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# AN *IN VITRO* RELEASE STUDY OF DRUG – DRUG INTERACTION BETWEEN LUMEFANTRINE AND AMOXICILLIN

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## ABSTRACT

An *in vitro* investigation of the drug – drug interaction between a commercial brand each of an artemisinin combination therapy (ACT) antimalarial (Sample A) containing 120 mg Lumefantrine (LMF) and 20 mg Artemeter (ATM) tablet and an antibiotic, amoxicillin (Sample B), containing 500 mg amoxicillin trihydrate (AMO) capsules on the drug release profile of LMF and AMO by dissolution studies was conducted. Lumefantrine release profile was conducted in fresh preparations of the following media (a) a surfactant, 1% w/v benzalkonium chloride (BZK) in 0.1 M Hydrochloric acid, HCl (pH 1.63) and (b) 1% w/v BZK and AMO in 0.1 M HCl . Amoxicillin release profile was conducted in medium of (a) 0.1 M HCl (pH 1.5), (b) 1% w/v BZK in 0.1 M HCl and (c) LMF and 1% w/v BZK in 0.1 M HCl. USP II method for dissolution testing was used. Reverse Phase Liquid Chromatography (HPLC) was used in analysis of withdrawn drug samples. Results show a three fold release of AMO in media containing 1% w/v BZK and 0.1 M HCl than in 0.1 M HCl alone. A similar result was obtained in the medium containing LMF and 1% w/v BZK and this can be attributed to the presence of the surfactant. The HPLC retention time was higher for LMF (7.13 min) than AMO (1.21 min). Lumefantrine release was significantly reduced in amoxicillin (25.02%) at the time for 30% of drug release ($T_{30}$) while there was an increment of 16.32% at $T_{50}$ (time

for 50% drug release) suggesting the existence of a non time dependent interaction between LMF and AMO. However the level of interaction could not be ascertained in the presence of the 1% w/v BZK.

**KEYWORDS:** Lumefantrine, amoxicillin, surfactant, drug – drug interaction, dissolution studies, USP (United States Pharmacopoeia).

## INTRODUCTION

Therapeutically, the concurrent use of two, three or more drugs is often essential and sometimes mandatory to achieve a desired therapeutic goal or to treat co-existing diseases.[1] Malaria, a disease transmitted amongst humans by mosquitoes remains one of the important diseases of the developing world, killing 1 – 3 million people and causing disease in 300 – 500 million people annually. [2] Africa has 90% of all malaria cases in the world with a high mortality rate amongst children and pregnant women. Nigeria is an endemic area of malarial infection. [3] Antimalarial drugs are used to treat, manage and prevent the mortality and morbidity caused by the disease. Resistance to antimalarial drugs within the last two decades has led to high treatment failure and the introduction of Artemisinin Combination Therapy (ACTs) by the World Health Organization (WHO) for treatment of uncomplicated malaria in sub Saharan Africa. [4] Some times malarial infection is accompanied by other infections such as typhoid fever and respiratory tract infections which would necessitate the concurrent use of an antimalarial, antibiotic, analgesic and haematinic. Such concurrent use of multiple drugs and combination products in medical practice has resulted in an ever increasing potential for drug – drug interactions.[5] Drug – drug interaction can be defined as the modification of the pharmacological activity of one drug by prior or concomitant administration with another drug. Some of such interactions can be synergistic and of benefit to the patient while others can have adverse effects of great concern. [6] Artemeter and Lumefantrine combination is synergistic *in vitro* against *Plasmodium falciparum*.[7]Drug – drug interactions can be broadly classified into pharmacokinetic and pharmacodynamic interactions. Pharmacokinetic interactions involve interference with and/or alteration of the absorption, distribution, biotransformation or excretion processes of other drugs. Pharmacodynamic interactions involve action of the drugs on the same target site resulting in clinical manifestation as either synergism or antagonism. Amoxicillin, a beta – lactam antibiotic stable in acidic medium is designed both for oral and parenteral use. Orally it is completely absorbed with over 85% bioavailability. Its half life is 80 min and food does not

interfere with its absorption. Its volume of distribution is 0.3 L/kg in humans and it is widely distributed to many tissues.[8] Clinically, the combination of artemether and Lumefantrine has proved to be highly effective and well tolerated in *P. falciparum* malaria treatment in children and adults including those infections caused by multi-drug resistant *P. falciparum*.[8, 9, 10]

Dissolution testing is an important tool of pharmaceutical quality control studies and can be used to predict bioavailability, and sometimes to determine bioequivalence especially in cases where it is difficult to conduct clinical studies.[11]

## MATERIALS AND METHOD

### Equipment

High Pressure Liquid Chromatography (HPLC) model: Agilent Technologies 1200 series, UV/Vis spectrophotometer (Parker Elmer Lamba 25), Dissolution apparatus (Electrolab TDT – 08L (USP), Analytical balance (Denver).

**Materials :** Acetonitrile HPLC grade, Dimethylformamide, Ethylacetate, Sodium dihydrogen phosphate, Hydrochloric acid, Acetone, Silica gel (all obtained from BDH, UK), Amoxicillin powder (Fidson Lab.,Nigeria), Lumefantrine powder (LUTH, Nigeria), Sample A tablets(commercial brand of 120 mg Lumefantrine and 20 mg arthemeter) and Sample B capsules(commercial brand of 500 mg amoxicillin capsules).

**Method:** Sample A tablets and B capsules were purchased from a local retail pharmacy outlet in Ibadan, Nigeria. Both drug formulations were subjected to uniformity of weight and content of active ingredient tests.

### Uniformity of weight

Twenty tablets and capsules of samples A and B were randomly selected and weighed individually to ascertain any variation in weight.

### Lumefantrine Assay

Twenty tablets of sample A were collectively weighed into a Wedgewood mortar, triturated to fine powder and 28.43 mg, a quantity equivalent to the weight of one tablet weighed into a 5 ml volumetric flask. A 1.5 ml volume of Tetrahydrofuran (THF) was added and shaken to dissolve the LMF, then acetonitrile was added to make up to the 5 ml mark. A concentration of 3 mg/ml of solution equivalent to 3000 µg/ml of LMF and 500 µg/ml of arthemeter was

sonicated in an ultrasonic bath for 15 min to enhance dissolution. Filtration was done using aerodisc syringe filters of 0.45 µm pore size. Further dilutions were done to obtain concentrations of 1500 µg/ml of LMF and 250 mg/ml of arthemeter. 15 mg and 2.5 mg of reference(standard) powders of LMF and ATM respectively were subjected to the above procedures. 20 µL of the resulting test and reference (standard) solutions were injected into the HPLC column and the peak areas recorded. Assay conditions were at 30˚C, flow rate of 1 ml/min and wavelength of 265 nm. Mobile phase consisted of phosphate buffer (pH 3.0, 0.025 M): methanol : acetonitrile (50:25:25).

**Amoxicillin Assay**

Determination of content of amoxicillin in sample B capsules was done by HPLC and the International Pharmacopoeia (IP) 2006 methods. In the HPLC method, ten sample B capsules were emptied together, triturated and 5mg of powder taken. A 1 mg/ml stock solution of sample was made to obtain a 100 µg/ml solution with acetonitrile. The same was done with a reference(standard) amoxicillin sample. 20 µL solution of both sample and reference material were injected into the HPLC and the assay conducted. [10]

**Standard Calibration Curve for Amoxicillin**

A 5 mg quantity of LMF reference(standard) powder was weighed into a 5 ml volumetric flask. It was dissolved and made up to the 5 ml mark with freshly prepared solution of 1% w/v BZK in 0.1 M HCl to obtain a 1 mg/mL stock solution. Dilutions were made with the same medium to obtain a 5, 10, 25, 50, 75 and 100 µg/ml solutions. 20 µL of each dilution was injected into the HPLC column at a flow rate of 1ml /min and peak areas of concentration noted at 278 nm. Determinations were done in replicates. The mobile phase was acetonitrile: water : 25 mM $KH_2PO4$ (pH 3.65) at a ratio of 67.5 : 10 : 22.5 at 30˚C.

**Validation of Analytical Methods For Test and Reference materials**

Three concentrations of 10, 50, and 100 µg/ml of both test and reference material were injected into the columns and determined by HPLC. Instrumental response and concentrations for a day were used to determine intraday validity while that of three days was used for interday. Determinations were done in replicates. Results were used to determine precision and accuracy.

## DISSOLUTION TESTS

USP II method for the dissolution tests for capsules and tablets was adopted. A 900 ml of freshly prepared 1% w/v BZK maintained at 37 ± 1˚C and paddle set to rotate at 100 ± 1 rpm was used. The test period was for 60 min and sampling was at 10 min intervals. Aliquot samples collected were filtered using 0.45 µm aerodisc syringe filter and assayed by HPLC. Five (5) ml of dissolution medium maintained at 37˚C was used to replace withdrawn samples after each sampling. Amoxicillin test and control dissolution profiles were determined in replicates. Sample A test was done without a control because of solubility problems associated with LMF in aqueous media such as 0.1 M HCl.

### Statistical evaluation

Results were evaluated using standard estimation of means (SEM) and the student t test.

## RESULTS AND DISCUSSION

### Weight variation tests:

Test results of both sample A tablets and sample B capsules (Tables 1 and 2 respectively) conformed to official specifications of ± 7.5 % of the labeled tablet or capsule weight [12]

**Table 1 : Uniformity of weight determinations of Lumefantrine (Sample A) tablets.**

| S/N | Weight of tablet (g) | Absolute deviation | Relative deviation | Percentage deviation (%) |
|---|---|---|---|---|
| 1 | 0.227 | 0.0005 | 0.0022 | 0.22 |
| 2 | 0.226 | 0.0015 | 0.0066 | 0.66 |
| 3 | 0.229 | 0.0015 | 0.0066 | 0.66 |
| 4 | 0.229 | 0.0015 | 0.0066 | 0.66 |
| 5 | 0.226 | 0.0015 | 0.0066 | 0.66 |
| 6 | 0.226 | 0.0015 | 0.0066 | 0.66 |
| 7 | 0.226 | 0.0015 | 0.0066 | 0.66 |
| 8 | 0.227 | 0.0005 | 0.0022 | 0.22 |
| 9 | 0.229 | 0.0015 | 0.0066 | 0.66 |
| 10 | 0.228 | 0.0025 | 0.0022 | 0.22 |
| 11 | 0.230 | 0.0005 | 0.0110 | 1.10 |
| 12 | 0.228 | 0.0025 | 0.0022 | 0.22 |
| 13 | 0.228 | 0.0025 | 0.0022 | 0.22 |
| 14 | 0.226 | 0.0015 | 0.0066 | 0.66 |
| 15 | 0.227 | 0.0005 | 0.0022 | 0.22 |
| 16 | 0.230 | 0.0025 | 0.0110 | 1.1 |
| 17 | 0.226 | 0.0015 | 0.0066 | 0.66 |
| 18 | 0.226 | 0.0015 | 0.0066 | 0.66 |
| 19 | 0.228 | 0.0025 | 0.0022 | 0.22 |
| 20 | 0.228 | 0.0025 | 0.0022 | 0.22 |

Mean tab.weight = 0.2275g, SD = 0.001603, Coefficient of variation(CV) = 0.70%

**Table 2 : Uniformity of weight determination for amoxicillin capsules (Sample B)**

| S/N | Weight of amoxicillin capsules(g) | Weight of powder content (g) | Absolute deviation | Relative deviation | Percent deviation(%) |
|---|---|---|---|---|---|
| 1 | 0.642 | 0.541 | 0.0123 | 0.0223 | 2.22 |
| 2 | 0.652 | 0.551 | 0.0023 | 0.0042 | 0.42 |
| 3 | 0.642 | 0.541 | 0.0123 | 0.0222 | 2.22 |
| 4 | 0.653 | 0.555 | 0.0017 | 0.0031 | 0.31 |
| 5 | 0.662 | 0.554 | 0.0007 | 0.0013 | 0.13 |
| 6 | 0.655 | 0.558 | 0.0047 | 0.0085 | 0.85 |
| 7 | 0.657 | 0.560 | 0.0067 | 0.0121 | 1.21 |
| 8 | 0.645 | 0.544 | 0.0093 | 0.0168 | 1.68 |
| 9 | 0.655 | 0.558 | 0.0047 | 0.0085 | 0.85 |
| 10 | 0,658 | 0.558 | 0.0047 | 0.0085 | 0.85 |
| 11 | 0.638 | 0.540 | 0.0133 | 0.0240 | 2.40 |
| 12 | 0.650 | 0.550 | 0.0033 | 0.0060 | 0.60 |
| 13 | 0.642 | 0.546 | 0.0073 | 0.0132 | 1.32 |
| 14 | 0.660 | 0.563 | 0.0097 | 0.0175 | 1.75 |
| 15 | 0.651 | 0.553 | 0.0003 | 0.0005 | 0.05 |
| 16 | 0.656 | 0.558 | 0.0047 | 0.0085 | 0.85 |
| 17 | 0.660 | 0.562 | 0.0087 | 0.0157 | 1.57 |
| 18 | 0.660 | 0.558 | 0.0047 | 0.0085 | 0.85 |
| 19 | 0.653 | 0.556 | 0.0027 | 0.0049 | 0.49 |
| 20 | 0.657 | 0.560 | 0.0067 | 0.0120 | 1.21 |

Mean powder content = 0.553g, SD = 0.0079, Coefficient of variation(CV) = 1.43%

**Assay of Lumefantrin in Sample A tablet by HPLC method**

Table 3 shows results of assay of lumefantrine in sample A tablets. A value of 99.46 ± 0.89 % was obtained. This is found to conform to IP 2006 draft proposal which states that not less than 98.5% and not more than 101.1% of LMF should be contained. [13]

**Table 1 : Assay results of Lumefantrine in sample A tablets**

| Peak area of sample A(1500µg) | Peak area of reference(1500µg) | Peak area ratio | Conc. LMF | Percentage LMF (%) |
|---|---|---|---|---|
| $11.551 \times 10^4$ | $11.719 \times 10^4$ | 0.9857 | 118.284 | 98.57 |
| $12.345 \times 10^4$ | $12.365 \times 10^4$ | 0.9983 | 119.796 | 99.83 |
| $12.398 \times 10^4$ | $12.401 \times 10^4$ | 0.9997 | 119.97 | 99.97 |

Mean % content = 99.46 % and conc. of LMF in sample A = 119.34 ± 0.89 mg

**Table 2 : Assay results of amoxicillin capsules.**

| Peak area of sample B (100µg) | Peak area of reference(100µg) | Peak area ratio | Conc. of amoxicillin (mg) | Percentage of amoxicillin(%) |
|---|---|---|---|---|
| 215.970 | 227.012 | 0.951 | 475.5 | 95.1 |
| 211.651 | 215.731 | 0.981 | 490.5 | 98.1 |
| 223.352 | 229.140 | 0.975 | 487.5 | 97.5 |

Mean % content = 96.88 % and conc of amoxicillin in sample B = 484.5 ± 1.60 mg

**Assay of amoxicillin in Sample B capsule by HPLC method**

Results of assay tests for amoxicillin trihydrate is shown in Table 2. Content of AMO values obtained were between 95.06% to 98.11% and were found to conform to British Pharmacopoeia (BP) specifications which states that product must contain between 92.5 to 110.0% of the labeled amount. [14] Using the International pharmacopoeia (IP) method, assay result gave a mean value of 98.68% which also conforms to the IP specification of 95.0 to 102.0%.[15]

**Calibration Curves for Amoxicillin and Lumefantrine**

The correlation coefficient ($R^2$) of the plots showed values of 0.994 and 0.971 for AMO and LMF reference materials respectively. This indicates linearity between concentration and peak area in milliabsorbance units.

**Validation of analytical results**

The intraday precision and accuracy results for 10, 50, and 100 µg/ml of LMF the intraday precision and accuracy for 10, 50, 100 µg/ml were 6.94, 5.44, 9.42 % and 103.97 ± 8.42, 85.73 ± 4.85 and 95.01 ± 9.18 while the interday precision and accuracy was 4.59, 6.24, 4.20 % and 118, 85 and 101.36% respectively. For AMO the result were 3.99, 1.58, 4.67 and 89.88 ± 8.11, 99.51 ± 1.43 and 89.45 ± 4.34% respectively while the interday precision and accuracy for 10, 50, and 100 µg/ml were 6.94, 5.44, 9.42 % and 89.06 ± 7.14,104.23 ± 6.74 and 94.85 ± 4.81% respectively.

**Dissolution profiles of Amoxicillin and Lumefantrine**

The dissolution profiles of amoxicillin in (a) 0.1 M HCl, (b)1% w/v BZK in 0.1 M HCl, and (c) Lumefantrine in 0.1 M HCl and 1% BZK is shown in Fig.1. In 0.1 M HCL, up to 57.43% of amoxicillin was released within 10 min (a burst effect), after which there was a gradual increase in its release. At 40 min ($T_{40}$) 80.17 ± 5.47% (445.86 ± 27.37 mg) was released. At $T_{50}$ and $T_{60}$ there was a steady decline in drug release. The release profile of amoxicillin in the medium containing 0.1 M HCl with 1% w/v BZK shows 140.84 ± 7.79% release at 10 min which steadily increased to 241.00 ± 7.17% (1205.04 ± 29.25 mg) at 40 min. There was a decline in drug release at 50 and 60 min respectively. This indicates that 1% w/v BZK (surfactant) greatly affected its solubility. The medium containing LMF and 1% w/v BZK in 0.1 M HCl showed a maximum release of 240.76 ± 7.18% at 40 min. The pattern of amoxicillin release was similar to that in 1% w/v BZK. Thus LMF did not significantly

increase amoxicillin release when compared with the surfactant. Also more than 50% of amoxicillin was released before 10 min in the three media (Fig.1).

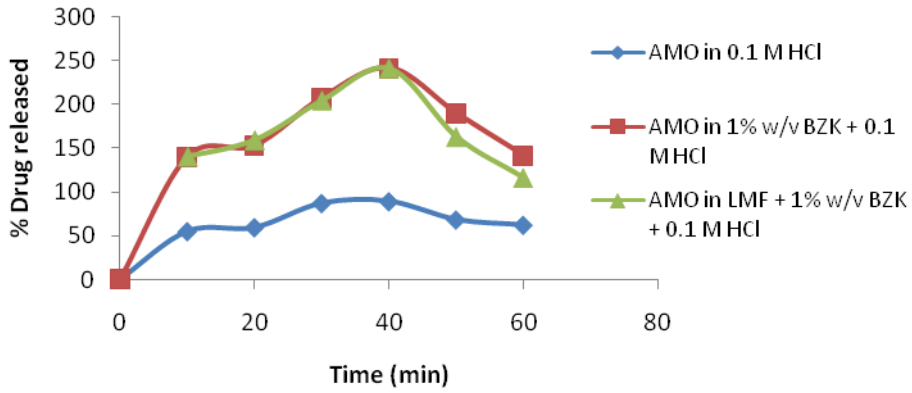


**Fig.1 : Dissolution profile of amoxicillin in 0.1 M HCl, 1% w/v BZK in 0.1 M HCl and Lumefantrine + 1% w/v BZK in 0.1 M HCL**

The dissolution profile of Sample A tablets in two different media viz 1% w/v BZK in 0.1 M HCl, and AMO with 1% w/v BZK in 0.1 M HCl are shown in Fig 2.

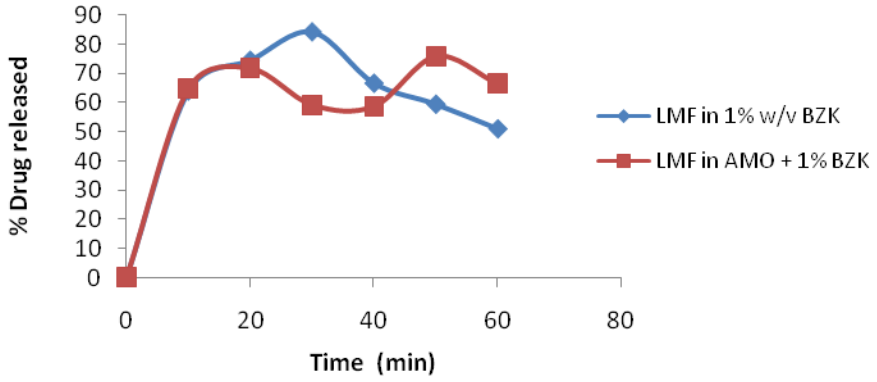


**Fig.2 : Dissolution profile of Lumefantrine(LMF) in 1% w/v BZK and in Amoxicillin(AMO) + 1% w/v BZK in 0.1 M HCl**

Up to 63.03% of LMF was sharply released upon hydration within 10 min after which there was a gentle but steady increment cumulating to 84.20 % in 30 min. There was a steady decline in release between 30 and 60 min. The dissolution profile of LMF in the presence of AMO using 1% w/v BZK in 0.1 M HCl was similar to that in medium containing LMF alone within 0 – 20 min. However, there was a decline or depression at between 30 and 40 min before peaking at 50 min and decreasing thereafter till 60 min . This double peak is indicative of an interaction between amoxicillin and lumefantrine. The amount released at 45

min was 63.00 ± 1.30 % in the medium containing surfactant alone and 66.60% in the medium containing amoxicillin and surfactant in 0.1 M HCl. These values fall within the USP (United States Pharmacopeia) dissolution tolerance which states that not less than 60% of the labeled amount of lumefantrine be dissolved in 45 min.[16]

## CONCLUSION

From the methods adopted which involved both dissolution and HPLC studies, it was observed that there was a drug – drug interaction between lumefantrine, an antimalarial and amoxicillin, an antibiotic. However results of greater than 100% release for amoxicillin in the presence of 1% w/v benzalkonium chloride, a surfactant could be an indication of interference with the dissolution process by other highly polar materials from the surfactant. Alternatively, it could be suggestive that amoxicillin release is potentiated *in vitro* by lumefantrine especially in the presence of a surfactant. It is recommended that further studies be conducted to establish the nature of interaction between these two drugs.

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