3 REVIEW OF LITERATURE:

3.1 Review on background of disease:
Substantial information is assessable regarding seriousness of the disease and its treatment. Efforts were made to cover as much important information about the backbone and severity of the disease.

**Miller LH, Good MF, Millon G. (1994)** [37]

Had given idea about the cause of the disease and its pathogenesis. According to them major reason for malaria is repeated multiplication of plasmodium species in erythrocytic phase. Under favorable conditions they invade human body and start multiplying without initiating immune response. In such case it can lead to cerebral malaria or can produce anemic condition. The severity of disease depends upon physiological condition patient as well as parasite. Severity of disease can increase during pregnancy. Another problem arise due to resistance in available treatment.

**Pukrittayakamee S, Imwong M, White NJ and et al (2004)** [38]

Had suggest the occurrence of resistance. In one of the major city they studied effect of various antimalarial agent on the species of plasmodium vivax Hospital for Tropical Diseases. From this studies they found that there are major 8 antibacterial or antimalarial agent which can prove to be effective in the treatment. (Doxycycline, azithromycin tetracycline, clindamycin). They postulated that artemether is one of the most effective agent in treatment while primaquine is least effective. They even found effectiveness of combination of sulfadoxine and pyrimethamine


Had widespread occurrence of the disease and species responsible for that according to their review, majorly five species have been identified till date which can infect human host: P. vivax, P. falciparum, P. malariae, P. ovale, and P. knowlesi. This disease is the major cause of death by causing almost few millions of death worldwide and infects as many as around 500 million population. One of the major problem is least availability of safe regimens for the treatment of the disease and condition get worsen when problem of resistance is in place. Due to limited opportunities by commercial factor chance
of introduction of newer antimalarial agent is least. So the current approach to overcome this problem is chemical libraries and evaluate them against concerned parasite and by using validated biomarkers

**Nosten F, White NJ. (2007) [40]**

They gave review about the artemisinine based therapy. For the treatment of no complicated malaria combinations containing artemisinine derivatives are widely used. Effectiveness is identified by making combination of any of the available artemisinine derivatives like artesunate or artemether with agents like Mefloquine or Lumefantrine. Efficacy of such regimens is over 90%. When combined with sulfadoxine or Amodiaquine, effectiveness decreases. Artesinisin derivatives are considered as safer agent except for occasional hypersensitivity.

**Jullien V, Valecha N, Shrivastava B and et al (2014) [41]**

Had given details about efficacy of fixed combination of artesunate and Mefloquine in treatment of acute uncomplicated plasmodium falciparum. The study had included fixed dose combination of 200/400 mg of artemetate and Mefloquine respectively. Study on 77 patients showed excellent clinical significance of the combination at the mentioned dose.

**Bukiwara H, Unnikrishnan B, Kramer B and et al (2014) [42]**

Had suggested used of pyronaridine as supportive therapy along with artesunate. They gave advantage of Artesunate- Pyronaridine therapy over conventional artemether Lumefantrine therapy with less chance of relapse chance.

**Plucinski MM, Talundzic E, Morton L. et al. (2014) [43]**

Had performed study in the mentioned regions of angola and give glimpse of having chance of resistance in single line therapy of Lumefantrine over combined therapy of artemether and Lumefantrine.

Conclusion:

From the above literature review it can one can conclude that following agent are used in the treatment of plasmodium falciparum

1. Artemisinin Derivatives (Artesunate, Arteether, Artemether)

2. Amodiaquine (Supportive agents)
3. Lumefantrine (Combination therapy with Artemether)

4. Mefloquine (Supportive Agents)

5. Pyronaridine (New Combination Therapy with Artemether).

None of the agents are used as single line treatment they are used in combination to avoid the chances of resistance. It has been not only the problem of resistance but frequent problems has been reported regarding resistance.

3.2 Overview on available analytical methods for selected drugs:

Review of literature was majorly performed on spectrometric, chromatographic methods and hyphenated techniques for estimation of artimisinine derivatives like artemether, artesunate, artemether either single alone or from synthetic mixture. Review was also extended to combination of artemether and Lumefantrine and Mefloquine and artesunate.


A method was developed for separation of dihydroartemisinine derivatives from plasma along with their metabolites. They separated components on C\textsubscript{18} spherisorb column by water–acetonitrile (50:50, v/v) as mobile phase in 30 min. They applied method successfully for pharmacokinetic studies of mentioned drugs.

**Muhia DK, Mberu EK, Watkins WM (1994)** [45]

Had separated artemether from their metabolite dihydroartemisinine initially by differential solvent extraction. Derivatized extracts were estimated by RP HPLC on a 5-μm ODS column with acetonitrile–water (60:40v/v) as solvent system and detected at 254 nm. This developed method has advantage of early retention times than previous reported method.


They developed RP HPLC method for determination of artimisinine (as internal standard) artemether and dihydroartemisinine on Lichrocart 100 CN column by using acetonitrile-0.05 M acetic acid (15:85, v/v) pH 5.0 as mobile phase. They used electrochemical detection in reduction mode. They proved that method can be used in clinical studies.
Artemether was separated from its metabolite in plasma by RP HPLC. By using artemisinine as internal standard. LLE was used to separate contents from plasma followed by evaporation by drying. The reconstituted samples were analyzed by hyphenated liquid chromatography in SRM mode using APCI as an interface. Separation was achieved on C_{18} RP column using glacial acetic acid 0.1%- acetonitrile (34:66 v/v) as a mobile phase. Linearity of method was ranging from 5–200 ng/ml.

Gabriels M, Plaizier-Vercammen JA (2003) [48]
They developed TLC for estimation of artemether and arteether. Both components were separated on silica gel as stationary phase and chloroform as mobile phase. As its lacks Chromophore, it detection was performed after spraying with 1% 4-Methoxybezaldehyde in H_{2}SO_{4} and finally diluted with alcohol. Drying was performed at little above 100°C. After 12 minutes stability of spots was highest. At two levels precision of method was less than 5%. Method was found to be linear in the range of 0.5-8 mcg/ml. They demonstrated the presence of isomers by performing pre derivatization. They roved that this method can separate and quantify artemether and arteether with sufficient ease and with low cost from bulk or from pharmaceutical formulation

Naik H, Murry DJ, Kirsch LE, et al. (2005) [49]
They developed same method as above but extraction was carried using solid phase extraction cartridges. Development was made on a Shimadzu LCMS-in SRM mode using APCI as an interface. The m/z ration for α- and β-DHA was found to be 221 and 283 m/zfor artemisinine. Separation was performed on a RP-Synergi Max, 75 mm × 4.6 mm column by using combination of methanol, acetonitrile and glacial acetic acid (15.5: 46.5: 38 v/v/v) as a Eluent. Flow rate was kept at 0.5 ml/minute. In total 20 minutes of runtime elution time for components was 11.8, 13.4, 17.5 and 18.7 minutes. Linearity of method was between 1–3000 ng/mL

Artresunate was estimated from bulk and formulation by a HPTLC method. They separated the drug on silica gel G F_{254} plate by using toluene: ethyl acetate: acetic acid (2:8:0.2) as mobile phase. Vanillin (1%) in sulphuric acid (5%) in
ethanolic solution gave prominent well-resolved pink color spot for artesunate, which was stable for more than a day. Components were measure at 520 nm by densitometry. Symmetrical, well-resolved, well-defined peaks were obtained. The Rf value of artesunate was found to be 0.44. The linear detector response for artesunate was observed between 100-600 ng per spot and the regression method showed linearity with, \( r^2 = 0.9989 \). Finally method was applied for estimation of artesunate from pharmaceutical formulation

**Atemnkeng MA, Marchand E, Plaizier-Vercammen J. (2007)** [51]

They separated artemether and combined derivatives (methyl Paraben, propyl Paraben) in pharmaceutical dosage form (Dry Suspension). They separated artemether on C\(_{18}\) nucleosil column (5 microm, 125 mm x 4 mm, ID) with a mixture of acetonitrile: potassium phosphate buffer pH 5.0 (0.05 M): water [48:32:10 (v/v/v)] as mobile phase. Gradient wavelength programming was used for detection where artemether was estimated at 215 nm and Paraben were at 254 nm. And in both methods, flow rate was 1.0 ml/min. components showed linearity at concentration ranges of 4-16, 1-4 and 1-10 mcg/ml for methyl Paraben, propyl Paraben and artemether respectively. Interference from matrix was negligible as much diluted sample were utilized for analysis.

**Lai CS, Nair NK, Mansor SM, et al. (2007)** [52]

They developed a RP HPLC method for separation of artesunate, Mefloquine and DHA from human plasma. Extraction of components from plasma was performed on SUPELCLEAN C\(_{18}\) cartridges. Separation of components was performed on Hypersil C4 column by using mixture of 0.05 M acetic acid and acetonitrile (58:42 v/v) as mobile phase. Electrochemical detection was performed by keeping in reduction mode. Above method can be successfully applied for determination of mentioned components in clinical samples.


They developed LC-MS for the estimation of artesunate from vials. The content were estimated 3 times by utilizing methanol as solvent. Concentration of sample was kept at 1000 ng/ml. APCI was used as ionization method for artesunate and mass detection was performed after separation on a Hypersil column (100 x 4.6 mm). Separation was achieved by using 10 mM Ammonium acetate: methanol (30: 70 v/v) by keeping flow rate at 1 ml/minute
Tayade NG, Nagarsenker MS (2007) [54]
They developed HPTLC technique for estimation of artemether from bulk as well as pharmaceutical formulation. They separated components on silica gel 60F254 and combination formic acid, ethyl acetate and toluene as mobile phase. Reflectance mode was utilized for estimation of artemether. Rf value of artemether was found to be 0.50 ± 0.03. Linearity was in between 200–1000 ng/band with 0.9904 as value of r². Method was applied successfully for quantification of artesunate from solid oral as well as parenteral formulation.

Unites states pharmacopoeia and National formulary (2007) [55]
They have suggested separation of hydroxychlorquine on C18 column by using water and methanol containing sodium pentane sulponate as ion pairing reagent. Separation was performed at detection lamda of 254 nm and utilizing 1 ml/minute flow rate.

César IC, Andrade Nogueira FH, Antônio Pianetti G (2008) [56]
They separated and estimated lumefantrine and artemether from combined tablets by RP HPLC. Chromatographic condition however are not mentioned but method shows good linearity with r²=0.99. The content of artemether was found in between 98.61% to 103.35%, while for lumefantrine it was 97.92–100.48%.

Esimone CO, Omeje EO, Okoye FB, et al. (2008) [57]
Authors had developed ultraviolet absorption spectroscopy to establish the wavelength of maximum absorbance for pure powder of artesunate and then the Beer's plot generated. This was validated and used to assay nine brands. Distinctive ultraviolet absorption at 287 nm of pure sample of Artesunate in simulated intestinal fluid (SIF) afforded the most reliable method for the analysis of nine different brands of Artesunate marketed in Nigeria. Linearity of method was in between of 10-200 mg%. The limits of detection (sensitivity) and quantitation were found to be 0.471 mg/ml and 1.27 mg/ml respectively. Based on these convincing data, simple ultraviolet spectroscopy at 287 nm could be used to assay artesunate in formulations.

Hanpithakpong W, Kamanikom B, Dondorp AM, et al. (2008) [58]
Authors had developed method for the quantification of artesunate and DHA from plasma using SPE. The separated components from LC were detected by
positive tandem mass spectroscopy. Guidelines of FDA were followed for validation purpose. The developed method was extremely sensitive quantitation for artemether as well as its metabolite. Interday and intraday precision was found to be lower than 7%. Quantity of plasma used for analysis for each time was 50 µl. They further ensured that the analysis out of the range can be accurately performed with dilutions if they are outside the range. Further they proved that the method was completely free from interference from plasma both qualitatively as well as quantitatively.

**Green MD (2009)** [59]

Author holds the patent application for determination of artesunate from its formulation by colorimetric analysis. Patent application has described the method to test artimisinine derivative before its administration to check its integrity. This reaction was based on rapid decomposition of artimisinine derivative at 25 °C with inclusion of solvents consisting of polar compounds and acids with pKa of less than 3.5. After allowing it to stand for specified period of time, reaction mixture turns into colored solution. A kit was developed consisting of all above mentioned components and formulation containing artesunate from formulation or from bulk at a time. (Type of Utility Patent).


They developed method for separation and quantitation of artemether and Lumefantrine from their formulation. Stationary phase used was silica gel G and mobile phase consisting of n hexane: Ethyl acetate 8:2 (v/v). Separated contents were estimated at 357 nm. Assay values of artemether was between 98.50 to 102.45% and for lumefantrine from 97.80 to 100.64%. LOD and LOQ was found to be 50 and 150 ng/spot for artemether. For lumefantrine it was found to be 300 and 900 ng/spot. ICH guidelines were followed to validate method. This method was applied for quantification of Artemether and Lumefantrine from their tablet formulation.

**Sunil J, Sanjithnath M, Sambhamurthi U. (2010)** [61]

Authors had developed simple accurate and precise RP HPLC method for quantification of Lumefantrine and artemether. Separation was performed on C18 column, 250 x 4.6 mm, by isocratic elution. Separation was achieved by combination of buffer and ACN in proportion of 40:60 (v/v), pH 3, at flow rate of
1.5 ml/mi. Detection was carried out in dual wavelength mode i.e., 210 and 303 nm. Elution time for artemether was 13 minutes and for Lumefantrine was 7.2 minutes. Accuracy was found to be 98.87 and 99.78% for Artemether and Lumefantrine.

**Indian Pharmacopoeia (2010)**[62]

Monograph of artesunate from Indian pharmacopoeia 2010 has suggested RP HPLC method for its assay from bulk. Separation is indicated on stainless steel column 15cm x 4.6 mm by utilizing 30 parts of a solution having 3.85 g of ammonium acetate + 1% TEA, pH 5.5 with acetic acid and 70 volumes of methanol as mobile phase. Separation was monitored at 216 nm with pump rate at 0.6 ml/min.

**Shrivastava A, Issarani R, Nagori BP. (2010)**[63]

Artemether was estimated from capsule and bulk by a newly developed RP-HPLC method. Artemether was separated on to column by buffer and acetonitrile in proportion of 35 to 65 with pH 6.5. Linearity of method was in between 250-750 mcg/ml with 0.9996 as value of $r^2$. LOD for artemether was found to be 21.83 mcg/ml.

**Arun R, Smith AA. (2010)**[64]

They developed UV method for quantification of Lumefantrine from bulk and formulation. He developed method by taking 234 nm as analytical wavelength. Linearity of method was in between 8-16 mcg/ml.

**Prasanna R. (2010)**[65]

He developed simple and precise RP HPLC method for estimation of Lumefantrine from its solid dosage form. He utilized methanol and acetonitrile in 50:50 v/v as eluent and pump was set at 2 ml/min. separation was achieved on C$_{18}$ column. Linearity of method was in between 50-150 mcg/ml with recovery of 99.76% over the range.


Authors had developed a Hyphenated tandem mass spectrometry with electron spray as ionization interface method for the simultaneous quantitation of artemether and lumefantrine in plasma. Artesunate was utilized as an IS. PPT method was employed for extraction of components from human plasma. The components were estimated on Ciano column by utilizing combination of
methanol, ammonium acetate buffer. Eluent was monitored in MRM mode to identify components at m/z 316 → m/z 267 for artemether, m/z 530 → m/z 348 for Lumefantrine and m/z 402 → m/z 267 for artemunate. Regression lines were plotted between 10–1000 ng/mL for artemether and 10–18,000 ng/mL for lumefantrine. LLOQ was found to be 10 ng/mL for both components. The validated method was successfully applied to determine the plasma concentrations of artemether and lumefantrine in healthy volunteers, in a one-dose pharmacokinetic study, over the course of 11 days. Concentration of both components from was measure over time of 11 days by developed method

**Duthaler U, Keiser J, Huwyler J. (2011)** [67]

(LC-MS/MS) method was reported to estimate artemether, artesunate, and their complex metabolites from plasma of sheep. Separation of components from sheep plasma was achieved by protein precipitation method. Extracted components were separated on an Atlantis C18 analytical column by using ammonium formate and acetonitrile as mobile phase in gradient mode. Mass detection was performed in SRM mode. Ionization was performed by EI source. Positive ions were selectively detected at 267 m/z and 163 m/z All components except artemether gave linear response in the range of 10-1000 ng/ml, while 90-3000 ng/ml for artemether. LLOQ was found to be 10 and 90 ng/ml, respectively. Components were stable during freeze and thaw cycles. Even found to be stable for 30 hours in auto sampler. After performing validation it was demonstrated that method can be applied for pharmacokinetic studies of artesunate and its metabolite.

**Kalyankar TM, Kakde RB (2011)** [68]

Artemether and lumefantrine were simultaneously determined from combined formulation by RP HPLC method. Components were separated on Hypersil ODS C18 (250mm×4.6mm×5µ particle Size) column, with TEA buffer pH 2.8 and methanol (20:80 v/v) as eluent. Eluents were monitored at 215 nm at flow rate of 1.5 ml/minute. Artemether was eluting at 11 minutes and Lumefantrine was eluting at 6 minute. Linearity of method was in between 20-120 mcg/ml for

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+++ m/z = mass to charge ration in mass spectrometry is the ratio of mass of formed fragment to the charge of formed fragment. Generally it indicates the molecular mass of the formed fragment/ion.
artemether & 120-720 mcg/ml for lumefantrine. Method was free from the interference of non-active components

Khalil IF, Abildrup U, Alifrangis LH, et al. (2011)\[69\]
They developed RP-HPLC method estimation of lumefantrine and its desbutyl metabolite from plasma. As an IS Halofantrine was used. For extraction of components from plasma LLE was utilized by combination of ethyl acetate and hexane. Separated components were quantified on a 250 mm-RP column. Elution was performed by utilizing combination of ammonium acetate (0.1 M) and ACN having pH of 4.9. **Elution process was monitored at 335 nm by keeping 360 nm as reference.** Recovery from plasma was found to be 88% and for metabolite it was 90, respectively. Inter- and intraday coefficient of variation for LUM and DL were ≤10%. LLOQ was found to be 12.5 and 6.5 ng/mL for principle and metabolite component. Sample of various patient were analyzed in laboratory by application of above method after validation.

Nogueira FHA, Goulart LPL, César IC, et al. (2011)\[70\]
Mefloquine was estimated from tablet formulation by a chromatographic method. The estimation was carried out on an Xterra RP\textsubscript{18} (250 x 4.6 mm id, 5 pm particle size) analytical column. **The eluent was 0.05 M monobasic potassium phosphate buffer (pH 3.5)-methanol (40 + 60, v/v).** The pump rate was set to 1 mL/min and elute was monitored at 283 nm. The method was specific for mefloquine in the presence of stress induced degradation products. It was also linear, precise, accurate, and robust, being suitable for routine QC analyses and stability studies. The developed HPLC method was compared to a previously described spectrophotometric method.

Okwelogu C SB, Azubike C, Babatunde K (2011)\[71\]
Authors had developed simple two step method for determination of artesunate from formulations. Artesunate is made to react with basic solution followed by neutralization. The reaction mixture is then made acidic and spectrum is recorded which gave higher absorptivity. This mixture was chromatographed on ODS C\textsubscript{18} column by using combination of ACN and phosphate buffer in same proportions. Two separate peak were observed which may correspond to derivative of principle component
Olajire Adegoke A, Afolake Osoye O. (2011) [72]
Authors had developed and validated colorimetric method for determination of artesunate and dihydroartemisinine in bulk samples as well as pharmaceutical formulation. The method involves the reaction of the reactive methylene centres generated in situ from the acid decomposition of the artemisinin derivatives with p-dimethylaminobenzaldehyde (DMAB). DMAB was reduced to the purple-Colored alcohol and this was quantitatively used to estimate the concentrations of the artemisinin derivatives. The new procedure was carried out at 60 °C in less than 30 minutes with excellent calibration data. They showed that the method could find application as an alternative in-process and after manufacture method for the quality control of artesunate and dihydroartemisinine.

Artemether was analyzed by a RP HPLC method. Combination of methanol, water and acetonitrile was utilized as eluent proportion of 30:35:35 v/v/v. Chromatography was performed on RP C18, 4.6 x 150mm, 3.5 μm and detector of UV at 210 nm, 1.0 mL/min as a Flow rate, 30 μL as an Injection volume. The chromatogram of Artemether its impurities namely α-artemether, dihydroartemisinin and artemisinin were found. An accelerated forced degradation study on Artemether significant degradation was observed when Artemether sample solution exposed to acid at room temperature, base and peroxide. Rapid degradation observed when Artemether samples exposed to acid at 600C and UV light. The assay values obtained for the solid state in the range of 92.1- 99.9%.The proposed method was validated as per requirements.

Arun R, Smith A. (2011) [74]
They developed HPLC UV method for determination of artemether and Lumefantrine in fixed dose combinations. They developed quick method as the retention times were around 2 and 6 minutes for artemether and Lumefantrine respectively. Separation was carried out by using mobile phase containing acetonitrile and 0.01M potassium dihydrogen orthophosphate buffer (70:30), at the pH 4.0 and flow rate of 1 ml/min. detection was carried out at 254 nm where both of the drug were possessing significant absorbance. They developed
method in the concentration range of 32-192 mcg/ml of artemether and 20-120 mcg/ml of Lumefantrine with good regression coefficient.

**Pawar PY, Chavan MP, Bhosle HP, et al. (2011)** [75]
They reported simple spectrophotometric method for determination of artemether from its capsule. They employed 1 N HCl as solvent and used to derivatize drug. Linearity of method was in between 5-40 mcg/ml with good regression coefficient. The LOD and LOQ for artemether was found to be 2.30 and 4.08 mcg/ml respectively. They demonstrated utility of developed method by analyzing marketed formulation.

**Grace Gbotosho O, Happi CT, Omowunmi L, et al. (2012)** [76]
For purpose of therapeutic monitoring chromatographic method was developed for estimation of Mefloquine from saliva and eluent was monitored at 220 nm. Concentration were confirmed by comparing concentration from plasma and from saliva. For IS, verapamil was preferred choice. Components were separated on a Hypersil ODS column. The eluent consisted of phosphate buffer-acetonitrile-methanol (40:30:30 v/v/v) with 1% triethylamine adjusted to a pH 2.8 with concentrated phosphoric acid. The pumping rate of mobile phase was 1.0 ml/min. and elution time was around 4 minutes for Mefloquine.

**Meena S, Sandhya S. (2012)** [77]
They had developed sensitive chromatographic method for quantitation of Mefloquine hydrochloride from finished dosage forms but however the chromatographic condition and other required information is not mentioned in the procured reference.

**Tembhurkar NB, Jadhav SB, Chaudhari PD. et al. (2012)** [78]
Authors had developed and validated a stability indicating HPLC method for determination of Mefloquine hydrochloride in bulk and finished formulation. Separation was achieved on to C18 column (250 x 4.6mm). Combination of TEA, methanol, water and ACN was utilized as eluent. pH of eluent was set at 3 with OPA. Pumping rate of eluent was 1ml/min. Elution process was monitored at 288nm. Elution time of Mefloquine hydrochloride was 4.5 min. Mefloquine hydrochloride was subjected to various stress conditions. Linearity of method was achieved between 10-50µg/ml. It was concluded that drug is unstable
under all stress conditions. Degradants peak were clearly separated from the parent peak with adequate resolution

**Wang LZ, Ong RY, Chin TM et al (2012)** [79]

They developed hyphenated tandem technique for determination of hydroxychloroquine in blood using its stable labeled isotope, hydroxychloroquine-d4 as the internal standard. Components were separated by water containing 0.1% formic acid-acetonitrile (94:6, v/v) on C8 column (50mm×2.1mm). Elutes were detected by Quadrupol mass analyzer.

**Weisheng Biolog Pharmaceuticals Co Ltd (2013)** [80]

The current review of literature describes a patent which had been filed by Weisheng Biolog Pharmaceuticals Co Ltd from China. Zhang M, Peng X, Zhao J and Yanan Q had invented new sensitive analytical method for quantifying artemether from injectable by HPLC. Chromatographic column adopts octadecyl silane as a filler, a mobile phase is acetonitrile-water (75:25 v/v), the pump rate is 1.0ml/min, and the column temperature is 40 ºC and the detection wavelength is 216nm. Solution of artemether were prepared in methanol and trichloromethane (9:1 v/v). Contents of artemether were calculated by external standard method. The method is easy and simple to operate, precise and reliable in determination result, high in specificity, and short in detection time, and the precise and reliable in content detection method is provided for quality control of the artemether injection.


Authors had developed UV spectrometric method for determination of artesunate and Mefloquine from pharmaceutical formulation. Simultaneous equation method has been designed and performed by using methanol as solvent. Linearity data were taken for both of the drugs at 240 and 222 nm. The linearity range of Artesunate and Mefloquine were 10–60 and 20–120 μg/mL with r2 value of 0.999 for each drug. Both components can be estimated without any intrusion from inactive ingredients.

**Sapakal AD, Mohit SK, Magdum CS (2013)** [82]

They developed simple, accurate, precise and economical UV-method for estimation of Artesunate and Mefloquine HCL and was validated. For analysis of Artesunate and Mefloquine HCl, 242nm and 256nm wavelengths were used.
respectively. The linearity range for Artesunate and Mefloquine HCl were 20-140 µg/ml & 50-350µg/ml. Accuracy for ART were 98.33-100.33 & for MEF 101.33-101.92. The LOD was found to be 0.13 and 1.53 µg/ml for ART and LOQ was 0.39 & 2.21µg/ml for MEF  

**Marion S, Bruno M, Pascal H (2014)** [83]  
They developed hyphenated tandem technique for quantification of hydroxychlorquine and its three major metabolites from human blood. They separated impurities namely monodesethylhydroxychloroquine, desethylchloroquine and bisdesethylchloroquine. But major drawback of the method was the accuracy which was below 90 percentage.

### 3.3 Conclusion from review of literature:

Above mentioned literature review has revealed that till now, no derivative spectroscopic method is available for quantification of Mefloquine and artemesunate from their combined dosage form. In addition no HPTLC method is available for estimation of Mefloquine and artemesunate from its pharmaceutical formulation. Further more recently published HPLC method for determination of Mefloquine and artemesunate are suffering from the problem of stability during the analysis.

Literature survey has revealed that one densitometry is available for determination of artemether and Lumefantrine from its pharmaceutical formulation which suggest determination of both the separated components at 357 nm. Review of literature has suggested that there is no chromophoric group available by which artemether can show absorbance at 357 nm. So there is a need of more reliable densitometric method which can easily separate artemether and Lumefantrine with appropriate detection condition.

Review of literature has suggested many method which can separate dihydroartemisinine derivatives by RP HPLC from formulation as well as from biological matrix. All of the methods as such an environment in which the drugs cannot remain stable,[84- 85] (i.e. highly acidic, presence of moisture, elevated temperature.). Even most of the reported method requires longer runtime and hence are more time consuming. So it is a preliminary requisite that, one method should separate all dihydroartemisinine derivatives from mixture in short
time period in such an environment, in which all can remain stable and their stability can be checked by mass detection and characterization.

Furthermore there is also a need of a stability indicating method which can effectively separate hydroxychlorquine from its impurities in reverse phase mode along with its degradation products