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Review Article

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A REVIEW ON CHARACTERISTICS AND ANALYTICAL METHODS OF ATOVAQUONE – A POTENT ANTIMALARIAL AGENT

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ABSTRACT
Drugs used in the treatment of malaria that is caused by various plasmosium species i.e. *P. falciparum, P. vivax* are most irresistible disease throughout the world. The different medications are utilized in the treatment of malaria incorporated the Aryl aminoalcohol mixes: Quinine, Quinidine, Chloroquine, Mefloquine; Antifolate compound: Pyrimethamine, Proguanil, Chlorproguanil, Trimethoprim and Atovaquone. Atovaquone is most effective drug utilized in the treatment of malaria. It must be given in single or in mixture with different antimalarials. An enormous number of methodologies including High Performance Liquid chromatography (HPLC), UV–Visible spectroscopy and Liquid Chromatography-Mass Spectroscopy (LC-MS) are utilized for the determination of atovaquone. Various analytical methods are used for the analysis of pharmaceutical products and these methods were validated according to ICH guidelines (Q1A R2). Thus, this technique can be safely used for the standard quality control analysis of atovaquone.

INTRODUCTION

Malaria is a mosquito-borne infection brought about by plasmodium parasites. Patients with malaria generally experiences flu-like manifestations. In serious cases, the ailment can advance to neurological disturbances, unconsciousness and death. Symptoms generally start ten to fifteen days after nabbed by a tainted mosquito. Malaria is endemic in tropical and subtropical localities and cause approximately one million death every year [1].

Malaria parasites have a place with the variety Plasmodium (*Phylum* apicomplexa). In people, malaria is brought about by *P. falciparum, P. malariae, P. ovale, P. vivax and P. knowlesi*.

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Among those tainted, *P. falciparum* is the most well-known species distinguished (~75%) trailed by *P. vivax* (~20%). Although *P. falciparum* customarily represents most of deaths, late proof proposes that *P. vivax* malaria is related with conceivably perilous conditions about as frequently likewise with a finding of *P. falciparum* infection. *P. vivax* relatively is increasingly basic outside Africa. There have been filed human pollutions with a couple of sorts of plasmodium from higher gorillas; regardless, beside *P. knowlesi* which is a zoonotic animal varieties that causes malaria in macaques [2]. Just female mosquitoes feed on blood, male mosquitoes feed on plant nectar and don't transmit the sickness. Females of the mosquito class anopheles went to encourage around evening time. They as a rule start scanning for a supper at nightfall and proceed during that time until they succeed. Malaria parasites can likewise be transmitted by blood transfusions, in spite of the fact that this is uncommon [1].

In 2018, According to WHO survey report the mostly malaria cases were in Africa (93%), East Asia Region (3.4%), Eastern Mediterranean Region (2.4%). The *P. falciparum* found mostly spread malaria in African Region (99.7%) of reported malaria cases in 2018, similarly as in the WHO South East Asia Region (a large portion of), the WHO Eastern Mediterranean Region (71%) and the WHO Weastern Pacific Region (65%) [3, 4].

Between 2015 and 2018, just 31 nations, where malaria is as yet endemic, diminished case frequency fundamentally and were on track to decrease occurrence by 40% or more by 2020. Without quickened change, the Global specialized procedure for malaria 2016–2030 (GTS) achievements for horribleness in 2025 and 2030 won't be accomplished [4].

Now a days, resistance to antimalarials are the topic of discussion. The outgrowth of chloroquine resistance has been accomplice with a theatrical increase in malaria in endemic regions [6,7]. Chloroquine resitant *P. falciparum* malaria occurred from chromosomal mutation. In chloroquine obstruction, there is change in the chloroquine collection in the digestive vacule in intraerythrocytic tropozoite [8]. In chloroquine resistance, there is multiple mutation of PfCRT, a protein that behaves like transporter in parasite's digestive vacuole membrane [3, 4]. Huge chloroquine resistance for both *P. vivax* and *P. falciparum*. Among both, *P. vivax* was more fetal to young children. There is great need to study about resistance for chloroquine and its further treatment [9, 10].

The analytical techniques are used for the qualitative and quantitative analysis of drugs in bulk as well as pharmaceutical formulations. The techniques are very much useful in past and present scenario. The popular analytical techniques are UV-Visible spectroscopy, High Performance Liquid Chromatography (HPLC), Mass spectrometry, Hyphenated techniques like GC-MS, LC-MS, LC-NMR etc. In this review, we have discussed some analytical techniques of atovaquone in bulk as well as formulations

Malaria life cycle

Sporozoites from the mosquito salivary glands quickly enter the circulation after a nibble and limit through openresponse occasions in hepatocytes, where they change, grows and form into tissue schizonts. This fundamental asymptomatic tissue (pre-erythrocytic or exoerythrocytic) period of infection continues for 5 to 15 days, relies on the plasmodium species. Tissue schizonts then split each discharge thousands merozoites that enter the circulation, attack erythrocytes and start the erythrocytic cycle.

At the point when the tissue schizonts burst in *P. falciparum* and *P. malariae* infections, no types of the parasite stay in the liver. Be that as it may, in *P. vivax* and *P. ovale* diseases, tissue parasites (hypnozoites) continue that can deliver backslides of erythrocytic contamination months to years after the essential assault. When plasmodia enter the erythrocytic cycle, they can't reinvade the liver, along these lines, there is no tissue phase of contamination for jungle fever shrunk by transfusion. In erythrocytes, most parasites experience agamic improvement from youthful ring structures to trophozoites lastly to develop schizonts. Schizont-containing erythrocytes burst, each discharging 6 to 32 merozoites relying upon the plasmodium species. It is this procedure that produces febrile clinical assaults. The merozoites attack more erythrocytes to proceed with the cycle, which continues till the very end of the host or adjustment by drugs or procured halfway insusceptibility. The periodicity of parasitemia and febrile clinical signs relies upon the planning of schizogony of an age of erythrocytic parasites. For *P. falciparum, P. vivax* and *P. ovale*, it takes around 48 hours to finish this procedure; for *P. malariae*, around 72 hours is required [11-14].

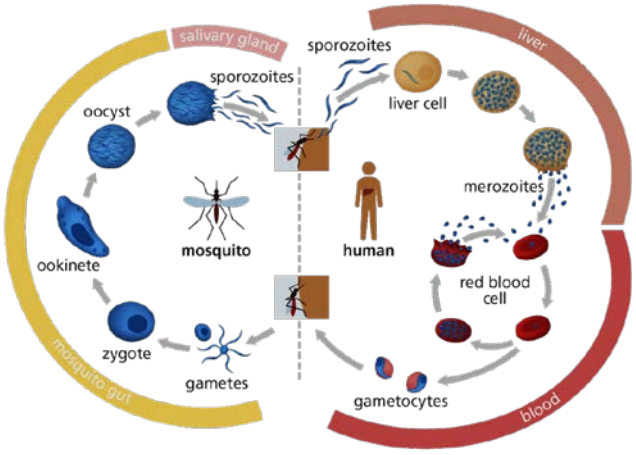

Figure 1: Life cycle of malaria [15]

Antimalarial drugs are used for treating malaria. The aim of treatment is to prevent mortality from severe malaria illness and to cure fever, chills, sweating like symptoms that are produced during plasmodia release into blood stream [15-16]. All the antimalarials have their different mode of action for malaria disease. Some are active in pre-erythocytic stage and some are active in erythrocytic stage. Some drugs are given for malaria prophylaxis [17-18]. The drugs which are active against *P. falciparum* are also active against other species of malaria that effects the human beings. The focused strategies goes on development of fast acting blood schizontocidal antimalarial drugs and resistant *P. falciparum* antimalarials in all over the world [19-20].

Table 1: Various classes of antimalarial drugs [16].

Class	**Drugs**
4-Amino quinolines	Chloroquinine, Amodiaquine, Piperaquine
Quinoline methanol	Mefloquine
Cinchona Alkaloid	Quinine, Quinidine
Biguanides	Proguanil, Chloroproguanil
Diamino Pyrimidine	Pyrimethamine
8-Amino quinoline	Primaquine, Bulaquine
Sulfonamide and Sulfones	Sulfadoxine, Dapsone
Tetracycline	Doxycycline
Sesquiterpene lactones	Artesunate, Artemether, Arteether
Amino alcohols	Halofantrine, Lumefantrine
Mannich base	Pyronaridine
Napthaquinone	Atovaquone

Atovaquone is a hydroxy napthaquinone or an analogue of ubiquinone which is highly lipophillic in nature and used for treatment and prevention of chloroquine–resistant *P. falciparum* in combination with proguanil. Atovaquine is a potent anti-malarial drug and plays vital role in disease management of malaria because of drug resistance, intolerable side effects of other anti-malarials [21-35].

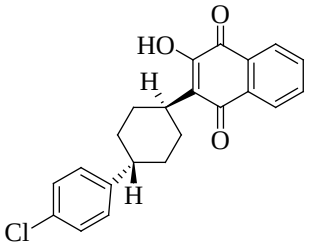

Figure 2: Chemical structure of atovaquone

IUPAC name	2-hydroxy-3-{(1R,4R)-4-(4-chlorophenyl)cyclohexyl}-1,4-dihydronapthalene-1,4-dione
Molecular formula	$C_{22}H_{19}ClO_3$
Solubility	Ethanol, Methanol, slightly soluble in water
LogP	5.9
Half life	2.2 -3.2 days
Molecular weight	366.8 g/mol
Melting point	216–219 ºC

Mechanism of action

Atovaquone is a competitive inhibitor of ubiquinol. It inhibits mitochondrial electron transport chain at the bc1 complex that leads to loss of mitochondrial function.Atovaquone hinder mitochondrial ETC (Electron Transport Chain) particularly at cytochrome bc1 complex [21,34]. During intra-erythrocytic phase of infection, a key job of the parasite in mitochondria is to give orotate for pyrimidine through the action of dihydro-orotate dehydrogenase (DHODH). Inhibition of bc1 complex by atovaquone impacts on the concentraton of metabolites in the pyrimidine biosynthesis pathway [28, 32, 36, 37].

Absorption: The bioavailability of atovaquone is low and is profoundly subject to formulation and diet. Bioavailability of the suspension expands two-crease when administered with suppers. When administered with food, bioavailability is around 47%. Without food, the bioavailability is 23% [33, 34, 26].

Volume of distribution: 0.60± 0.17 L/kg [24, 34].

Protein binding: 99% plasma proteins [24, 34].

Excretion: The half-life of atovaquone is long becouse of enterohepatic cycling and fecal disposal. There was no excretion of atovaquine *via* urine (0.6%) [24].

UV methods for Atovaquone

Varsha HC developed an extremely basic and selective UV-Visible spectroscopic method and validated for the estimation of Atovaquone in pure form and the nanosuspension. The instrument used for spectrophotometric observations was Varian Cary C50. Atovaquone was assessed to be at 494 nm in pH 8 (Phosphate Buffer and IPA in the ratio of 60:40 v/v). The linearity was found between the concentration ranges of 20-140 µg/ml. The developed method was then validated as per ICH guidelines [38].

Srujani C *et al.*, developed a method for atovaquone by using hydrotropic solubilization technique. Hydrotropic solubilization technique is utilized for the inadequately water-soluble drugs. Piperazine, urea, salicylate, sodium benzoate etc. are used as hydrotropic solvents in their different concentrations. The Shimadzu UV-1800 spectrophotometer with UV-probe software was used for the whole work process. 1M piperazine was used for enhancing water solubility of atovaquone. Wavelength is assessed to be 274 nm. Linearity was found in the range of 4-20 µg/ml. The mean percent label claim of tablet formulation of atovaquone was found to be 98.75%. The proposed method was validated as per ICH guidelines [39].

Kalpesh NP *et al*., developed a simple and precised method for the estimation of atovaquone in bulk and tablet formulations. Wavelength was assessed to be 251 nm using methanol as solvent. The linearity range was within 1-10 µg/ml. The validation of method was done as per ICH guidelines and the method was used for investigation of atovaquone marketed formulation. The label claim ± standard deviation was founded 99.14±1.14%. This methodic strategy was applied in routine analysis in bulk drugs as well as formulation of Atovaquone [40].

HPLC methods for Atovaquone

Lakshmana Rao A *et al*., developed a simple and precise RP-HPLC method for the simultaneous estimation of Proguanil and Atovaquone in pharmaceutical dosage form. Chromatographic separation was carried out by using kromasil C18 column (150mm × 4.6mm, 5µm) utilizing mobile phase 0.1% OPA:ACN in ratio 50:50 having 1ml/min flow rate with 287nm UV detection. The retention time were 2.15 and 2.48 min for k respectively. The developed method was validated as per ICH guidelines. Linearity of the method was good over the concentration range 25-150 µg/ml for proguanil and 6.25-375 µg/ml for atovaquone. The percent mean recovery of proguanil and atovaquone was with the range of 98.86 – 99.97%. This method is suitable for the routine analysis of atovaquone in bulk as well as tablet formulation [41].

Naazneen S. and Sridevi A. developed a precised and rapid RP-HPLC method for the estimation of atovaquone and proguanil in tablet formulations. The method was carried out by using gradient HPLC on C18 column (250mm × 4.6 mm, 5µ) and mobile phase comprised of 10 mM ammonium formate, pH 3.5 and 90:10 v/v acetonitrile-methanol in ratio of 30:70 v/v. The flow rate was 0.9 ml/min and effluent were monitored at 254 nm. The retention time of atovaquone and proguanil were 7.3 and 3.8 min respectively. The method was validated as per ICH guidelines. Linearity was in the range of 2.5 µg/ml- 20 µg/ml for proguanil and 6.25 µg/ml to 50 µg/ml for atovaquone. The percent recoveries of both drugs were from 98.38-101.09% for proguanil and 98.62-100.99%. This method was introduced for regular analysis and also applied for forced degradation studies of tablet formulations [42].

Viplava K and Haritha PV developed a simple and precise HPLC method for the determination of atovaquone in bulk drugs. Atovaquone was found to be degraded under different set of conditions as followed according to ICH guidelines and degradants so formed along with atovaquone were separated using Thermo Hypersil BDS C18, 250mm×4.6mm×5µm columns utilizing Buffer:Acetonitrile (20:80) as mobile phase. The flow rate was 1.5 ml/min with wavelength of 283 nm. The retention time was seen as 4.9 min. The technique was approved according to ICH guidelines. The strategy is utilized for the examination of atovaquone within the degraded items formed under different stress condition [43].

Hyphenated techniques for atovaquone

Sanjay G *et al*., developed LC-APCI method that described for human plasma determination of atovaquone utilizing lapachol as internal standard. Plasma extraction of atovaquone was finished by single step precipitation technique accomplishing mean recovery of 94.17% (CV 8%) without trading off sensitivity

(Limit of Quantitation 50.3 ng/mL) or linearity (50.3 ng/mL-23924.6 ng/mL). Warmed nebulizer in negative various reaction observing mode was utilized with transitions m/z 365.2 → m/z 337.1 and m/z 240.9→ *m/z* 185.7 for atovaquone and lapachol. Chromatographic separation on a Synergi 4 µm Polar-RP 80A (150 × 2.0 mm) column using 100 µL of plasma extraction. Injection volume was 10 µL analysis run time within minute. The created analytical method can be effectively applied to pharmacokinetic studies on atovaquone suspension regulated in sound volunteers or HIV-infected patients [44].

Allison BC *et al.*, built up an analytical evaluation of an UPLC-MS/MS strategy for Atovaquone measurement in plasma. The depicted method was adequately delicate for Atovaquone evaluation in plasma to help preclinical and clinical preliminaries. UPLC-MS/MS is the method used for the analysis of atovaquone in plasma samples. Protein precipitate with the drug atovaquone and the drug was extracted from 25µL K2-EDTA. Test solution was the separated on 2.5µm Polar-RP100 A (100 × 2 mm) column synergi. Atovaquone and its internal standard were detected over 1.3 min on an API 4000 mass analyzer using an electrospray ionization source. The method was validated in accordance with the Food and Drug Administration (FDA). From the pharmacokinetic parameters the two calibration curves were obtained ranges from 250-5000 ng/ml and 5000-50000 ng/ml. QC levels for both lower and higher concentration ranges prepared at low (750 ng/mL, 12000 ng/mL), mid (2000 ng/mL, 22500 ng/mL) and high (4250 ng/mL, 42500 ng/mL) concentrations. The precision and accuracy were founded ≤9.1% and≤±9.4%. The dilution, stability and matrix effects were studied and results were within limits [45].

CONCLUSION

In summary, the atovaquone is a novel napthaquinone derivative with great clinical applications is widely used in many antimalarial formulations. The above described analytical methods were regularly used for the quantification and identification of atovaquone.

FINANCIAL ASSISTANCE

Nil

CONFLICT OF INTEREST

The authors declare no conflict of interest

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