World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 6.129

THE SHELF LIFE OF ARTEMETHER+ LUMEFANTRINE TABLETS AT THE DISTRIBUTION CHANNELS OF SUDAN

Dr. Abdrhman Mahmoud Gamil*

Associate Professor of Pharmaceutics, Al-Neelain University, Sudan.

Corresponding Author: Dr. Abdrhman Mahmoud Gamil
Associate Professor of Pharmaceutics, Al-Neelain University, Sudan.

Article Received on 05/07/2020 **Article Revised on 26/07/2020** **Article Accepted on 16/08/2020**

ABSTRACT

Distribution and storage conditions are the critical factors in prediction of a pharmaceutical product shelf-life. Artemether and Lumfantrine combination tablets is one of the most important pharmaceutical product in Sudan as it is used in the treatment of the endemic disease, Malaria. To evaluate the distribution and storage conditions and their impacts on the shelf life of this product, samples with dataloggers were analyzed initially then transported and stored at five selective cities for 12 months while logging temperature and humidity every 90 minutes. samples were returned back to the centre and subjected to chemical analysis at intervals I month, 6 months and 12 months. The Reference Thermal Exposure is nearly 8000 hours. The active contents of the tablets were obtained at each time interval using gradient HPLC and then the LSRLs were plotted and the results were computed according to the ICH guidelines using Woolf's Equation for inverse prediction to obtain X at a given Y. Lumefantrine was found to be stable and its shelf life can be extended to 3 years, but the other part of the tablets, the Artemether component, is the determinant component for the shelf-life. Artemether was found that, it may lose 2 – 6 months from its labelled shelf life because it had been subjected to thermal exposure above 25℃ for 51% - 61% of the total exposure period. The product retains its physical and microbiological characters indicating GMP compliance.

KEYWORDS: *Lumefantrine stability, Artemether stability, Reference Thermal Exposure, Shelf life Estimation.*

INTRODUCTION

Shelf-life is the period time during which a pharmaceutical product is expected, if stored correctly, to comply with the specifications. It is the time interval that a drug product is expected to remain within the approved shelf-life specification provided that it is stored under the conditions defined on the label in the proposed containers and closure, (FDA, ICH, BPC1994). Of all the many environmental factors that can be involved in drug degradation, temperature is the most important one that cannot be controlled by package (Kommanoboyina and Rhodes – 1999). The stability of a drug product depends on the raw materials used, warehouse and transport facilities.

On highly relative humidity solid dosage form may sorbs water, leading to dissolution of the drug and to degradation. Water vapor may gain access to medicinal products through poorly-fitted closures or by direct permeation within the container's wall. (Florence and Attwood, 1998).

Effects of Temperature on drug stability

Temperature remains as the most important factor influencing drug degradation, which the product cannot be protected from. For many reactions an increase in temperature enhances the rate constant and the effect can often be described by Arrhenius equation. Information on the effect of temperature is important in prediction of the shelf life.

$$\text{Log K} = \log A - \frac{Ea}{2.303RT}$$

Log K versus 1/T, linear plot will be obtained. Then Ea which is the activation energy could be calculated because the intercept of plot with 1/T = 0 is log A. Ea usually between 50-96 Ks/mol.

If the reaction is determined by diffusion or photochemical reaction or if the decomposition is due to freezing, contamination by microorganisms or excessive agitation during transport, and soon, an elevated temperature study is obviously of little use in predicting the shelf-life (Sinko P.2006).

Stability of products in the channels of distribution

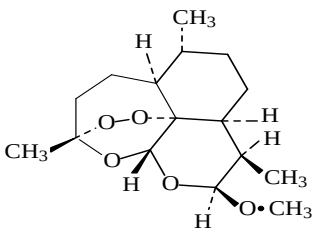
The retained samples at the manufacturer stores for stability testing are not dropped off the back of the truck; they are not left on a blazing sun on a loading dock; nor

exposed to dusty weather. Thus it is somewhat unrealistic to expect retained samples to reflect accurately the stability status range of products those in channels of distribution (Rhodes T, 2000). The manufacture can generate the quality of a product up to the time designated as its expiration date only if the product has been stored in the original container under recommended storage conditions usually stated on the label (USP,2016). Mean kinetic temperature should be calculated for any separate area of the warehouse. Validated, available temperature and humidity-monitoring technologies can be used to monitor the overall environment effect on articles during shipment and distribution (USP,2016). The USP subcommittee on packaging, storage and distribution conduct mailing study from Rockville to various destinations in USA. The results showed that 16% were subjected to temperature above 30 °C and 21% of them experienced excessive heat over 40 °C (Rhodes, 2000). HPLC is the method of choice for stability testing used by the pharmaceutical industry to assay the intact drug and its degradation products (Jeffs P, 1998). WHO recommended that shelf-life is always determined in relation to storage conditions.

Stability of Medicines across Sudan

A basket of medicinal products had been transported to Sudan from Netherlands. Then the samples were returned back to IDA laboratories by air. The study resulted in that, three products were instable. One of them is already instable at room temperature, ergometrine, Suxomethonium chloride and lignocaine+ adrenaline (Hogenzil, 1991). The study did not report the storage conditions. Also stated staying 8 months at Port Sudan which is not a familiar case.

Arthemether: Figure (1) Artemether structural formula.

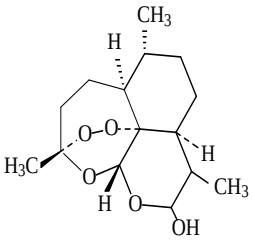


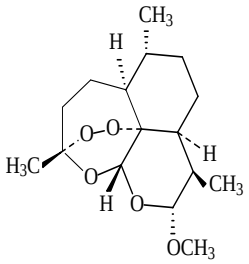
$C_{16}H_{26}O_5$ = 298.4

Dihydroartemesinin methyl ether, "12-b-dihydroartemesinin methyl ether". Artemether is the methyl ether of dihydroartesinin, which is derived from artesinin the isolate substance from the extract of leafy portion of Artemisia annua (Ging Hao) family asteraccae. It acts through the endoperoxides group as schizontocidal destructing the asexual erythrocytes form of *Plasmodium filciparum* and *P. vivax* (Ch. ph1995).

For tablets the Int. Ph specifies that it should contain not less than 90% and not more than 110% of the labelled amount of $C_{16}H_{26}O_5$.

1. Dihydroartemisinin Figure (2) 2. α-artemether figure (3) and unknown related substance.





Long-term storage at 35 °C and 30 °C RH 60% indicates a decrease in the content after 24 months but without dropping below 90% and increase in the related substances dihydroartmesinin and other related substances. At 40 °C and 50 °C for 12 and 6 months, an increase of unknown decomposition products is observed. The result is that, artemether stability is good at ambient temperature. The product need to be protected from light and protected from temperature above 30°C to avoid loss and degradation. Being packed in PVC/PVDC blister packs and stored at temperature not above 30 °C, the product shall have shelf-life of 24 months (Manufacturer).

Lumefantrine

Lumefantrine is a synthetic racemic fluorine mixture. It is an antimalarial drug; it is also named Benflumetol. It is a yellow crystalline powder, insoluble in water, freely soluble in dimethylformamide and ethyl acetate, soluble in dichloromethane, slightly soluble in ethanol and methanol. Molecular weight: 528.94, $C_{30}H_{32}Cl_3NO$.

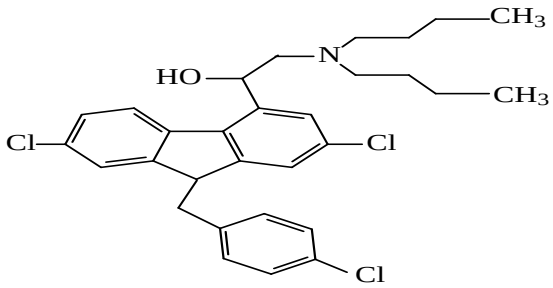


Figure 4: Lumefantrine.

When dried it loses weight not more than 0.2% (Ch. ph 2005). α-(dibutylaminomethyl)-2,7-dichloro-9-(p-chlorobenzenemethylene)-4-fluorene methanol. The absorption of lumefantrine in the fasting states is relatively poor (Walther H 4/2004). Bioavailability of lumefantrine increased markedly by taking a normal good meal (Ezzet *F l.*2000). Using HPLC method of assay, the results indicated that no degradation of the drug substance observed. In the long-term stability in solid state, lumefantrine was found to be stable and no increase in the content of the related substances. In the finished product, the tablets dosage form in combination with artemether in study of 40°C/12 months, 50°C/6 months, 25°C/75%RH, 30°C/80%RH 6 months, there

was no degradation for lumefantrine. The shelf-life had been stated as two years.

MATERIALS AND METHODS

Artemether+lumefantrine 20/120mg tablets. Sample had been manufactured by Swiss company each 8 film coated tablets are blistered in PVC/AL blister, each 3 blisters are packed in carton box. Donated by Novartis Scientific Office, Dubai.

Reagents

HPLC grade reagents

Acetonitrile - Sotex pharmaceuticals –UK, Potasium Dihydrogen orthophosphate – UK, Dilute acetic acid, Dibasic sodium phosphate. BDH- Germany, Phosphoric acid. BDH- Germany, Methanol - BDH- Germany, Hexane sulphonic acid sodium salt. Sotex. U.K, Ethanol BDH- Germany, Propanol – Sotex- U.K., Purified water, Eurowater.

Analytical reagents

Sulphanilic acid solution – Sotex, U.K, Aminonaphthalenesulponic acid – Sotex U.K, 0.1 Hydrochloric acid - BDH- Germanyn, Nitrite standard solution 20 PPm - BDH- Germany, Sodium Dihydrogen phosphate Monohydrate- BDH- Germany.

Reference standards

Artemether Reference Standard: (Chinese Ph. 2005). Herbstars Co. – China. Concentration is 99.7%; each 100 grams powder contains 99.7 grams artemether based on dried substance.

Lumefantrine Reference Standard: (Chinese Ph. 2005). Lot No., 060701, Herbstars Co. – China.

Concentration, each 100 grams powder contains 99.69 grams lumefantrine.

Staphylococeus Aureus specimen, Candida albicans specimen, E. coli specimen.

Culture Media: Fluid Soya bean- Casein Digest medium, Medium B, B.P, Soya bean. Casein Digest Agar Media, HIMEDIA – India, Sabouraud –Glucose Agar Medium. HIMEDIA – India, Buffered Sodium Chloride – peptone solution pH 7, Lactose monohydrate broth) B.P, Enrichment broth B.P, (Entrobacteria enrichment broth – Mossel), (Crystal violet, neutral red, bile agar with glucose), Mac Conkey Broth, Mac Conkey Agar, Tetrathionate bile brilliant green broth, Deoxycholate citrate agar, xylose, lysine, deoxycholate agar, Triple sugar – iron agar, Cetrimide agar, Baird Parker agar, Fluid thioglylcolate Medium . (Oxoid CM 173), Soy Bean Casein Digest Medium (Oxiod CM 129), Peptone (oxiod), Mannitol – salt Agar medium USP.

Instruments: Temperature and humidity data logging devices: Total of five electronic devices are used, from SATO KEIRYOKI MFG. CO. LTD. TOKYO 101 – 0037 JAPAN SK- L 200II series data loggers No. 8171 – 00 model SK – L 200 TH II. With Probes sensitive for temperature of thermistor type and a high polymeric resistance change humidity sensor. Software deriver.

Chromatographic Instruments

Gradient HPLC with U. V detector: by Knauer advanced scientific Instruments .Germany. Model, Smart line HPLC consists of degasser S.N, 92382 – Smart line manger 5000. Pump S.N. 92603 – Smart line pump 100 stainless steel. Injection valve S.N 91157, stainless steel. Chamber S.N 92379. Programmable detector S.N. 90662- U.V detector 2500. Column thermostat test stream 2 –plus. With software and PCL.

Columns: Lichro cart 250 mm – 4 lichorospher 100 RP - 18 (5 micrometer) HPLC cartridge. Packed with silica gel bounded to octadecylsilyl Groups, Lichrocart 4-4 Purosher RP-18 (5- micrometer) HPLC guard column. Merck K GaA – D 64271, Frankfurter- Germany. **Grant Ultrasonic bath**, type, MXB 6. S.N. : CG0709004. – England. **pH meter,** by HNNA Instruments – Portugal, Measuring range, 0.01 pH Unit. **Weight Balance:**: AB 204 Metter Toledo in a weighting cabinet. of measuring range 0.01. **Hardness Tester:** ERWEKA – Apparatuses BAU GHBH – Germany**. Disintegration Tester;** ERWEKA Disintegrator – Germany. **Mixers :** Fisher Scientific Mixer with Magnetic Stirrer, Stuart (Auto vortex Mixer)–UK. Stirrer, Scientific Industrial incorporation, Bohemia N.Y- USA. **Stop watch:** Q&Q – indicate 0.01 second. **Laminar Flow Cabinets:** Laminar Flow Cabinet with U.V lamp, Labconco ® Class II Safety Cabinet. USA. Laminar Sterile air flow: BS – 57 26 Class I, Model, ROBINAIR – VACUMASTER. **Pipettes:** Micro pipette :10,20, 50, 100 nl. Pipettes : 2, 5, 10 ml. **3.2.14 Ovens :** Incubator 33 C^0 Nŭve , EN 400. Incubator 25C^0 VVWR ≡ **Oven Glass ware sterilizer**, Fisher model S.N 501 –137. Isotemp oven (35 C^0 - 250 C^0). **Autoclaves:** GCA 67013 – 12 AR – 3, **Glass ware**: Test tube and racks for test tubes, petri dishes of a 9 cm diameter, beakers, conical flasks, measuring cylinders, **Miscellaneous**: Water bath; SB 410, Nŭe, Turkey S.N 01 .311, Leonard refrigerator, Bunsen flame, porcelain mortar and pastille, aluminum foil and sterile cotton Swab. **Microscope:** Olympic Binocular microscope – 25 v. The magnifying power is 10.

Methods

Study Design

The study was designed to evaluate the distribution, transportation and storage of the antimalarial tablets across Sudan which lies between 3^0 -4^0 N and 21 -22^0 N latitude. Sudan climate differ from one region to another, and the warehouses RTE will be measured. The international reports were based on Khartoum only using the meteorological data. The main city was selected as a representative station for such smaller climatic zones. Then the samples were transported to the designated cities via the usual route of transportation of the hospital

drugs. To Omdurman by small car after wandering in Khartoum streets, to Dungola by passenger buses 520 km to the north, to Al- Fashir by airplane 1355 km to the west, to Al_Damazin by trucks 780 km to the south east and to Portsudan by distribution trucks 1200 Km to the Red Sea.

Setting Dataloggers: They are readily calibrated and tested, each with software CD, USB, and calibration certificate. They are thermistor type which is recommended by USP. They had been adjusted to log data every 90 minutes intervals, up- to more than 5000 points in 52 weeks. The readings had been barred by adjusting the screen not to display the recorded data.

Storage at the sites: The samples were stored in the normal storage conditions at the hospital pharmacies among the stored medicinal products intended to be dispensed to patients. The usual actual conditions are not altered and the samples and dataloggers were stored with the current stock side by side on the shelves.

Duration of the study: 52 weeks
Frequencies of testing: Initial testing or zero-time: After transportation (one month): Samples from each city were returned back for analysis. Mid – point testing: Chemical assay for the active ingredients were performed after 6 months. Final testing: at the end of 52 weeks.

Chemical Analysis
A. Principle: Gradient HPLC, programmed U.V detection Smart line knauer HPLC chromatographic system with automatic programmable pump and detector.

B. Reagents: HPLC grade reagents**,** Hexane sulphonic acid sodium salt. Sodium dihydrogen phosphate monohydrate. Phosphoric acid. Acetonitrite. 1- propanol.

C. Chromatographic conditions

1. Ion- pair reagent: dissolve (5.65 gm) of hexanesulphonic acid sodium salt and 20 mmol (2.75 gm) of sodium dihydrogen phosphate monohydrate in 800 ml water. Adjust the pH to 2.3 using phosphoric acid. Dilute to 100 ml with water and filter.
2. Solvent: Mix 100 ml of ion pair reagent with 30 ml of water, add 100 ml of propanol and dilute with acetonitrile to 500 ml.
3. Mobile Phase: Solution A: 700 ion pair + 300 acetonitrile, Solution B: 300 ion pair + 700 acetonitrile,
4. Column: Neucleosil C 18, 15 cm x 4.6 mm – Merck. Packed with 5 μ 3. Flow rate: 1.3 ml per minute.
5. Detection: UV 210 nm and 380 nm. Set the detector for the first 30 minutes to 210 nm, then switch to 380 nm. 5. Ambient temperature. 6. injection volume: 20 ul, 7. Program the pump.

D. Assay preparation: Standard Reference Solution: Weigh 20 mg of artemether reference standard powder and 120 mg of lumefantrine reference standard in 100 ml volumetric flask. Dissolve and dilute to volume with solvent. Test solution: Disintegrate 10 tablets, equivalent to 200 mg of artemether and 1200 mg of lumefantrine, with 60 ml of water in 100 ml volumetric flask. Add 200 ml of 1- propanol and sonicate for 15 min. Add 200 ml of ion pair reagent and 400 ml of acetonitrile and sonicate again for 30 minutes. Dilute to volume with acetonitrile. Withdraw 10 ml of the suspension and centrifuge for 5 minutes. Use the clear solution for the assay.

E. Procedure: Set the gradient program. Set the detector as described. Inject each of the standard preparation for three replicate between each replicate 15 minutes running of mobile phase and do the same for the samples tested. Record the results and print out the chromatograph.

F. Calculations: Calculate the content of artemether from the formula:

$$C_A = \frac{M_{RA} \times PA_{TA} \times 5}{PA_{RA}}$$

C_A : Percent content of artemether, M_{RA}: Mass in mg of artemether, PA_{TA} :Reference Standard,

PA_{RA}: Peak area for artemether in Reference Standard.

Calculate the content of lumefantrine accordingly.

Validation of Method (Huber 2010).

Testing for linearity
Procedures described in the methods were repeated for each method using five dilutions for each sample and three replicate injections for each dilution.

The following concentration are used, Table (1)

Table 1: Validation of Method.

Con	Artemether$_+$ Lumefantrine	
	Artemether Mg/100ml	Lumefantmg/100ml
1	1.25	7.5
2	2.5	15
3	5	5
3	10	60
5	20	120

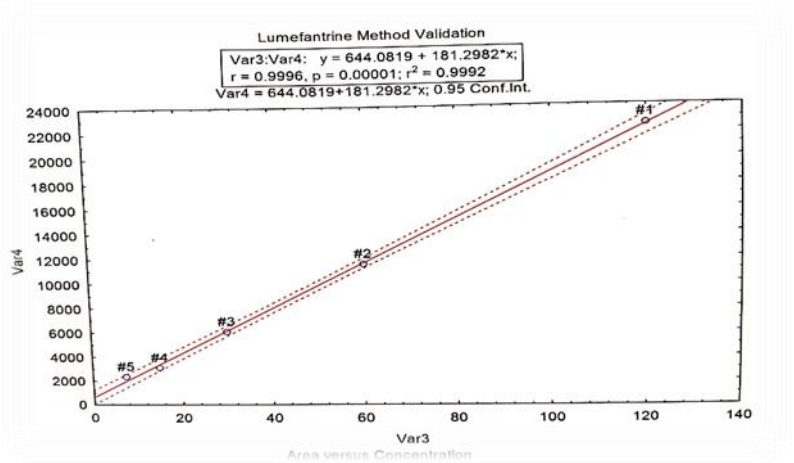


Figure 5: Linearity of lumefantrine method of Analysis.

Treatment of data

The mean of the three replicate is obtained; the standard deviation is calculated. The mean of area under the peak is used for each dilution.

Statistical analysis: Statistica (statsoft version 8) is uses to analyze the data obtained. Regression analysis is used to plot the peak area against the concentration. The correlation coefficient, the regression equation, the slope and intercept were obtained. P. value and results of the positive linearity were obtained.

Total viable count: not more than 10^3 /gm bacteria and not more than 10^2 /gm fungi.

Absence of specific organism: (B.P 2007, Appendix XVID, Microbiological quality of pharmaceutical preparation, ph. Eur general text 5.1.4, categeory 3, A). Preparation for oral and rectal administration, the required tests are the following: Absence of salmonella. Absence of E. coli. Absence of staphylococcus aureus. Not more than 10^2 Enterobacteria.

Reference Thermal exposure

RTE is an unofficial approach for calculating the total exposure temperature as a function of temperature and period of exposure. The approach was proposed by pharmaceutical manufacturing association in USA–1980.

The time in hours for each range of temperature emphasis to compare the results with those of the mean kinetic temperature with the potency of drugs, not less than 5000 reading for each station for temperature, humidity, date and time had utilized in the calculation. SPSS software and Excel statistical packages were used.

Analysis of results

Evaluation of the results according to QE, ICH guideline. Regression analysis for the potency versus time for every station was performed taking 5% lower level of confidence and the P of replicates 0.05. Using Statistica software version 16 and/ or SPSS version 14 statistical packages and Microsoft Excel spread sheet. The shelf life of each sample at each station was calculated and compared to that of the label. The effect of storage conditions on the expiration date was obtained. The storage conditions being measured was compared with that stated on the label and thekinetic order of reaction and rate constant we computed.

RESULTS

Physical tests

Appearance: Yellow coloured, flatted film coated tablets, diameter of 8.5 mm with notched rounded ages and marker line across the tablet with a fine touch.

mean	0.240885 gm
STD	0.001323

Uniformity of weight

Table (2) Initial weight uniformity test of Coartem tablets

Then the test was done again at the end of the storage period for product stored at different stations and the results could be presented in the following table (3).

Table (3): Weight uniformity of tablets after storage at different stations.

station	Al-Fashir	Damazin	Khartoum	Dungola	Portsudan
Mean	0.241545	0.242525	0.24178	0.24127	0.24321
STD	0.365507	0.439611	0.439068	0.364593	0.359166

Resistance to crushing of tablets (Hardness): using Erweka Hardness Tester.

Table (4): Hardness testing for tablets.

Maximum: 92 Newtons. STD: 8.8, Minimum: 67 Newtons, RSD:11.5, Mean: 76.5 Newtons.

Then the test had been done at the end of the storage period as shown on table (5).

Table (5): Hardness testing for Tablets after storage at different stations.

station	Al-Fashir	Damazin	Khartoum	Dungola	Portsudan
Min	68	52	60	57	60
Max	91	84	99	94	86
Mean	76.7	71	75.5	79.3	72.3

Loss on drying: Before distribution the moisture content was 3.85 W/W %, The moisture contents after the storage period at different stations could be tabled as follows:

Table (6): Moisture contents after the storage period.

station	Al-Fashir	Damazin	Khartoum	Dungola	Portsudan
Moisture contents	1.65	1.65	1.67	1.67	2.87

Disintegration Time test: Six tablets were subjected to test on Erweka disintegration tester of paddle type. Disintegration time was 4 minutes and 24 seconds. Then the test was repeated after the storage period and the results are presented in table (7)

Table (7): Disintegration time for tablets after storage at different stations.

station	Al-Fashir	Damazin	Khartoum	Dungola	Portsudan
Disintegration time in min	4: 55	4: 53	4: 55	4: 40	4: 39

Determination of Lumefantrine and Artemether Contents in tablets during storage at different stations

Samples were assayed initially for lumefantrine and Artemether contents. Then they were reanalyzed after transportation (one month), after six months and then finally on 12 months.

Lumefantrine contents

Table (8): Initial contents percentage of Lumefantrine in the tablets.

3 Replicates	Area of the standard	Area of the sample	Content percent.
Mean	165.4747	165.914	100.2655 %
St. d	1.339517	0	0

Table (9): Contents of lumefantrine after transportation.

Station	RS	Portsudan	Dongola	Khartoum	Damazin	AlFashir
P. area	10727	11022	10763	10810	10718	10896
Contents%	100	102.7	100.3	100.8	99.9	101.5

Table (10): Content of lumefantrine in Coartem after six months at different stations.

RS	Station	Portsudan	Dongola	Khartoum	Damazin	Al-Fashir
17458.77	P. area	17697.79	18168.92	16973.67	17602.86	16935.48
16523.72	Contents%	104.15%	106.93%	99.89%	103.59%	99.67%

16991.25	Mean	

Table (11): Final contents of lumefantrine Coartem after 12 months.

station	RS	Portsudan	Dongola	Khartoum	Damazin	Al-Fashir
Mean	21502.8	21278.33	22218	22390.67	22103.33	22140.67
Content%	100 %	98.95 %	103.3	104.1	102.8	102.9

Artemether contents in Coartem:

Table (12): Initial contents percentage of Artemether in Coartem.

3 replicates	Area of the standard	Area of the sample	Content percent.
Mean	18.332	18.74403	102.2476
St. d	0.050074	0.0272	0.148

Table (13): Contents percentage of Artemether in Coartem after transportation.

Station	RS	Portsudan	Dongula	Khartoum	Damazin	Al-Fashir
P. area	189.472	164.4	190	206	182	175.1
Contents%	100	86.8	100.3	108.7	96.1	92.4

Table (14): Concentration of Artemether in Coartem after six months of storage.

RS	Station	Portsudan	Dongula	Khartoum	Damazin	Al-Fashir
242.479	P. area	267.013	236.374	243.89	227.131	208.246
240.148	Contents%	110.6498	97.95308	101.0677	94.12279	86.29687
241.3135	Mean					

Table (15): Final contents of Artemether in Coartem after 12 months of storage.

station	RS	Portsudan	Dongula	Khartoum	Damazin	Al-Fashir
Mean	346.6727	323.6667	332	325	329.3333	299.6667
R. st. d	2.98317	3.86	1.02	2.82	1.48	1.26
Content	100	93.4 %	95.76 %	93.74 %	94.99 %	86.43 %

Microbiological Tests

Total viable count and absence of specific organism were carried out before and after distribution.

Initial count of bacteria: Results are presented as in table (16).

Control Test

Experiment	Observation of set I	Observation of set II	Result
Buffer + medium B	No c.f.u	No c.f.u	Negative
Buffer + Medium B + Staph. aureus	Many c.f.u	Many c.f.u	Positive

Table (16): Intial Count for Bacteria in different dilutions.

Dilution	1×10^{-1}	1×10^{-2}	1×10^{-3}	1×10^{-4}	1×10^{-5}
Sample+ medium B	No c.f.u	No c.f.u	No c.f.u	No c.f.u	No c.f.u
Sample + medium B + Staph.areus	Many c.f.u	Many c.f.u	Many c.f.u	Many c.f.u	Many c.f.u

Result: Total viable aerobic count for bacteria is < $I0^3$/ gram of tablets.

Initial count for fungi

The results could be presented as in table (17).

Control Test

Experiment	Observation of set I	Observation of set II	Result
Buffer + medium C	No c.f.u	No c.f.u	Negative
Buffer + Medium C + Candida Alb.	Many c.f.u	Many c.f.u	Positive

Table (17): Initial Count for Fungi in different dilutions.

Dilution	1×10^{-1}	1×10^{-2}	1×10^{-3}	1×10^{-4}	1×10^{-5}
Sample+ medium C	No c.f.u	No c.f.u	No c.f.u	No c.f.u	No c.f.u
Sample + medium C + Candida	Many c.f.u	Many c.f.u	Many c.f.u	Many c.f.u	Many c.f.u

Result: Total viable aerobic count for Fungi is < $I0^2$/ gram of tablets.

Final bacterial count

Five serial dilutions of the powdered product were used for every station. Medium B was used and cultured at 33 °C for 30 hours and the results could be presented in table (18)

Table (18): Bacterial Count at different stations.

Station	Dilution 1×10^{-1}	Dilution 1×10^{-2}	Dilution 1×10^{-3}	Result
Portsudan	-	3- 0 c.f.u	-	15×10^1/gram
Dongola	-	-	-	< $I0^3$/ gram
Khartoum	-	-	-	< $I0^3$/ gram
Damazin	-	-	-	< $I0^3$/ gram
Fashir	-	-	-	< $I0^3$/ gram

Result: All samples passed the test.

Final Fungal Count: Five serial dilutions of the powdered product were used for every station. Medium C was used and cultured at 23 °C for five days and the results are as follows:

Table (19): Total Fungal Count at different stations.

Station	Dilution 1×10^{-1}	Dilution 1×10^{-2}	Dilution 1×10^{-3}	Result
Portsudan	-	-	-	< $I0^2$/ gram
Dongola	-	-	-	< $I0^2$/ gram
Khartoum	0.5 c.f.u	-	-	0.5×10^1/gram
Damazin	-	-	-	< $I0^2$/ gram
Fashir	-	-	-	< $I0^2$/ gram

Result: All samples pass the test.

DISCUSSION

Thermal Exposure Periods

Table (20): Thermal Exposure Periods for the Five Stations.

Station	Total Exposure Hours	Period Over 25°C %	Period Over 30°C %
Damazin	9003 hours	65%	0.4%
Dongola	5869.5 hours	55%	19.5%
Fashir	7927.5 hours	60.9%	25.2%
Khartoum	7773.5 hours	51%	10.2%
Portsudan	8700 hours	57%	3%

From the above table, it could be concluded that all samples were expoesd at all stations to temperature more than 25°C % for the most period of storage with a considerable excursions over 30°C % and this is contradicting to the labeled storage instructions for the product. The FDA specifies two excursions up to 30°C % after which the storage facility will be disqualified.

Physical Stability

Appearance: There were no significant changes in shape, colour or appearance.

Uniformity of weight: The B.P Appendix XII G *(Ph. Eur. Method 2.9.5)* specifies that for tablets containing more than 80 mg and less than 250 mg, not more than two tablets deviate from the average weight of 20 tablets by more than 5% and none deviates by more than 10%. All samples pass the test initially and at the end of the storage period at all stations with standard deviation of about 0.001. This indicates GMP measures and accurate and precise machinery systems.

Hardness testing: The results of minimum, maximum and mean showed a little variation initially and increased at the end of the storage period at all stations. This indicates the regular force being applied to tablets during compression to give uniform tablet hardness.

Loss on drying: The initial moisture content was 3.85%, finally it decreased in samples stored at all stations to 1.67% except samples stored at Portsudan giving 2.87%, due to high RH%.

Disintegration Time: The *USP*, *B.P* and *Ph. Eur* specify 30 minutes as limit for disintegration failure. The results obtained indicates that the product passes the disintegration test at the beginning and at the end of the storage period having a short uniformed time (4: 10 - 4:55 minutes).

Content of Lumefanterine component

Samples at all stations yielded results that were over 90 % potency, although the labeled expiration date had been passed four months ago from the end of the storage period.

Table (21): Content of Lumefantrine during the storage period.

Time month	Portsudan	Dongola	Khartoum	Damazin	Al-Fahir
0	100.3	100.3	100.3	100.3	100.3
0	100.3	100.3	100.3	100.3	100.3
1	102.7	100.3	100.8	99.9	101.5
6	104.15	106.93	99.89	103.59	99.67
12	96.4014	102.7959	101.1961	101.7449	103.5912
12	99.26614	103.0052	105.0607	103.3633	101.6426
12	101.2008	104.1771	106.1304	103.2703	103.6656
12	98.95609	103.3261	104.1291	102.7928	102.9665

There are insignificant differences between concentrations at different stations, sign. 0.087.

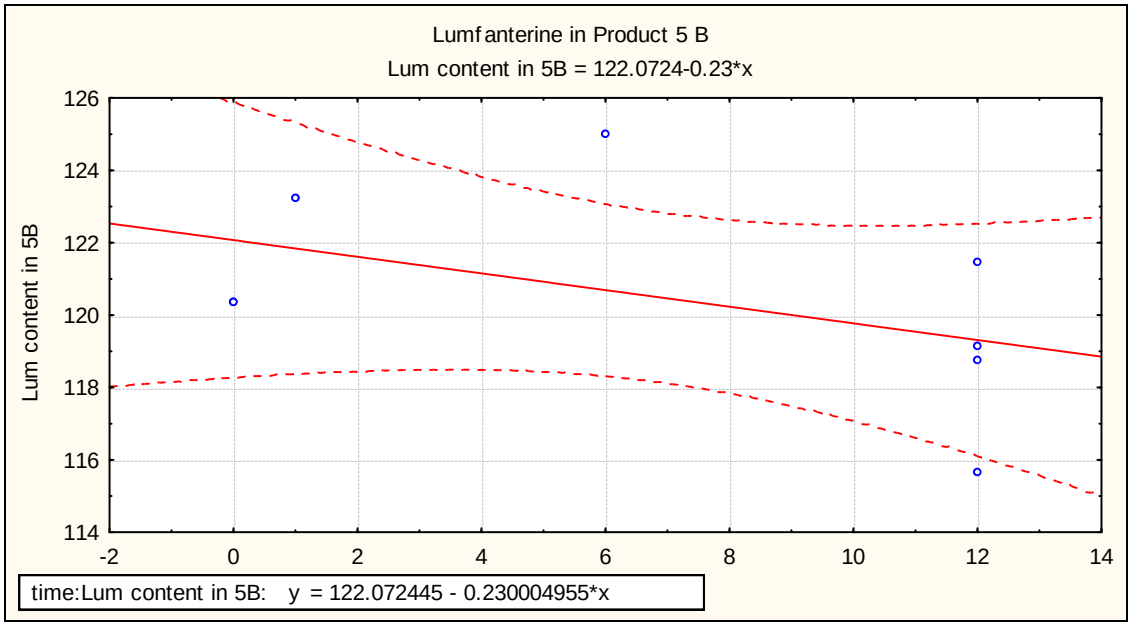


Figure (6): Content of lumefantrine during 12 months at Portsudan.

The LS regression equation is: C = 122.072445 – 0.230004955 × t, Thus, $t_{(90)}$ = 61.18 months.

Estimation of expiration date based on Lumefantrine

The ICH, FDA and EMRO stated that 5% one- sided lower CI was appropriate enough for drugs known to decrease with time. The 5% lower one-sided confidence interval for 108 mg intercepts the time axis could be given by Woolfs equation.

$$\frac{X-g\bar{x} \pm [t(Syx)/b]\,[\sqrt{(1-g)/N+(X-\bar{x})^2/\sum(x-\bar{x})^2}]}{1-g}, \text{ Where, } Syx = \sqrt{\frac{\sum y^2 - (\sum y)^2/N - b^2\sum(x-\bar{x})^2}{N-2}}$$

$g = t^2(S^2yx) / b^2\sum(x-\bar{x})^2$, and t value of 5% one sided CI at d.f 6 is 1.94.

LCI for $t\ (90)$ = 26.13 months. So, the product could be given a shelf life of two years from the begging of the study which was 11 months before the labelled expiration date. Thus, based on lumefantrine component, the shelf life may be extended for 15 months.

Content of Artemether component: Table (22) Content of Artemether during 12 months.

Time	fashir	damazin	khartoum	dongola	portsudan
00	102.2%	102.2%	102.2%	102.2%	102.2%
1	86.8%	87.6%	100.3%	108.7%	92.4%
6	110.6%	98.1%	97.9%	101%	86.2%
12	93.4%	102%	95.7%	93.7%	86.4%

Transportation to and from Portsudan was very harmful, that the concentration decline to less than 90%. Samples at Al- Fashir showed a marked decline in potency after 6 months and continued to decline throughout the storage period. All concentrations at the end of the storage period were above 90% for all stations except Al-Fashir.

Estimation of shelf life of Artemether at Dongola
Table (23): Computation of Results.

Product 5 G	content mg	X^2	Y^2	$(X-x)^2$
0.000	20.432	0.000	417.467	28.891
0.000	20.482	0.000	419.512	28.891
0.000	20.432	0.000	417.467	28.891
1.000	20.060	1.000	402.404	19.141
6.000	17.258	36.000	297.839	0.391
12.000	17.132	144.000	293.505	43.891
12.000	17.190	144.000	295.496	43.891
12.000	17.536	144.000	307.511	43.891
43.000	130.462	469.000	2448.797	237.875

On plotting contents content versus time, graph ( ) could be obtained.

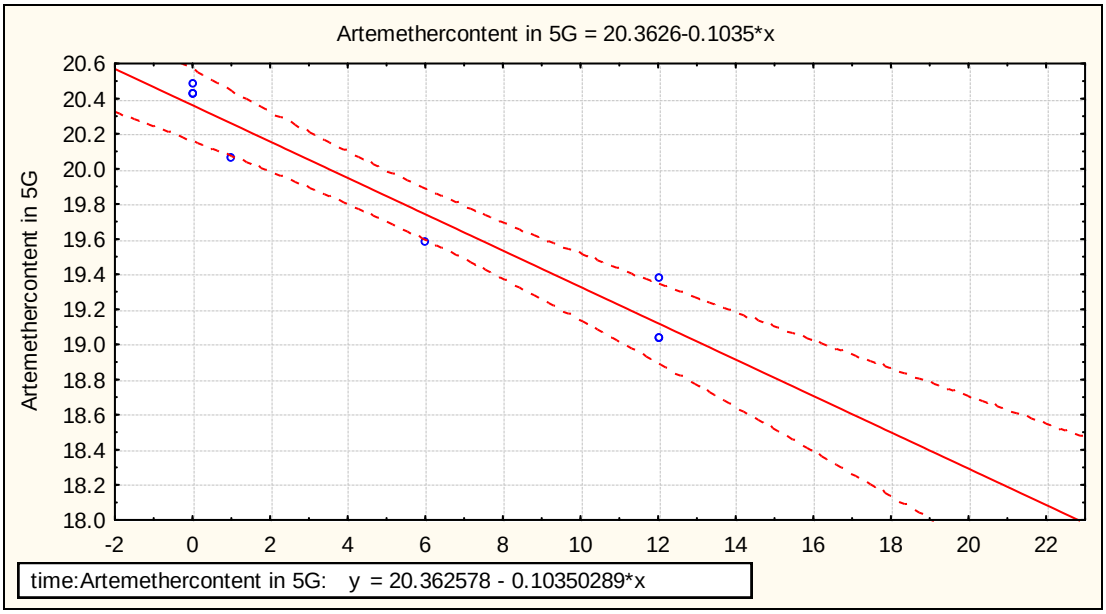


Figure (7): Artemether Content at Dongla versus Time.

The LSRLequation is: C = 20.362578 – 0.103502289 × t , So, the time required for the concentration to reach 18 mg was 22.8 months. The 5% lower two-sided CI incepts content 18 mg at 19 months. The 5% lower one-sided confidence interval for 18 mg intercepts the time axis could be given by Woolfs equation. $X\text{-}g\bar{x} \pm [t(Syx)/b] [\sqrt{(1-g)/N+(X-\bar{x})^2/\sum(x-\bar{x})^2}]$ 1-g,Where, $Syx = \sqrt{\sum y^2 - (\sum y)^2/N - b^2\sum(x-\bar{x})^2 /N}$-s Thus, *t(90)* = 19.84598868 months. So, additional six months from the labeled shelf life.

AL- Fashir

The LSR line equation is: C = 19.8885265 – 0.236423542 × t, Thus, *t (90)* = 7.99 months

The 5% lower one-sided confidence interval for 18 mg intercepts the time axis could be given by Woolfs equation.

$$\frac{X\text{-}g\bar{x} \pm [t(Syx)/b] [\sqrt{(1-g)/N+(X-\bar{x})^2/\sum(x-\bar{x})^2}]}{1-g}, \text{Where, } Syx = \sqrt{\frac{\sum y^2 - (\sum y)^2/N - b^2\sum(x-\bar{x})^2}{N-2}}$$

$g = t^2(S^2yx) / b^2\sum(x-x^{-}) ^2$, and t value of 5% one sided CI at d.f 6 is 1.94.

$$\frac{(8-0.19088168\times5.373)-1.94\times0.80911832/0.2364235 \sqrt{.80911832/8+6.89/234.875}}{0.80911832}$$

LCI for *t (90)* = 7.19148488 months. So, the product could be given a shelf life of 7 months, resulting in shelf life two months less than the labelled on.

Estimation of shelf life of Artemether at Portsudan

The LSR line equation is: C = 20.8159 – 0.1482 × t

From the equation, the time required for concentration to reach 18 mg=19 months.

The 5% lower band of the two-sided CI intercept the content of 18 mg at about 10 months. But, the ICH, FDA and EMRO stated that 5% one- sided lower CI was appropriate enough for drugs known to decrease with time. The 5% lower one-sided confidence interval for 18 mg intercepts the time axis could be given by Woolfs equation.

Thus, $t_{(90)}$ = 7.54729887 months and this was almost similar to that of Fashir.

Reaction rate constant; Results were used to calculate and compare reaction rate constant Using substitution method to define the order of reaction table (24)

Table (24): comparison of the rate constant values.

t	ko	ki	Kii
6	0.0365	0.001796	0.76103501
12	0.130833	0.006663	0.05307856
12	0.10225	0.005161	0.06791633
12	0.188833	0.009796	0.03677552
mean	0.114604	0.005854	0.22970135
St.d	0.063315	0.003323	0.35445067
CV	55.24639	56.77188	154.30935275

The order of reaction seems to be pseudo-zero order with a Ko = 1.14×10^{-1}.

Microbiological stability of Product 5

The total viable count was within the B.P limits which are 10^3 for aerobic bacteria and 10^2 for fungi. As no bacterial contamination was detected, so no evidence of presence of specific organism.

These results indicate the adherence to GMP measures of the manufacturer and the packaging system of the product was efficient enough to prevent microbiological contamination during its presence in the distribution channels of Sudan.

CONCLUSION

The conditions of the distribution channels in Sudan are inconvenient for medicinal products. The RTE over 25°C is 51 -61% and 25% over 30°C of the storage period. This reflects a considerable impact on the shelf-life of medicines as the labelled storage conditions are not met. The components of the antimalarial tablets Artemether + lumefantrine which should be stored below 30 C were subjected to this drastic conditions of transportation and storage and shows a quite different profile. The Lumefantrine component is stable enough to withstand these conditions giving content percentage 102% - 98.9% at the end of the storage conditions. it may be given shelf life of 37 months from the date of manufacture.

The shelf life of the product depends on the critical component, Artemether. The artemether concentration at the end of the storage period is above the 90%, the pharmacopoeial limit, except at Portsudan and this may be attributed to the high RH% and to the drastic transportation conditions which the medicine had faced in Al-Fashir and Portsudan. The RTE for the samples at al-Fashir is 60.9% over 25°C and 25.2% over 30°C. At Portsudan RTE is 57% over 25C and 3% over 30°C but the average relative humidity is 52% and during transportation, temperature reached over 45°C. The total exposure period is 7927 hours. However, in the other stations the product is satisfactory stable and its shelf –– life can be extended for extra 6 months.

The degradation reaction follows pseudo-zero order with a Ko = 1.14×10^{-1}.

The product retains its physical characters and no significant changes were reported. The product is microbiologically stable and no contamination took place and the results were within the pharmacopoeial limits indicating GMP compliance.

REFERENCES

1. B.P crown copyright volume III and IV.
2. BPC, PP 227-330,336,731. Published by the British Pharmaceutical Press, 1994.

3. *Bolton S and Charles Bones, Pharmaceutical Statistics Practical and Clinical, fourth edition, by Marcel Dekker, Transformation of Outlier PP 295. Regression analysis, 2010; 173-200.*
4. *Chinese Pharmacopoeia, 1995.*
5. *Ezzet F; et,al 2000, American Society for Microbiology, 44(3): 297-700.*
6. *Floence A and Atwood, School of Pharmacy, University of London, Physiochemical Principles of Pharmacy, Mc,illan Press Londom, Third edition, 1998; 101,151,136,140,142,146.*
7. *Hogerzil, Goeje and AbuRaid, Inland Stability Study (Sudan), WHO/DAP/914, WHO Action Programme on Essential Drugs and Vaccines, 1991.*
8. *Huber, Pharmaceutical Process Validation, Third edition By Marcel Dekker, 2003; 514.*
9. *International pharmacopoeia, third edition, 2003; 5: 187-198.*
10. *International pharmacopoeia, 10/2006; 43.*
11. Jeffs P, International Stability testing, published by By Mazzo, 1998.
12. *Kinghub Lin, Elsorevier, 2005.*
13. *Kommanoboyina B and Rhodes, Department of applied Pharmaceutical Sciences, University of Rhode Island, Kingstone. Trends in Stability Testing, with Emphasis on Stability During Distribution and Storage, Drug Development and Industrial Pharmacy, 1999; 25(7): 857-868).*
14. *Kommanoboyina B and Rhodes, Department of applied Pharmaceutical Sciences, University of Rhode Island, Kingstone. Effects of Temperature Excursions on Mean Kinetic Temperature and Shelf-life, Drug Development and Industrial Pharmacy, 1999; 25(12): 1301-1306.*
15. Rhodes C.T, University of Rhode Island, Drug Stability Priciples and Practices, chapter 1, Reasons for Stability Testing, PP10, 11, By Marcel Dekker, 2000.
16. Sinko P, Park-Davis, Prof. in pharmaceutics, Ernest Mario School of Pharmacy, Neo Jersy, USA, Martin's Physical Pharmacy and Pharmaceutical Sciences, by Lippin-cott Williams and Wilkin-N.Y. USA, Chapter 15, Chemical Kinetic and Stability, 2006; 397-423, 403, 42, 411.
17. *USP 30, 1/8/2007 for Windows by U.S pharmacopeia 12601 Twinbrook Pakway, Rockville,MD 20852 USA. Authentication No. 03-DXG15H1401.*
18. *WHO 7/ An update on quality assurance and procurement through WHO for improving access to artesmisinin based combination treatment for malaria. Malaria control department, Geneva, 2003.*
19. Walter Susan, International Stability Testing, 1999; 265-290.