

The Development and Validation of the HPLC Method for Determination of Artesunate and Amodiaquine in Novel Antimalarial Formulations

1. Payal Deokar*, 2. Santosh Payghan, 3. Prof. Amol Wadhve 4. Mrs. Ashwini Pundkar

1. Research Scholar, Rajesh Bhaiyya Tope College of Pharmacy, Chh.Sambhajinagar.

2. Dept. of Pharmaceutical Science, Rajesh Bhaiyya Tope College of Pharmacy, Chh.Sambhajinagar.

ABSTRACT

ASAQ combination drug used in treating malaria is bitter in taste, resulting in poor adherence and difficulty in swallowing by children. To mitigate this issue, taste masking is achieved by coating the drug with polymers to improve the taste of the dosage form. However, this produces a novel drug product which must undergo analytical evaluation to ensure that it is safe and drug content matches the label claim. The aim of this study is to develop and validate a novel analytical methodology which is suitable for this purpose. Thus, a reversed phase high performance liquid chromatography for the quantitative determination of AS and its impurities was developed. The method was validated according to ICH guidelines for linearity, precision, accuracy, and specificity, limit of detection (LOD) and limit of quantification (LOQ). The method was found accurate and precise with an average retention time of 8.2 min. Good linearity was observed in the concentration range of 0.25 – 0.75 mg/ml with regression coefficient of $r^2 = 0.999$. The assay of the proposed method was found to be 101.5%. The recoveries of artesunate were found to be within 99% - 102%. The % RSD for precision was found to be

Keywords: ASAQ, ICH guidelines, LOQ, LOD, RSD. Reversed phase, analytical methodology.

Introduction

Malaria is a life-threatening disease found around the world and is especially dominant in Africa. Malaria is spread to human beings by the female Anopheles mosquitos which are infected with these kinds of parasites. The parasites Plasmodium falciparum, P. vivax, P. ovale, and P. malariae are the primary cause of malaria in humans. Of these, P. Falciparum is the most harmful, leading to severe infections and potentially death. As per data recorded by the WHO, in 2020 there were an estimated 241 million cases of malaria, with around 627,000 recorded fatalities. These data indicate that Africa has a large share of the global malaria burden, with 95% of recorded infections being found in this region. Of the deaths within this region, 80% occurred in children below five years of age, making them the most at risk for contracting the disease [1]. Malaria is an acute, fever-based illness. For a less immune person, the symptoms of disease are normally observed between 10-15 days after the bite of a parasite infected mosquito. The primary symptoms observed are generally fever, headache, and chills which may be acute and not easy to identify as malaria. Young children with severe malaria may possess more than one symptom, including, anaemia, respiratory troubles, metabolic acidosis, cerebral malaria and multiorgan failure [2]. Malaria is an avertable and curable disease. Malaria is controlled by early detection and treatment, which also lowers additional transmission and prevents fatalities. There are several antimalarial drugs available, which include quinine sulphate, atovaquone/proguanil, doxycycline, primaquine phosphate, chloroquine mefloquine, and artemisinin combinations. The best available first line treatment suggested by World health organisation (WHO), particularly for Plasmodium falciparum malaria, is artemisinin-based combination therapy (ACT) [1]. Some of the different ACT combination medicines used for the treatment. Artemisinin derivatives act quickly to reduce the number of malaria parasites in the blood, leading to rapid clinical improvement. Partner drugs with different mechanisms of action work synergistically with artemisinin, enhancing overall treatment efficacy and reducing the likelihood of treatment failure. The combination of two or more drugs with different mechanisms of action helps to prevent the development of resistance. ACT regimens are usually short (typically 3 days), improving patient compliance compared to longer treatment courses. Better compliance leads to more effective treatment and reduces the risk of resistance development. This combination therapy is proven to be more effective in treatment of acute, uncomplicated stage, because it is highly effective whilst having fewer adverse effects. The drugs used in this fixed combination are artesunate (AS) and amodiaquine (AQ), which can be administered to patients orally [3]. Artesunate is a prodrug that is converted to its active form dihydroartemisinin

(DHA). This process involves hydrolysis of the 4-carbon ester group via plasma esterase enzyme. It is hypothesized that the cleavage of end peroxide bridge in the pharmacophore of DHA generates reactive oxygen species which increase stress and cause malarial protein damage via alkylation. The chemical structure of articulate is given in Figure 1[4]. A disadvantage of artesunate is its short half-life. Rapid conversion to dihydroartemisinin takes place in vivo, with half-life of about 26 mins. Hence it is given in combination with other antimalarial drugs such as amodiaquine. Figure 1: Structure of Artesunate C₁₉H₂₈O₉ The chemical structure of amodiaquine is given in Figure 2[5]. Figure 2: Structure of Amodiaquine C₂₀H₂₂ClN₃O Table 1: Properties of artesunate and amodiaquine Artesunate Amodiaquine dihydrochloride dihydrate Description White crystalline powder Yellow crystalline powder Molecular weight 384.4 g/mol[4] 355.86 g/mol[5] Solubility soluble in organic solvents like ethanol, sparsely soluble soluble in water, very faintly soluble in alcohol, benzene, in aqueous buffers. Log P 2.61 and ether. 3.8 [6] pKa 4.6 7.1 and 8.1[6] Amodiaquine is believed to limit the heme polymerase activity of parasite, just as other quinoline derivatives. This results in an increase of available heme, which is lethal to the parasites. This analyte connects with the free heme inhibiting the parasite, in turn changing to less lethal form and disrupts its membrane function [7]. The primary concern about AS-AQ combination is its bitter taste which results in poor adherence in children[8]. One such approach to overcome this, is by using a novel formulation approach to mask the bitter taste. By coating the active pharmaceutical ingredient with MicroCoat™ technology developed at Fluid pharma Ltd, it is hypothesised that the bitter flavour of the drug when consumed orally will be reduced. APIs will be spray layered onto separate micropellets and taste masking is done with polymer coating (when necessary) separately using a modified fluidized bed coater. Following components were used in the formulation. Table 2: Components used in formulation. Components Mweight in g/mol Chemical name Cellets ®100 Layer in formulation Function 370.35 PVP K30 Microcrystalline cellulose inner core Have uniform particle size of 100 - 200 µm and large surface area which helps in uniform drug loading 40000 Polyvinylpyrrolidone seal coating Methocel™ E5 It used as a binder, binding drug with Cellets and as a seal coating 1261.4 Ethocel™ standard 20 premium Hydroxypropyl methyl cellulose taste mask coating Can help in masking the taste of bitter APIs especially for water soluble drugs and a pore former 454.5 Ethyl cellulose taste mask coating It is water insoluble polymer and helps in controlling the drug release Figure 3: Schematic representation of different layers used in the formulation. 11 The main objective of any drug formulation is to be safe (e.g., to not cause any side effects to the consumer) and effective. To meet these criteria several safety characteristics must be assessed. The most important are strength, purity, and identity. The formulation to be developed by Fluid Pharma will differ from existing Winthrop AS-AQ tablet combination necessitating the analytical method development and validation to support novel development and safety testing. All analytical method development and validation is key part of pharmaceutical analysis it plays a significant part in the innovation, development, and mass production of drugs. These test procedures are used by quality control (QC) departments in establishing the uniqueness, purity, effectiveness, and performance of product essential for drug safety and therapeutic efficacy [9]. One method of choice for pharmaceutical analysis is high pressure liquid chromatography (HPLC) connected to an Ultraviolet (UV) detector, due to the advantages it offers over standalone UV in terms of specificity and sensitivity. The use of chromatography assists with the separation and identification of target analytes within complex mixtures. HPLC methods can therefore be used to identify, quantify analyte, and separate mixture of components present in a sample [10]. The HPLC method can be used to assess a range of analytes in combination dose forms due to the advantages of speed, selectivity, precision, ease of automation and accuracy[11]. Separation by HPLC is performed by flowing a mobile phase, with analyte dissolved within, over a stationary phase. Based on the chemical composition of the analyte, the molecules interact while moving through the stationary phase. The elution time of sample depends on the interaction of analyte with stationary phase. Thus, different components of a sample are eluted at various time points [11]. The detector unit (e.g., UV detector) detects the analytes when leaving the column. The signals are processed and verified by a computer software and subsequently displayed in a chromatogram. 12 In general, HPLC system contains the four major components. 1. Pump: The HPLC pump is called as solvent delivery system. The main function of pump is to deliver constant flow of liquid mobile phase all through the HPLC. 2. Injector: It is employed to inject the sample into the flow of mobile phase without affecting the system's flow rate or pressure. 3. Column: The column is the primary component responsible for separation of the analytes. When analytes present in the mobile phase move through the column, partition of analytes occur depending on their interaction with stationary phase. The most popular packing material for HPLC columns is silica. The long carbon chain of C18 provides a large surface area for interaction with analytes, leading to high selectivity and resolution in separations. This helps to achieve sharp and

well-defined peaks in chromatograms. C18 is highly hydrophobic due to its long alkyl chain (18 carbon atoms). This allows a strong hydrophobic interaction with non-polar compounds, making it suitable for reversed phase HPLC, where non-polar analytes are retained and separated effectively. C18 bonded phases are chemically stable and can withstand a wide range of pH values (typically pH 2 to 8), making them durable and suitable for various analytical conditions. Other kinds of substances for the support of the stationary phase include polymers and alumina [12]. columns have different length ranging from 30mm to 250mm and particle size vary from 3-5 μ . 4. Detectors: In HPLC a UV/Vis's spectrophotometer detection unit comprises of a flow cell that is positioned beneath the column. As samples moves from the column, they travel through the flow cell and take up UV light, changing the energy level in a quantitative way. A high-pressure xenon lamp, deuterium, or a mercury lamp could be the source of the radiation.[13]. Multi-wavelength detectors can determine the absorbance at different wavelengths altogether at the same time. In DAD (diode array multi-wavelength detector) the uninterrupted radiation is projected over the Analyte cell. The photodiode array 13 picks up each wavelength that has been independently settled. These detectors gather data on absorbance across the entire UV-visible spectrum.

MATERIALS AND METHODS

Artesunate (AS- lot no: 230001) is obtained from Huevepharma (Huevepharma, Italy) and Amodiaquine Hydrochloride (AQ- lot no: 210016) is obtained from IPCA (IPCA, India). HPLC grade solvents methanol (lot no: 1914578), acetonitrile (lot no: A9964), 96% ethanol (lot no: 1991216), potassium dihydrogen phosphate, sodium octane sulfonate, sodium acetate, PTFE, PVDF and polypropylene filters was procured from Fisher Scientific. Cellulose acetate and glass filters were sourced from Chromatography Direct, Runcorn UK). AS and AQ reference standards are obtained from Hueve pharma. anhydrodihydroartemisinin, Impurity reference dihydroartemisinin, standards of acetaminophen, artemisinin, 4,7- Di chloroquinoline (4,7- DCQ), 7- choro-4-hydroxy phenylamino quinoline and 4 acetamido-2-diethylaminomethylphenol were obtained from LGC, UK. Phosphoric acid, perchloric acid and hydrochloric acid are purchased from Honeywell. Uncoated AS micro pellets formulations one with (Artesunate, Cellets, Syloid and 1.2% PVP K30 and one with (Artesunate, Cellets, Syloid and 0.8% klucel), coated AQ micro pellets formulation with (Amodiaquine, Cellets, Syloid, Methocel, Ethocel and magnesium stearate) are obtained from Fluid Pharma.

2.2. METHOD DEVELOPMENT FOR ASTESUNATE & ITS IMPURITIES

2.2.1. PREPARATION OF SAMPLES

Buffer:

Accurately weighing 1.36 g of potassium dihydrogen phosphate, it was then transferred to a volumetric flask, dissolved in 900 mL of water, and then brought to pH 3.0 by adding phosphoric acid while stirring at 200 RPM with a bibby stirrer. Finally, the volume was brought to 1000 mL by adding deionized water. Then, a 0.45 μ nylon filter membrane was used to filter this solution.

Mobile phase: It was prepared from this buffer by combining sixty-six volumes of acetonitrile and thirty-four volumes of buffer. This was used as mobile phase after filtration and degassing.

Artesunate stock solution: It is prepared by weighing 5 mg of analyte and dissolve it in 5 mL of methanol then sonicate it for 5 mins, before making to volume with methanol and vortex it for 10 sec to get a final concentration of 0.5 mg/mL.

Artesunate impurities stock solution: DHA, artemisinin, anhydrodihydro-artemisinin were prepared by weighing 1 mg of sample and dissolve it in 5 mL of methanol then sonicate it for 5 mins, before making up the volume to 10 mL with methanol and vortex it for 10 sec to get a final concentration of 0.1 mg/mL.

Amodiaquine hydrochloride stock solution: It was prepared by weighing 13.5 mg of analyte and dissolve it in methanol and sonicate for 5 mins and make up the volume up to 10 mL with methanol to get a final concentration of 1.35 mg/mL.

Amodiaquine impurities stock solution: weigh 1.35 mg of acetaminophen, 4,7- Di chloroquinoline, 7-chloro-4-(4-hydroxy phenyl amino) quinoline and 4-acetamido-2 diethylaminomethylphenol and dissolve it in 5 mL of methanol

and sonicate it for 5 mins then make up the volume up to 10 mL with methanol to get a concentration of 0.00675 mg/mL.

Forced degradation studies:

Acid degradation: AS stock solution was prepared at a concentration of 2.5 mg/mL from the stock take 5 mL of solution and add 4 mL of 0.01M HCL and heat it in water bath at 50 0C for 30 mins. Samples were then made up to volume by addition of sufficient methanol to reach 25 mL before being filtered and injected on the HPLC system.

Base degradation: AS stock solution was prepared at a concentration of 2.5 mg/mL from the stock take 5 mL of solution and add 4 mL of 0.01M NaOH and heat it in water bath at 50 0C for 30 mins. Samples were then made up to volume by addition of sufficient methanol to reach 25 mL before being filtered and injected on the HPLC system.

Oxidative degradation: Studies were done similarly 2.5 mg/mL stock solution was prepared then take 4 mL of the sample from stock solution and add 4 mL of 10 % H₂O₂ which was left for 24 h before being made to volume, filtered, and injected onto the HPLC system.

Photolytic degradation: A stock solution of 0.5 mg/mL was treated with light for 5 h by use of UV light 24 h. These samples were then injected onto the HPLC system.

Heat Degradation: A stock solution of 0.5 mg/mL sample was heated in water bath at 50 0C for 24 h. These samples were then injected onto the HPLC system.

Placebo preparation: Placebo mixture was prepared by weighing all the excipients (Cellets, Polyvinylpyrrolidone K30, Syloid) used in the formulation without adding API and add it to 50 mL volumetric flask. Then add $\frac{3}{4}$ of methanol and stir it by placing a magnetic stirrer bar in the sample for 60 mins. After 60 mins make up the volume to 50 mL with methanol and filter the sample.

RESULT AND DISCUSSION

RESULT FOR ARTESUNATE & ITS IMPURITIES METHOD

SIMULTANEOUS DETECTION METHOD FOR ARTESUNATE AND AMODIAQUINE HYDROCHLORIDE

Initial method developed focus on the methodology provided in Section 2.2.2, based upon a publication by [30]. This method aimed to separate all analytes of interest. Artesunate at 0.5 mg/mL and its impurities DHA, artemisinin, anhydro dihydroartemisinin at 0.1 mg/mL, amodiaquine at (1.35 mg/mL) and its impurities acetaminophen, mannich base, 4,7-DCQ, 7-chloro-4(4-hydroxy phenyl amino) quinoline was injected onto the system and separation was assessed. Chloroquine phosphate was included in the separation for system suitability test because chloroquine and amodiaquine are both 4-aminoquinoline compounds. Their structural similarity confirms that the chromatographic behaviour of chloroquine can work as a reliable indicator of how amodiaquine will behave under similar conditions. Chloroquine phosphate is commonly available and relatively inexpensive compared to some other potential reference standards. This makes it a functional choice for regular analysis in quality control laboratories. All the peaks were identified by injecting the individual standards of each analyte separately.

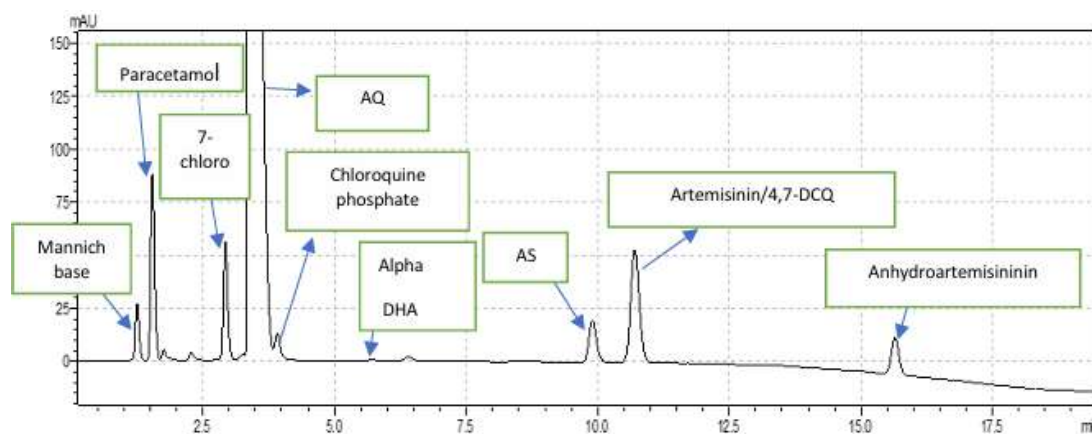
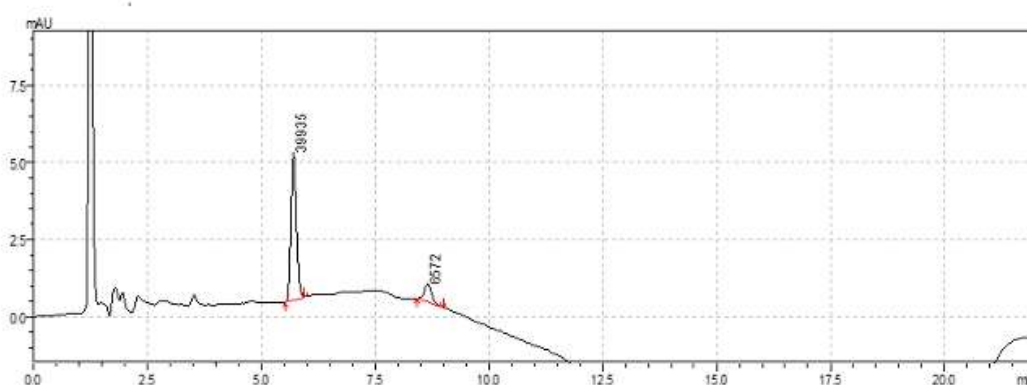


Figure 4: Separation of AS/AQ and its impurities using a mixture of analytes dissolved in buffer: acetonitrile.

Table 4: Retention times of AS, AQ and its impurities based on simultaneous method.

S.no	Analyte	Peak Area	Retention time in (mins)
1	Acetaminophen (paracetamol)	443719	1.54
2	7-chloro 4- hydroxy phenyl amino quinoline	325178	2.92
3	Amodiaquine	40086017	3.47
4	Chloroquine phosphate	50250	3.91
5	Alpha DHA	21402	5.71
6	Beta DHA	8585	8.65
7	Artesunate	196644	9.89
8	Artemisinin	609044	10.70
9	4,7-DCQ	609044	10.70
10	Anhydroartemisinin	175356	15.64



HA peaks.

Whilst the separation of most analytes was achieved, co-elution was observed between artemisinin and 4,7-di chloroquinoline. The octane sulfonate acts as an ion pairing agent. In reversed-phase HPLC, hydrophobic compounds are retained longer than hydrophilic ones. Artesunate and amodiaquine, being antimalarial drugs, may have ionizable groups. AQ is in an ionic form, so not sufficiently retained. By adding a hydrophobic ion-pairing agent like octane sulfonate, ionic analytes form neutral or less polar ion-pairs that have greater affinity for the hydrophobic stationary phase. This enhances the retention of AQ in a reversed phase system simultaneously to AS allowing for better separation, but the octane sulfonate used is high cost (60£ per 10 g). Hence the decision was made to develop two separate analytical for artesunate and its impurities and amodiaquine and its impurities because there are many analytes to separate.

INTERNATIONAL PHARMACOPEIA METHOD DEVELOPMENT FOR ARTESUNATE

The conditions given in Section 2.2.3 were used to assess the separation of a standard mixture containing AS and its three impurities as shown in Figure 6. Peaks were identified by injection of individual standards of analytes. Amodiaquine hydrochloride is also present in the final formulation therefore the impact of AQ and its impurities on the AS separation was evaluated by injection of mixture of AQ and its impurities.

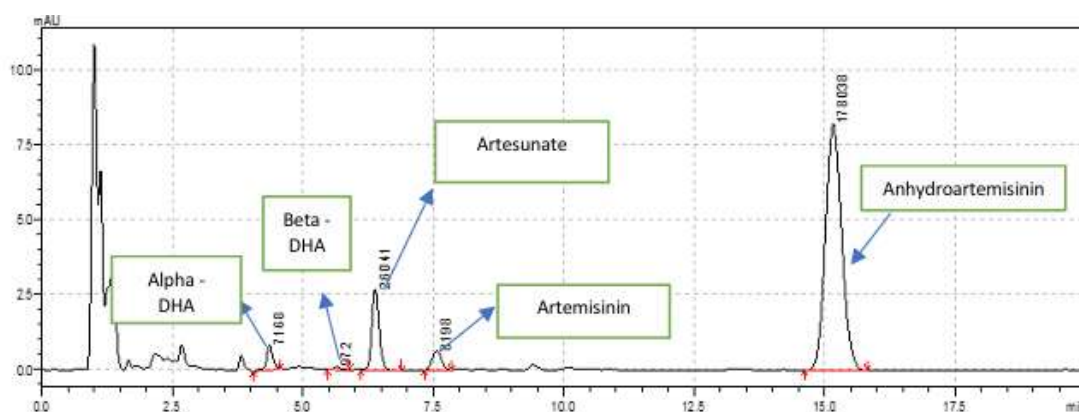


Figure 6: Artesunate and its 3 impurities dissolved in methanol at a concentration of 0.5 & 0.1 mg/mL to check their separation.

Table 5: Retention times of artesunate and its impurities

S.no	Analyte	Peak Area	Retention time in (mins)
1	Alpha DHA	7168	4.36
2	Beta DHA	972	5.65
3	Artesunate	26041	6.38
4	Artemisinin	8198	7.56
5	4,7-DCQ	8198	7.56
6	Anhydroartemisinin	178038	15.16

Whilst AQ and its impurities will not be assayed using this method, it must be demonstrated that they should not interfere with the AS and its impurities peaks. AQ and its impurities (acetaminophen, Mannich base, 7-chloro-4-(4-hydroxy phenyl amino quinoline) were not observed to interfere with AS or impurity elution. However, the elution of 4,7-DCQ took place at the same retention time as that of artemisinin, an AS impurity. This is shown in figure - 7.

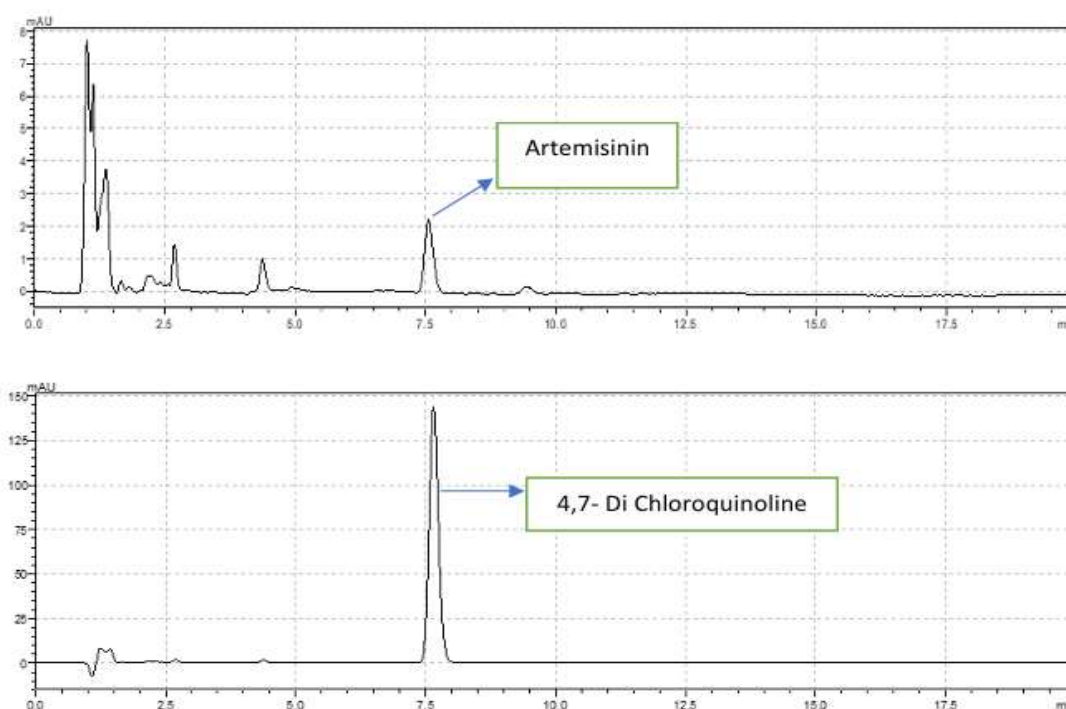


Figure 7: Co-elution of artemisinin retention time: 7.56 mins and 4,7- Di chloroquinoline retention time: 7.56 mins.

Due to this co-elution several approaches were used to separate all components by changing the mobile phase composition 50:50 with 0.8 mL/min flow rate, 56:44 buffer: acetonitrile with 0.8 mL/min flow rate, 52 % acetonitrile and 48 % buffer with 0.6 mL/min flow rate but, both analytes were coeluting when tried with these mobile phase ratios and columns (brand- Varian, thermo Hypersil with ODS or BDS material), at lengths of 10 cm, 15 cm and 30 cm at particle sizes of 3 and 5 μ m. When 66 % acetonitrile and 34 % buffer with a flow rate of 0.4 mL/min is tried on 15 cm Varian column with 3 μ m particle size acceptable separation is seen for all analytes. The diluent was also changed from acetonitrile to methanol because the impurity standard dihydroartemisinin peak shape is not good, when we have used acetonitrile as a diluent and different injection volumes like 50, 75 μ L were also tried but peak shape was not good, because in reversed phase HPLC, the stationary phase is typically non polar (e.g., C18). When acetonitrile used, its elution strength might not adequately balance the interactions between DHA and the stationary phase this can lead to strong retention and peak tailing. Acetonitrile has limited hydrogen bonding capabilities compared to solvents like methanol. Since DHA contains functional groups capable of hydrogen bonding, methanol can form stronger hydrogen bonds with polar analytes like DHA, improving solubility and reducing peak tailing by facilitating more consistent elution from the stationary phase. However acceptable separation was achieved by using the conditions mentioned in section 2.2.3 by using 15 cm Varian column with 4.6 mm diameter and 3 μ m particle size by using 66 % acetonitrile and 34 % buffer at a lower flow rate of 0.4 mL/min and injection volume is 20 μ L. AS separation was now acceptable with resolution observed between all the impurities.

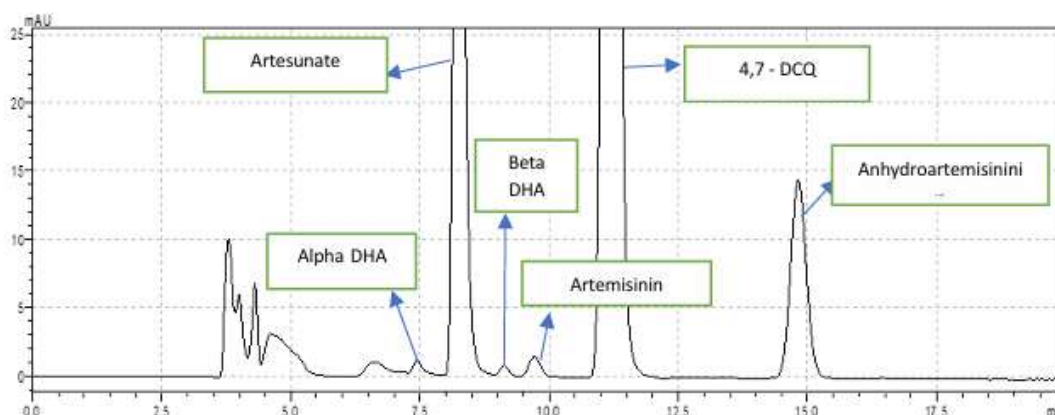


Figure 8: Chromatogram showing separation of AS from its impurities (DHA, artemisinin, anhydrodihydro artemisinin) and an AQ impurity 4,7- DCQ, at impurity concentration of 0.01 mg/mL for AS impurities, 0.0675 mg/mL for 4,7-DCQ and AS concentration at 0.5 mg/mL.

Table 6: Retention times of AS and its impurities.

S.no	Analyte	Peak Area	Retention time in (mins)
1	Alpha DHA	11712	7.44
2	Artesunate	1065382	8.2
3	Beta DHA	8286	9.12
4	Artemisinin	25370	9.71
5	4,7- DCQ	34130	11.18
6	Anhydroartemisinin	3229830	14.82

EFFECT OF FILTERS ON BACKGROUND PEAKS

Background peaks in the chromatogram were observed. Following investigation, by injection of a methanol blank with and without filtration with a PTFE filter, these extraneous peaks were identified as coming from the filtration process. It was hypothesised that use of an alternative filter material would remove these background peaks. Therefore, several filters containing different materials (nylon, polypropylene, glass fibre, cellulose acetate, PVDF and PTFE) were assessed by filtration of methanol blanks and subsequent injection onto the system. Filter materials containing PVDF and PTFE were found to give the cleanest chromatograms, as evidenced in Figure 9(B) & (C).

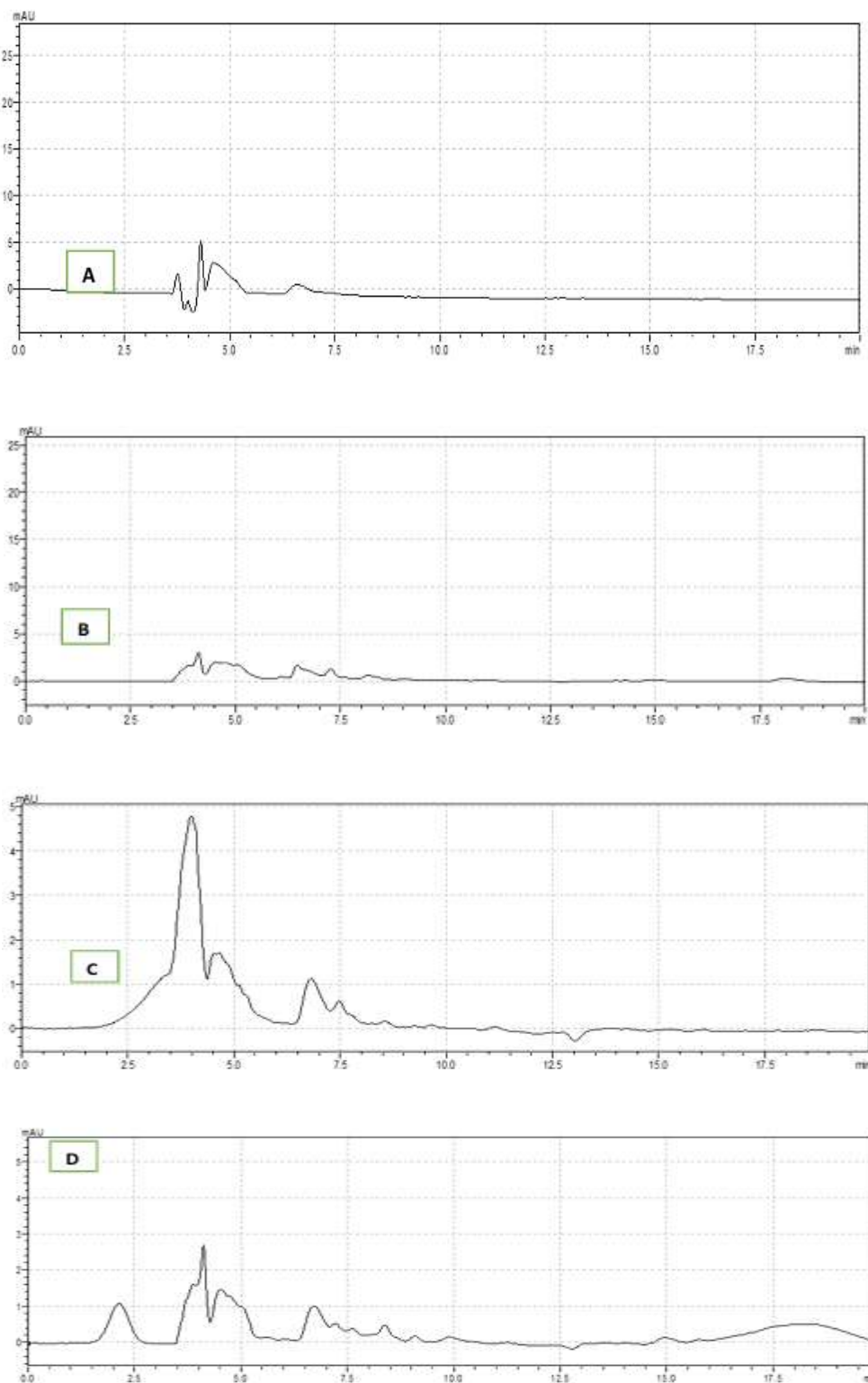


Figure 9: (A)-Chromatogram of methanol without filtration, (B)-methanol with filtration by PTFE filters (second image), (C)-methanol with filtration by PVDF filters (third image), (D)-methanol with filtration by polypropylene filters (fourth image).

The data generated suggested that both figure 9 (B) - PTFE and figure 9(C) - PVDF filter material were suitable for preparation of samples to the presence of the fewest extraneous peaks, coupled to this interference being found at very low levels such that it was unlikely to interfere with detection of the analytes of interest. PTFE was selected and used for sample preparation moving forward.

EXTRACTION METHOD OF ARTESUNATE FROM FORMULATION

Extraction of AS from two formulations as mentioned in section 2.1 uncoated AS micro pellets formulations are one with Artesunate, Cellets, Syloid and 1.2% PVP K30 and another with Artesunate, Cellets, Syloid and 0.8% klucel composition. One dose of each formulation containing 25 mg of AS was weighed and transferred to a 50 mL volumetric flask. This flask was filled with methanol such that it was $\frac{3}{4}$ full and a magnetic stirrer added. Stirring took place for either 30 or 60 mins to evaluate the effect of agitation before the flask was made to volume with methanol. The solution within the flask was then vortexed. A guard column is a short column placed between the injector and the analytical column because the final ASAQ formulation contains polymers. It helps to adsorb and trap strongly retained substances or chemical impurities that could irreversibly bind to the analytical column thus prevents clogging and maintaining the integrity of analytical column. Sample is filtered before being injected and onto the HPLC system at a final concentration of 0.5 mg/mL for each PVP K30 and Klucel separately. Recovery of the sample was calculated based upon the theoretical API content of the formulation. The results of this are given in Table-7 below.

Table 7: Percentage Recovery of API by stirring at two-time points.

Formulation	Extraction Time	% Recovery	%RSD
1.2% PVP Formulation	30 min	98.62	4.52
	60 min	101.52	0.29
0.8% Klucel Formulation	30 min	103.81	7.89
	60 min	101.66	1.88

For both formulations, recovery was near 100 %. However, the % RSD of extraction was lower at 60 mins than at 30 mins. Therefore, a 60 mins extraction timepoint was used to reduce the variability of the method.

RESULTS FOR VALIDATION OF ARTESUNATE AND ITS IMPURITIES USING HPLC

The developed method was validated in accordance with the ICH guidelines [17]. The finalized chromatographic conditions developed are given in section 2.2.3. The analytical methods were evaluated for specificity, linearity, precision, and accuracy in accordance with the International Conference on Harmonization (ICH) guidelines (ICH, 2005). The linearity, precision, accuracy, and system suitability parameters for artesunate are summarized in Table 8. The analytical methods implemented, as detailed in Section 2.2.4, were deemed to pass all specification criteria as defined by the ICH guidelines with suitable LOD and LOQ levels for the quantification of small amounts of artesunate in samples.

Specificity:

Results for Specificity:

- Methanol (diluent): No extra peaks observed within the blank that will interfere with analysis of AS or related substances Figure 10(A).
- Placebo mixture: No interference of any peaks presents in the placebo mixtures (AQ micropellets, AS micropellets and AS dry powder blend) for AS or related substances peak analysis Figure 10(C & D).
- Forced degradation samples: separation was observed for all known and unknown degradants of artesunate Figure 11.
- The suitability of method for assessment of AS and related substances was determined in method specificity.

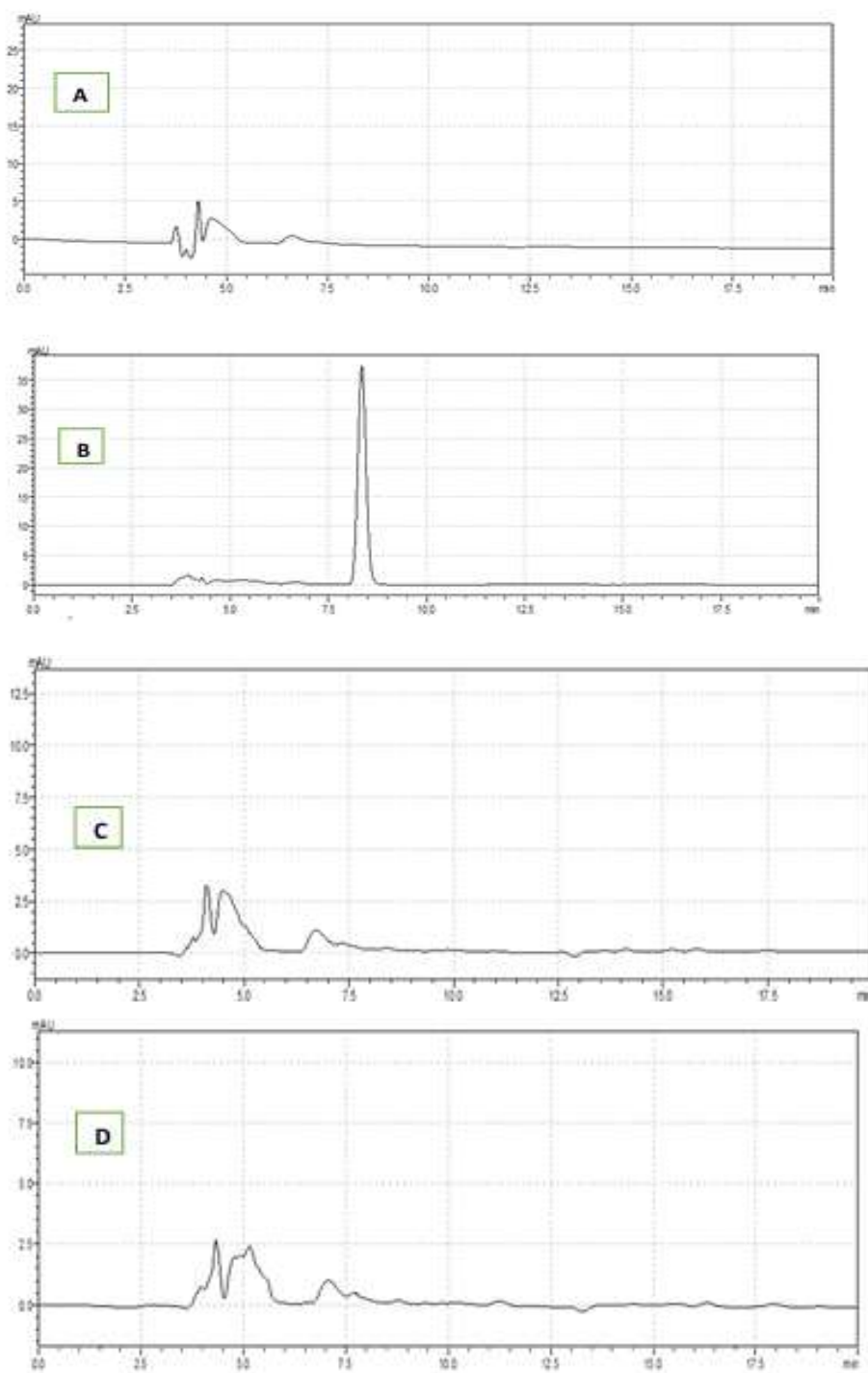


Figure 10: (A) Blank diluent (methanol), (B) AS reference standard at 0.5 mg/mL, (C) AQ placebo (3rd image) and (D) AS placebo (4th image).

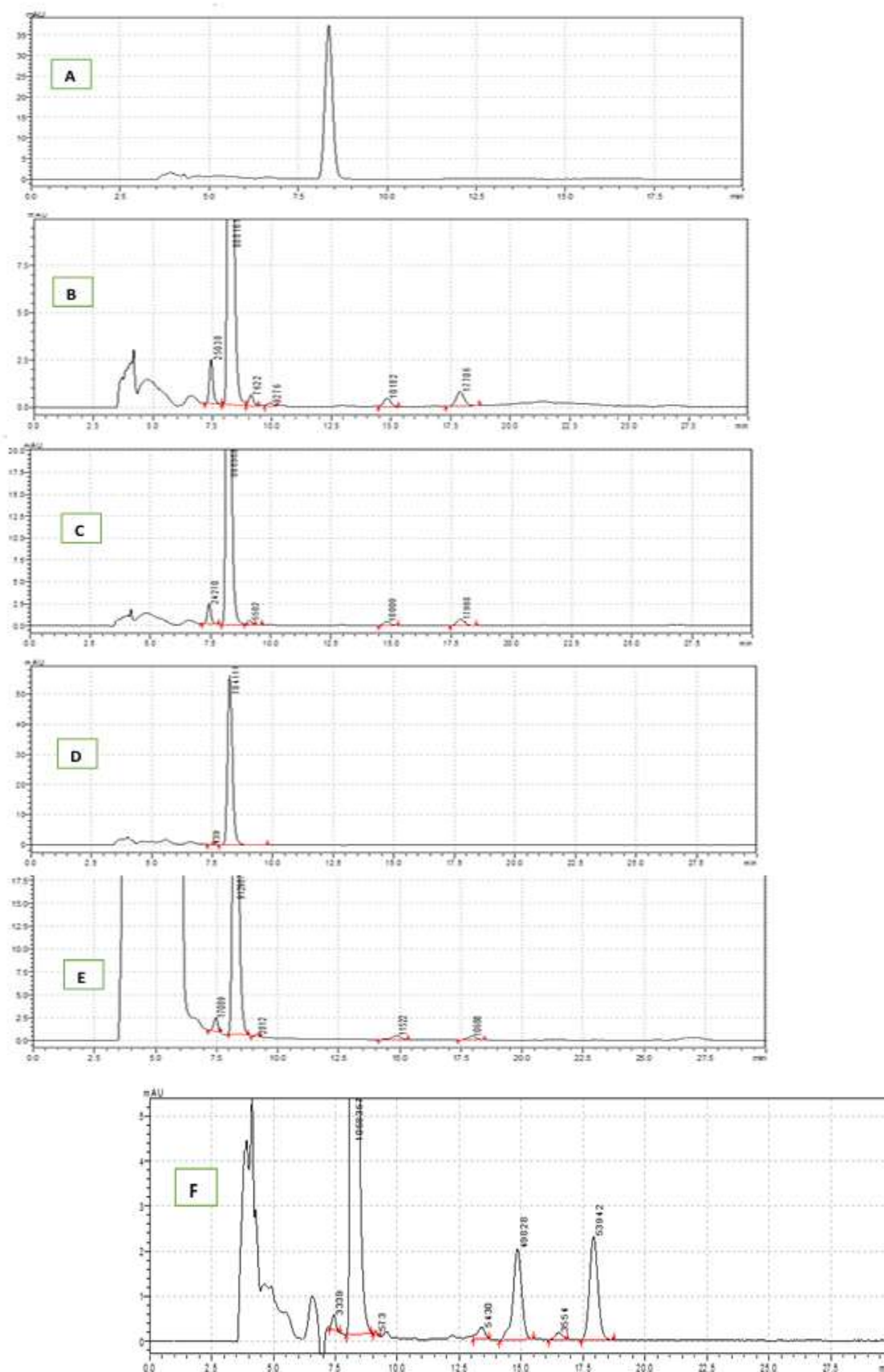


Figure 11: Artesunate control in MeOH (A), forced degradation of an AS solution at 0.5 mg/mL using 0.01 M HCl (B), 0.01M NaOH (C), degradation with light for 5 h (D), oxidation with 10% H₂O₂ for 24 h (E), water bath at 50°C for 24 h (F)

SUMMARY & CONCLUSION

The ideal objective was to develop a simultaneous method for AS, AQ and its impurities. Due to the complexity as some analytes were coeluting, separate method for AS with impurities and AQ with impurities was developed. In total three analytical methods were developed for the monitoring of AS and its impurities, AQ and its impurities and HPLC method for dissolution samples for a novel taste mask coated ASAQ formulation developed by Fluid Pharma Ltd. Acceptable recovery of API from the formulation was found when using methanol as an extraction solvent for artesunate and methanol: water (80:20) as an extraction solvent for amodiaquine. Validation took place, with all tested parameters were found to be within the limits stated in the ICH guidelines. The current approach is firmly established as being unique compared to the previous methods. Focused on AQ, as forced degradation for AQ and its impurities method was done for different parameters like oxidation, acid and base hydrolysis, thermal, photolytic degradations. Within this phase of work some unknown peaks were observed in the chromatogram and requires detailed analysis to identify the degradation products by using LCM-MS, NMR spectroscopy. The study on the effect of polymers on AS-AQ in taste masking formulations holds significant potential to enhance patient compliance. The future scope can be comprehensive research involving polymer selection, formulation development, and rigorous evaluation to achieve effective and safe taste masking solutions for AS-AQ.

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