EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 10
Issue: 6
142-166
Year: 2023

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF LUMEFANTRINE AND ARTEMETHER IN BULK AND TABLET DOSAGE FORMS

Chadalavada Swathi*

Student Kodandaramapuram Buchireddypalem Nellore Nellore Andhra Pradesh India.

***Corresponding Author: Chadalavada Swathi**
Student Kodandaramapuram Buchireddypalem Nellore Nellore Andhra Pradesh India.

Article Received on 27/3/2023 **Article Revised on 17/4/2023** **Article Accepted on 07/5/2023**

1. INTRODUCTION

Typically, analytical methods are necessary to determine the identification, potency, quality, and assay of drug material, drug product, and contaminant. Examining pharmaceutical and biological samples may aid bio-pharmacokinetics researchers in identifying the presence of drugs and their metabolites.[1]

Lumefantrine is a member of the fluorenes family and has the formula 9-(p-chlorobenzylidene)-9H-fluorene. It contains chlorine atoms at locations 2 and 7, as well as a 2-(dibutylamino)-1-hydroxyethyl group at position 4. Used in combination with artemether to treat resistant falciparum malaria. It is beneficial because it prevents malaria. It is a member of the tertiary amine, monochlorobenzene, secondary alcohol, and fluorene families.

Artemether, an artemisinin derivative, is artemisinin with the lactone substituted by lactol methyl ether. It is an antimalarial used in concert with lumefantrine to treat resistant strains of falciparum malaria. It is beneficial because it prevents malaria. It consists of artemisinin, a semisynthetic sesquiterpenoid, cyclic acetal, organic peroxide, and a semisynthetic acetal.

Innovations made to the rp-hplc technique

High-performance liquid chromatography (HPLC) is a potent and simple analytical technique used to measure the quality, safety, and effectiveness of pharmaceutical drugs.[4]

The following approach optimises analytical operations like related substances and assay testing.

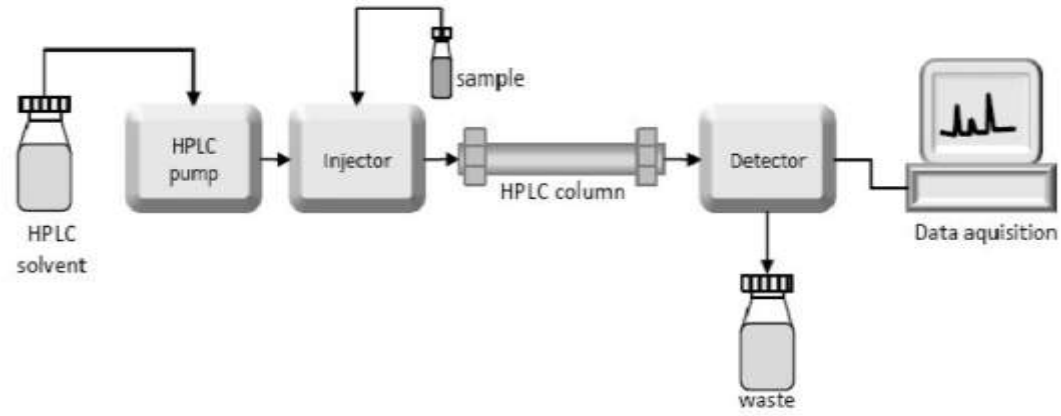


Figure 1: HPLC process works.

1.2. Method development aspects

During method development, several factors must be studied to produce best chromatographic results.

1.2.1 Choosing mobile phases

By optimising and choosing the mobile phase, organic contaminants, process-related impurities, and stress condition degradants may be reduced. The analysis

requires this. Several analytical buffers have been considered.[5]

1.2.2 Buffer pH rise

The buffer pH is crucial for contaminant separation in chromatograms. The buffer's pH controls the ionisation of the active medicinal component, which affects analyte elution. The pKa of the active medicinal component is used to establish peak retention periods.[6] Every HPLC column's pH should be between 2.0 and 8.0 for appropriate mobile phase setup. Always keep this range. Optimal buffer pH separates the primary analyte peak from process-related pollutants.

1.2.3 Selecting a High-Performance LC Column

When establishing a procedure, the following factors must be considered when choosing HPLC columns:

i. Column length and diameter
ii. Porousness
iii. Packaging or stationary phase material
iv. Floor space
v. Stop "capping"
vi. Small-to-large particles.
vii. Stationary carbon loading

Most normal phase HPLC columns employ silica packing.[7] Isopropyl alcohol and n-hexane are non-polar organic solvent phases (mobile phase composition).

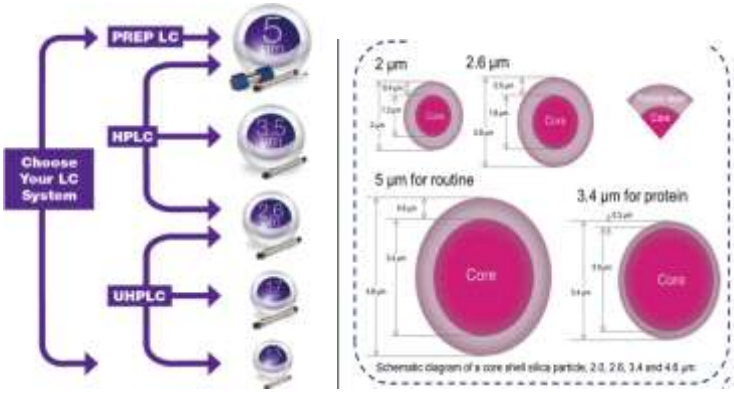


Figure 2: Depicts column particle size.

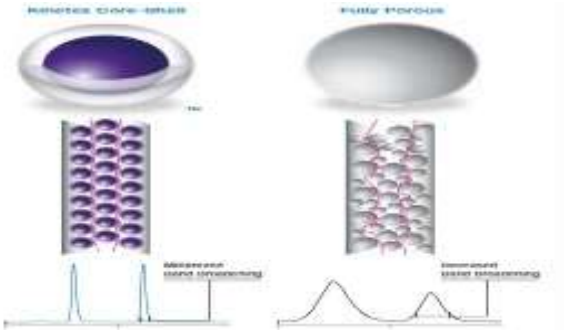


Figure 3: Smaller particles enhance system back pressure.

The following is a list of silica-based HPLC columns with different types of cross-linking arranged in ascending order of polarity.[8]

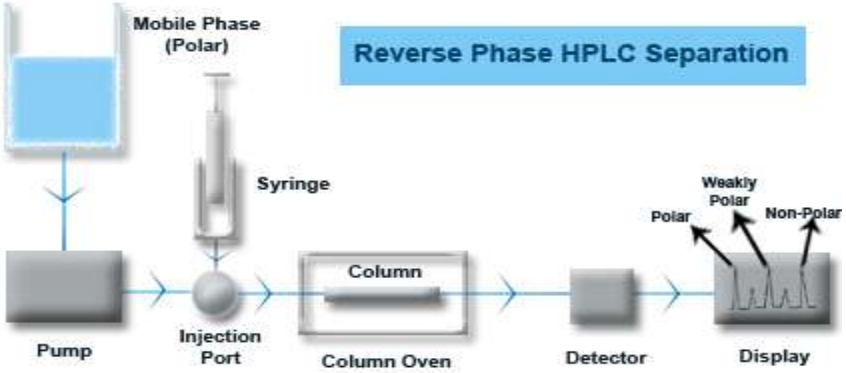


Figure 4: Schematic diagram of Reverse Phase HPLC.

1.2.4 Selection of the Detector and the Wavelength

There are various different types of detectors that can be used, and which one is used depends on the chemical make-up of the molecule.

i. UV/VIS
a. One and only wavelength
b. Altering the wavelength of the light
c. Detector array made of photodiodes Waters
d. Refraction Index of the Glass (RI Detector)
ii. Fluorescence metre
iii. Light scattering due to evaporation (ELSD)
iv. Mass metre (LC-MS)

The detector selection and wavelength tuning are crucial phases in the SIM analytical process. Pharmaceutical businesses use UV and PDA detectors to analyse water quality. Inject API standard stock solutions into the HPLC PDA system, then collect spectra (Fig. 1.5). Choose a high-absorbance wavelength for the main analyte.

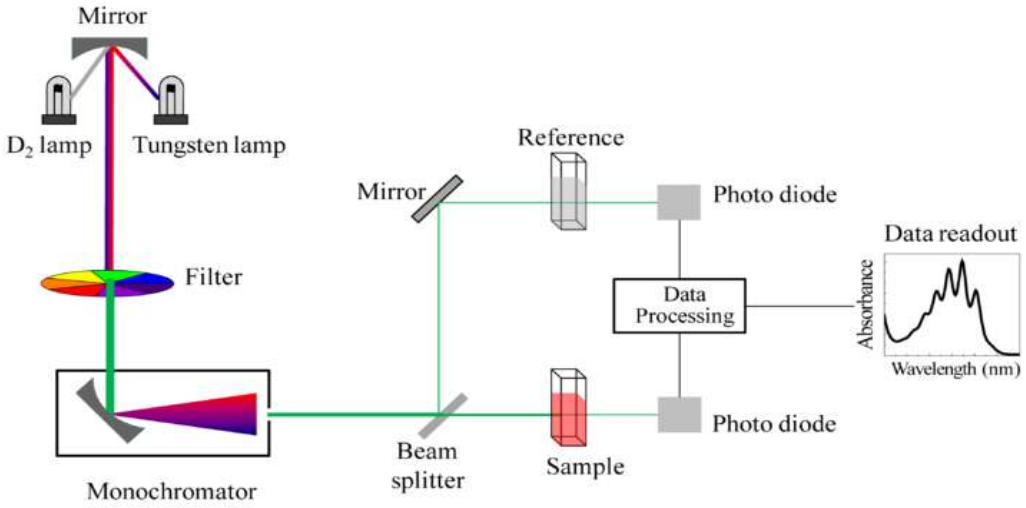


Figure 5: Schematic of UV – Visible spectrophotometer.

1.3 Forced degradation/exposure study

Stress testing is vital for accuraDSPy's pharmacological stability in ICH-recommended recommendations. It involves rapid testing, heat (in increments of 10-15 °C), humidity, oxidation, and photostability.

The analytical department uses SIM, or reverse Phase Stability Indicating Technique.

- Determine the shelf life of various dosage forms of finished medicines or provide suggestions.
- Suggest optimal packaging and warehousing conditions.
- Analyze a specification's amount and calculate a maximum value for a number of pharmaceutical chemical entities.

The API is broken down at higher temperatures than those employed for accelerated stability testing, creating the stability indicator combination (SIM). After 10%, the API may experience secondary degradation. In stress tests, medicines are exposed to acidity, alkalinity, oxidation, humidity, and light. These research aim to find possible drug degradation pathways and pollutants.[9] This provides formula-creation data.

Test samples must be distinguished from analyte peaks. To prove that contaminants, degradation products, and excipients do not interact with the analyte, forced degradation experiments (FDS) were conducted under the following circumstances.

To obtain the appropriate degree of degradation, the tablet powder or capsule should be put to acid and base stress of up to 5N strength for 12 hours, and then neutralised. If deterioration is required, the sample cannot be stressed directly with chemicals (Degradation followed by extraction of drug substance) First, dilute the sample so it may be investigated for deterioration (drug extraction followed by degradation), then determine the stressing agent concentration.

If required, treat capsules and tablet powder with 10 or 30% hydrogen peroxide to test for oxidative stress. If sample deterioration is needed, peroxide solution cannot be used directly (Degradation followed by extraction of drug substance) After dilution for the degradation investigation (drug extraction followed by degradation), the stressing agent's normality should be determined.[10]

1.3.1 Causes of Stress Factors

- Heat 0.1M HCl at 60°C for 30 minutes to relieve acid tension.
- Heat 0.1N NaOH at 60°C for 30 minutes to create base stress.
- Use 60°C water for 24 hours for water stress.
- 1 percent hydrogen peroxide for oxidative stress (Hydrogen When possible, follow these FDS requirements:
- Heat strain: straining at 105-150°C API melting point determines temperature.
- Humidity: 7 days with 90% RH and 25°C.

- UV Light: Seven days in an ultraviolet cabinet or 200 watts hour/m2 in a photostability chamber.
- Visible Light: 1,000,000-200,000 Lux hours in a photostability chamber or 7 days in a UV cabinet

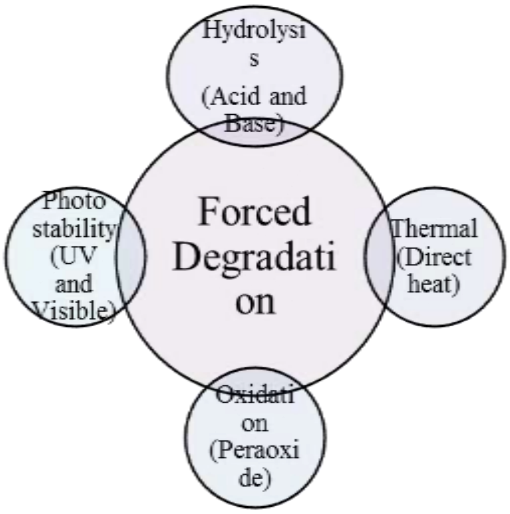


Figure 6: Graphical representation of FD studies.

By evaluating the analyte's peak purity with the help of chromatographic empower software, you can demonstrate that there is no interference from impurities on the analyte peaks.

1.4 Validation of analytic method

The suggested analytical technique must be verified according to ICHQ2 (R1) criteria (Validation of analytical procedure: Text and Methodology).[11]

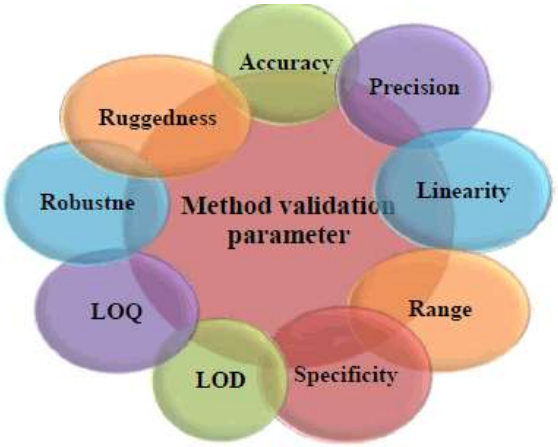


Figure 7: Graphical representation of method validation.

Check the validation settings for the procedure that are shown below.

System compatibility Test, often known as SST

Create a system-appropriate solution for the analytical operation, then inject it into the HPLC or UPLC chromatographic device. Assess USP 621 general chapter system suitability using the technique's criteria (s). If all SST values are within the range, the chromatographic column, system (UPLC/HPLC), and optimised procedure parameters may be used in the lab.

By injecting a single solution into liquid chromatography, one may examine both the active peak's (API) and degrading impurities' linearity.

When there's a substantial analyte peak at the test concentration, linearity must be evaluated (RRt 0.90 to 1.10 from the main peak). We created six sample solutions ranging from the lowest allowable concentration (LOQ) to the highest acceptable concentration (maximum). These sample solutions were injected into an analytical system using an optimised standard test procedure to confirm linearity for the principal analyte and known contaminants.

Bias is intercept (b) multiplied by area response at 100% target concentration.

1.5.3 Accuracy of the method

Make sure the testing technique is done correctly by developing a medical product mix that is consistent (such as solid oral Tablets & Capsules). in order to produce a

predetermined quantity of standard stock solutions. The standard procedure for conducting the test mandates the preparation of at least six (six) sample solutions by making use of the blend sample that was previously indicated. [12] Examine the precision of the testing technique by applying it to the drug product's level of release specification. Determine the relative standard deviation of each of the six preparations, as well as the weight-to-weight percentage of each impurity.

1.5.4 Recoverability and Accuracy:

Recovery experiments were conducted at dosages that ranged from fifty percent to one hundred fifty percent of the objective concentration.

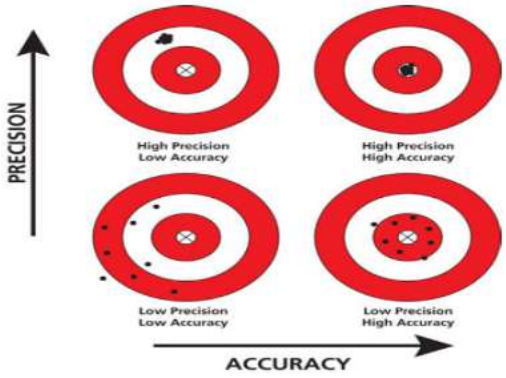


Figure 8: Graphical representation of Precision and Accuracy.

1.5.5 Upper and Lower limits for Detection and Quantification:

By injecting sample solution with a diluent or placebo, LOD and LOQ for the active peak (API) and a known contaminant may be determined (s). Experiment using the sample test's medication and placebo. Three setup methods exist.

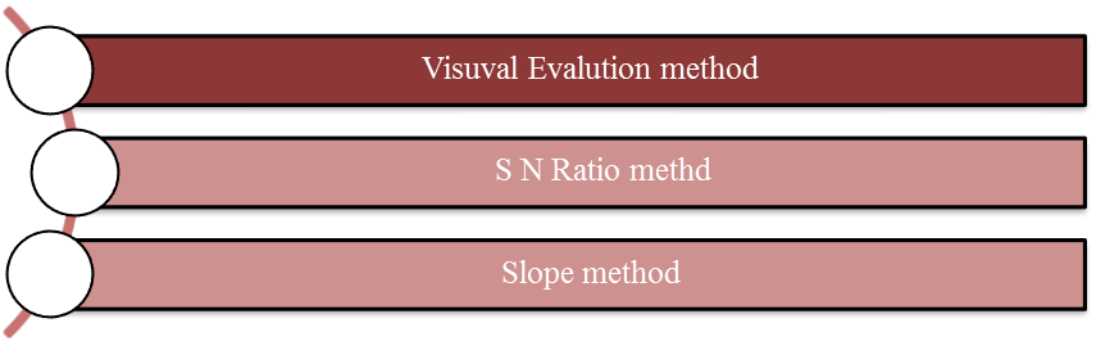


Figure 9: Graphical representation of LOD and LOQ.

1.5.6 LOQ Accuracy

After the test preparation is spiked with contaminants and the samples are run through the chromatograph, six test solutions are generated. Find the RSD and impurity percentages.

1.5.7 Range

When defining the concentration range where the technique is linear, precise, and accurate, consider the linearity, LOQ, and accuracy sections.

Figure 10: Graphical representation of range.

1.5.8 Precision

1.5.8.1 Intermediate Accuracy

Variability studies may be done alone or with chemist-to-chemist and column-column study. Determine the percentages of impurities (s). Changing one variable at a time lets you examine the outcome if it doesn't meet approval standards.

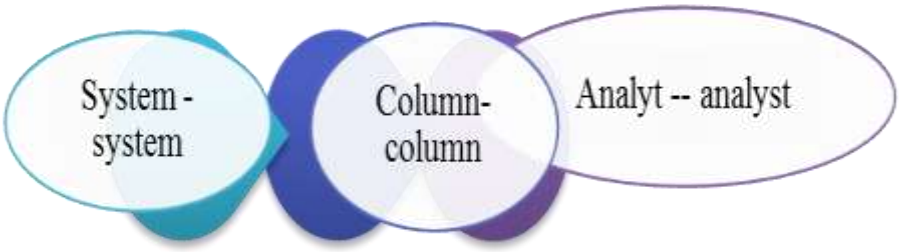


Figure 11: Graphical representation of ruggedness.

1.5.8.2 Lab conditions for the stability of the mobile phase:

Prepare the Mobile phase on a closed workstation using the Test method. Inject mobile phase, SST, and test solution into chromatographic apparatus for one week, preserving batch uniformity. Find the RRT and pollutant impurity percentages (s). Calculate the difference between daily impurity and starting values. If the mobile phase isn't stable after a day, repeat the experiment with shorter timer intervals. As shown below, mobile phase stability, standard preparation, and test preparation may be evaluated concurrently. Store mobile phase standard test solution on a workstation. Refrigerate test and standard solutions. After utilising the stored mobile phase on Days 1, 2, 5, and 7, inject new standard and test solutions. Then, standard and test solutions are injected. If the mobile phase you're employing doesn't satisfy the standards, use a new one to ensure standard stability.

1.5.9 Accuracy

In order to show the nature of the stability, interference testing was performed using a blank, a placebo, and an impurity.

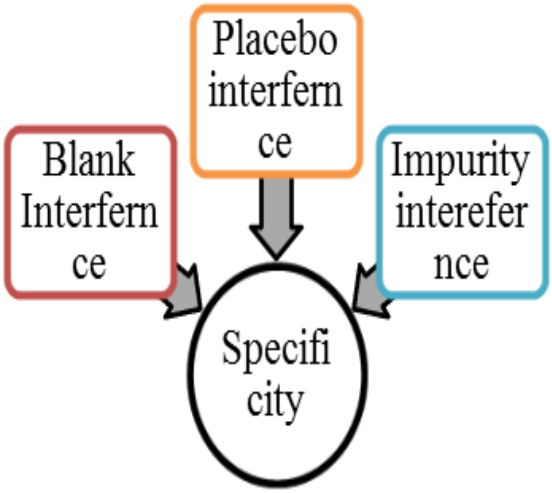


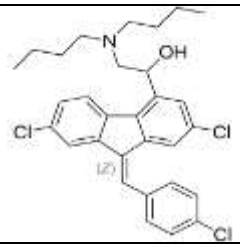
Figure 12: Graphical representation of specificity.

1.6 Checking the appropriateness of the method the order of parameters[13]

- System suitability
- A focus on detail (Placebo interference and Impurity Interference)
- Accuracy of the method
- Grit and tenacity (Stability of solutions and mobile phase)
- LOD &LOQ
- The linearity of the ideal technique and its prerequisites
- Preciseness
- Range (Minimum to maximum range)
- The doggedness of the process (Variations)
- Stability (Filter validation)
- Stability (Variations)

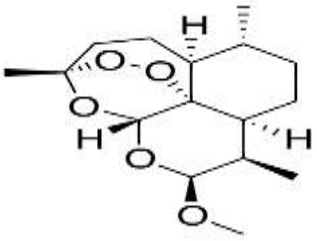
Drug profile

Table 1: Lumefantrine drug profile.[14]

Name of the drug	Lumefantrine
Chemical structure	
Chemical name	2-(dibutylamino)-1-[(9Z)-2,7-dichloro-9-[(4-chlorophenyl)methylidene]-9H-fluoren-4-yl]ethan-1-ol
Chemical formula	$C_{30}H_{32}Cl_3NO$
Molecular weight	528.9
Category	antimalarial drug
Dosage form	Tablet
Dose	120&480mg
Brand Name	Coartem

While lumefantrine has been shown to be effective against malaria, the specific mechanism by which this is achieved is not known. However, current evidence suggests that lumefantrine inhibits nucleic acid and protein synthesis in addition to preventing the creation of -hematin by building a complex with hemin.(15)

Table 2: Artemether drug profile.[16]

Name of the drug	Artemether
Chemical structure	
Chemical name	(1*R*,4*S*,5*R*,8*S*,9*R*,10*S*,12*R*,13*R*)-10-methoxy-1,5,9-trimethyl-11,14,15,16-tetraoxatetracyclo[10.3.1.0^{4,13}.0^{8,13}]hexadecane
Chemical formula	$C_{16}H_{26}O_5$
Molecular weight	298.3
Category	Antimalarial drug
Dosage form	Injection
Dose	20-80mg/ml
Brand Name	Larither, Rezart M, Arteo, Biomal, Malafi, Rmther, Malither, Larmal

It involves a reaction with ferriprotoporphyrin IX ("heme") or ferrous ions in the acidic parasite food vacuole, which makes cytotoxic radical species.

Most people agree that peroxide antimalarials work by interacting with heme, a byproduct of haemoglobin degradation that is made when haemoglobin is broken down into smaller pieces. It is thought that this interaction leads to the creation of a number of oxygen- and carbon-centered radicals that could be harmful.[17]

EXPERIMENTAL METHODS

Materials:

- Potassium dihydrogen phosphate
- Methanol
- Sodium hydroxide
- Phosphoric acid
- Hydrochloric acid

Drugs:

- LUMEFANTRINE(LUM)
- ARTEMETHER(ARM)

Tablets:

Product tablet name: Lumerax-80

- Claimed content: LUM – 480mg and ARM – 80 mg

Apparatus:

- HPLC system: make company - Waters alliance

- Photodiode array detector: make company - Waters alliance

Mobile phase
Transfer 1000ml of HPLC water into 1000ml of beaker and KH2PO4 adjust pH 4.5. Transfer the above solution 500ml KH2 PO4 of, 500ml of Methanol is used as mobile phase.

Lum & Arm Assaying conditions for liquid chromatographic determination:
Stock Lum & Arm solution:
Dissolving 480 mg of LUM and 80 mg of ARM, respectively, in 100 ml of a suitable diluent yielded stock solutions with concentrations of 480 µg /ml and 80 µg /ml, respectively.

A working LUM and ARM solution (LUM - 480 µg/ml & ARM - 80 µg/ml) was obtained by emulsifying 1 ml of stock solution (LUM - 480 µg/ml & ARM - 80 µ g/ml) in 10 ml of the recommended diluent.

Solution one linearity (LUM concentration – 240 µg/ml & ARM concentration – 40 µg/ml): With 9.5 ml of the selected diluent, 0.5 ml of stock LUM and ARM solution (LUM - 480 µg/ml and ARM - 80 µg/ml) was liquefied.

- **Solution two linearity (Conc. LUM- 360 µg/ml & ARM-60 µg/ml):**

0.75 ml of stock LUM & ARM solution (LUM - 480 µg/ml & ARM - 80 µg/ml) was dissolved in 9.25 ml of the selected diluent**.**

Solution three linearity (Conc. LUM- 480 µg/ml & ARM- 80 µg/ml): With 9.0 ml of the specified diluent, 1.0 ml of stock LUM & ARM solution (LUM - 480 µg/ml & ARM - 80 µg/ml) was liquefied.

- **Solution four linearity (Conc. LUM- 600 µg/ml & ARM- 100 µg/ml):** With 8.75 ml of the prescribed diluent, 1.25 ml of stock LUM & ARM solution (LUM - 480 µg/ml & ARM - 80 µg/ml) was liquefied.
- **Solution five linearity (Conc. LUM- 720 µg/ml & ARM- 120 µg/ml):** 1.50 ml of the stock LUM & ARM solution (LUM - 480 µg/ml & ARM - 80 µg/ml) was reconstituted with 8.50 ml of the selected diluent.

Evaluation of developed method validation:
The analytical approach for assessing LUM and ARM in Leemether tablets was evaluated using International Conference on Harmonization validation standards.

LUM and ARM stock solutions (LUM – 480 µg/ml & ARM – 80 µg/ml) were made by dissolving 480 mg LUM and 80 mg ARM in 100 ml of a certain diluent.

1 ml of stock LUM and ARM solution (LUM – 480 µg/ml & ARM- 80 g/ml) was dissolved in 10 ml of the specified diluent to produce a practical LUM and ARM solution (LUM – 480 g/ml & ARM- 80 g/ml).

The stock LUM and ARM solution for tablet estimation (LUM - 480 µg/ml & ARM - 80 µg/ml) was made by ultrasonically dissolving 996 mg of powdered Leemether tablets (equal to 480 mg DSP and 80 mg EST) for 20 minutes in 100 ml of the appropriate diluent.

10 ml of stock LUM and ARM tablet solution (LUM-480 µg/ml & ARM-80 µg/ml) in 100 ml of the prescribed diluent yielded a LUM and ARM solution suitable for chromatographic estimation (LUM-480 µg/ml & ARM-80 µg/ml).

Ten microliters of LUM & ARM tablet solution were pushed via a YMC C18 column with the dimensions 150 x 4.6 mm x 5 m. Using the chromatographic estimate parameters, the LUM & ARM contents of contraceptive pills were determined.

Lum & Arm stability evaluation
LUM & ARM stability testing was carried out by subjecting samples to the following conditions:

LUM & ARM stability in 0.1N hydrochloric acid: In a 100 ml flask, 10 ml of LUM & ARM tablet stock solution was dissolved in 10 ml of 0.1N hydrochloric acid after 30 minutes of ultrasonication. After thirty minutes, 100 millilitres of the appropriate diluent were added to the flask. Following the proper application of 0.1N HCl, 10 litres of tablet solution were pumped onto a YMC C18 column. The parameters outlined in the section under " LUM & ARM COMBINATION TESTING CONDITIONS " were used to determine the LUM & ARM concentrations in the 0.1N HCl solution.

Stability of LUM & ARM in 0.1N NaOH: After thirty minutes of ultrasonication in a 100-milliliter flask containing liquid, ten millilitres of LUM & ARM tablet stock solution were dissolved in one millilitre of 0.1N NaOH. After thirty minutes, 100 millilitres of the appropriate diluent were added to the flask. Following the proper application of 0.1N NaOH, 10 litres of tablet solution were pumped onto a YMC C18 column. The parameters described in the section entitled "LUM & ARM COMBINATION TESTING CONDITIONS " were used to determine the LUM & ARM concentrations in 0.1N NaOH.

Stability of LUM and ARM in 30% Hydrogen peroxide:
The stability of LUM and ARM in 30 percent peroxide was evaluated by dissolving 10 millilitres of LUM and ARM tablet stock solution in 10 millilitres of 30 percent peroxide after 30 minutes of ultrasonication in a 100-milliliter flask. After thirty minutes, 100 millilitres of the appropriate diluent were added to the flask. Ten millilitres of tablet solution were put onto a YMC C18 column following a peroxide treatment at a concentration

of thirty percent. Using the parameters provided in the section headed " LUM and ARM COMBINATION ASSAYING CONDITIONS ", the LUM and ARM concentrations in the 30 percent peroxide were determined.

Stability of LUM and ARM in the sun:
A 10-milliliter stock tablet solution of LUM and ARM (10 micrograms per millilitre of LUM and 40 micrograms per millilitre of ARM) was exposed to the sun for six hours. After the allocated time had elapsed, the remaining quantity was stated to be 100 millilitres.

After the treatment (consisting of six hours in the sun) was finished, a 10microliter volume of tablet solution was injected into the YMC C18 column. The criteria indicated in the section headed " LUM & ARM COMBINATION TESTING CONDITIONS " were used to analyse the content of LUM and ARM tablet solutions that had been treated with 30% peroxide.

Stability of LUM and ARM at 105 ^{0} Celsius:
For thirty minutes, the stock tablet solution (10 ml) of LUM and ARM (10 µg/ml LUM & 40 µg/ml ARM) was kept at 105 degrees Celsius. After thirty minutes, the desired diluent was used to reduce the capacity of the flask to one hundred millilitres. The YMC C18 column received ten microliters of a dry solution containing LUM and ARM tablet that had been subjected to heat. By following the criteria stated in the section entitled " LUM & ARM COMBINATION TESTING CONDITIONS," we were able to determine the LUM and ARM content of the dried, heat-treated LUM and ARM tablet solution.

Conditions applied	Vol. of sample (ml)	Vol. of reagent (ml)		Vol. of diluent added (ml)	Theoretical conc. (µg/ml)
Acid hydrolysis	10ml	10ml 0.1N HCL	Sonicated for 30min at room temperature and then filtered	80	LUM – 480; ARM – 80
Base hydrolysis	10ml	10ml 0.1N NaoH		80	LUM – 480; ARM – 80
Peroxide Oxidation	10ml	10ml 30% peroxide		80	LUM – 480; ARM – 80
Thermal degradation	10ml	Exposed to 60°c in oven and then filtered		90	LUM – 480; ARM – 80
Photo degradation	10ml	Exposed to sun light for 6hr and then filtered		90	LUM – 480; ARM – 80

Table 3: Lumefantrine and Artemether combination testing conditions – Stability Evaluation.

RESULTS AND DISCUSSION

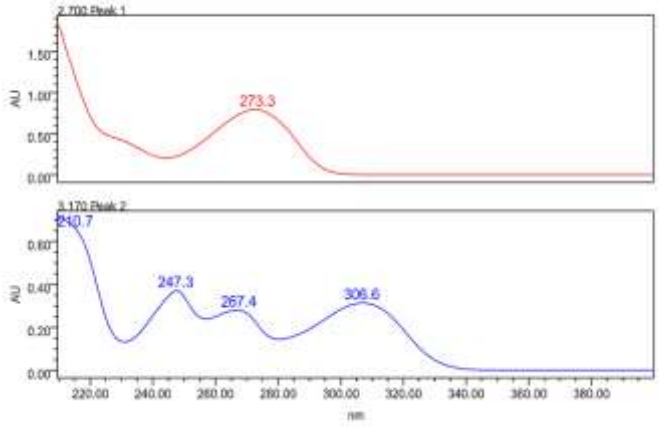


Figure 13: UV graph.

Development and Trails

Trail: 1

Mobile Phase : K_2HPO_4: Methanol (70:30)

Column : SUPELCO, C18, 150X4.6mm, 5µm

Flow Rate : 1.0ml/Min

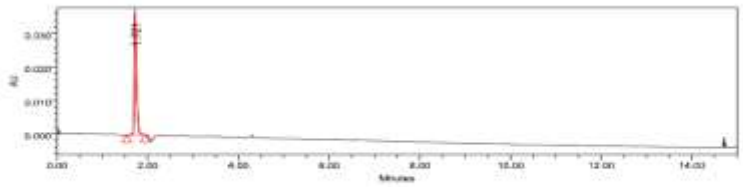
Column Temperature: 25˚C

Sample Temperature: 25˚C

Volume : 10µl

Run time : 15min

Detector : PDA



	Name	Retention Time	Area	% Area	Height	Int Type	USP Resolution	USP Tailing	USP Plate Count
1		1.721	125695	100.00	36076	bb		1.72	6295

Observation: One peak was detected & peak shape is not good.

Reason: may be column efficiency is low.

Corrective Action: Change the column.

Figure 14: Readings of suitability testing and chromatogram of trail one.

Trail: 2

Mobile Phase : K_2HPO_4: Methanol (70:30)

Column : WATERS, C8, 150X4.6mm, 3.5µm

Flow Rate : 1.0ml/Min

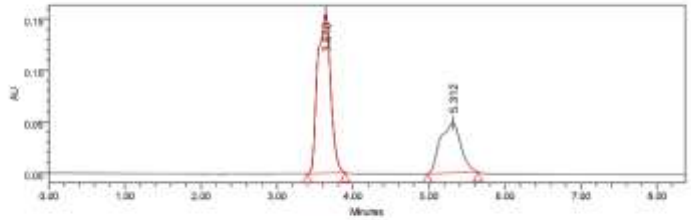
Column Temperature: 25˚C

Sample Temperature: 25˚C

Volume : 10µl

Run time : 9min

Detector : PDA



	Name	Retention Time	Area	% Area	Height	Int Type	USP Resolution	USP Tailing	USP Plate Count
1		3.640	1636921	66.84	155632	BB		0.98	2341
2		5.312	812219	33.16	48919	BB	4.27	0.98	1815

Observation: Two peak was detected & peak shape is not good.

Reason: may be Buffer not set column efficiency is low.

Corrective Action: Change the column and Buffer.

Figure 15: Readings of suitability Testing and Chromatogram of trail two.

Trail: 3

Mobile Phase : KH_2PO_4: Methanol (60:40)

Column : INERTSUSTAIN, C8, 150X4.6mm, 3µm

Flow Rate : 1.0ml/Min

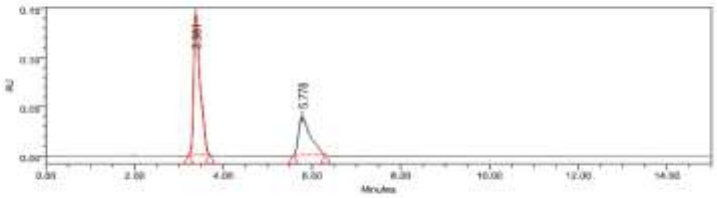
Column Temperature: 25˚C

Sample Temperature: 25˚C

Volume : 10µl

Run time : 15min

Detector : PDA



	Name	Retention Time	Area	% Area	Height	Int Type	USP Resolution	USP Tailing	USP Plate Count
1		3.351	1460534	67.11	142799	BB		1.72	2625
2		5.778	715879	32.89	39927	BB	6.72	1.92	2372

Observation: Two peak was detected & peak shape is not good.

Reason: may be column efficiency is low.

Corrective Action: Change the column.

Figure 16: Readings of suitability Testing and Chromatogram of trail three.

Trail: 4

Mobile Phase : KH_2PO_4: Methanol (60:40)

Column : YMC, C18, 150X4.6mm, 5µm

Flow Rate : 1.0ml/Min

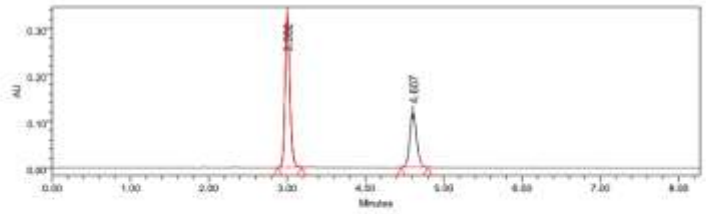
Column Temperature: 25˚C

Sample Temperature: 25˚C

Volume : 10µl

Run time : 9min

Detector : PDA



	Name	Retention Time	Area	% Area	Height	Int Type	USP Resolution	USP Tailing	USP Plate Count
1		3.002	1406904	66.09	529612	BB		1.22	11916
2		4.607	722005	33.91	117915	BB	11.62	1.14	13208

Observation: Two peaks were detected but resolution and RT high.

Reason: may be composition is not suitable.

Corrective Action: Change the composition and Flow Rate.

Figure 17: Readings of suitability Testing and Chromatogram of trail four.

Trail: 5

Mobile Phase : KH_2PO_4: Methanol (60:40)

Column : YMC, C18, 150X4.6mm, 5µm

Flow Rate : 0.9ml/Min

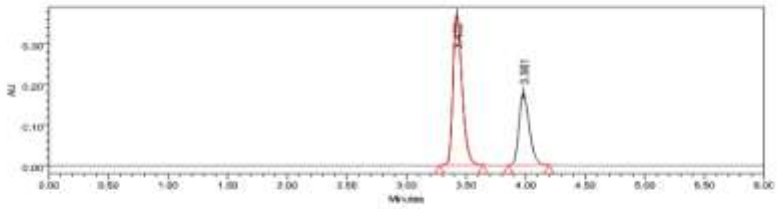
Column Temperature: 25˚C

Sample Temperature: 25˚C

Volume : 10µl

Run time : 6min

Detector : PDA



	Name	Retention Time	Area	% Area	Height	Int Type	USP Resolution	USP Tailing	USP Plate Count
1		3.422	1967411	64.74	371090	BB		1.35	9744
2		3.981	1071507	35.26	178503	BB	3.69	1.29	10232

Observation: Two peaks were detected but resolution and RT high.

Reason: may be composition is not suitable.

Corrective Action: Change the composition.

Figure 18: Readings of suitability Testing and Chromatogram of trail five.

Trail: 6

Mobile Phase : KH_2PO_4: Methanol (50:50)

Column : YMC, C18, 150X4.6mm, 5µm

Flow Rate : 0.9ml/Min

Column Temperature: 25˚C

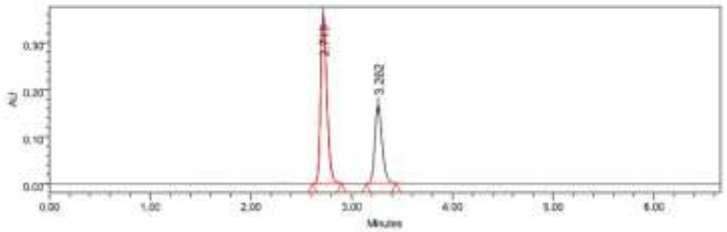
Sample Temperature: 25˚C

Volume : 10µl

Run time : 6min

Detector : 287

pH :4.5



	Name	Retention Time	Area	% Area	Height	Int Type	USP Resolution	USP Tailing	USP Plate Count
1		2.719	1538911	64.82	356236	BB		1.32	9493
2		3.262	835090	35.18	166378	BB	4.37	1.24	8935

Observation: Two peaks eluted and all the system suitability parameters are within the limit.

Figure 19: Readings of suitability Testing and Chromatogram of trail six.

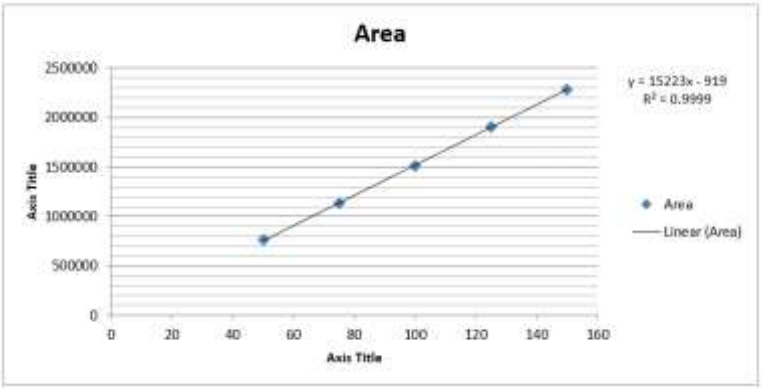
Validation of developed method by utilising ich: linearity:

- **Solution one linearity (LUM- 240 µg/ml & ARM-40 µg/ml concentrations):** With 9.5 ml of the selected diluent, 0.5 ml of stock LUM and ARM solution (480 µg/ml LUM and 80 µg/ml ARM) was liquefied.
- **Solution two linearity (Conc. LUM- 360.00 µg/ml & ARM-60 µg/ml):** With 9.5 ml of the selected diluent, 0.5 ml of stock LUM and ARM solution (480 µg/ml LUM and 80 µg/ml ARM) was liquefied.
- **Solution three linearity (Conc. LUM- 480.00 µg/ml & ARM- 80 µg/ml):** With 9.0 ml of the specified diluent, 1.0 ml of stock LUM and ARM solution (480 µg/ml LUM and 80 µg/ml ARM) was liquefied.
- **Solution four linearity (Conc. LUM- 600 µg/ml & ARM- 100 µg/ml):** With 8.75 ml of the selected diluent, 1.25 ml of stock LUM & ARM solution (480 µg/ml LUM & 80 µg/ml ARM) was liquefied.
- **Solution five linearity (Conc. LUM-720 µg/ml & ARM-120.00 µg/ml):** 1.50 ml of stock LUM & ARM solution (480 µ g/ml LUM & 80 µg/ml ARM) was dissolved in 8.50 ml of the selected diluent.

The gathered results demonstrated that the methodology was sufficiently linear for LUM and ARM analysis.

Table 4: Peak Area and Concentration for the Lumefantrine and Artemether.

LUM		ARM	
Response peak area	**µg/ml amount**	**Response peak area**	**µg/ml amount**
766204	240	415096	40
1136411	360.00	623097	60
1513332	480.00	832020	80
1907344	600	1022689	100
2283612	720	1230079	120.00



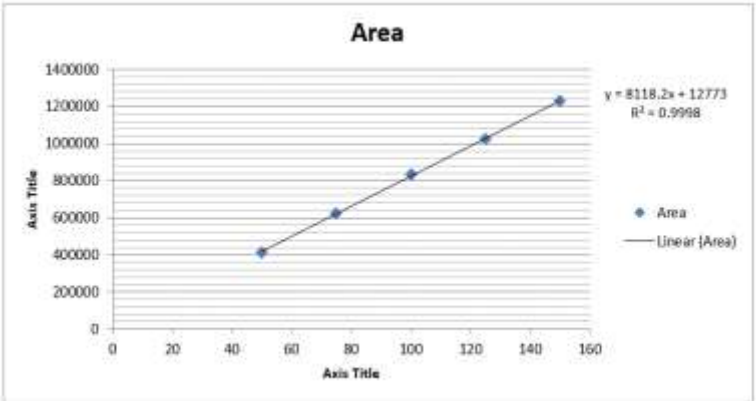


Figure 20: Lumefantrine and Artemether calibration curves.

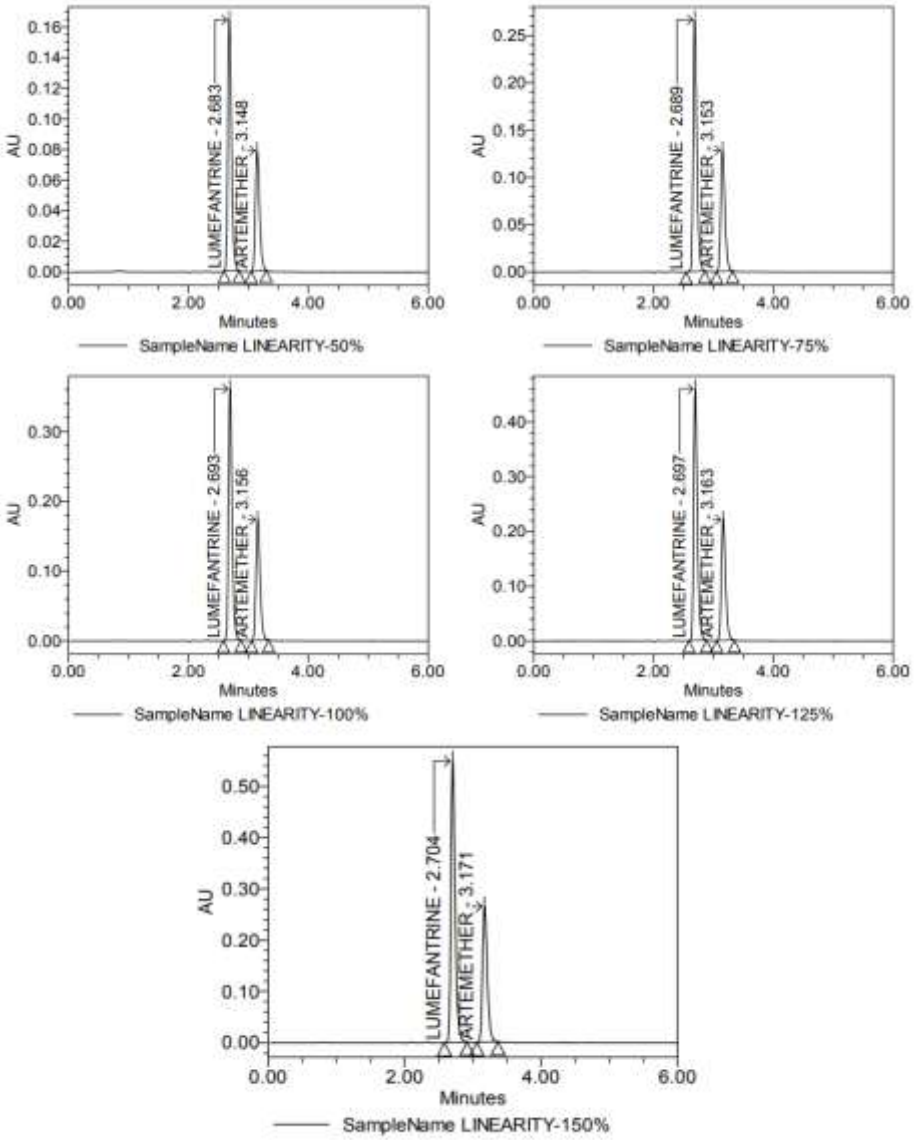


Figure 21: Lumwfantrine and Artemether chromatogram linearity readings.

Detection limit:

Using the standard deviation method and formula, the following are accomplished:

The detection limit corresponds to 3.3% of the peak area or slope of the drug calibration curve.

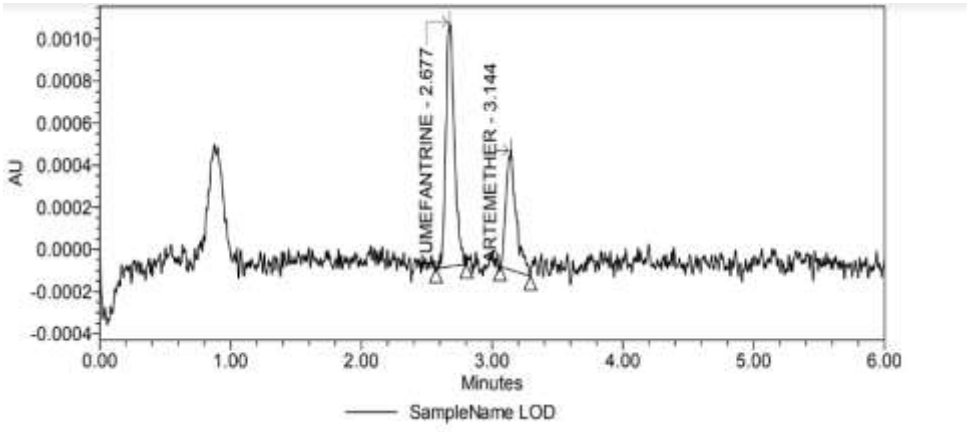


Figure 22: Reading of Lumefantrine and Artemether chromatograms - sensitivity - detection limit.

LUM detection limit: 0.860 µg/ml
ARM detection limit: 0.299 µg/ml
LUM has a signal-to-noise ratio of 1674.8 at µg/ml.
ARM has a signal-to-noise ratio of 802.2 at µg/ml.
According to the collected data, the method is sensitive enough to be employed for LUM and ARM analyses.

Limit of quantification:
The following method and formula were applied to calculate the standard deviation:The quantitative limit is equivalent to 10 standard deviations off the slope or peak area of the drug calibration curve. This limit is determined by the drug's effect.

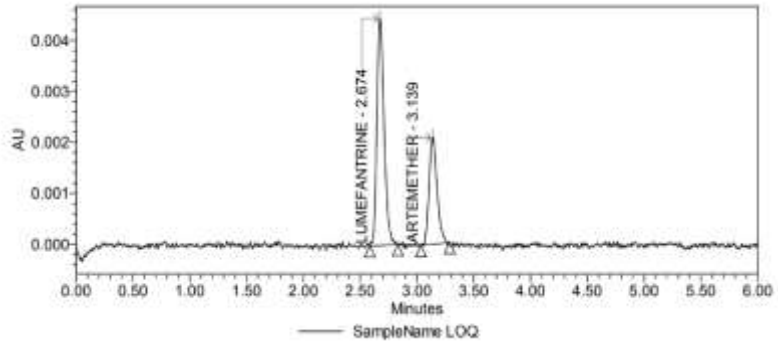


Figure 23: Readings from the Lumefantrine and Artemether chromatograms, sensitivity Levels and Quantitation limits.

LUM measurement limit: 2.866 µg/ml
The quantitative limit of ARM is 0.997 µg/ml.
LUM has a signal-to-noise ratio of 1674.8 at µg/ml.
At µg/ml, the signal-to-noise ratio for ARM is 802.2
The data acquired revealed that the technique is sensitive enough for LUM and ARM analysis.

Precision:
On a YMC C18 column measuring 5 metres in length and 150 X 4.6 mm in diameter, 480 µg/ml and 80 µg/ml solutions of LUM and ARM, respectively, were applied. Using the criteria stated in the section labelled " LUM & ARM ASSESSMENT CONDITIONS," it was found that both the LUM and ARM replies had peak areas. Six distinct LUM and ARM response peak locations had their respective standard deviation and corresponding % standard deviation calculated. Both the LUM and ARM evaluation results suggest that the approach is acceptable. The specific results of the investigation are presented in Table 7 for both LUM & ARM.

Table 5: The specific results of the investigation for both Lumefantrine and Artemether

Response peak area	
LUM	
1518791	**Mean response peak area**
1513902	**1**515213
1517517	**Standard response Deviation**
1519347	3868.9
1510763	**RSD**
1510956	**0**.3
ARM	
832234	**Mean response peak area**
831394	831868
832168	**Standard response Deviation**
831626	324.8
831990	**RSD fraction**
831794	0.0

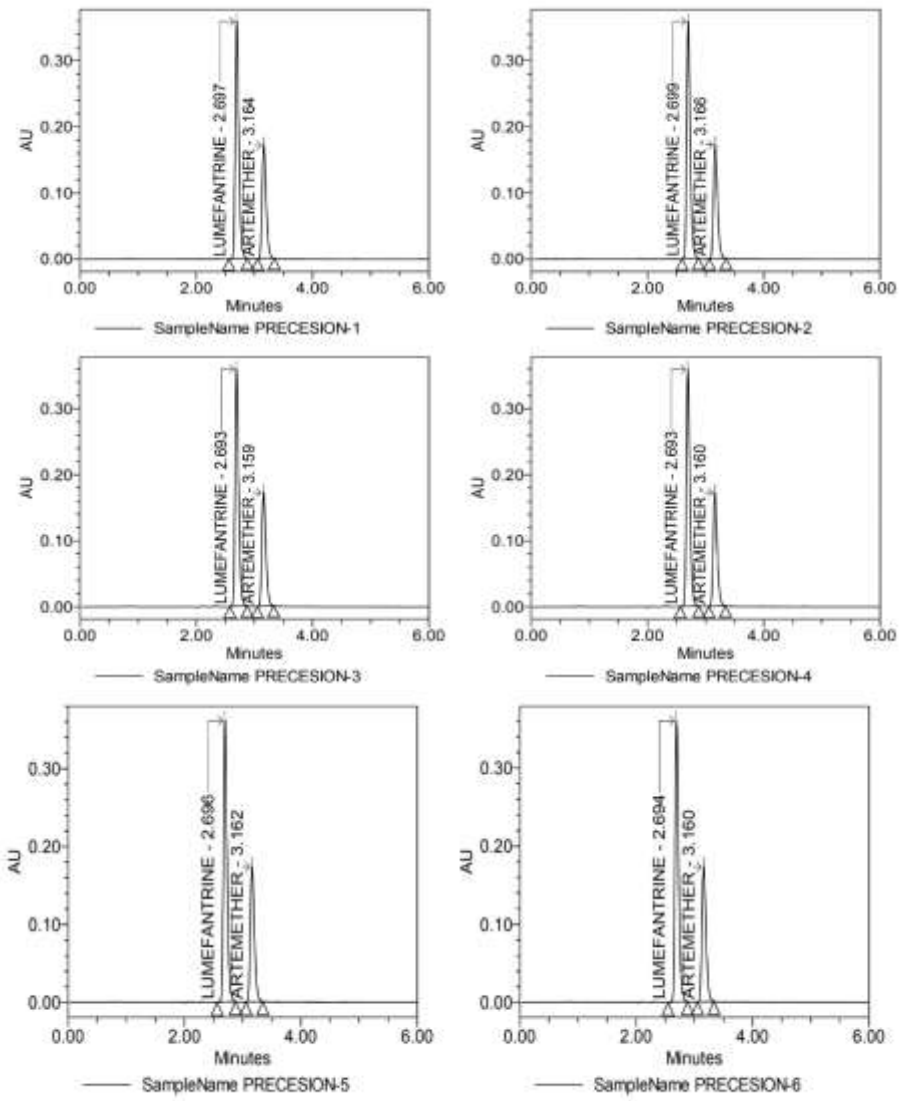


Figure 24: Precision in interpreting Lumefantrine and Artemether chromatograms.

Accuracy:

Six injections of a solution containing LUM and ARM at concentrations of 40 and 4 micrograms per millilitre, respectively, were made into a 5-meter-long, 250-millimeter-wide, and 4.6-millimeter-diameter YMC C18 column. The requirements mentioned in the section labelled " LUM & ARM COMBINATION ANALYSIS CONDITIONS " served as the foundation for the data analysis. Throughout the examination, the percentages of LUM and ARM were recorded. Six distinct LUM and ARM injections were statistically analysed, and the mean, standard deviation, and relative percent standard deviation for each were computed. The findings gave solid proof that the LUM and ARM analysis approach was correct.

The findings of the examinations into the accuracy of LUM and ARM are presented in Table 6.

Table 6: Accuracy of Lumefantrine and Artemether.

µg/ml amount considered	µg/ml amount quantified	Assay percent	
LUM			
230.949	238.83	103	**Mean percent assay** 100 **Standard assay Deviation** 0.5 **RSD** 0.5
230.949	238.55	103	
230.949	238.53	103	
470.400	470.71	100	
470.400	470.48	100	
470.400	470.10	100	
705.600	708.10	100	

705.600	712.83	101	
705.600	710.48	101	
AAAAAAAAAAAAAAAAAAAR ARMM			
38.884	39.66	102	**Mean percent assay 100 Standard assay Deviation 0.4 RSD 0.4**
38.884	39.60	102	
38.884	39.61	102	
79.200	79.47	100	
79.200	79.71	100	
79.200	79.45	100	
118.800	118.26	100	
118.800	118.19	99	
118.800	118.50	100	

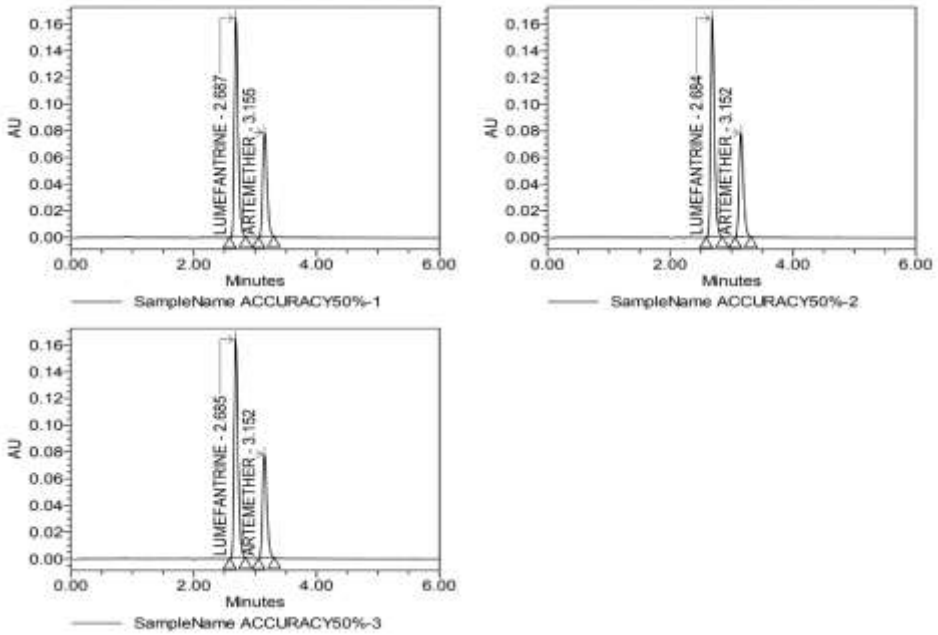


Figure 25: Accuracy of Lumefantrine and Artemether chromatogram reading (50%).

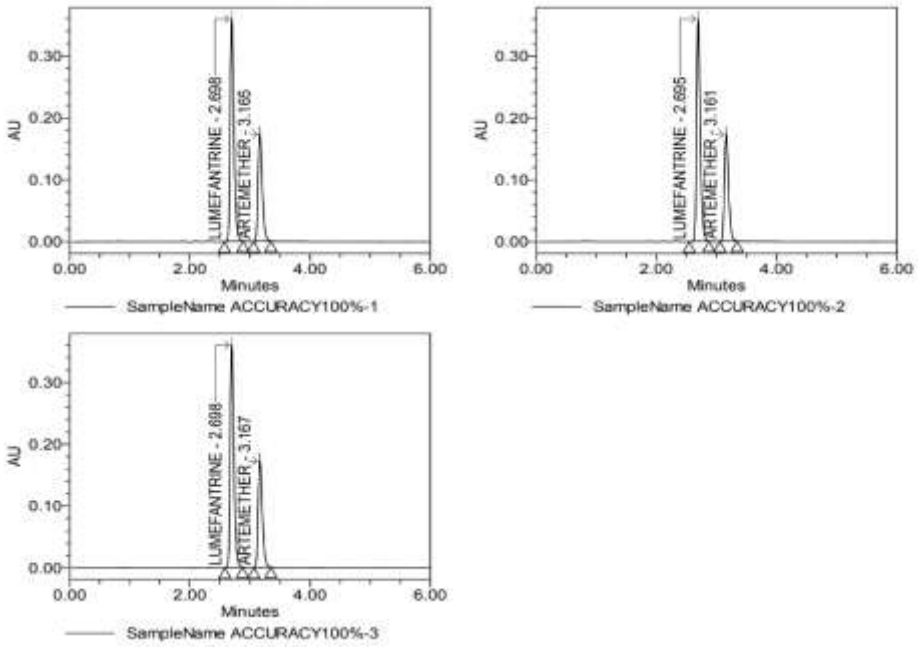


Figure 26: Accuracy of Lumefantrine and Artemether chromatogram reading (100%).

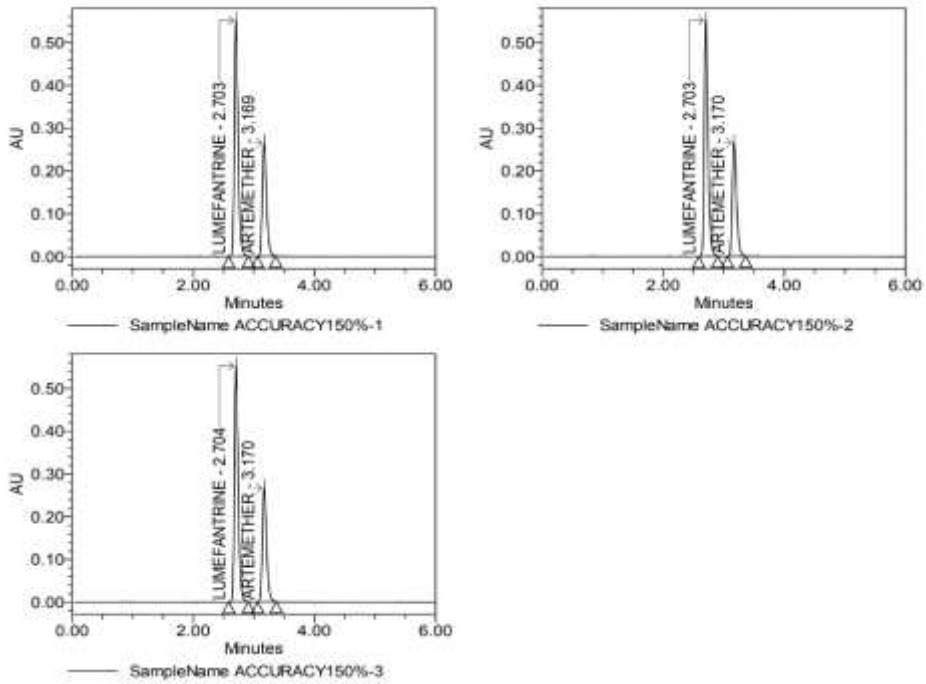


Figure 27: Accuracy of Lumefantrine and Artemether chromatogram reading (150%).

Selectivity:

Using the traditional addition method, we were able to determine the experiment's selectivity by calculating the percent recovery of LUM and ARM. 50 percent (LUM-240 µg/ml and EST-40 µg/ml) 100 percent (DSP-480 µg/ml and EST-80 µg/ml) and 150 percent (DSP-720 µg/ml and EST-120 µg/ml) stock solutions of LUM and ARM were produced. The samples were then analysed three times in accordance with the criteria outlined in the section labelled " LUM and
ARM COMBINATION TESTING CONDITION.

Table 7: Lumefantrine selectivity investigation results.

µg/ml amount considered	µg/ml amount quantified	Recovered percent	
50% additional			
230.949	238.83	103	**Mean percent recovered** 103
230.949	238.55	103	
230.949	238.53	103	
100% additional			
470.400	470.71	100	**Mean percent recovered** 100
470.400	470.48	100	
470.400	470.10	100	
150% additional			
705.600	708.10	100	**Mean percent recovered** 101
705.600	712.83	101	
705.600	710.48	101	

Table 8: Artemether selectivity investigation results.

µg/ml amount considered	µg/ml amount quantified	Recovered percent	
50% additional			
38.884	39.66	102	**Mean percent recovered** 102
38.884	39.60	102	
38.884	39.61	102	
100% additional			
79.200	79.47	100	**Mean percent recovered** 100
79.200	79.41	100	
79.200	79.45	100	
150% additional			

118.800	118.26	100	**Mean percent recovered** 100
118.800	118.19	99	
118.800	118.50	100	

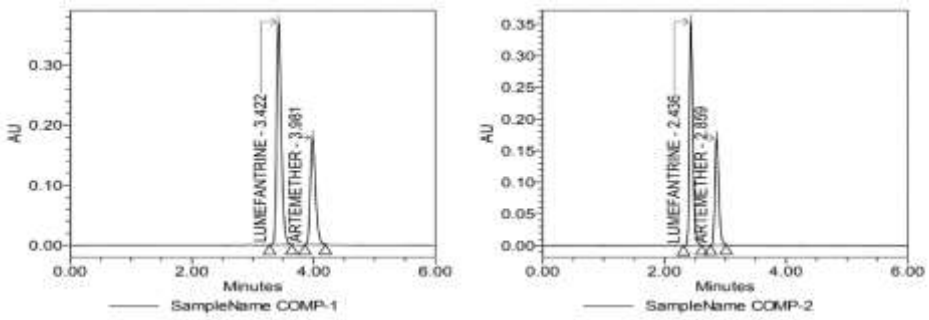
The collected data demonstrated the method's selectivity for LUM and ARM analyses (no influence from **Leemether** tablet excipients).

Robustness:

Analyses of LUM and ARM solutions (LUM-480 µg/ml and ARM-80 µg/ml) were conducted with only minor modifications to the criteria described in the part of the article titled " LUM and ARM COMBINATION TESTING CONDITIONS." The device settings were determined to be compatible with both LUM and ARM. The collected findings demonstrated that the approach may be used to LUM and ARM analyses.

The first modification was to optimise the methanol ratio at 50% volume.

The modified values were 55% (comp 1) and 45% (comp 2)



Peak Name: ARTEMETHER

	Sample Name	Peak Name	RT	Area (µV*sec)	USP Resolution	USP Plate Count	USP Tailing
1	COMP-1	ARTEMETHER	3.981	1071507	3.69	10232	1.29
2	COMP-2	ARTEMETHER	2.859	750200	3.80	9759	1.25

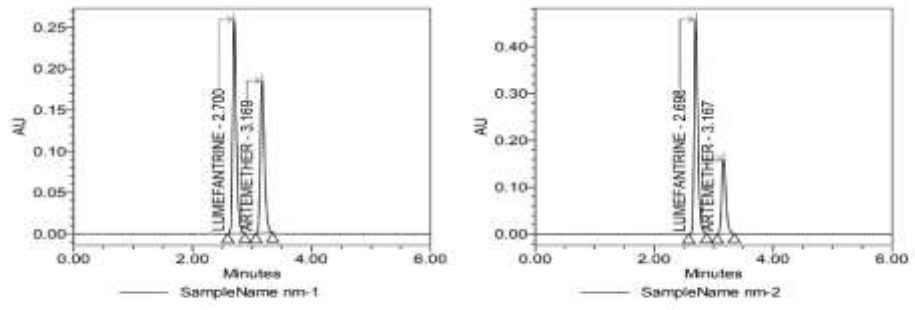
Peak Name: LUMEFANTRINE

	Sample Name	Peak Name	RT	Area (µV*sec)	USP Plate Count	USP Tailing
1	COMP-1	LUMEFANTRINE	3.422	1967411	9744	1.35
2	COMP-2	LUMEFANTRINE	2.436	1386634	9304	1.30

Figure 28: Suitability test Results and Chromatograms of robustness (Methanol modification).

Second, the optimal nm was 287nm.

The modified wavelengths were 285nm (nm 1) and 289nm (nm 2)



Peak Name: ARTEMETHER

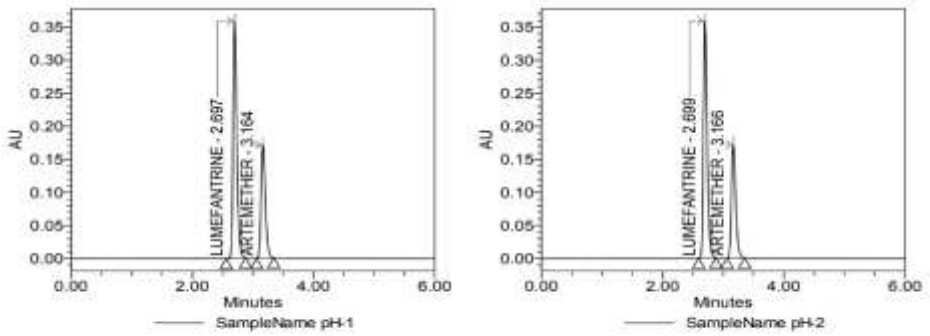
	Sample Name	Peak Name	RT	Area (µV*sec)	USP Resolution	USP Plate Count	USP Tailing
1	nm-1	ARTEMETHER	3.169	900837	3.83	9933	1.29
2	nm-2	ARTEMETHER	3.167	778124	3.83	9906	1.29

Peak Name: LUMEFANTRINE

	Sample Name	Peak Name	RT	Area (µV*sec)	USP Plate Count	USP Tailing
1	nm-1	LUMEFANTRINE	2.700	1116157	9365	1.35
2	nm-2	LUMEFANTRINE	2.698	1959900	9435	1.35

Figure 29: Suitability test Results and Chromatograms of robustness (nm modification).

Third modification:
The ideal pH was 4.5.
The corrected values were 4.7 (pH 1) and 4.3 (pH 2).



Peak Name: ARTEMETHER

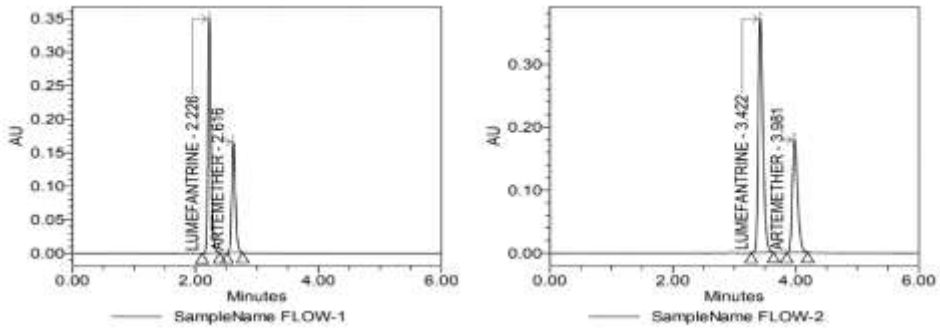
	Sample Name	Peak Name	RT	Area (µV*sec)	USP Resolution	USP Plate Count	USP Tailing
1	pH-1	ARTEMETHER	3.164	837234	3.81	9877	1.30
2	pH-2	ARTEMETHER	3.166	837394	3.82	9942	1.29

Peak Name: LUMEFANTRINE

	Sample Name	Peak Name	RT	Area (µV*sec)	USP Plate Count	USP Tailing
1	pH-1	LUMEFANTRINE	2.697	1538791	9381	1.35
2	pH-2	LUMEFANTRINE	2.699	1533902	9450	1.35

Figure 30: Suitability test Results and Chromatograms of robustness (pH modification).

Fourth modification
Flow optimized rate was 0.9ml/Min
Modifiedalues were 0.7, 1.1



Peak Name: ARTEMETHER

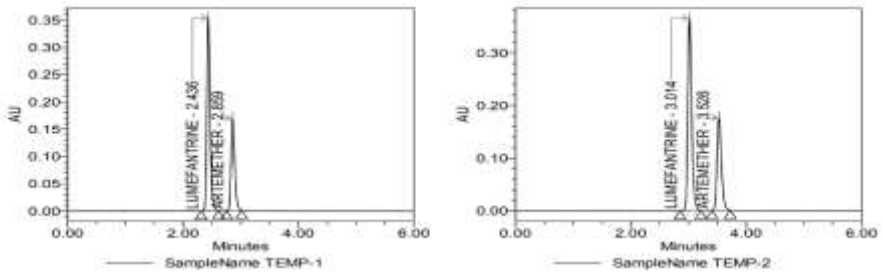
	Sample Name	Peak Name	RT	Area (µV*sec)	USP Resolution	USP Plate Count	USP Tailing
1	FLOW-1	ARTEMETHER	2.616	684582	3.80	9484	1.23
2	FLOW-2	ARTEMETHER	3.981	1071507	3.69	10232	1.29

Peak Name: LUMEFANTRINE

	Sample Name	Peak Name	RT	Area (µV*sec)	USP Plate Count	USP Tailing
1	FLOW-1	LUMEFANTRINE	2.226	1262493	9184	1.28
2	FLOW-2	LUMEFANTRINE	3.422	1967411	9744	1.35

Figure 31: Suitability test Results and Chromatograms of robustness (Flow modification).

Modification Five:
Temp optimized rate was 25^0c
Modified values were 20^0c and 30^0c



Peak Name: ARTEMETHER

	Sample Name	Peak Name	RT	Area (µV*sec)	USP Resolution	USP Plate Count	USP Tailing
1	TEMP-1	ARTEMETHER	2.859	750200	3.80	9759	1.25
2	TEMP-2	ARTEMETHER	3.526	940693	3.81	10241	1.28

Peak Name: LUMEFANTRINE

	Sample Name	Peak Name	RT	Area (µV*sec)	USP Plate Count	USP Tailing
1	TEMP-1	LUMEFANTRINE	2.436	1386634	9304	1.30
2	TEMP-2	LUMEFANTRINE	3.014	1727927	9801	1.33

Figure 32: Suitability test Results and Chromatograms of robustness (Temp modification).

LUM AND ARM STABILITY REPORTS The results of tests to determine the LUM and ARM's stability after being subjected to 0.1N HCl, 0.1N NaOH, peroxide, sunshine, and 105 degrees Celsius are presented in the table below.

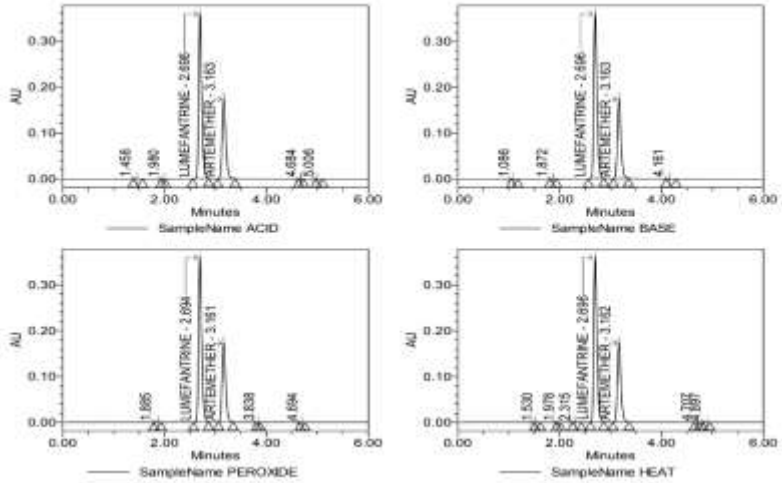
Table 9: Lumefantrine and Artemether stability.

Drug exposed	LUM			ARM		
	Response Area	Perished (percent)	Survived (percent)	Response Area	Perished (%)	Survived (%)
0.1N HCl	1382725	89.74	10.26	754337	90.03	9.97
0.1N NaOH	1423972	92.42	7.58	789342	94.21	5.79
30% H_2O_2	1444463	93.75	6.25	809600	96.62	3.38
105 ^oC	1392757	90.39	9.61	744646	88.87	11.13
Sunlight	1423927	92.42	7.58	787079	93.94	6.06
Untreated	1521540	98.75	1.25	832111	99.31	0.69

LUM stability: untreated>30% H2O2 > Sunlight > 0.1N NaOH > 105 degrees Celsius
> 0.1N HCl
Direction of ARM stability: untreated > 30% H2O2 > 0.1N NaOH > Sunlight > 0.1N HCl > 105 degrees Fahrenheit

Specificity indicating stability:

Below are the chromatograms of the LUM and ARM stability reports after exposure to 0.1N HCl, 0.1N NaOH, peroxide, sunlight, and 105 degrees Celsius. The resolution (different RT values) that may be reached between LUM and ARM for deteriorated compounds is both exceptional and adequate. The gathered findings demonstrated the technique's consistency as well as its specificity with respect to LUM and ARM analyses.



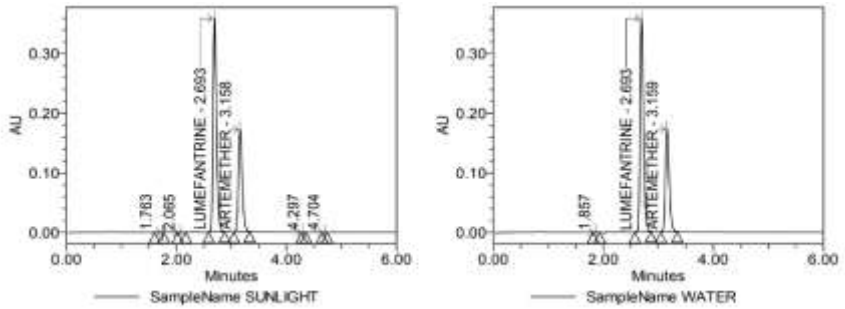
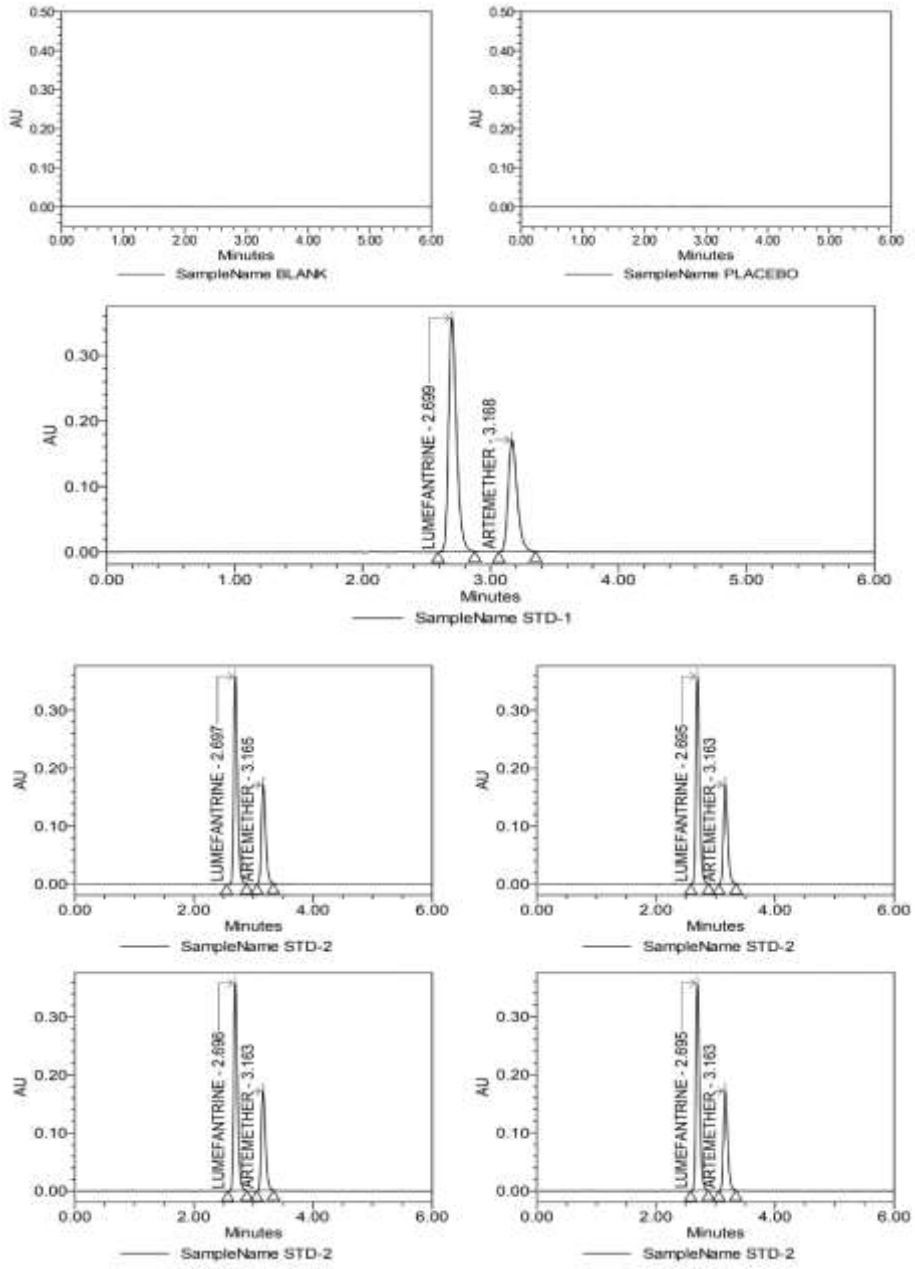


Figure 33: Chromatogram readings illustrating stability, Nature and Specificity.

Selectivity:

Lumether solution with LUM quantity of 480 mg/ml, ARM quantity of 80mg/ml, diluent [KH 2 PO 4 : Methanol are combined in a 50:50 vol/vol fraction] and Working LUM and ARM solution. Peak chromatograms for LUM and ARM were calculated. Peaks associated with excipients and diluents were not found during any of the LUM and ARM retention periods. The test results for LUM and ARM combo analysis were extremely selected.



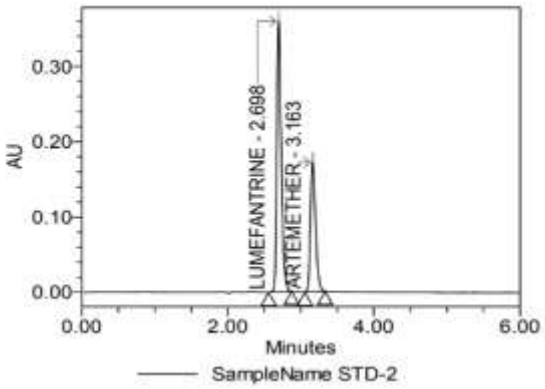


Figure 34: Chromatograms –Lumefantrine and Artemether selectivity.

CONCLUSION

The quantification of LUM and ARM was performed using an RP-HPLC-based method that is stability-indicating, selective, needs the bare minimum run time, is precise, specific, robust, and cost-effective; this was thoroughly verified.

ACKNOWLEDGEMENT

I would like to express my special thanks of gratitude to my guide and to my parents who gave me the opportunity to do this project.

BIBLIOGRAPHY

1. Narayan S, Choudhary M. A review on stability studies of pharmaceutical products.Int Jr of Appl Pharm & Bio Res, 2017; 2(3): 67-75.
2. National Center for Biotechnology Information (2022). PubChem Compound Summary for CID 6437380, Lumefantrine. Retrieved August, 2022; 19. from https://pubchem.ncbi.nlm.nih.gov/compound/lumefantrine.
3. National Center for Biotechnology Information (2022). PubChem Compound Summary for CID 68911, Artemether. Retrieved August, 2022; 19. from https://pubchem.ncbi.nlm.nih.gov/compound/Artemether.
4. Watson DG. Pharmaceutical analysis E-book: a textbook for pharmacy students and pharmaceutical chemists. Elsevier Health Sciences, 2020; 10.
5. Snyder LR, Kirkland JJ, Glajch JL. Practical HPLC method development. John Wiley & Sons; 2012 Dec 3.
6. Chandrul Kaushal K, Srivastava B. A process of method development: Achromatographic approach. J. Chem. Pharm. Res, 2010; 2(2): 519-45.
7. Kagan M, Chlenov M, Melnikov S, Greenfield A, Gross J, Bernotas RC.Optimization of normal-phase chromatographic separation of compounds with primary, secondary and tertiary amino groups. Journal of Chromatography A, 2008; 13, 1194(1): 80-9.
8. Rathnasekara R, Khadka S, Jonnada M, El Rassi Z. Polar and nonpolar organic polymer-based monolithic columns for capillary electrochromatography and high-performance liquid chromatography. Electrophoresis, 2017; 38(1): 60-79.
9. Shah BP, Jain S, Prajapati KK, Mansuri NY. Stability indicating HPLC method development: A Review. International Journal of Pharmaceutical Sciences and Research, 2012; 1, 3(9): 2978.
10. Sengupta P, Chatterjee B, Tekade RK. Current regulatory requirements and practical approaches for stability analysis of pharmaceutical products: A comprehensive review. International journal of pharmaceutics, 2018; 30, 543(1-2): 328-44.
11. Bakshi M, Singh S. Development of validated stability-indicating assay methods—critical review. Journal of pharmaceutical and biomedical analysis, 2002; 15, 28(6): 1011-40.
12. Borman P, Elder D. Q2 (R1) validation of analytical procedures. ICH Qualityguidelines, 2017; 29, 5: 127-66.
13. LoBrutto R, Patel T. Method validation. HPLC for pharmaceutical scientists, 2007; 16, 1: 455-502.
14. Acemic aryl alcohol originally synthesized in the's by the Academy of Military Medical Sciences in Beijing, China. Inhibits hemozoin formation. Prepn: R. Deng *et al*, 1970.
15. Lumefantrine: Uses, Interactions, Mechanism of Action | DrugBank Online
16. Artemether - Drugs.com". *www.drugs.com. Archived from the original on, 2016; 20. December. Retrieved, 2016; 7.*
17. Mutabingwa TK, Adam I: Use of artemether-lumefantrine to treat malaria during pregnancy: what do we know and need to know? Expert Rev Anti Infect Ther, 2013; 11(2): 125-35. doi: 10.1586/eri.12.169.
18. Arun, R. & Arul Gnana Dhas, Anton Smith. Development of Analytical Method for Lumefantrine by UV Spectrophotometry. International Journal of Research in Pharmaceutical Sciences, 2010; 1.
19. Swain, Dr. Suryakanta & Jena, Bikash & Ghose, Debashish & Maharana, Rajib & Duddukuri, Sravani. New UV spectrophotometric Method for

the estimation of Lumefantrine in pharmaceutical formulations, 2021; 12: 40955-40960. 10.24327/ijrsr.2021.1202.5799.
20. Baokar, Shrikrishna. New Spectrophotometric Method Development and Validation of Lumefantrine. PHARMATUTOR MAGAZIN, 2014; 2: 148-154.
21. Huang L, Lizak PS, Jayewardene AL, Marzan F, Lee MN, Aweeka FT. A modified method for determination of lumefantrine in human plasma by HPLC-UV and combination of protein precipitation and solid-phase extraction: application to a pharmacokinetic study. Anal Chem Insights, 2010; 29, 5: 15-23. doi: 10.4137/aci.s4431. PMID: 20448843; PMCID: PMC2865164.
22. William O Foye, Principles of medicinal chemistry, 6, Lippincott Williams and Wilkins, Baltimore, 2008; 1098-1099.
23. Sisowath, C., J. Stromberg, A. Martensson, Msellem, C. Obondo, A. Bjorkman and J.P. Gil, In vivo selection of Plasmodium falciparum pfmdr1 86 N coding alleles by artemether- Lumefantrine (Coartem). J. Infect. Dis, 2005; 191: 1014-7.
24. César, I. C., de Aquino Ribeiro, J. A., de Souza Teixeira, L., Bellorio, K. B., de Abreu, F. C., Moreira, J. M., Pianetti, G. A. Liquid chromatography-tandem mass spectrometry for the simultaneous quantitation of artemether and lumefantrine in human plasma: Application for a pharmacokinetic study. Journal of Pharmaceutical and Biomedical Analysis, 2011; 54(1): 114–120. https://doi.org/10.1016/j.jpba.2010.07.027
25. Ghosh, Somsubhra & Shirisha, V. & Banji, David. Analytical method development & validation of Lumefantrine in its bulk dosage form by using RP-HPLC method as per ICH guidelines,. Journal of Chemical and Pharmaceutical Research, 2015; 7: 372-378.
26. S. Toovey, A. Jamieson, and G. Nettleton, "Successful co-artemether (artemether-lumefantrine) clearance of falciparum malaria in a patient with severe cholera in Mozambique," *Travel Medicine and Infectious Disease*, 2003; 1, 3: 177–179.
27. Yadav, Shanti & Shah, Jaivik & Shah, Priyanka & Patel, Pavan & Sanyal, Mallika & Patel, Mukesh & S, Shrivastav. Bioanalytical Method Development and Validation for the Estimation of Lumefantrine and Desbutyl Lumefantrine in Human Plasma by LC-MS/MS. JOURNAL OF ADVANCEMENT IN MEDICAL AND LIFE SCIENCES, 2016; 3: 1-10. 10.15297/JALS.V3I4.02.
28. Sultan Suleman, Yannick Verheust, Ann Dumoulin, Evelien Wynendaele, Matthias D'Hondt, Kirsten Vandercruyssen, Lieselotte Veryser, Luc Duchateau, Bart De Spiegeleer, Gas chromatographic method for the determination of lumefantrine in antimalarial finished pharmaceutical products, Journal of Food and Drug Analysis, 2015; 23: 3.
29. Hamrapurkar P, Phale M, Pawar S, Patil P, Gandhi M. A simple and precise method for quantitative analysis of lumefantrine by planar chromatography. Pharmaceutical Methods, 2010; 1(1): 44-48. DOI: 10.4103/2229-4708.72230. PMID: 23781415; PMCID: PMC3658015.
30. Vinodh et al: ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF ARTEMETHER AND LUMEFANTRINE IN PURE AND PHARMACEUTICAL DOSAGE FORM USING RP-HPLC METHOD Malaysian Journal of Analytical Sciences, 2013; 17, 3: 348 – 358.
31. Mannur Vinodh*, Mastiholimath Vinayak, Patware Pankaj, Kharya Rahul and Mascarenhas Renita. Analytical method development and validation for the simultaneous estimation of artemether and lumafantrine in pure form and pharmaceutical dosage form. Malaysian Journal of Analytical Sciences, 2013; 17 (3): 348 – 358.
32. Shamshad, Komal & Gohar, Umer & Mukhtar, Hamid & Zafar, Asma & Mubarik, Shamroza. High Performance Liquid Chromatography Method Development and an Innovative Technique for Validation of Artemether and Lumefantrine Oral Dry Suspension, 2022; 10.26717/BJSTR.2020.32.005234.
33. Sridhar, Balasubramaniam et al. "A VALIDATED REVERSE PHASE HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF ARTEMETHER AND LUMEFANTRINE IN PHARMACEUTICAL DOSAGE FORMS.", 2010.
34. Ezhilvani, A Analytical Method Development and Method Validation of Artemether and Lumefantrine in Powder for Oral Suspension Dosage Form by RPHPLC, 2017.
35. Arun, R. & Arul Gnana Dhas, Anton Smith. Simultaneous HPLC-UV method for the estimation of artemether and lumefantrine in tablet dosage form. Int J Pharma Biomed Res, 2011; 2.
36. César, Isabela & Pianetti, Gerson. Quantitation of artemether in pharmaceutical raw material and injections by high performance liquid chromatography. Brazilian Journal of Pharmaceutical Sciences - BRAZ J PHARM SCI, 2009; 45. 10.1590/S1984-82502009000400018.
37. Christian, Jenee R. et al. "Optimizing derivatization conditions using an experimental design and simultaneous estimation of artemether and lumefantrine by ratio first order derivative spectrophotometric method." Journal of Taibah University for Science, 2017; 11: 729 - 740.
38. Shrivastava A, Issarani R, Nagori BP. Stability indicating HPLC method for the estimation of Artemether in capsule dosage forms, Quality assurance, 2010; 2, 1: 79-84.
39. Esu, E., Effa, E. E., Opie, O. N., Uwaoma, A., Meremikwu, M. M., National Center for Biotechnology Information, 2014; 2.
40. Sukanya, Bandari & Goud, Mohan & Anitha, P. Stability indicating Ultra Performance Liquid

Chromatography method development and validation for simultaneous estimation of artemether and lumefantrine in bulk and pharmaceutical dosage form. Journal of Drug Delivery and Therapeutics, 2019; 9: 217-221. 10.22270/jddt.v9i2.2408.

41. Phadke, Manisha et al. "HPLC study of related substances in artemether and Lumefantrine tablets." Analytical chemistry, an Indian journal 8 (2009): n. pag.
42. SIMULTANEOUS ESTIMATION OF ARTEMETHER & CURCUMIN BY RP-HPLC METHOD - Pharmacophore (pharmacophorejournal.com)
43. César Ida C, Andrade Nogueira FH, Antônio Pianetti G. Simultaneous determination of artemether and lumefantrine in fixed dose combination tablets by HPLC with UV detection. J Pharm Biomed Anal, 2008; 4, 48(3): 951-4. doi: 10.1016/j.jpba.2008.05.022. Epub 2008 May 28. PMID: 18602241.
44. Yabré M, Ferey L, Sakira AK, Bonmatin C, Fauré C, Somé TI, Gaudin K. Green Analytical Methods of Antimalarial Artemether-Lumefantrine Analysis for Falsification Detection Using a Low-Cost Handled NIR Spectrometer with DD-SIMCA and Drug Quantification by HPLC. Molecules, 2020; 27, 25(15): 3397. doi: 10.3390/molecules25153397. PMID: 32727052; PMCID: PMC7435840.
45. Sunil, Junapudi & Nath, M & Moorthy, U & Sambamoorthy, Unnam. HPLC method development and validation for simultaneous estimation of Artemether and Lumefantrine in pharmaceutical dosage forms. International Journal of Pharmacy and Pharmaceutical Sciences, 2010; 2: 93-96.
46. Vandercruyssen K, D'Hondt M, Vergote V, Jansen H, Burvenich C, De Spiegeleer B. LC-UV/MS quality analytics of paediatric artemether formulations. J Pharm Anal, 2014; 4(1): 37-52. doi: 10.1016/j.jpha.2013.03.006. Epub 2013 Apr 25. PMID: 29403867; PMCID: PMC5761056.
47. Jadhav, Vaishali & Singh, Kamalinder. Development and validation of HPLC-UV method for quantification of artemether in plasma. Journal of Pharmacognosy and Phytochemistry, 2018; 7: 75-78. 10.22271/phyto.2018.v7.isp6.1.17.
48. César Ida C, Andrade Nogueira FH, Antônio Pianetti G. Simultaneous determination of artemether and lumefantrine in fixed dose combination tablets by HPLC with UV detection. J Pharm Biomed Anal, 2008; 4, 48(3): 951-4. doi: 10.1016/j.jpba.2008.05.022. Epub 2008 May 28. PMID: 18602241.
49. Meka.Srinivasa rao Simultaneous estimation of artemether and lumefantrine in combination drug products by RP-HPLC method European Journal of Molecular & Clinical Medicine, 2022; 7: 3. ISSN 2515-8260.