MS method was used to study 14 antimalarial drugs, which are the components of the current first-line combination treatments for malaria (artemether, artesunate, dihydroartemisinin, amodiaquine, N-desethyl-amodiaquine, lumefantrine, desbutyl-lumefantrine, piperaquine, pyronaridine, mefloquine, chloroquine, quinine, pyrimethamine and sulfadoxine). The best conditions for mass spectrometry analysis were optimized (Hodel et al., 2009) and the method developed was implemented, and used to analyse samples from an *in vivo* study with 125 Southeast Asian patients from two regions of Cambodia: one region with a high level of antimalarial drug resistance and another region with moderate levels of drug resistance (Hodel et al., 2010). The 14 antimalarial drugs were measured in plasma samples from the patients, and results showed that for half of them, an antimalarial drug was detected namely mefloquine, piperaquine, chloroquine or quinine. However all patients reported either not having taken any antimalarial before or not knowing to have taken. These results are important, as they show that it is urgent to ensure appropriate use of antimalarials among the populations.

Tafenoquine (8-[(4-amino-1-methylbutyl)amino]-2,6-dimethoxy-4-methyl-5-(3-trifluoromethyl-phenoxy) quinoline succinate) (Fig. 6) was measured in human plasma from patients and healthy volunteers during a clinical efficacy trial, using a LC-MS/MS equipment.(Doyle et al., 2002)

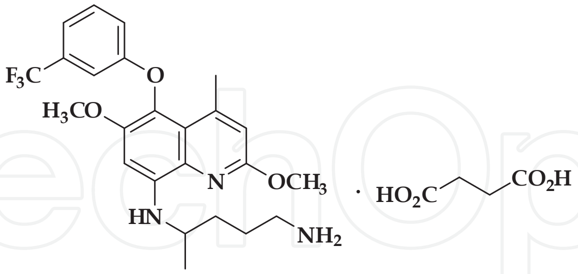


Figure 6. Tafenoquine

For NPC1161 (Fig. 7), an 8-aminoquinoline analog *(8-[(4-amino-1-methylbutyl)amino]-5-[3,4-dichloro-phenoxyl]-4-methyl-quinoline)* and their metabolites, a LC-MS method using an electrospray ionization source and a TOF analyzer, was implemented.

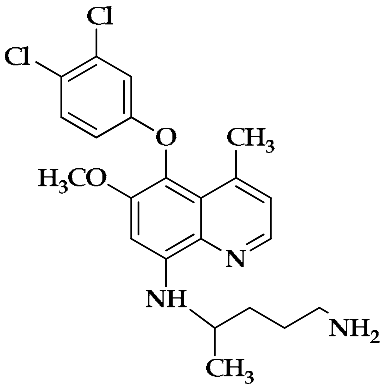


Figure 7. NPC1161, an 8-aminoquinoline analog

Using mass spectrometry other antimalarial molecules as α-/β-diastereomers of arteether (AE), sulphadoxine (SDX) and pyrimethamine (PYR) (Sabarinath et al., 2006) and three novel trioxane antimalarials (Fig. 8) (Singh et al., 2008) were determined in rat plasma. A *N*-alkylamidine compound, M64, and its corresponding bioprecursors were measured in human and rat plasma (Margout et al., 2011) (Fig. 9). Artemisinin class compounds and its active *in-vivo* metabolites were analysed in monkey plasma (Singh et al., 2009).

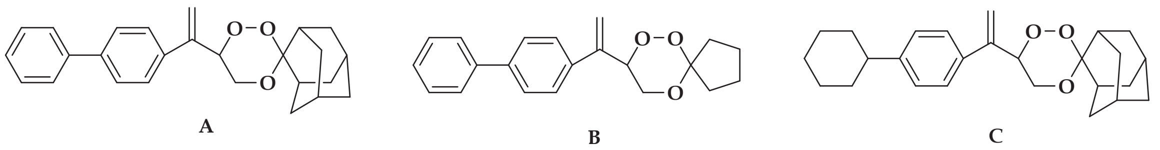
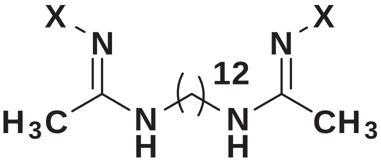


Figure 8. Chemical structures of three novel trioxane antimalarials



M64, X=H; M64AH, X=OH; M64-S-Me, X=OSO_2CH_3

Figure 9. Chemical structures of compound, M64, and its corresponding bioprecursors

A method was also developed and validated according to FDA guidelines for simultaneous determination of two mono-thiazolium compounds in plasma, whole blood and red blood cells from human and rat (Taudon et al., 2008). More recently a rapid (3 min analysis) and sensitive UPLC-MS/MS method using a triple quadrupole tandem mass spectrometer in positive ESI mode, has been implemented for the analysis of ARB-89 (7α-hydroxy artemisinin carbamate) (Fig. 10) in rat serum, for pharmacokinetics studies, (Pabbisetty et al., 2012).

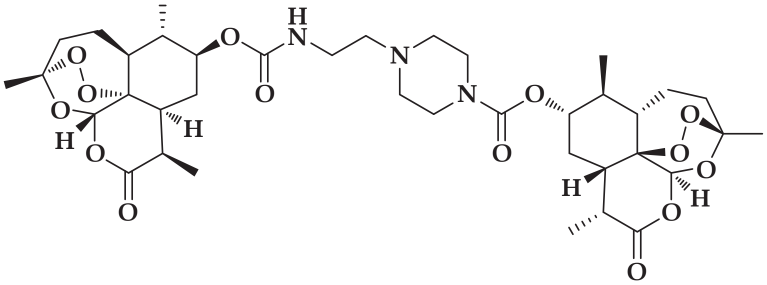


Figure 10. ARB-89 (7α-hydroxy artemisinin carbamate)

Mass spectrometry has proven to be particularly useful in identifying complex metabolites as those arising from phase I (e.g. those involving cytochrome P450 monooxygenases) and phase II (e.g. conjugation with glucuronic acid, sulfonates, glutathione or amino acids) reactions. In a study published by Liu et al., 2011, metabolites of artemisinin, also known as Qing-hao-su (QHS), and its active derivative dihydroartemisinin (DHA) were identified in *in vitro* and *in vivo* biological samples using a LTQ-Orbitrap mass spectrometer in tandem with H/D exchange. The authors were able to show that artemisinin drugs mainly undergo hydroxylation and loss of oxygen in the phase I metabolic pathway and can form glucuronides in the phase II processes, as shown in Fig. 11. Based on MS data it was proposed a metabolic pathway for these metabolites.

Piperaquine was synthesized for the first time about 50 years ago, but seems to be a suitable partner drug in artemisinin-based combination treatments. In a paper published by Tarning et al., 2006, the main metabolites of piperaquine were characterized in a 16-h human urine, after a single p.o. administration of a fixed combination of dihydroartemisinin-piperaquine, with a fatty meal. A LC method in tandem with a QTRAP system was used to analyse piperaquine and its metabolites and a FT-ICR/MS equipment was used to obtain mass spectra

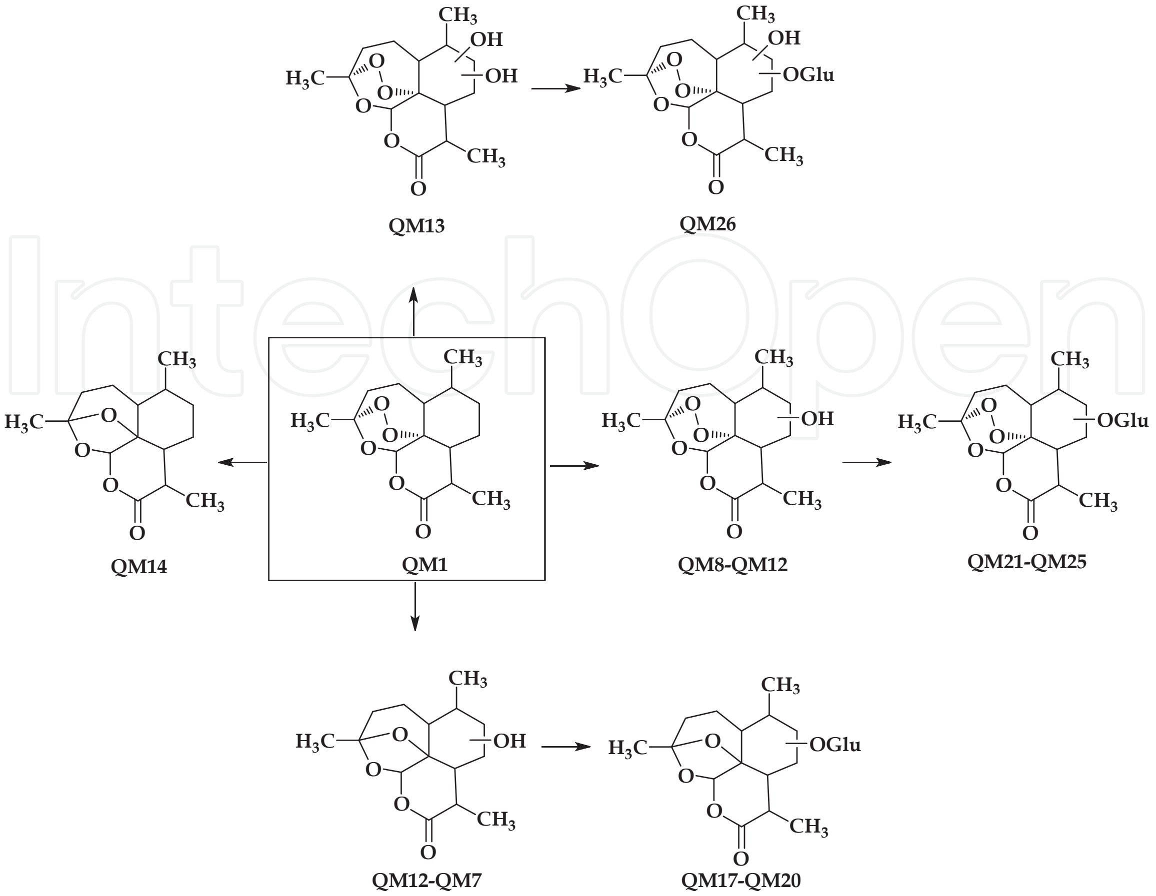


Figure 11. Proposed metabolic pathways for QHS *in vitro* and *in vivo* (Liu et al., 2011)

of the five metabolites of piperaquine in urine samples. Two of the metabolites (a carboxylic and a mono N-oxidated piperaquine metabolite) were considered as the most relevant as they were detected in the serum/plasma samples collected up to 93 days, and also in urine 123 days after administration of the drug. Other two monohydroxylated metabolites and a di-N-oxidized metabolite were also detected in urine samples.

4.3. Detection of impurities

A major impurity (8-(4-amino-4-methylbutylamino)-6 methoxyquinoline) associated with primaquine drug samples (Fig.12), obtained from European Pharmacopoeia and other commercial sources, was detected by gas chromatography-electron impact-mass spectrometry (GC–EI-MS) (Dongre et al., 2005).

4.4. Chemical stability studies

Mass spectrometry can also be conducted in order to contribute to study the properties of compounds, through knowledge of their stability and fragmentation mechanisms, under the gas-phase conditions of a mass spectrometer. This type of studies can have, in the future, important implications in drug analysis and development.

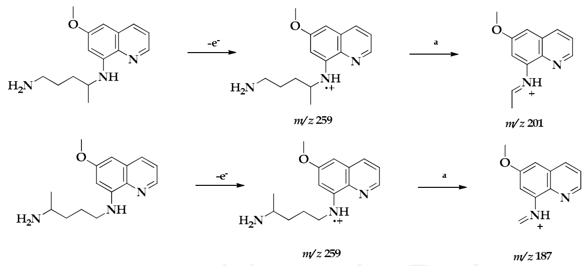
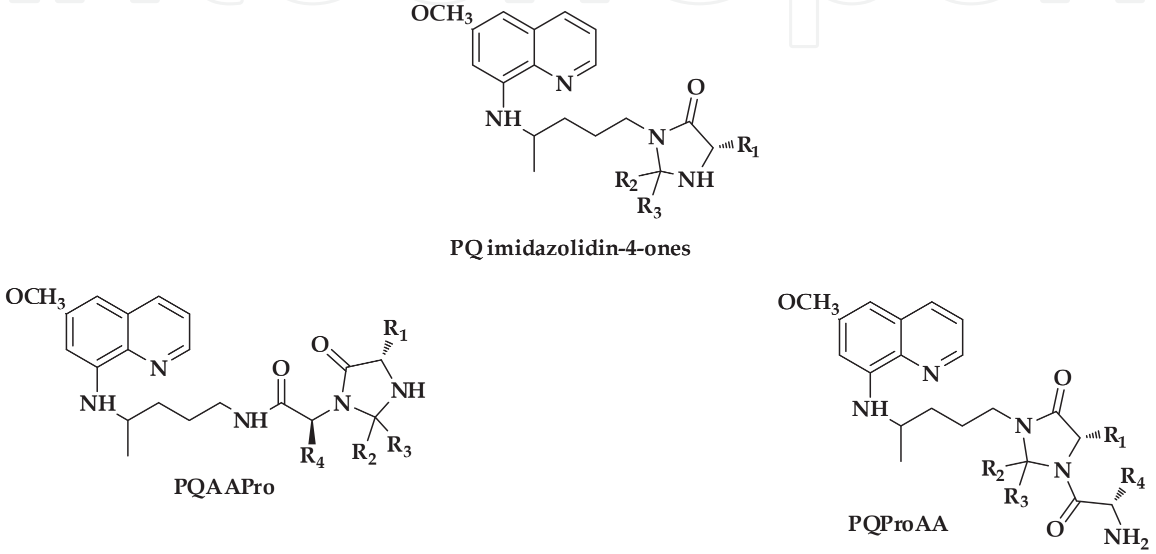


Figure 12. Fragmentation pattern of primaquine and the corresponding impurity



PQ imidazolidin-4-ones

PQAAPro

PQProAA

Figure 13. PQ imidazolidin-4-ones, PQAAPro and PQProAA mimetic derivatives of primaquine

Studies on primaquine derived imidazolidin-4-ones using an ESI-ion trap mass spectrometer have allowed to find a correlation between the stability of the ions in the nozzle-skimmer region during the CID (Collision Induced Dissociation) analyses and reactivity in both isotonic buffer and human plasma (Vale et al., 2008). The same authors (Vale et al., 2008a) studied imidazolidin-4-one peptidomimetic derivatives of primaquine, PQAAPro and PQProA (Fig. 13) and they also found the parallelism between compound reactivity to hydrolysis and stability during CID analysis. Using CID and MS/MS experiments to study the peptidomimetic imidazolidin-4-one derivatives of primaquine, it was possible to conclude that CID spectra reflected the reactivity of compounds under physiological conditions, and the relative abundances of MS/MS generated fragments were correlated with the Charton steric parameters associated to amino acid side chain in the molecule (Vale et al., 2009).

From the results obtained, ESI-MS proved to be a reliable tool for stability prediction of compounds towards hydrolysis at physiological pH and temperature although the mechanisms in water and in the gas-phase are not comparable. This type of studies were, for the first time, approached by these authors.

4.5. Detection of counterfeit drugs

The quality of commercially available drugs varies among countries. The WHO/International Medical Products Anti-Counterfeiting Taskforce estimates that in some less developed countries, the counterfeit drugs are up to 50% of the total drugs supplied to the populations(Hall et al., 2006). Due to the lack of regulations and poor quality control practices, the amount of the active ingredient may be incorrect, as a result of chemical degradation that occurs due to poor storage conditions, especially in warm and humid tropical environments. In some cases, expired drugs are repackaged with new expiration dates and put in the market. Also some drugs can be contaminated or replaced by other substances and people consume sawdust, paint and other toxic or inert substances (Kaur et al., 2009).

The report published by WHO in 2011, about the quality of antimalarial drugs in 6 countries of sub-Saharan Africa (Cameroon, Ethiopia, Ghana, Kenya, Nigeria and the United Republic of Tanzania) resumes the results obtained from the analysis of 935 samples and gives an idea about the quality and counterfeits that more often occur.

As an incorrect intake can result in a low bioavailability of the drug in the individual, leading to drug-resistance strains, provoking a therapeutic failure, reliable methods of analysis must be available to determine the quality of antimalarials commercialized, and LC coupled with mass spectrometry can be used. However this technique is expensive, requires training, technological support, and sample preparation. Actually, DART (Fernández et al., 2006) and DESI (Haiss et al., 2007) methodologies are often used, as they produce results rapidly, because they do not require sample preparation. Results obtained from a DESI MS method were used to validate Fourier-transform infrared imaging for characterization of counterfeit antimalarial pharmaceutical in tablets (Ricci et al., 2007). A DESI MS method was also applied for the quantitative screening of counterfeit antimalarial tablets containing artesunate (Nyadong et al., 2008), and more recently DESI and DART methods were used to validate results from the application of FT-Raman spectroscopy for *in situ* screening for potentially counterfeit artesunate antimalarial tablets (Ricci et al., 2008).

4.6. Rapid diagnosis of malaria

The rapid diagnosis of malaria infection can also be performed by mass spectrometry. Hemozoin, the malaria pigment, can be detected by laser desorption mass spectrometry (LDMS) in human blood. (Scholl et al., 2004) Detection of malaria in 45 asymptomatic pregnant Zambian women was performed by this technique. Detection of *Plasmodium falciparum* malaria during pregnancy is complicated by sequestration of parasites in the placenta reducing peripheral blood microscopic detection. LDMS was able to detect <10 parasites/uL cultured in human blood and provided a more rapid mean of screening for infection than the technique used currently for this purpose, light microscopy (Nyunt et al., 2005).

5. Conclusions

The information obtained from the use of mass spectrometry in the study of antimalarials agents is important, in order to understand all the mechanisms of the illness, malaria, and the way the different drugs interact in the human organism. Due to its characteristics (sensitivity, speed, possibility to be automated, possibility to combine with separation techniques) and diversity of equipments available, mass spectrometry can be used in the structural identification of new molecules, in the study of many phases of drug discovery, for assessment of compound stability, pharmacokinetic studies monitoring the concentrations of antimalarials and metabolites in biological matrices, the studies of cell permeability and plasma protein binding, and finally, in the quality control of commercial drugs.

The use of mass spectrometry to predict stability of compounds in physiological conditions may become an important tool.

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References

[1] Arnaud, C. H. (2007). Taking mass spec into the open: Open-air ionization methods minimize sample pre and widen range odf mass spectrometry applications. Chemical & Engineering News , 85(41), 13-18.

[2] Biot, C, Castro, W, & Navarro, M. (2012). The therapeutic potential of metal-based antimalarial agents: Implications for the mechanism of action. Dalton Transactions , 41(21), 6335-6349.

[3] Capela, R, Cabal, G. G, Rosenthal, P. J, Gut, J, Mota, M. M, Moreira, R, Lopes, F, & Prudêncio, M. (2011). Design and Evaluation of Primaquine-Artemisinin Hybrids as a Multistage Antimalarial Strategy. Antimicrobial Agents and Chemotherapy , 55(10), 4698-4706.

[4] Denisov, E. Damoc; Lange, O. & Makarov, A. ((2012). Orbitrap mass spectrometry with resolving powers above 1,000,000. International Journal of Mass Spectrometry 325-327(0), 80-85.

[5] Donato, P. Cacciola; Tranchida, F.P.; Dugo, P. & Mondello, L. ((2012). Mass spectrometry detection in comprehensive liquid chromatography: basic concepts, instrumental aspects, applications and trends. Mass Spectrometry Reviews , 31(5), 523-559.

[6] Dongre, V. G, Karmuse, P. P, Nimbalkar, M. M, Singh, D, & Kumar, A. (2005). Application of GC-EI-MS for the identification and investigation of positional isomer in primaquine, an antimalarial drug. Journal of Pharmaceutical and Biomedical Analysis 39(1-2), 111-116.

[7] Doyle, E, Fowles, S. E, Summerfield, S, & White, T. J. (2002). Rapid determination of tafenoquine in small volume human plasma samples by high-performance liquid chromatography-tandem mass spectrometry. Jounal of Chromatography B Analytical Technologies Biomedical Life Sciences , 769(1), 127-132.

[8] Eisenstein, M. (2012). Drug development holding Holding out for reinforcements. Nature 484(7395): SS18., 16.

[9] El-Aneed, A, Cohen, A, & Banoub, J. (2009). Mass Spectrometry, Review of the Basics: Electrospray, MALDI, and Commonly Used Mass Analyzers. Applied Spectroscopy Reviews , 44(3), 210-230.

[10] Feng, W. Y. (2004). Mass spectrometry in drug discovery: a current review. Current Drug Discovery Technologies , 1(4), 295-312.

[11] Fernández, F. M, Cody, R. B, Green, M. D, Hampton, C. Y, Mcgready, R, Sengaloundeth, S, White, N. J, & Newton, P. N. (2006). Characterization of Solid Counterfeit Drug Samples by Desorption Electrospray Ionization and Direct-analysis-in-real-time Coupled to Time-of-flight Mass Spectrometry. ChemMedChem , 1(7), 702-705.

[12] Glish, G. L, & Vachet, R. W. (2003). The basics of mass spectrometry in the twenty-first century. Nature Reviews Drug Discovery , 2(2), 140-150.

[13] Haiss, W, Thanh, N. T, Aveyard, J, & Fernig, D. G. (2007). Determination of size and concentration of gold nanoparticles from UV-vis spectra. Analytical Chemistry , 79(11), 4215-4221.

[14] Hall, K. A, & Newton, P. N. Green, M.D; De Veij, M; Vandenabeele, P.; Pizzanelli, D.; Mayxay, M.; Dondorp, A. & Fernandez, F.M. ((2006). Characterization of counterfeit artesunate antimalarial tablets from southeast Asia. The American Journal Tropical Medicine and Hygiene , 75(5), 804-811.

[15] Hobbs, C, & Duffy, P. (2011). Drugs for malaria: something old, something new, something borrowed. F1000 Biology Reports, 3(24), 1-9.

[16] Hodel, E. M, Genton, B, Zanolari, B, Mercier, T, Duong, S, Beck, H. P, Olliaro, P, Decosterd, L. A, & Ariey, F. (2010). Residual antimalarial concentrations before treatment in patients with malaria from Cambodia: indication of drug pressure. Journal of Infectious Diseases , 202(7), 1088-1094.

[17] Hodel, E. M, Zanolari, B, Mercier, T, Biollaz, J, Keiser, J, Olliaro, P, Genton, B, & Decosterd, L. A. LC-tandem mass spectrometry method for the simultaneous determination of 14 antimalarial drugs and their metabolites in human plasma. Journal of Chromatography B , 877(10), 867-886.

[18] Hoffmann, E, & Stroobant, V. (2002). Mass Spectrometry: Principles and Applications, John Wiley & Sons, 3rd Edition, 978-0-47003-310-4HB), Great Britain.

[19] Hofstadler, S. A, Sannes-lowery, K. A, Crooke, S. T, Ecker, D. J, Sasmor, H, Manalili, S, & Griffey, R. H. (1999). Multiplexed screening of neutral mass-tagged RNA targets against ligand libraries with electrospray ionization FTICR MS: a paradigm for high-throughput affinity screening. Analytical Chemistry , 71(16), 3436-3440.

[20] Kantele, A, & Jokiranta, T. S. (2011). Review of Cases With the Emerging Fifth Human Malaria Parasite, Plasmodium knowlesi. Clinical Infectious Diseases , 52(11), 1356-1362.

[21] Kaur, H, Green, M. D, Hostetler, M. D, & Fernández, F. M. Paul N Newton ((2009). Antimalarial drug quality: methods to detect suspect drugs. Therapy , 7(1), 49-57.

[22] Liu, T, Du, F, Wan, Y, Zhu, F, & Xing, J. (2011). Rapid identification of phase I and II metabolites of artemisinin antimalarials using LTQ-Orbitrap hybrid mass spectrometer in combination with online hydrogen/deuterium exchange technique. Journal of Mass Spectrometry , 46(8), 725-733.

[23] Margout, D, Gattacceca, F, Moarbess, G, & Wein, S. Tran van Ba, C.; Le Pape, S.; Berger, O.; Escale, R.; Vial, H. J. & Bressolle, F. M. ((2011). Pharmacokinetic properties and metabolism of a new potent antimalarial N-alkylamidine compound, M64, and its corresponding bioprecursors. European Journal of Pharmaceutical Sciences 42(1-2), 81-90.

[24] Masselon, C, Anderson, G, Harkewicz, R, Bruce, J. E, Pasa-tolic, L, & Smith, R. D. (2000). Accurate Mass Multiplexed Tandem Mass Spectrometry for High-Throughput Polypeptide Identification from Mixtures. Analytical Chemistry , 72(8), 1918-1924.

[25] Nitin, M, Rajanikanth, M, Lal, J, Madhusudanan, K. P, & Gupta, R. C. (2003). Liquid chromatography-tandem mass spectrometric assay with a novel method of quantitation for the simultaneous determination of bulaquine and its metabolite, primaquine, in monkey plasma. Journal of Chromatography B , 793(2), 253-263.

[26] Nyadong, L, Late, S, Green, M. D, Banga, A, & Fernández, F. M. (2008). Direct Quantitation of Active Ingredients in Solid Artesunate Antimalarials by Noncovalent Complex Forming Reactive Desorption Electrospray Ionization Mass Spectrometry. Journal of the American Society for Mass Spectrometry , 19(3), 380-388.

[27] Nyunt, M, Pisciotta, J, Feldman, A. B, Thuma, P, Scholl, P. F, Demirev, P. A, Lin, J. F, Shi, L, Kumar, N, & Sullivan, D. J. (2005). Detection of Plasmodium falciparum in pregnancy by laser desorption mass spectrometry. The American Journal of Tropical Medicine and Hygiene , 73(3), 485-490.

[28] Pabbisetty, D, Illendula, A, Muraleedharan, K. M, Chittiboyina, A. G, Williamson, J. S, Avery, M. A, & Avery, B. A. (2012). Determination of antimalarial compound,

ARB-89 (7β-hydroxy-artemisinin carbamate) in rat serum by UPLC/MS/MS and its application in pharmacokinetics. Journal of Chromatography B 889-890(0), 123-129.

[29] Ricci, C, Nyadong, L, Fernandez, F. M, Newton, P. N, & Kazarian, S. G. (2007). Combined Fourier-transform infrared imaging and desorption electrospray-ionization linear ion-trap mass spectrometry for analysis of counterfeit antimalarial tablets. Analytical and Bioanaytical Chemistry , 387(2), 551-559.

[30] Ricci, C, Nyadong, L, Yang, F, Fernandez, F. M, Brown, C. D, Newton, P. N, & Kazarian, S. G. (2008). Assessment of hand-held Raman instrumentation for in situ screening for potentially counterfeit artesunate antimalarial tablets by FT-Raman spectroscopy and direct ionization mass spectrometry. Analytica Chimica Acta , 623(2), 178-186.

[31] Rodrigues, T, Moreira, R, & Lopes, F. (2010). New hope in the fight against malaria? Future Medicinal Chemistry , 3(1), 1-3.

[32] Rodrigues, T, Prudêncio, M, Moreira, R, Mota, M. M, & Lopes, F. (2012). Targeting the liver stage of malaria parasites: a yet unmet goal. J Med Chem , 55(3), 995-1012.

[33] Rosenthal, P. J. (2001). Antimalarial chemotherapy: mechanisms of action, resistance, and new directions in drug discovery, Springer-Science+Business Media, LLC, New Jersey.

[34] Sabarinath, S, Singh, R. P, & Gupta, R. C. (2006). Simultaneous quantification of α-/β-diastereomers of arteether, sulphadoxine and pyrimethamine: A promising anti-relapse antimalarial therapeutic combination, by liquid chromatography tandem mass spectrometry. Journal of Chromatography B , 842(1), 36-42.

[35] Scholl, P. F, Kongkasuriyachai, D, Demirev, P. A, Feldman, A. B, Lin, J. S, Sullivan, D. J, & Kumar, N. (2004). Rapid detection of malaria infection in by laser desorption mass spectrometry. The American Journal of Tropical Medicine and Hygiene, 71(5), 2004, 546-551.

[36] Singh, R. P, Sabarinath, S, & Gautam, N. Gupta, R. C & Singh, SK. ((2009). Liquid chromatographic tandem mass spectrometric assay for quantification of 97/78 and its metabolite 97/63: a promising trioxane antimalarial in monkey plasma. Journal of Chromatography B Analytical Technolologies and Biomedical Life Sciences , 877(22), 2074-2080.

[37] Singh, R. P, Sabarinath, S, Singh, S. K, & Gupta, R. C. (2008). A Sensitive and selective liquid chromatographic tandem mass spectrometric assay for simultaneous quantification of novel trioxane antimalarials in different biomatrices using sample-pooling approach for high throughput pharmacokinetic studies. Journal of Chromatography B, 864(1-2), 52-60.

[38] Sinha, S. N. & V. K. Dua (2004). Fast atom bombardment mass spectral analysis of three new oxidative products of primaquine. International Journal of Mass Spectrometry , 232(2), 151-163.

[39] Takats, Z, Wiseman, J. M, et al. (2005). Ambient mass spectrometry using desorption electrospray ionization (DESI): instrumentation, mechanisms and applications in forensics, chemistry, and biology. Jounal of Mass Spectrometry , 40(10), 1261-1275.

[40] Tarning, J, Bergqvist, Y, Day, N. P, Bergquist, J, Arvidsson, B, & White, N. J. Ashton, M & Lindegårdh, N. (2006). Characterization of human urinary metabolites of the antimalarial piperaquine. Drug Metabolism and Disposition , 34(12), 2011-2019.

[41] Taudon, N, Margout, D, Calas, M, Kezutyte, T, Vial, H. J, & Bressolle, F. M. (2008). A liquid chromatography-mass spectrometry assay for simultaneous determination of two antimalarial thiazolium compounds in human and rat matrices. Journal of Pharmaceutical and Biomedical Analysis , 48(3), 1001-1005.

[42] Vale, N, Matos, J, Moreira, R, & Gomes, P. (2008). Electrospray ionization-ion trap mass spectrometry study of PQAAPro and PQProAA mimetic derivatives of the antimalarial primaquine. Journal of the American Society for Mass Spectrometry , 19(10), 1476-1490.

[43] Vale, N, Moreira, R, & Gomes, P. (2008). Characterization of primaquine imidazolidin-4 ones with antimalarial activity by electrospray ionization-ion trap mass spectrometry. International Journal of Mass Spectrometry 270(1-2), 81-93.

[44] Vale, N, Matos, J, Moreira, R, & Gomes, P. (2009). Electrospray ionization mass spectrometry as a valuable tool in the characterization of novel primaquine peptidomimetic derivatives. European Journal of Mass Spectrometry , 15(5), 627-640.

[45] Vale, N, Moreira, R, & Gomes, P. (2009). Primaquine revisited six decades after its discovery. European Journal of Medicinal Chemistry , 44(3), 937-953.

[46] World Health Organization (2010). World Malaria Report 2010, WHO Press, Geneva, Switzerland.

[47] World Health Organization (2011). Survey of the quality of selected antimalarial medicines circulating in six countries of sub-Saharan Africa, Quality Assurance and Safety: Medicines Essential Medicines and Pharmaceutical Policies, WHO Press, Geneva, Switzerland.