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A Review in Analytical Method for Determination of Anti Malarial Agents

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ABSTRACT: Malaria is an life threatening infectious caused by a plasmodium parasite transmitted by the bite of infected mosquitoes .Malaria mostly spreads to people through the bites of some infected female Anopheles mosquitoes. Blood transfusion and contaminated needles may also transmit malaria. Left untreated, P. falciparum malaria can progress to severe illness and death within 24 hour This study is a review of various analytical .methods used for the determination of antimalarial agents reported in the literature. This review encompasses methods such as spectrometry (UV), high-performance liquid chromatography (HPLC), ultra-performance liquid chromatography (UPLC), thin-layer chromatography (TLC), High performance thin-layer chromatography (HPTLC), liquid chromatography-mass spectrometry (LCMS) ,and gas chromatography (GC). It was found that all the above-mentioned methods were used for the estimation of antimalarial agents. UV spectrometry and HPLC were the most commonly used.
KEY WORDS : Malaria , Anti -malarial drugs , Analytical methods, Validation parameters.

I. INTRODUCTION

Malaria is a parasitic infection transmitted by the Anopheles mosquito that leads to acute life-threatening disease and poses a significant global health threat. Two billion people risk contracting malaria annually, including those in 90 endemic countries and 125 million travelers, and 1.5 to 2.7 million people die in a year.[1] The Plasmodium parasite has a multistage lifecycle, which leads to characteristic cyclical fevers. With timely treatment, most people experience rapid resolution of symptoms; however, significant complications may occur, including cerebral malaria, severe malarial anemia, coma, or death. Preferred antimalarial therapeutic and chemoprophylactic regimens get dictated by species, geography, susceptibility, and patient demographics. Latent or reactivating infections may be reported years following exposure.

Etiology

The incubation period, and therefore time to symptom development, varies by species: 8 to 11 days for P. falciparum, 8 to 17 days for P. vivax, 10 to 17 days for P. ovale, 18 to 40 days for P. malariae (though possibly up to several years), and 9 to 12 days for P. knowlesi. [1] The periodicity of the Plasmodium lifecycle creates the classic "malarial paroxysm" of rigors, followed by several hours of fever, followed by diaphoresis, and a drop to normal body temperature (P. vivax infection establishes a 48-hour cycle), though this is less common seen today due to rapid identification and treatment.[1]

Epidemiology

Forty percent of the global population resides in or visits malaria-endemic regions annually.[1] P. falciparum is present in Western and sub-Saharan Africa and displays the highest morbidity and mortality of the Plasmodia species.[2] P. vivax is present in South Asia, the Western Pacific, and Central America.[2] P. ovale and P. malariae are present in Sub-Saharan Africa.[2] P. knowlesi is present in Southeast Asia.[2] As many as 500 million malaria cases occur annually, with 1.5 to 2.7 million deaths.[1] Ninety percent of fatalities occur in Africa.[1] Those at highest risk include children under age 5, pregnant women, and disease naïve populations, including refugees in Central and Eastern Africa, non immune civilian and military travelers, and immigrants returning to their place of origin.[2] Of the 125 million travelers who visit endemic locations each year, 10000 to 30000 develop malaria, and 1% of these will die from

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complications of their disease.[2][3] Rising average global temperatures and changes in weather patterns are projected to expand the burden of malaria; a rise of 3 degrees Celsius is postulated to increase malaria incidence by 50 to 80 million.[1]

Types:
There are five main types of malaria, each caused by a different species of the Plasmodium part

Plasmodium falciparum (P. falciparum)

Most severe and deadly form, Accounts for 80% of malaria cases and 90% of malaria deaths, Primarily found in Africa. Symptoms: severe fever, chills, flu-like symptoms, organ failure.

Plasmodium vivax (P. vivax)

Most widespread geographically ,Found in Asia, Latin America, and Africa. Symptoms: mild to moderate fever, chills, flu-like symptoms Can relapse from liver stages similar to vivae.

Plasmodium ovale (P. ovale)

Found in Africa and Asia. Symptoms: mild fever, chills, flu-like symptoms Can relapse from liver stages.

Plasmodium malariae (P. malariae)

Mild symptoms Found in Africa, Asia, and Latin America. Symptoms: mild fever chills, flu-like symptoms, organ failure.

Plasmodium knowlesi (P. knowlesi)

Rare and primarily found in Southeast Asia Can cause severe symptoms. Symptoms: fever, chills, flu-like symptoms, respiratory distress.

Life cycle of malaria

1. Infection: An infected female Anopheles mosquito bites a human, injecting sporozoites into the bloodstream.
2. Sporozoites: Travel to the liver, where they multiply and develop into merozoites.
3. Merozoites: Infect red blood cells, causing them to rupture and release more merozoites. Stage 2: Mosquito Host

1. Gametocytes: Some merozoites develop into male and female gametocytes in the human bloodstream.
2. Mosquito Ingestion: When an Anopheles mosquito feeds on infected blood, gametocytes are ingested.
3. Fertilization: Male and female gametocytes fuse, forming a zygote.
4. Oocyst: The zygote develops into an oocyst, which releases sporozoites.
5. Sporozoites: Migrate to the mosquito's salivary glands, ready to infect another human. Interrupting the Cycle

Prevention and treatment strategies focus on:

1. Mosquito control (nets, repellents, insecticides)
2. Eliminating standing water
3. Wearing protective clothing
4. Vaccination
5. Antimalarial medications

Understanding the life cycle is crucial for developing effective interventions against this disease.

Medications:
The most common antimalarial drugs includes:

- Chloroquine phosphate,
- Artemisinin based combinations therapies(ACTS)
- Eg:Artemether-Lumefantrine,Artesunate-Mefloquine.
- Atovaquone-proguanil(Malarone)
- Quinine sulfate with doxycycline
- Primaquine phosphate

Classification of antimalarial drugs

Table 1

Class	Name of Drugs
4 amino quinolones	Chloroquine,amodaquine,piperaquine,Hydroxychlorquine.
8 amino quinolines	Primaquine,pamaquine,tafenoquine.
Sesquiterpene lactones	Artemether ,arteether,artesunate,arteroline

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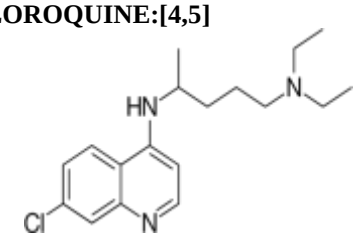
4 quinoline methanol	Mefloquine
Biguanides	Proguanil
Cinchona alkaloids	Quinine,quinidine
Pyrimidine analogue	Pyrimethamine
Polycyclics(antibiotics)	Doxycycline,clindamycin,halofantrine
Sulfonamides	Sulfadoxine,sulfamethopyrizine
Dihyrdo triazines	Cycloguanil
9amino acridine	Quinacrine
Hydroxy naphthaquinone	Atovaquone
Amino alcohol	Lumefantrine
Newer antimalarial agents	Artemisinin,fosmidomycin.
Miscellaneous	Metaloamine,sulphadoxine

1. 4AMINO QUINOLINES
A] CHLOROQUINE:[4,5]



IUPAC name : 7 chloro -4(4-dimethylamino -1-methyl butyamino) – quinolone
Molecular formula : C18H26ClN3 **Molecular weight :** 319.872g/ml **Physical properties:**
Appearance : chloroquine is a white slightly yellow , odorless, crystalline powder that tastes better **Solubility :**
slightly soluble in water ,but soluble in dilute acids, chloroform and ether.
Boiling point:460.6°C
Melting point : 87°C
Chloroquine is a medication used to prevent and treat malaria a disease caused by mosquito borne parasites .It is used for other condition ,such as amoebic dysentery,rheumatoid arthritis.

TYPES:
1. Chloroquine phosphate (oral) 2.Chloroquine hydrochloride(injectable) 3.Chloroquine sulfate (topical)

MECHANISM:
The parasite digests the host cell's hemoglobin to obtain essential amino acids.The process releases large amounts of heme,which is toxic to the parasite.To prevent itself the parasite ordinarily polymerizes the heme to non toxic hemeozoin which is sequestered in the parasite's food vacuole.Chloroquine prevents the polymerization to hemozoin. The accumulation of heme results in analyis of both the parasite and the red blood cell.-U

ANALYTICAL METHODS :
HPLC:
Several HPLC methods have been described for the measurement for the chloroquine.

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1. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH VARIABLE WAVELENGTH DETECTOR[VWD]:

The chromatographic equipment consisted of a series 1100 HPLC system with manual injector, variable wavelength detector (VWD) and Vacuum degasser (Agilent Technologies Deutschland GmbH, Waldron, Germany). An Eclipse C18 (Agilent, Germany) column (150mm x 4.6mm, 5µm particle size) was used for the separation. HPLC Conditions for Chloroquine diphosphate Analysis: Mobile phase: Phosphate Buffer: Acetonitrile (40:60v/v), the pH of the phosphate buffer was adjusted to 6.60 with sodium perchlorate Flow rate: 1.500mL/min. Column: Eclipse, XDB-C18 (150mm, 4.6mm, 5µm). Detection wavelength: 330nm Good selection of mobile phase combination may shorten the retention time.[4]

2. HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH DIODE ARRAY DETECTION[DAD]:

The HPLC analyses were carried out on an Acella system from Thermo Scientific (Waltham, MA, USA), composed of quaternary pump, auto sampler and diode array detector (DAD). The column was an ACE C18 (100 × 4.6 mm id; 5 µm particle size) from ACT (Aberdeen, Scotland), maintained at 25°C. UV detection was performed at 260 nm and injection volume was 10 µl. The mobile phase was composed of acetonitrile (A) and 0.1% aqueous triethylamine, pH 3.0 adjusted with phosphoric acid (B), at a flow rate 1 mL/min. The gradient elution programmme was 10% A from 0–1.9 min, 10-40% A from 1.9-2.0 min and 40% A from 2.0-3.3 min. For column re-equilibration, 10% A was maintained from 3.3-5.00 min.[5]

ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY WITH DIODE ARRAY DETECTION [DAD]

The UPLC analyses were carried out on an Acella system from Thermo Scientific (Waltham, MA, USA), composed of quaternary pump, auto sampler and DAD. The column was a Hypersil C18 (50 × 2.1 mm id; 1.9 µm particle size) from Thermo Scientific (Waltham, MA, USA), maintained at 25°C. UV detection was performed at 260 nm. UV spectra from 200 to 400 nm were on line recorded for peak identification. The injection volume was 7.0 µl. The mobile phase was composed of acetonitrile (A) and 0.1% aqueous triethylamine, pH 3.0 adjusted with phosphoric acid (B), at a flow rate 0.6 mL/min. The separation of chloroquine and primaquine was evaluated in different proportions of these solvents and, for each condition, retention time and resolution (R) were calculated. The optimized condition was achieved using a gradient elution programme: 10% A from 0–0.45 min, 10-40% A from 0.45-0.47 min and 40% A from 0.47-1.30 min. For column re-equilibration, 10% A was maintained from 1.30-4.00 min.[5]

METHOD DEVELOPMENT:

INSTRUMENTS	COLUMN	FLOW RATE	WAVELENGTH	MOBILE PHASE	DETECTOR	REFERENCE
HPLC	1.HPLC-VWD EclipseC18 Column(150mm x 4.6mm)	1.500mL/min.	330nm	Phosphate Buffer: Acetonitrile (40:60v/v),	Variable wavelength detector(VWD)	4
	2.HPLC-DAD ACE C18 (100 × 4.6 mm)	1 mL/min.	260 nm	Acetonitrile (A) and 0.1% aqueous triethylamine	Diode array detector (DAD)	5
UPLC	Hypersil C18 (50 × 2.1 mm	0.6 mL/min.	260 nm	Acetonitrile (A) and 0.1% aqueous triethylamin	Diode array detector (DAD)	5

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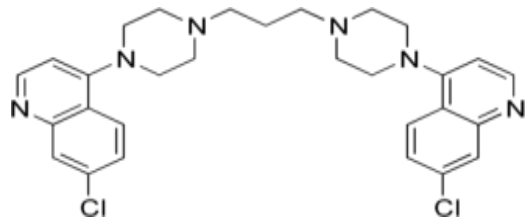
VALIDATION PARAMETERS:

INSTRUMENTS	LINEARITY	ACCURACY	PRECISION	LOD	LOQ	REFERENCE
HPLC-VWD	0.953	33.2	43.1	25.5	247.8	4
HPLC-DAD	60	99.83	1.59	Limit the relation of 10.	Limit the relation of 10	5

B] PIPERAQUINE.[6,7]



IUPAC NAME:7-chloro-4-[4-[3-[4-(7-chloroquinolin-4-yl)piperazin-1-yl]propyl]piperazin-1- yl]quinoline
MOLECULAR FORMULA : C29H32Cl2N6
MOLECULAR WEIGHT : 535.5 g/mol
PHYSICAL PROPERTIES :
APPEARANCE : Piperaquine is a white or pale-yellow crystalline powder that is odorless and tasteless. Its color changes readily in light.
SOLUBILITY : Piperaquine is a drug that is highly soluble in chloroform, slightly soluble in ethanol, and almost insoluble in water Piperaquine is also highly fat-soluble
MELTING POINT :199-204^{o}c
MECHANISM OF ACTION: The mechanism of piperaquine inhibition of the haem detoxification pathway is unknown but is expected to be similar to that of Chloroquine.
THERAPEUTIC USES: Piperaquine is an antiparasitic drug used in combination with dihydroartemisinin to treat malaria.

ANALYTICAL METHODS:
HPLC:
Several HPLC methods have been described for the measurement for the piperaquine.

1. HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH DIODE ARRAY DETECTION[DAD]:

The liquid chromatography system was a Lachrom Elite-Hitachi (Merck) consisting of an L-2200 auto sampler, L- 2130 (2 pumps), a L-2350 column Oven and a L-2455 DAD. Data acquisition was performed using EZchrom Elite version 3.18 HPLC System Manager Software (Merck-Hitachi Japan). The analytical compounds were analysis on a Poroshell 120 C8-end-capped (100 × 4.6 mm, 2.7 µm) column protected by 2.7 µm guard cartridges 12.5 × 4.6 mm (Agilent Technologies, USA). The SPE process was carried out on the manual SPE system, VAC Master 96 sample processing Manifold (IST-Biotage, Sweden) using Oasis-MCX 30 mg cartridges or 96 well plates (Waters, USA). The Manifold system uses vacuum to get the liquid through the SPE column or plate. The Speed Vac system (ThermoFisher Scientific) consisting of a condensation vacuum combined with a centrifugator was used to evaporate the eluates.The injection volume was 50 µL.[7]

2. **HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH ULTRAVIOLET[UV] ABSORBANCE DETECTION:**

The HPLC system consisted of a Waters Millipore Solvent Delivery System coupled to an HP 1100 isocratic pump, auto sampler and variable wavelength UV detector (Agilent Technology, Waldbronn, Germany). Separations were achieved on a WatersXTerra RP 3.5 18 mm, 4.63100 mm HPLC column with a Waters Symmetry C 5 18 mm, 3.9320 mm noise ratio of 2.5. The mobile phase of 7% (v/v) acetonitrile in water (containing 0.025% trifluoroacetic acid, v/v, 0.1% NaCl, w/v,

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and 0.008%triethylamine, v/v), was pumped at 1.2 ml/min, and analytes were detected by their UV absorbance at 340 nm. Analysis and quantification of chromatograms (peak areas) were undertaken using CHEM- STATION software (Agilent Technology, Waldbronn Germany).[7]

MASS SPECTROMETRY:

Sciex API2000 tandem mass spectrometer was coupled with a Perkin Elmer 200 series micro LC system. The LC column was Gemini C18 (50×2.0 mm, 5µm) fitted with a guard column (4×2.0 mm, 5µm) (Phenomenex Inc., Torrance, CA, USA), eluted with 10 mM NH4OH (A) and MeCN (B) in a gradient mode at a flow rate of 0.6 mL/min. ElectroSpray ionization in positive mode (ESI+) was used as the ion source with multiple reaction monitoring (MRM) of m/z 535/288 for PQ and m/z 541/294 for the IS (PQ-d6) for quantitation. PQ stock solution was prepared in MeCN- water (1:9, v/v) containing 0.5% formic acid. Calibration standard samples (10, 25, 50, 100, 250, 500, and 1000 ng/mL) and QC samples (30, 200, and 800 ng/mL) were prepared in blank plasma from two different stock solutions. Plasma samples (25 µL) were mixed with 25 µL 30 ng/mL PQ-d6 in MeCN-water (1:9, v/v) containing 0.5%FA, added 150 µL MeOH, briefly vortex-mixed, and centrifuged at 25,000g for 5min. Transferred ~100µL supernatant to plastic sample vials or 96-well plate in autos ampler. Injection volume was 10 µL.[6]

METHOD DEVELOPMENT:

INSTRUMENTS	COLUMN	FLOW RATE	WAVELENGTH	MOBILE PHASE	DETECTOR	REFERENCE
HPLC	1.HPLC-DAD C8-end-capped (100 × 4.6 mm, 2.7 µm)	1 ml/min	347nm	Methanol:water (80:20**)**	Diode array detector (DAD)	7
	2.HPLC-UV Waters Symmetry C5 18 mm	1.2 ml/min	340 nm	7% (v/v) acetonitrile in water (containing 0.025% trifluoroacetic acid, v/v, 0.1% NaCl, w/v, and 0.008%triethylamine, v/v)	variable wavelength UV detector[UVD]	7
Mass spectrometry	Gemini C18 (50×2.0 mm, 5µm)	0.6 mL/min	------	MeCN-water (1:9, v/v)	Array detector	6

VALIDATION PARAMETERS

INSTRUMENTS	LINEARITY	ACCURACY	PRECISION	LOD	LOQ	REFERENCE
HPLC-DAD	70	99.97	5.76	-	-	7
HPLC-UV	0.998	109	100	25	20	7

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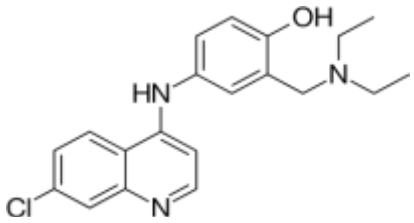
C]AMODAQUINE:[8,9,10,11]



IUPAC NAME: 4-[(7-chloroquinolin-4-yl)amino]-2-(diethylaminomethyl)phenol
MOLECULAR FORMULA : C20H22ClN3O
MOLECULAR WEIGHT : 355.9 g/mol
PHYSICAL PROPERTIES :
APPEARANCE : Yellow, crystalline powder with a bitter taste and little to no odor **SOLUBILITY :** Soluble in water and ethanol, but not in benzene, chloroform, or ether **BOILING POINT :** The boiling point of amodiaquine hydrochloride is 478°C at 760 mmHg. **MELTING POINT:** 206 - 208^{o}c
MECHANISM OF ACTION: Amodiaquine is an antimalarial drug that works by binding to parasitic DNA and preventing the production of DNA, RNA, and proteins
THERAPEUTIC USES :Amodiaquine is an aminoquinoline used for the therapy of malaria. Amodiaquine has been linked to severe cases of acute hepatitis which can be fatal, for which reason it is recommended for use only as treatment and not for prophylaxis against malaria.

ANALYTICAL METHODS:
HPLC
Several HPLC methods have been described for the measurement for the amodaquine

1. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH ULTRAVIOLET[UV]ABSORBANCE DETECTION:

The chromatographic columns used in this study were Xbridge BEH C18 (150 mm x 3 mm, 5 µm) purchased from Waters (Milford, MA, USA). All analyzes were performed in gradient mode. UV spectra were systematically recorded from 200 to 400 nm for peak identification. To ensure a satisfactory sensitivity, peak integrations were performed at ,and 254 nm for AQ and its impurities. For all experiments, the sample injection volume was 10 µL. An experimental design was used to vary simultaneously gradient slope, pH of mobile phase A, and column temperature. Mobile phase A was an aqueous solution with pH modifiers to reach the desired pH. For method development, pH 2.9 was obtained by mixing 10 mM formic acid with 10 mM ammonium formate while pH 3.5 was fixed using a mixture of 10 mM acetic acid and 10 mM ammonium acetate. A pH of 3.2 was obtained with a 20 mM acetic acid solution. At the working point, a pH of 3.35 was fixed using 10 mM acetic acid. Mobile phase B was composed of 96% EtOH containing the same proportion of pH modifiers as mobile phase A to prevent ionic concentration change during the gradient. The gradient profile consisted in a linear ramp from 5% to 80% of mobile phase B, which corresponded to a variation of 72% in pure ethanol from 4.8% to 76.8%. Since an UHPLC system was used and for a suitable transfer of the method to a classical HPLC apparatus, an isocratic plate of 3 min consisting in 5% of mobile phase B was added, at the beginning of the gradient for all analyses. Flow rate was set 0.4 mL/min.[8]

2. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH VARIABLE WAVELENGTH DETECTOR[VWD]:

The chromatographic equipment consisted of a series 1100 HPLC system with manual injector, variable wavelength detector (VWD) and Vacuum degasser (Agilent Technologies Deutschland GmbH, Waldron, Germany). An Eclipse C18 (Agilent, Germany) column (150mm x 4.6mm, 5µm particle size) was used for the separation. The spectroscopic equipment consisted of a UV/VIS 1650PC (Shimadzu, Japan); with 2 mm slit width and 1 cm cuvette sample holder was used. HPLC Conditions for Amodiaquine are Mobile phase is Phosphate Buffer: Methanol (45:55v/v), the pH of the phosphate buffer was adjusted to 3.5 with perchloric acid. Flow rate is1.50mL/min. Column :Eclipse, XDB-C18 (150mm, 4.6mm, 5µm). Detection wavelength is 345nm.[9]

LIQUID CHROMATOGRAPHY MASS SPECTROMETRY [LC-MS]:

The LC system was an Agilent 1260 infinity system consisting of a binary LC pump, a vacuum degasser, a temperature-controlled microwell plate auto sampler set at 4 ◦C and a temperature-controlled column compartment set at 40 ◦C (Agilent technologies, CA, USA). Data acquisition and processing were performed using

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Analyst 1.6.2 (Sciex, MA, USA). The analytes were separated on a Zorbax SB-CN 50 mm × 4.6 mm, I.D. 3.5 µm (Agilent Technologies),with a pre-column CN AJO-4305 4 mm × 3 mm, I.D. 3.5 µm (Phenomenex, Torrance, California, USA), at a flow rate of 700 µl/min. The mobile phase consisted of (A) acetonitrileammonium formate 20 mM with 1% formic acid pH ~ 2.6 (15–85, v/v) and (B) methanol–acetonitrile (75–25, v/v). The mobile phase gradient was A: 0–2 min, B: 2.2–3.7 min and A: 3.9–6.5 min (with 0.2 min linear gradient switch), resulting in a total runtime of 6.5 min per sample. The injection volume was 2 µl. An API 5000 triple quadrupole mass spectrometer (Sciex, MA, USA) with a TurboV ionization source interface, operating in the positive ion mode, was used for the MS/MS analysis. Ion spray voltage was set to 5500 V, with a drying temperature at 650 ◦C. The curtain gas (CUR) was 25 psi and the nebulizer (GS1) and auxiliary (GS2) gases 60 psi. All used collision energy of 29 V.[10]

UV VISIBLE SPECTROPHOTOMETRY:

A Shimadzu UV-1700 UV/VIS Spectrophotometer loaded with the UV Probe software, spectral bandwidth of 1 nm and wavelength accuracy of ±0.3 nm was used for the spectral measurements. For weighing and dissolving, 1 mg sensitive electronic balance (Vibra DJ-150S-S, Shinko Denshi, Japan) and bath sonicator (Sarthak SUC-322) were used, respectively.[11]

METHOD DEVELOPMENT:

INSTRUMENT	COLUMN	FLOW RATE	WAVELENGTH	MOBILE PHASE	DETECTOR	REFERENCE
HPLC	1.HPLC-UV Xbridge BEH C18 (150 mm x 3 mm, 5 µm)	0.4 mL/min	254 nm	Aqueous solution with pH modifiers.AND 96% EtOH containing the same proportion of pH modifiers	Ultraviolet[uv] Absorbance Detector.	8
HPLC	2.HPLC-VWD Eclipse C18 column(150m m x 4.6mm, 5µm particle size).	1.50mL/min	345nm	Phosphate Buffer: Methanol (45:55v/v),	variable wavelength Detector.	9

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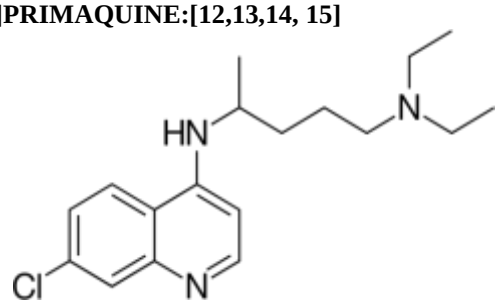
VALIDATION PARAMETERS:

INSTRUMENT	LINEARITY	ACCURACY	PRECISION	LOD	LOQ	REFERENCE
HPLC-UV	1.03	12.64	1.44	------	-----	8
HPLC-VWD	0.9554	36.3	80.7	140.2	431.2	9
LC-MS	85	101	9.11	0.57	1.08	10

2. 8AMINO QUINOLINES
A]PRIMAQUINE:[12,13,14, 15]



IUPAC NAME: 4-N-(6-methoxyquinolin-8-yl)pentane-1,4-diamine
MOLECULAR FORMULA: C15H21N3
MOLECULAR WEIGHT : 259.35 g/mol
PHYSICALPROPERTIES:
APPEARANCE: Viscous liquid
SOLUBILITY: tablets should be stored in well-closed, light-resistant containers at a temperature less than 40 °C, preferably between 15-30 °C
BOILING POINT : 175-179 °C
MELTING POINT: < 25 °C
MECHANISM OF ACTION : Primaquine acts by interfering with a part of the parasite (mitochondria) that is responsible for supplying it with energy. Without energy the parasite dies. This stops the infection from continuing and allows the person to recover.

ANALYTICAL METHODS :
HPLC
Several HPLC methods have been described for the measurement for the primaquine.

1. **HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH ULTRAVIOLET[UV]ABSORBANCE DETECTION:**

The HPLC analysis was performed on a Shimadzu LC-10A chromatographic system equipped with an LC-10AD pump, UV-Vis detector, and a SCL-10AVP system controller. The data were acquired and processed using CLASSVP 6.14 software program. The column used was an RP-C18A Merck (150 x 4,6 mm particle size of 5 µm). The mobile phase composition was acetonitrile, methanol, 1 M perchloric acid and water (33:6:1:87). The flow rate was of 1.0 mL/min and the injection volume was 20 µL for all standards and samples. The detection was investigated at 254 nm. A simple, precise, accuracy and convenience HPLC method was developed and validated for quantitative determination of PQ in extended release tablets.[12]

2. **HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH DIODE ARRAY DETECTION[DAD]&UV ABSORBANCE DETECTION:**

Analyses were performed by HPLC-DAD-UV using the Shimadzu Class-VP (liquid chromatographer coupled to a Shimadzu UV detector with the diode array SPD M10A VP equipped with a SCL 10A VP controller, DGU14A degasser, 10ADVP LC binary pump, CTO 10ASVP oven, and SIL10AF auto injector). The chromatograms were evaluated using Shimadzu Class VP® software, version 6.1. Various combinations of acetonitrile, methanol, and ammonium acetate buffer were used as mobile phases. All buffer solutions were filtered through a 0.45-µm pore PVDF filter (Merck-Millipore) before use. HPLC columns were silica-based C18 (250mm × 4.6mm i.d. × 5µm, ODS Hypersil, Thermo, Waltham, MA, USA) and modified-silica

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cyanopropyl (250mm × 4.6mm i.d. × 5µm, Supelcosil LC-CN; Supelco, St. Louis, MO, USA). The injection volume was 20µL for all analyses.[13]

ULTRA-HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY–TANDEM MASS SPECTROMETRY (UHPLC-MS/MS):

This system (UltiMateTM 3000 HPLC Systems and TSQ Quantum Access MAX) comprised Rapid Separation (RS) pump, vacuum degasser, RS autosampler, RS column compartment, and triple-stage quadrupole mass spectrometer. The separation was performed using a Hypersil GOLDTM aQ C18 column (100 × 2.1 mm, particle size 1.9 µm) with a C18 guard column ((4 mm × 3 mm) from Thermo Fisher. The column temperature was maintained at 25 °C. An isocratic mode of mobile phase A (0.1% of formic acid in methanol:water (40:60, v/v)) and mobile phase B (0.1% of formic acid in acetonitrile) flowed in a ratio of 80:20 at 0.4 mL/min. The injection volume was 1 µL.[14]

LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY:

Sample preparation and solid-phase extraction was performed on an automated Freedom Evo 200 platform (TECAN, Männedorf, Switzerland). Te LC system was an Agilent 1200 system consisting of a binary LC pump, a vacuum degasser, a temperature-controlled micro well plate auto-sampler set at 4 °C and a column compartment set at 20 °C (Agilent technologies, Santa Clara, CA, USA). Te mass spectrometry equipment was an API 5000 triple quadrupole system (Applied Biosystems/ MDS Sciex, Foster City, CA, USA), with a TurboV Ionization Source (TIS) interface operated in the positive ion mode. Data acquisition was performed using Analyst 1.5 (Applied Biosystems/MDS Sciex, Foster City, CA, USA). PRQ, PRQ-D3, CPRQ and CPRQ-D3 enantiomers were separated on a Chiralcel OD-3R column (150 mm×4.6 mm, I.D. 3 µm; Chiral Technologies Inc., West Chester, PA, USA), protected by a Chiralcel OD-3R guard column (4 mm×10 mm, I.D. 3 µm), at a fow rate of 1.0 mL/min. Mobile phase A contained 20 mM OD-3R guard column (4 mm×10 mm, I.D. 3 µm), and mobile phase B contained methanol:acetonitrile (75:25, v/v). Te following gradient program was employed for separation of enantiomeric PRQ and CPRQ and to remove phospholipid residues from the column: 0–15 min (100% mobile phase A), 15–16.0 min (100% mobile phase A to 100% mobile phase B), 16.0–20.8 min (100% mobile phase B), 20.8–21 min (100% mobile phase B to 100% mobile phase A), and 21.0–26.5 min (100% mobile phase A). Te MS/MS conditions were optimized by infusing (±)-PRQ (10 ng/mL) and (±)-CPRQ (20 ng/mL) at 10 µL/min, using a Harvard infusion pump connected directly to the MS. Additional MS/MS tuning was performed by continuous infusion of (±)-PRQ (25 ng/mL) and (±)-CPRQ (50 ng/mL) at a fow rate of20 µL/min via a "T"-connector into the post-column mobile phase at a fow rate of 1.0 mL/min. TIS temperature was maintained at 700 °C and the TIS voltage was set to 4500 V. Te curtain gas was set to 30 psi and the ion source gas 1 (GS1) and ion source gas 2 (GS2) at 50 and 60 psi, respectively. Te CAD gas in the collision cell was set to 5 psi. Quantifcation was performed using multiple reaction monitoring (MRM) for the transitions m/z 260–175 and m/z 263–86 for PRQ and PRQ-D3, respectively, and m/z 275–175 and m/z 278–178 for CPRQ and CPRQ-D3, respectively. Te declustering potential (DP) was set to 60 V for all analytes and stable isotope-labelled internal standards.[15]

METHOD DEVELOPMENT:

INSTRUMENTS	COLUMN	FLOW RATE	WAVELENGTH	MOBILE PHASE	DETECTOR	REFERENCE
HPLC	1.HPLC-UV: RP-C18A Merck (150 x 4,6 mm particle size of 5 µm)	1.0 mL/min	254 nm	Acetonitrile, methanol, 1 M perchloric acid and water (33:6:1:87).	UV Absorbance Detector.	12

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	2.HPLC-DAD-UV silica-based C18 (250mm × 4.6mm × 5µm)	1.1mL/min	251nm	Acetonitrile, methanol ammonium acetate buffer.	UV detector with the diode array detector.	13
UHPLC-MS/MS	C18 guard column (4mm×3mm, particle size 1.9 µm)	0.4 mL/min	225nm	(0.1% of formic acid in methanol:water (40:60, v/v)AND (0.1% of formic acid in acetonitrile)	------	14
LC-TMS	Chiralcel OD- 3R guard column (4 mm×10 mm, 3 µm)	1.0mL/min	226nm	Methanol:acetonitrile (75:25V/V)	Mass spectrometer	15

VALIDATION PARAMETERS:

INSTRUMENTS	LINEARITY	ACCURACY	PRECISION	LOD	LOQ	REFERENCE
HPLC-UV	0.9989	95.5-101	2	1.36	4.13	12
HPLC-DAD-UV	0.9997	115	100.4	1	3.5	13
LC-TMS	17	6	10	0.286	0.571	15

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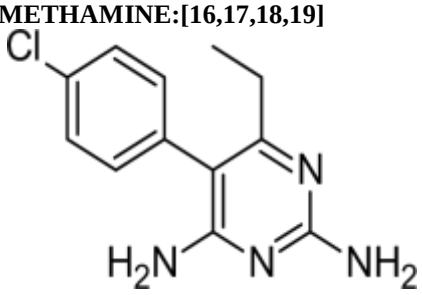
3.PYRIMIDINE ANALOGUE : A]
PYRIMETHAMINE:[16,17,18,19]



IUPAC NAME: 5-(4-chlorophenyl)-6-ethylpyrimidine-2,4-diamine
MOLECULAR FORMULA: C12H13ClN4
248.71 g/mol
Pyrimethamine is an odorless white crystalline powder. Tasteless. An antimalarial drug.
451 to 453°F

MECHANISM OF ACTION :

Pyrimethamine interferes with the regeneration of tetrahydrofolic acid from dihydrofolate by competitively inhibiting the enzyme dihydrofolate reductase.Tetrahydrofolic acid is essential for DNA and RNA synthesis in many species, including protozoa. It has also been found to
reduce the expression of SOD1, a key protein involved in amyotrophic lateral sclerosis

ANANALYTICAL METHORDS :
HPLC -UV SPECTROSCOPY :

All absorbance measurements were made with a Double beam UV-1800 (SHIMADZU, Japan) ultraviolet–visible spectrophotometer provided with matched 1-cm quartz cells and also temperature controller was used for the spectrophotometric measurements. pH meter model pH211 (HANNA, Italy). Thermostatically controlled water bath type RE 220 (LAUDA, Germany)

UV SPECTROSCOPY /DUAL WAVE LENGTH :

1800 UV-Vis spectrophotometer (Shimadzu) and a set of Personal Computer (PC) equipped with UV-Probe 2:42 , Microsoft Excel and SPSS 20, Matlab® version R2016a, cuvette 1 cm, glass tools (Oberoi), mortar and pestle , a rubber ball, analytical balance (Boeco), sonicator (Branson 1510), a pH meter (Hanna) as well as other tools that are required in sample preparation and solution.

RP HPLC:

Agilent 1260 infinity HPLC module, HPLC column C18 inertsil column (250mm× 4.6mm i.d, 5µm particle size) were used for the method development and validation, Kroma Tech (KL-1.5) sonicator was used for sonication, PH meter (LAB INDIA), UV 1800 (Schimadzu), Digital balance (Conitech).

RP HPLC :

Analytical grade PYR (purity ≥ 99%) and SLP (purity ≥ 99%) were obtained as gift samples from RL Fine Chemicals, Bangalore, India as a reference standard. Lari-500 (pyrimethamine-25 mg and sulfamethoxypyrazine- 500 mg) tablets manufactured by IPCA laboratories Ltd., were purchased from a local pharmacy.

Acetonitrile (HPLC grade) was purchased from Merck, India. Potassium dihydrogen orthophosphate used in the preparation of buffer, was procured from Sigma Aldrich, Mumbai. Milli pore HPLC grade water was obtained from Milli-Q system (Direct- Q).

METHOD DEVELOPMENT :

INSTRUMENT	COLUMN	FLOW RATE	WAVELENGTH	MOBILE PHASE	DETECTOR	REFERENCE
HPLC –UV	C18	2ml/min	480NM	80%GLACIAL ACITIC ACID (1:100) 20% ACETO NITRILE 500 µg TRIETHYLAMINE	UV	16
DUAL WAVE-LENGTH	-		**SDN :** 274.2nm			

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SPECTROPHOTOMETRY METHOD			291.6nm **PTN :** 284.8nm 28.8nm	methanol	UV	17
RP HPLC (PYRIMETHAMINE/ SULFAMETHAMINE)	C18	1min /ml	221nm	Potassium dihydrogen phosphate (10mmol) Acetonitrite (70:30) Ph :3.5 Orthophosphoric acid	Uv visible detector	18
RP HPLC (PYRIMETHAMINE / SULFAMETHOXY PYRAZINE)	C8	0.8ml/min	254nm	Acetonitrite /potassium hydrogen phosphate 60;40v/v	PDA DETECTOR	19

VALIDATION PARAMETERS:

HPLC -UV

INSTRUMENT	LINEAR RANGE	CORRELATION CO EFFICIENT	ACCURACY	PRESITION	LOD	LOQ	REFERENCE
HPLC UV	12-40	0.996	100.04-101.9	0.996	3.25	10.83	16

DUAL WAVE LENGTH SPECTROPHOTOMETRY METHOD

DRUG	ACCURACY %	PRESITION RSD (%)	r2	INTERDAY RSD (%)	INTRADAY RSD (%)	LOD	LOQ	REFERENCE
PTN	100.27	0.82	0.9997	1.29	0.47	0.39	1.18	17
SDN	101.03	0.57	0.9998	1.16	2.35	0.20	0.61	

RP HPLC :

DRUG	LINEARITY	ACCURACY	PRECITION	L O D	L O Q	REFERENCE
PYRIMETHAMINE	1702308	99.00	99.60	-	-	18
SULFAMETHOXYPYROZINE	6170716	99.50	99.20	-	-	

RP HPLC :

DRUG	LINEARITY	ACCURACY	PRESITION	LOD	LOQ	REFERENCE
PYR	2 TO 32	0.449	1.022	2.983	9.042	19
SLP		0.739	1.028	2.297	6.963	

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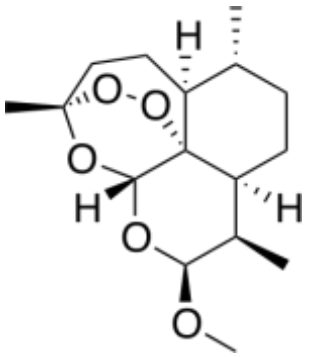
4. SESQUITERPENE LACTONES
A]ARTEMETHER :[20,21,22,23]



IUPACNAME:(1R,4S,5R,8S,9R,10S,12R,13R)-10-methoxy-1,5,9-trimethyl-11,14,15,16tetraoxatetracyclo[10.3.1.$0^{4,13}$.$0^{8,13}$]hexadecane

MOLECULAR FORMULA: C16H26O5
MOLECULAR WEIGHT : 298.37 g/mol
PHYSICAL PROPERTIES
APPEARANCE : Solid , Crystals **BOILING POINT :** 357.46 °C **MELTING POINT:** 86 - 90 °C

MECHANISM OFACTION :

Artemether is a semi-synthetic derivative of artemisinin that works against the malarial parasite Plasmodium falciparum by disrupting the parasite's cellular functions:Complexing with iron Artemether is activated when it complexes with iron in the parasite's hemoglobin. Producing free radicals The resulting compound produces carbon-centered free radicals and reactive oxygen species. Disrupting calcium transport.The free radicals and reactive oxygen species disrupt the parasite's calcium transport and other cellular functions. Inhibiting nucleic acid and protein synthesis
Artemether inhibits the parasite's nucleic acid and protein synthesis.Artemether is usually administered in combination
with lumefantrine for improved efficacy. The combination reduces the emergence of resistance.Artemether is lipid
soluble and can be toxic in high dosages. Adverse effects include gastrointestinal disturbances, dizziness, tinnitus, and
prolongation of the QT interval.

ANALYTICAL METHODS :
HPLC:

An HPLC method for the determination of artemether in injections is also mention in the European pharmacopoeia. The assay is determined using HPLC, reverse phase C18 column Supelco L1 C18 (4.6 mm × 250 mm, 5 µ) and UV detector set at a wavelength of about 216 nm. Mobile phase consisted of a mixture of acetonitrile and water (62:38, v/v) operated at a flow rate of 1.5 ml/min in ambient temperature. After preparation, solution of artemether was injected into the HPLC rheodyne (20 µl). Artemether peak was observed at 73 min, which is a very long retention time. To reduce the retention time, the proportion of mobile phase was changed as it is known that artemether is soluble in methanol and insoluble in water so the ratio of methanol in the mobile phase was increased. The new mobile phase ratio became as methanol and water (70:30). The retention time reduced to 32 min keeping the above mentioned conditions constant, which is a very long and time consuming retention time. Then the ratio of methanol in mobile phase was further increased and the new ratio of mobile phase became (80:20) for methanol and water, respectively. This time the retention time was 12 min which is considered as good retention time for an analysis. So, the mobile phase in the ratio of 80: 20 for methanol and water was finalized. New method for the estimation of artemether is linear, specific and sensitive and can be applied to analyze various injection formulations of artemether.

HPLC:

HPLC Apparatus (shimadzu) equipped with LC-10 ATvp, double reciprocating plunger pump and SPD-10 Avp UV detector, and deuterium lamp as a light source ranging 190-600 nm was used. Hypersil Octadecyl silane (ODS) column, (250 3 4) mm, 5 mm particle size of packing, pore size 120 A, Thermo Electron Corporation. Marketed formulations Larither and Falcidol capsules were purchased from a local market. Mobile phase selected was ACN and buffer in the ratio of 65:35 of pH 6.5 adjusted with tryethylamine. Wavelength selected was 210 nm. Standards and facilities were provided by Oasis Lab, Ahmedabad.

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METHOD DEVELOPMENT:

HPLC:

INSTRUMENT	COLUMN	FLOW RATE	WAVE LENGTH	MOBILEPHASE	DETECTOR	REFERENCE
HPLC	C18	1.5 ml/min	216NM	ACETONITRILE /WATER(62:38V/V)	UV	20
HPLC	C18	1.0ml/min	210NM	ACETONITRILE /BUFFER 65:35 PH : 6.5	UV	21

VALIDATION PARAMETERS:

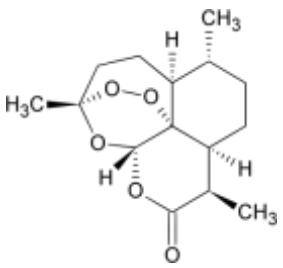
HPLC:

S.NO	PARAMETERS	LINEARITY		ACCURACY		PRECISION		LOQ (µg/ml)	LOD (µg/ml)	REFERENCE
		CONC. (MG/ML)	R2	CONC. (MG/ML)	%SD (MINMAX)	CONC. (MG/ML)	%RSD (MINMAX			
1.	ARTEMETHER	60-140	0.999	80 100 120	0.45	80 100 120	0.92	1.92	0.63	22

HPLC :

DRUG	LINEARITY	ACCURACY	PRECISION			r	LOD	LOQ	REFERENCE
			REPEATABILITY	INTRADAY	INTERDAY				
ARTEMETHER	250-750 µg/ml	99.36	0.256	0.642	0.712	0.9998	7.20445	21.8317	23

B]ARTESUNATE:[24,25,26,27,28,29]



MOLECULAR FORMULA : $C_{19}H_{28}O_8$
MOLECULAR WEIGHT : 384.4 g/mol
PHYSICAL PROPERTIES :
APPEARANCE : Fine white crystalline powder

SOLUBILITY : Soluble in organic solvents like ethanol, DMSO, and dimethyl formamide.
STABILITY: Stable for about 4 hours at 30°C
MELTING POINT : 131-135
IUPAC NAME ; 4-oxo-4-{[(4S,5R,8S,9R,10R,12R,13R)-1,5,9-trimethyl-11,14,15,16-tetraoxatetracyclo[10.3.1.0^{4},^{13}.0^{8},^{13}]hexadecane-10-yl]oxy}butanoic acid

ANALYTICAL METHODS :

HPLC:

Chemicals and reagents. Authentic samples of Artesunate (ASN) and Artemether (ATM) were obtained fromEmcure Pharmaceuticals Ltd (Pune, India) and used as such

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without further purification. Brand of tablets FALCIGO (Zydus Cadila, India), labeled to contain 50 mg ofASN were procured from the local market. Acetonitrile(HPLC grade) was purchased from Merck specialties Pvt.Ltd. (Mumbai, India). Acetic acid (HPLC grade), sodium acetate (Extra pure grade), o phosphoric acid (AR grade) and double distilled water were used in analysis.Instrumentation and chromatographic conditions. Jasco HPLC system, consisting of Jasco PU-2080 plus HPLC pump, Jasco UV-2075 plus UV/VIS detector and Jasco Borwin Ver 1.50 software was used for analysis.Separation was carried out on HiQ-SiL C8 (250 mm ×× 4.6 mm i.d.) column using acetonitrile : 1 M sodium acetate buffer (pH 3 adjusted with o-phosphoric acid);(70 : 30, v/v) as mobile phase at flow rate of 1.0 mL/min.1 M sodium acetate buffer was prepared by addition of 40 ml of 1 M acetic acid and 60 mL of 1 M sodium acetate solution to 1000 mL volumetric flask and volume was made up to the mark with double distilled water. Samples were injected using Rheodyne injector with 20 µL loop.ATM was used as internal standard and detection was carried out at 220 nm. All weighing were done on Shimadzu balance (Model AY-120).

LC MS :

The HPLC system was the Waters 2695 Separations Module (Waters, Zellik, Belgium), an integrated system consisting out of an autosampler, pump, column oven, and online degasser. Chromatography was performed on a XTerra |RP18 column (3.5 ~m, 2.1 • 100 ram) coupled to a guard column XTerra MS C18 (3.5 m, 2.1 x 10 mm) with the column oven set at 35 The binary gradient program consisted of 0.15% aqueous formic acid (A) and 0.15% formic acid in acetonitrile (B). The flow rate was set at 300 ~L/min, and the injection volume was 30 IJL. The separation was done using the gradient mode, programmed as follows: 60:40 A/B isocratic for 2 rain, change to 0:100 A/B over 3 min, hold for 9 min, change back to 60:40 A/B over 2 rain, and hold for 14 rain.Masses were acquired on a QuattroMicro MS from Micromass (Waters, Zellik, Belgium) operated in the APCI § mode.The corona was set at 5.00 I~, extractor at 2.00 V, RF Lens at 0.2 V, source temperature at 120~ desolvation temperature at 225~ and cone voltage was dependent on the ion monitored. The desolvation gas was nitrogen, produced by a nitrogen generator, and settings were 100 L/h for the cone gas flow and 400 L/h for the desolvation gas flow. The multiplier was set at 650 V. The gas cell pirani pressure was 10 .4 mbar. The data were recorded in the single ion recording mode (SIR). For alpha- DHA, beta-DHA, artesunate, and artemether, three ions were monitored: 221 (cone at 22.0 V), 267 (10.0 V), and 249 (22.0 V). For artemisinin, two ions were monitored: 283 (12.0 V) and 209 (22.0 V). The work station used to operate the LC-MS system was MassLynx version 3.5. A second HPLC pump, the K-1001 from Knauer (Sercolab, Merksem, Belgium) with ultrapure water/acetonitrile (50:50,v/v) as mobile phase, was connected to the diversion valve on the MS. The pump was operated at flowrate of 0.2 mL/min in order to maintain a stream of fluid through the capillary of the probe during the periods that the eluent from the column was directly guided to the waste.A diversion valve was set in place and was programmed as follows. The first 2 min of the HPLC run were diverted to waste, the period of 2-8.5 min was diverted to the MS, and the rest of the run (8.5-30 min) was diverted again to waste. The flow was directed to the MS during the time that the HPLC run was directed to waste; the remaining time, the flow was redirected to the mobile phase reservoir.

METHOD DEVELOPMENT :

INSTRUMENT	COLUMN	FLOW RATE	WAVE LENGH	MOBILE PHASE	DETECTOR	REFERENCE
HPLC	C8	1.0 ml /min	220nm	1MSODIAM ACE TATE BUFFER / O PHOSPHORIC ACID 70 : 30 (V/V)	UV / VIS DETECTOR	24
LC – MS	MS C18	300		ACETONITRILE /WATER 50:50	--------	29-24
HPLC	RP C18	0.2ml/min	250nm			

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VALIDATION PARAMETERS:

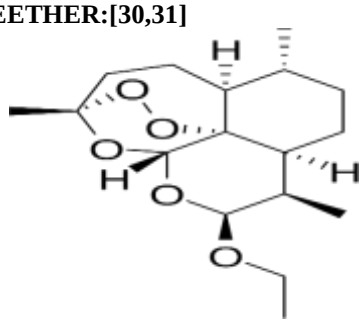
HPLC

INSTRUMENTS	LINEARITY	ACCURACY	PRESITION		LOD	LOQ	REFERENCE
			INTRADAY	INTERDAY			
HPLC	250 -2500	99.94-100.92	1.2867	1.6093	52.35	158.63	24

LC –MS

PARAMETERS DRUG NAME	LINEARITY		ACCURACY		PRECISION		INTRADAY PRECISION	INTERDAY PRECISION	REFERENCE
	Conc. (Mg/Ml)	R2	Conc. (Mg/M L)	%SD (Minmax)	Conc. (Mg/Ml)	%RSD (Minmax)			
ARTESUNATE	10 -50	**0.9999**	20 30 40 50	101.1 101.8 101.3 99.1	20 30 40	2.41 1.94 1.81	2.41 1.94 1.81	2.00 0.68 4.53	29

C]ARTEETHER:[30,31]



IUPACNAME:1S,4S,5R,8S,9R,10R,12R)-10-ethoxy-1,5,9-trimethyl-11,14,15,16-tetraoxatetracyclo[10.3.1.$0^{4,13}$.$0^{8,13}$]hexadecane
MOLECULAR FORMULA : C34H56O10
MOLECULAR WEIGHT : 624.8 g/mol
PHYSICAL PROPERTIES :
APPEARANCE : white crystalline solid
SOLUBILITY : Partly miscible **MELTING POINT :** 80–82°
MECHANISM OF ACTION: A possible mechanism of action is that artemisinin drugs exert their cidal action by inhibiting PfATP6. Since PfATP6 is an enzyme regulating cellular calcium concentration, its malfunctioning will lead to intracellular calcium accumulation, which in turns causes cell death . and in pharmacokinetics Absorption of artemether is improved 2- to 3-fold with food. It is highly bound to protein (95.4%). Peak concentrations of artemether are seen 2 hours after administration. Artemether is metabolized in the human body to the active metabolite, dihydroartemisinin, primarily by hepatic enzymes CYP3A4/5. Both the parent drug and active metabolite are eliminated with a half-life of about 2 hours. In the body, artemether is metabolized into the active metabolite dihydroartemisinin. The drug works against the erythrocytic stages of P. falciparum by inhibiting nucleic acid and protein synthesis.

THERAPEUTIC USES :

Artemether, artesunate, and artemisinin are used to treat severe malaria (White 2000). For non-oral administration, artemether is available as intramuscular injection, artemisinin as rectal suppositories, and artesunate as rectal, intramuscular, and intravenous preparations (WHO 2001).

ANALYTICAL METHODS :

UV – FLUORIMETER :

The research work is based on the development and validation of two different spectrophotometric methods (UV spectrophotometer and spectrofluorimeter) for estimation of α-β arteether. Two simple, accurate,precise, sensitive and economical methods has been developed, validated for the estimation of α-β arteether in bulk and pharmaceutical dosage form as per ICH guidelines Q2(R1). The solvent used for UV spectroscopy was methanol and HCl (8:2) and methanol was used for fluorimeter. For qualitative and quantitative analysis, 254 nm was used in UV spectroscopy and excitation and emission wavelengths were set at 354 nm and 697 nm, respectively for fluorimetry. Coefficients of correlation were found to be 0.993 and 0.992 for UV spectroscopy and fluorimetry respectively. Both methods show good accuracy and precision

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and were comparedstatistically by using two way ANOVA which shows no significant difference between thesemethods. So, the proposed methods were found to have equal applicability for estimation and routine analysis of arteether in pharmaceutical formulations.

HPLC:

The HPLC system consisted of a Plus Intelligent LC pump PU-2080 from Jasco (Tokyo, Japan) equipped with a Jasco UV-2075 Intelligent UV–Vis detector and a Rheodyne 7725 in-jector (Rheodyne, Cotati, CA, USA). The output signal was monitored and processed using a Jasco Chroma Pass Chromatography Data System Software (Version 1.8.6.1). Chromatographic separation was achieved on a 5-mm Agilent RP C18 Zorbax eclipse XDB column (4.6 -150 mm). The mobile phase employed comprised Solvent A:Water and Solvent B:Acetonitrile. Prior to use, water was filtered through a 0.45-mm filter membrane. Mobile phase was pumped through the column at a flow rate of 1.0 mL/min. The injection volume was 50 mL. The analytes were analyzed at a single wavelength of 216 nm for arteether and their associated degradation products.

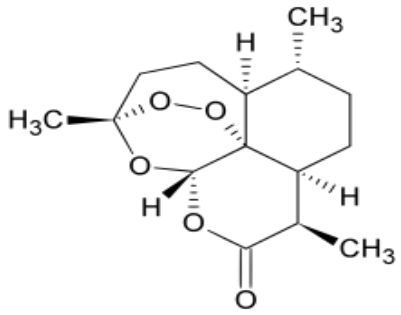
METHOD DEVELOPMENT :

INSTRUMENT	COLUMN	FLOWRATE	WAVE LENGTH	MOBILE PHASE	DETECTOR	REFERENCE
UV	_ ---------	_2.00ml/min	254nm	METHANOL (8:2)	UV spectroscopy _	30
FLUORIMETER			210nm			
HPLC	RP C18	1.0ml/min	216nm	WATER /ACETONITRILE 75:25	UV VIS	31

VALIDATION PARAMETER :

INSTRUMENT	LINEARITY	ACCURACY	PRECISION		LOD (µg/ml)	LOQ (µg/ml)	REFERENCE
			INTRADAY	INTERDAY			
UV	8-36	99.95	0.385	0.190	0.52408	1.58814	30
FLUORIMETER	697	99.95	0.378	0.243	18.77	61.94	
HPLC	10 - 5000	99.7	99.58		3.33	10.0	31

5. NEWER ANTIMALARIAL DRUG : ARTIMISININ [32]



IUPAC NAME: (1R,4S,5R,8S,9R,12S,13R)-1,5,9-trimethyl-11,14,15,16-tetraoxatetracyclo[10.3.1.04,13.08,13]hexadecan-10-one

MOLECULAR FORMULA : C15H22O5

MOLECULAR WEIGHT : 282.332 g/mol

PHYSICAL PROPERTIES :

APPEARANCE : white powder or colorless crystal

SOLUBILITY : sparingly soluble in water and oils, but it is soluble in organic solvents.

BOILING POINT : 389.9 °C

MELTING POINT : 156–157°C.

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MECHANISM OF ACTION : Artemisinin and its derivatives kill malaria parasites by producing carbon-centered free radicals that damage the parasite's proteins and membranes. This process is selective to malaria parasites because it involves heme.

ANALYTICAL METHODS :
HPLC :

m, 250 × 4.6 mm column, maintained at 40 °C, a mobile phase consisting of 0.05 M potassium phosphate buffer, pH 6.0 - acetonitrile (60:40) containing 5 mM hexane sulfonate in isocratic flow. The mobile phase flow rate was 1.0 ml/min while elution was monitored at 216 nm. The method satisfied the International Conference on Harmonization (ICH) validation criteria for linearity, accuracy, precision andsensitivity. The developed method is applicable in routine quality control of Artemisia annua crude extracts.µA simple, sensitive, accurate and precise high-performance liquid chromatography (HPLC) method for determination of artemisinin in crude plant material was developed and validated. Optimal separation of artemisinin from matrix components in the plant extracts was achieved using a Waters XTerra® RP18, 5

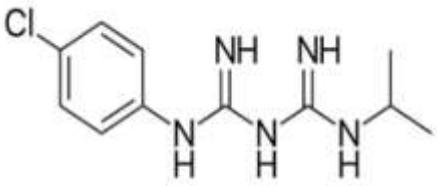
METHOD DEVELOPMENT

INSTRUMENT	COLUMN	FLOWRATE	WAVE LENGTH	MOBILE PHASE	DETECTOR	REFERENCE
HPLC	250 column	0.1ml/min	216nm	0.05 M potassium phosphate buffer	-	32

VALIDATION PARAMETER :

INSTRUMENT	LINEARITY	ACCURACY	PRECISION		LOD (µg/ml)	LOQ (µg/ml)	REFERENCE
			INTRADAY	INTERDAY			
HPLC	120	80	0.89	0.7	20	30	32

6 . BIGUANIDES
PROGUANIL[33,34,35,36]



Proguanil

IUPAC NAME:(1E0-1-[amino-4-chloroanilino)methylidene]-2-propan-2-ylguanidine
MOLECULAR FORMULA: $C_{11}H_{16}ClN_5$
MOLECULAR WEIGHT: 253.731 g/mol

PHYSICAL PROPERTIES:
1. Appearance: White or almost white crystalline powder
2. Odor: Odorless or faint odor
3. Solubility: Slightly soluble in water, soluble in ethanol and chloroform

MECHANISM OF ACTION : Proguanil inhibits the dihydrofolate reductase of plasmodia and thereby blocks the biosynthesis of purines and pyrimidines, which are essential for DNA synthesis and cell multiplication. This leads to failure of nuclear division at the time of schizont formation in erythrocytes and liver.

ANALYTICAL METHODS:
HPLC:
Several HPLC method have been employed for the measurement of proguanil.

1.HIGH PERFORMANCE LIQUID CHROMATOGRAPHY[UV-VISIBLE DETECTOR]

The Liquid chromatographic system used consisted of a Cecil 1100 series instrument (Cecil Instrument, Cambridge, England) made up of

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binary pumps fitted with a gradient mixer (Cecil instrument) with a system purge and a variable wavelength (200-800 nm) ultraviolet-visible detector model CE1200 (Cecil instrument) with a 18 µL flow cell. Injection was by a Rheodyne model 7725 valve (Cotati, California, U.S.A.) fitted with a 20 µL loop. The detector output is linked to a CTX Computer (made in Thailand) via a brainbox inter-phase (Cecil Instrument), which transforms signals from the detector to the computer that eventually records the chromatograms. The computer system is connected to an LX 300 printer (Epson). The column used was a Hypersil ODS (C-18) 5µm particle size and 250 x 4.6 mm I.D, reversed phase stainless steel (Alltech). A mobile phase consisting of methanol : acetonitrile : 0.5% ammonium acetate (40:5:55) containing 75 mM/L perchloric acid was pumped through the column at a flow rate of 1.2 ml/min. The pH of the mobile phase was 2.2, and the chromatogram was run at ambient temperature.[34]

2.HIGH PERFORMANCE LIQUID CHROMATOGRAPHY[UV-VISIBLE DETECTOR]

The analysis was carried out on a Shimadzu LC-10 series chromatographic system (Shimadzu Corporation, Kyoto, Japan) consisting of a SCL-10A controller unit, DGU-2A degasser unit, a LC-20AD quaternary gradient pump, SIL10AD autosampler with SPD-20A UV detector. System control, data acquisition and processing were performed with a PC-Pentium IV Processor personal computer operated with Microsoft Windows XP and Shimadzu LC solution 1.24 SP1 software. Standard substances were weighed on Sartorius analytical balance. A glass vacuum-filtration apparatus (Alltech Associates) was employed for the filtration of buffer solution using 0.45 µm filter obtained from Pall Life Sciences (Bangalore, Karnataka, India). Degassing of the mobile phase was performed by sonication in Oscar Micro clean-103 Ultrasonic bath. Thermo 995-Forma86C freezer (Thermo electron corporation, USA) was used to store the plasma samples. A model Genie-2 Spinix vortex mixer, a Heraeus refrigerated centrifuge (Thermo electron corporation, USA) and TurboVap LV Evaporator (Caliper Life Sciences, Hopkinton, MA, USA) were employed for sample pre-treatment. The chromatographic separation was achieved using a Thermo phenyl (150×4.6 mm ID×5 µm) column with mobile phase comprising of 15.0 mM potassium dihydrogen phosphate buffer, pH 7.2 (45%) and methanol (55%). The flow rate was 0.8 mL/min and column temperature was set to 18ºC. Ultra-violet measurements were done at 254 nm wavelength with total run time of 12.5 min.[35]

3.HIGH PERFORMANCE LIQUID CHROMATOGRAPHY[PHOTODIODE ARRAY DETECTOR]

HPLC instrument used was of WATERS HPLC 2695 SYSTEM with Auto Injector and PDA Detector. Software used is Empower 2. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2 mm and 10 mm and matched quartz was be used for measuring absorbance for Proguanil solutions. Degassing of the mobile phase was done by using an ultrasonic bath sonicator. A Shimadzu balance was used for weighing the materials. HPLC was connected with Kromasil C18 column (150 mm x 4.6 mm, 5 µm) as stationery phase. A mixture of 0.1%OPA and acetonitrile in the ratio of 50:50 v/v was prepared and used as mobile phase. 0.1%OPA was prepared by transferring 1 mL of OPA solution in a 1000 mL of volumetric flask adds about 100 mL of milli-Q water and final volume make up to 1000 mL with milli-Q water. Injection volume was 10 µL and flow rate was 1.0 mL/min and run time was 5.0 min. The column was maintained at ambient temperature and the eluent was monitored at 287 nm.[36]

4.REVERSE PHASE-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY[UV-VISIBLE DETECTOR]

RP-HPLC method was developed for of Proguanil Hydrochloride in pure form and marketed combined pharmaceutical dosage forms.A column having Develosil ODS HG-5 RP C18, 15cmx4.6mm, i.d. Columnin isocratic mode with mobile phase containing Methanol: Acetonitrile in the ratio of 85:15% v/vwas used. The flow rate was 1.0 ml/min and effluent was monitored at 258nm.[37]

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METHOD DEVELOPMENT

INSTRUMENT	COLUMN	FLOW RATE	WAVELENGTH	MOBILE PHASE	DETECTOR	REFERENCE
HPLC-UV Visible detector	Hypersil ODS (C-18)	1.2 ml/min.	254nm	Methanol : acetonitrile : 0.5% ammonium acetate (40:5:55)	Ultraviolet-visible detector	34
HPLC-UV Visible detector	Shimadzu LC-10	0.8 ml/min	254nm	15.0 mM potassium dihydrogen phosphate buffer	Ultraviolet-visible detector	35
HPLC-Photodiode array detector	Kromasil C18 column (150 mm x 4.6 mm, 5 µm)	1.0 ml/min	287 nm	Mixture of 0.1%OPA and acetonitrile in the ratio of 50:50 v/v	Photodiode array detector	36
RP-HPLC method	Develosil ODS HG-5 RP C18, 15cmx4.6mm	1.0 ml/min	258nm	Methanol: Acetonitrile85:15 % v/v	Ultraviolet-visible detector	37

VALIDATION PARAMETERS:

INSTRUMENT	LINEARITY	ACCURACY	PRECISION	LOD	LOQ	REFERENCE
HPLC-UV Visible detector	7-450	98.62	5.83	5.0	7.03	35
HPLC-Photodiode array detector	57502	98.86-99	190935	----	----	36
RP-HPLC method	229679	119571	------	----	----	37

7. NAPHTHAQUINONE ATOVAQUONE [37, 38, 39]

IUPAC NAME: trans-2-[4-(4-Chlorophenyl)cyclohexyl]-3-hydroxy-1,4-naphthalenedio

MOLECULAFORMULA:

MOLECULAR WEIGHT :

PHYSICAL PROPERTIES

APPEARANCE : Atovaquone also known as Mepron [pink] or Malorone [yellow],oval,film

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coated medication which Vary depending on the manufacturer.

SOLUBILITY : Atovaquone is a medication that treats and prevents Pneumocystis carinii pneumonia, a serious fungal infection. It comes as a liquid suspension that you take by mouth.

BOILING POINT : 542.2±50.0 °C at 760 mmHg

MELTING POINT : 216-2190C

MECHANISM OF ACTION: Atovaquone's mechanism of action is to inhibit the mitochondrial electron transport chain in parasites, Inhibits electron transport, Collapses mitochondrial membrane potential, Inhibits pyrimidine biosynthesis.

ANALYTICAL METHODS :

1. **REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY[PHOTODIODE ARRAY DETECTOR]**

The instruments used was HPLC Waters Model NO.2695 separation module consisting of 996 PDA detector along with Electronic balance and sonicator. : The mobile phase was prepared by taking Methanol: Water mixed in the ratio of 65:35 % v/v. They were mixed and degassed in a ultrasonic water bath for 10-15 min and then filtered through 0.45 µ membrane filter. The pH was adjusted with Acetonitrile. Stationary phase (column) Phenomenex Luna C18 (4.6×250mm) 5µm ,Mobile Phase Methanol :Water (65:35 % v/v) ,Flow rate 1.0 ml/min, Run time 7 min ,Column temperature (°C) 35˚C Volume of injection loop 10µl, Detection wavelength 220nm RT (min) 3.2 min for Atovaquone.[37]

2.UV-SPECTROSCOPY[VARIABLE WAVELENGTH DETECTOR]

Shimadzu 1800 double beam spectrophotometer with Shimadzu UV PC soft ware was used for all the spectrophotometric measurements and treatment of data. Zero-order absorption spectra were traced in 1 cm quartz cells over the range of 200–400 nm.[38]

3.LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY

Chromatographic separation was achieved with a Synergi 2.5-µm Polar-RP 100A (100 × 2 mm) column (Phenomenex) on an Acquity UPLC system (Waters). Mobile phase A (MPA) consisted of water and 0.1% formic acid, while mobile phase B (MPB) consisted of acetonitrile and 0.1% formic acid. Samples were eluted with a gradient with a total analytical runtime was 1.3 min. A flow rate of 0.75 mL/min was used throughout the run, with an initial solvent mixture of 20:80 MPA:MPB. At 0.3 min, the ratio was adjusted to 5:95 MPA:MPB and held for 0.6 min, followed by a return to 20:80 MPA:MPB at 1.2 min.[39]

METHOD DEVELOPMENT:

INSTRUMENT	COLUMN	FLOW RATE	WAVELENGTH	MOBILE PHASE	DETECTOR	REFERENCE
RP-HPLC-PDA	Phenomenex Luna C18 (4.6×250mm)	1.0 ml/min	220nm	Methanol :Water (65:35 % v/v)	Photodiode array detector	37
UV-Spectroscopy	------	1.2ml/min	254nm	Methanaol: water(8:2)	Variable wavelength detector(VWD)	38
LC-MS	Phenomenex Luna C18 (4.6×250mm)	0.75 ml/min	270nm	1.water and 0.1%formic acid 2.Acetonitrile and 0.1% formic acid	Mass spectrometry	39

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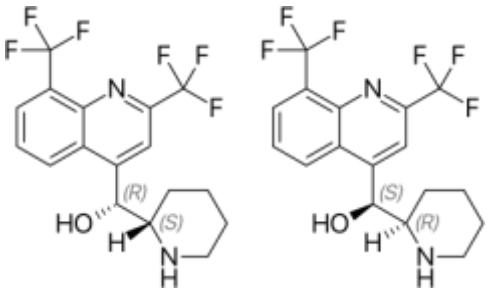
VALIDATION PARAMETERS:

INSTRUMENT	LINEARITY	ACCURACY	PRECISION	LOD	LOQ	REFERENCE
RP-HPLC-PDA	70	82	42	26	29	37
LC-MS	55	61	26	215	237	39

8. 1,4-QUINOLINE METHANOL MEFLOQUINE:



IUPAC NAME: (S)-[2,8-bis(trifluoromethyl)quinolin-4-yl]-[(2R)-piperidin-2-yl]methanol
MOLECULAR FORMULA : $C_{17}H_{16}F_6N_2O$
MOLECULAR WEIGHT : 378.31 g/mol
PHYSICAL PROPERTIES :
APPEARANCE : Round,yellow,film coated and marked with 'M'or the manufacturer'slogo.
SOLUBILITY : Mefloquine is very slightly soluble in water. Mefloquine is soluble in ethanol.
BOILING POINT : 415.7°C.
MELTING POINT : 250–254°C
MECHANISM OF ACTION: The mechanism of action of mefloquine is not completely understood. Some studies suggest that mefloquine specifically targets the 80S ribosome of the Plasmodium falciparum, inhibiting protein synthesis and causing subsequent schizonticidal effects.

THERAPEUTIC USES: lariamalsoknown as mefloquine is an anti-malarial tablet that can be taken to prevent malaria infections when visiting high-risk malaria countries.

ANALYTICAL METHOD:
HPLC

A simple, rapid and validated method for separation and determination of mefloquine enantiomers was developed. Mefolquine was separated and quantitated on cyclodextrin chiral column Quest-CM carboxymethylBCD (250x4mm i.d., 5µm particle size) using a mixture of acetonitrile: 1% triethylammonium acetate buffer (pH = 4.5) (20:80 v⁄ v) as a mobile phase at 20 o C and a flow rate of 1 mL /min. The UV-detector was set at 240 nm. The applied HPLC method allowed the separation and quantification of mefloquine enantiomers with good linearity (r > 0. 999) in the studied range. The relative standard deviations (RSD) were 0.865 and 0.907 for the mefloquine enantiomers with accuracy of 100.00 and 100.68. The limit of detection and limit of quantification of mefloquine enantionmers were found to be 5 and 15 µg/mL, respectively. The method was validated through the parameters of linearity, accuracy, precision and robustness. The HPLC method was applied for the quantitative determination of mefloquine in pharmaceutical formulations

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METHOD DEVELOPMENT :

INSTRUMENT	COLUMN	FLOWRATE	WAVE LENGTH	MOBILE PHASE	DETECTOR	REFERENCE
HPLC	chiral column	1ml/min	240nm	acetonitrile: 1% triethylammonium acetate buffer (pH = 4.5) (20:80 v⁄v)	UV	40

VALIDATION PARAMETER :

INSTRUMENTS	LINEARITY	ACCURACY	PRECISION		LOD	LOQ	REFERENCE
			INTRADAY	INTERDAY			
HPLC	50-500	99.82	0.32	0.56	0.6	0.78	40

UV

A simple, rapid, precise, and accurate UV-visible spectrophotometric method has been developed for the simultaneous determination of Artesunate in combination with Mefloquine. For developing the method, methanol was used as a solvent. Artesunate and Mefloquine showed $\lambda\lambda$max at 240 nm and 222 nm, respectively. We proposed method was validated as per ICH guideline. 廄e linearity range of Artesunate and Mefloquine were 10–60 and 20–120 $\mu\mu$g/mL, respectively. 99.91 ± 0.2740 and 99.56 ± 0.2067 these value represent the percent recovery of Artesunate and Mefloquine respectively 廄e correlation coeffcients of Artesunate and Mefloquine were 0.999, and 0.999, respectively. 廄e relative standard deviation for six replicates was always less than 2%. 廄e statistical analysis proves that the method is suitable for the analysis of Artesunate and Mefloquine as the bulk drugs and in pharmaceutical formulation without any interference from the excipients.

METHOD DEVELOPMENT :

INSTRUMENT	COLUMN	FLOWRATE	WAVE LENGTH	MOBILE PHASE	DETECTOR	REFERENCE
UV	-	1.5ml/min	222nm	Methanol	Diode array detector	41

VALIDATION PARAMETER :

INSTRUMENTS	LINEARITY	ACCURACY	PRECISION		LOD	LOQ	REFERENCE
			INTRADAY	INTERDAY			
UV	20-120	99.82	99.59	99.69	1.79	1.48	41

HPTLC

The HPLC (Shimadzu, Japan) instrument was equipped with a model series Shimadzu LC-10 ATVP pump, Rheodyne-7725 injector with 20 µl loop and a Shimadzu SPDM-20 A diode array detector. Separation was made on a Symmetry C18, 250 × 4.6 mm column (5 µm particle size). The detection wavelength was set at 220 and 313 nm. Data acquisition was performed on a model Class-VP software. In HPTLC, chromatographic separation of drugs was performed on Merck TLC plates (Germany) pre-coated with silica gel 60 F254 (10.0 × 10.0 cm with 250 mm layer thickness). Time for chamber saturation was optimized to 10 minutes. Sample and standard zones were applied to plates as bands by means of Camag 100 µl sample syringe (Hamilton, Switzerland) with a Linomat 5 applicator (Camag,

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Switzerland). The plates were left to equilibrate for 3 minutes in a 10.0 × 10.0 cm horizontal chamber (Camag, Switzerland) and then developed to a distance of 80 mm using toluene-ethyl acetate-acetone (2.5:1.0:0.5, v/v/v) as mobile phase. Separation was obtained within 10 minutes and before detection, the plates were dried at 60ºC for 4 minutes to eliminate mobile phase. Initially for detection of MEFQ, densitometric scanning was carried out using TLC scanner (Camag, Switzerland) in the absorbance / reflectance mode at 285 nm. Artesunate shows very weak UV absorbance property. Therefore subsequent to this scanning, TLC plates were derivatized with anisaldehyde-sulphuric acid reagent for 4 seconds and heated for 3 minutes at 110ºC. This post chromatographic treatment of artesunate spot was considered to produce lightabsorbing compound on the layer. Thus both MEFQ and ARTS can be developed and scanned in a single plate before and after derivatization. winCATS software (V 1.4.2, Camag, Switzerland) was used for scanner control and data processing. The sources of radiation used were deuterium-tungsten lamp. The whole procedure took not more than 30 minutes. The Rf values of MEFQ and ARTS were found to be 0.26±0.02 and 0.59±0.01 respectively.

METHOD DEVELOPMENT :

INSTRUMENT	COLUMN	FLOWRATE	WAVE LENGTH	MOBILE PHASE	DETECTOR	REFERENCE
HPTLC	C18, 250 × 4.6 mm column (5 µm particle size)	0.8ML/MIN	220 and 313 nm	toluene-ethyl acetate-acetone (2.5:1.0:0.5, v/v/v) as mobile phase.	Diode array detector	42

VALIDATION PARAMETER :

INSTRUMENTS	LINEARITY	ACCURACY	PRECISION		LOD	LOQ	REFERENCE
			INTRADAY	INTERDAY			
HPTLC	12.5-75	18.5	0.71	4.0	3.57	8.69	42

II. CONCLUSIONS

In this literature study,the most popular Spectrophotometry and chromatography analytical procedure have been listed. The development of this method involved the use of analytical equipment such as UV Visible spectrophotometer,High performance Liquid Chromatography,Reverse phase High Performance Liquid Chromatography and Ultra High Performance Liquid Chromatography. It was developed to describe particular antimalarial drugs.4Aminoquinolines,8Aminoquinolines,Pyrimidine,analogue,Sesquiterepne lactone, Dihydrotriazine, 4Quinoline methanol and Sulfonamides.

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