

**COMPARATIVE *IN VITRO* BIOEQUIVALENCE EVALUATION OF SIX BRANDS OF  
AMOXICILLIN CAPSULE MARKETED IN DUTSE, JIGAWA STATE, NIGERIA.**

**BY**

**Inuwa BELLO (B. Pharm. ABU., 2008)**

**P14PHMC8002**

**A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES,  
AHMADU BELLO UNIVERSITY, ZARIA  
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD  
OF A  
MASTER DEGREE IN PHARMACEUTICAL CHEMISTRY**

**DEPARTMENT OF PHARMACEUTICAL AND MEDICINAL CHEMISTRY,  
FACULTY OF PHARMACEUTICAL SCIENCES,  
AHMADU BELLO UNIVERSITY,  
ZARIA**

**JULY, 2017**

## DECLARATION

I declare that the work in this dissertation titled “Comparative *in vitro* Bioequivalence Evaluation of Six Brands of Amoxicillin Capsule Marketed in Dutse, Jigawa State, Nigeria” has been carried out by me in the Department of Pharmaceutical and Medicinal Chemistry. The information derived from the literature has been acknowledged accordingly in the text and a list of references provided. No part of this dissertation was previously presented for another Degree or Diploma in this or any other Institution.

Inuwa Bello

SignatureDate

\_\_\_\_\_

\_\_\_\_\_

### **Certification**

This dissertation titled “Comparative *in vitro* Bioequivalence Evaluation of Six Brands of Amoxicillin Capsule Marketed in Dutse, Jigawa State, Nigeria” meets the regulations governing the award of the degree of M.Sc. Pharmaceutical Chemistry of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

(Signature)\_\_\_\_\_

(Prof. Ibrahim Adamu Yakasai)  
Chairman, Supervisory Committee

Date\_\_\_\_\_

(Signature)\_\_\_\_\_

(Dr. M. A. Usman)  
Member, Supervisory Committee

Date\_\_\_\_\_

(Signature)\_\_\_\_\_

(Prof. A. M. Musa)  
Head of Department

Date\_\_\_\_\_

(Signature)\_\_\_\_\_

(Prof. S. Z. Abubakar)  
Dean, School of Postgraduate Studies

Date\_\_\_\_\_

## ACKNOWLEDGMENT

All Praise and adorations are due to Allah (SWT) the creator and sustainer of the universe who in His boundless compassion made this work a reality. May the Peace and blessings of Allah be on His noble Messenger, members of his household, his rightly guided companions and those who followed their path up to the last hour. My special appreciation goes to my supervisors, Dr. M.A. Usman and Prof. I.A. Yakasai. Thank you for your perseverance, guidance, support and commitment during the course of the work. My sincere appreciation also goes to Prof. Aliyu Musa Head of the Department of Pharmaceutical and Medicinal Chemistry. To our Lecturers I want to say big thank you sirs. However, my utmost appreciations go to my late Father Alh Bello Yahaya May Allah have mercy on your gentle soul, my Mother Malama Yahanasu Abdulkadir your prayers, support and encouragement were answered. To my beloved wife Fatima Abdullahi (Ummu Ammar) who is always there for me, Also my children Ammar, Mustapha and Aisha. I said a very big and warm thank you for being supportive. My Siblings Mustapha Bello (Yayana), his wife Aisha Abdullahi (Yayata), Yahaya Bello, Ayuba Bello (Madaki), Sulaiman Bello, Nasiru Bello, Adamu Bello, Abdullahi and my sisters Aisha Bello (Yaya Indo), Hauwa Bello (Yaya ta Zaria), Maryam Bello (Mairo), Rukayya Bello (Yayannan) and lastly Hauwa Bello (Gwaggo) you were very wonderful brothers and sisters, I also, acknowledged the support of my half brothers and sister namely; Abubakar Ibrahim (Habu), Kabiru Ibrahim (KB) and Juwairiyya Ibrahim (Yayannan). My appreciation will not be complete without acknowledging my good friends Pharm.(Malam). Salisu Awwalu, Pharm Hussaini M.A., Pharm. Muhammad S. A., Pharm. Simon Bitrus Gwary, A3, Yusuf, worthy to mention also are Sani Ado Yusuf, Nasuru Sani Musa, M Bala Riti, M. Nafi'u, Pharm. Hamza M Kakudi, Pharm. Hasiya A. L., and many others too numerous to mention here. My parent-in-laws, friends, colleagues and well-wishers will not be forgotten. Finally, my sincere appreciation goes to all the technical and the non-technical staff of the Department of Pharmaceutical and Medicinal Chemistry as well as anybody who in one way or the other has contributed to the success of this work. May the Almighty Allah bless your life all.

## ABSTRACT

Comparative *in vitro* bioequivalence study of biopharmaceutics class I and III drugs had gained prominence in recent times. *In vitro* bioequivalence offered many benefits compared to conventional *in-vivo* bioequivalence studies, due to its reduced cost and time of product release as well as avoiding unnecessary use of human volunteers. This study is aimed at evaluating and comparing the *in vitro* bioequivalence of branded and generic amoxicillin capsules available in Dutse, Jigawa State, Nigeria. These samples were randomly selected and evaluated for quality control studies via BP 2009 and USP 2009 specifications. Four UV Spectrophotometry methods for the determination of amoxicillin in simulated physiological media (pH 1.2, 4.5, 6.8, and 7.4), were developed and validated according to ICH guideline. Dissolution testing was conducted using USP apparatus I, sink volume of 900 ml, temperature  $37 \pm 0.5$  °C and 100 rpm, samples were withdrawn at an interval of 5, 15, 25, 35 and 45 minutes respectively. Bioequivalence of the samples were compared using different statistical methods; the difference factor ( $f_1$ ), similarity factor ( $f_2$ ) and the dissolution efficiency (% D.E.). From the result of quality control studies, all the brands were found to passed identification test as their IR spectra were superimposable with reference amoxicillin spectrum. Four of the six brands (A, B, C and E) passed the assay test, (90-120) USP 2009 while, brands D and F failed. The uniformity of weight test all the brands passed with percentage mean deviation  $<7.5\%$  (BP 2009). All the brands disintegrated in less than 15 minutes as required by BP 2009. The four methods developed for the determination of amoxicillin have  $\lambda_{max}$ . 229nm for buffer solutions (pH 1.2 and 4.5) and 228nm for (pH 6.8 and 7.4). The calibration curves were linear at the concentration range of 10-60  $\mu\text{g/ml}$  their correlation co-efficient were 0.999,

0.997, 0.997 and 0.996 for buffer solutions (pH 1.2, 4.5, 6.8 and 7.4) respectively. A regression equation of  $y = 0.0133x + 0.1847$ ,  $y = 0.0137x + 0.1967$ ,  $y = 0.0143x + 0.2113$ , and  $y = 0.0162x + 0.0807$  for (pH 1.2, 4.5, 6.8 and 7.4) respectively. The percentage recoveries of the developed methods were within the official range of 98-102%. Likewise, the intra-day and inter-day precision were within normal range of co-efficient of variation <15% (2.05% ,3.46%,2.48% ,0.66% and 9.15%,9.00%,5.82%,0.66%) for the simulated media (pH 1.2, 4.5, 6.8 and 7.4) respectively. The dissolution profiles obtained for each medium was subjected to bioequivalence comparison. The result of  $f_1$  for brands B and E were similar and within the acceptable range of  $\leq 15$  in each pHs. Similarly, the  $f_2$  values were  $\geq 50$  in pH 1.2 and 4.5 for B and pH 4.5 for E while in pH 6.8 and 7.4 for B and pH 1.2, 6.8 and 7.4 for E were below the acceptable range thus, failed  $f_2$  test. Also the % D.E. values of each of the simulated pHs for brands B and E were within the acceptable limit of  $\pm 10$  %. So, from the result they are considered bioequivalent with A. While, brands C, D and F failed both  $f_1$ ,  $f_2$  and % D. E. comparison as their values were outside the accepted range. Also, the analysis of variance (ANOVA) and dunnett multiple comparison results further, confirmed the observed difference among the brands obtained using  $f_1$ ,  $f_2$  and % D.E methods at ( $p < 0.05$ ). Therefore, brands C, D and F, are not bioequivalent with A. About 60 % of the samples may not be considered bioequivalent with innovator (A).

<b>Table of contents</b>	<b>Pg. №</b>
Title Page.....	i
Declaration.....	ii
Certification.....	iii
Acknowledgement.....	iv
Abstract.....	v
Table of contents.....	vii
List of Figures.....	xi
List of Tables.....	xii
List of Appendices.....	xiii
Abbreviations.....	xv
<b>CHAPTER ONE</b>	
<b>1.1</b>	
<b>INTRODUCTION.....</b>	<b>1</b>
<b>1.2 Statement of the Research Problem.....</b>	<b>7</b>
<b>1.3</b>	
<b>Justification.....</b>	<b>7</b>
<b>1.4 Research Hypothesis.....</b>	<b>8</b>
1.4.1 Null hypothesis.....	8
1.4.2 Alternate hypothesis.....	8

<b>1.5</b>	<b>Aim and Objectives of the</b>	
<b>Study.....</b>		<b>8</b>
1.5.1		
Aim.....		8
1.5.2		
Objectives.....		8

## **CHAPTER TWO**

<b>2.0</b>		<b>LITERATURE</b>
<b>REVIEW.....</b>		<b>9</b>
<b>2.1</b>	<b>Physicochemical Properties of</b>	
<b>Amoxicillin.....</b>		<b>10</b>
<b>2.2</b>	<b>Analytical Methods of</b>	
<b>Amoxicillin.....</b>		<b>11</b>
2.2.1	Compendial methods of	
analysis.....		11
2.2.2	Reported methods of analysis from literature.....	12
<b>2.3</b>	<b>Structure Activity</b>	
<b>Relationship.....</b>		<b>13</b>
<b>2.4</b>	<b>Pharmacokinetics.....</b>	<b>14</b>
<b>2.5</b>		
<b>Metabolism.....</b>		<b>15</b>
<b>2.6</b>	<b>Mechanism of Action of Amoxicillin.....</b>	<b>15</b>

<b>2.7</b>		<b>Therapeutic</b>	
	<b>Indications.....</b>		<b>16</b>
<b>2.8</b>	<b>Adverse Effects.....</b>		<b>16</b>
<b>2.9</b>	<b>Some Reported Literatures on <i>In vitro</i> Dissolution.....</b>		<b>17</b>
<b>2.10</b>	<b>Relationship of Dissolution test with Bioavailability.....</b>		<b>18</b>
<b>2.11</b>	<b>Advantages of <i>In vitro</i> Dissolution to Bioequivalence.....</b>		<b>20</b>
<b>2.12</b>	<b>Post Market Assessment.....</b>		<b>23</b>
<b>2.13</b>	<b>Multisource (Generic) Pharmaceutical</b>		
	<b>Products.....</b>		<b>24</b>
<b>2.14</b>	<b>Fake and Counterfeit</b>		
	<b>Drugs.....</b>		<b>25</b>
 <b>CHAPTER THREE</b>			
<b>3.0</b>	<b>MATERIALS</b>	<b>AND</b>	
	<b>METHODS.....</b>		<b>28</b>
<b>3.1</b>	<b>Materials.....</b>		<b>28</b>
3.1.1	Drugs.....		28
3.1.2	Glass wares and accessories.....		28
3.1.3	Equipment and instruments.....		28
3.1.4	Reagents.....		29
<b>3.2</b>	<b>Methods.....</b>		<b>30</b>
3.2.1	Sample survey and purchase of amoxicillin capsule and coding.....		30

3.2.2	Physical	inspection	of	NAFDAC	
	requirements.....				30
3.2.3	Identification	test	of	pure	amoxicillin
	powder.....				30
3.2.4	Quality	control	of	amoxicillin	
	capsules.....				31
3.2.5	UV	spectrophotometric		methods	
	development.....				32
3.2.6	Validation	of	the	developed	
	methods.....				32
3.2.7	determination	of	<i>in vitro</i>	dissolution	profiles of the
	samples.....				33
3.2.8	Determination	of	<i>in vitro</i>	bioequivalence.....	34

## CHAPTER FOUR

### 4.0

#### RESULTS.....37

#### 4.1 Labeling Characteristics Result.....37

#### 4.2 Quality control studies .....38

4.2.1	Identification	of	amoxicillin	standard	
	powder.....				38
4.2.2	Identification	test	of	amoxicillin	
	capsules.....				39
4.2.3	Uniformity of weight, assay and disintegration tests.....				45

**4.3 Analytical Methods.....48**

4.3.1 Wavelength of maximum absorption .....48

4.3.2 Validation parameters of the developed methods.....50

4.3.3. Calibration parameters.....56

**4.4 *In vitro* Bioequivalence Prediction Analysis.....57**

4.4.1 Dissolution profile of amoxicillin in simulated physiological media.....57

4.4.2 Difference and similarity factors ( $f_1$  &  $f_2$ ) and dissolution efficiency (% D.E.) of amoxicillin.....61

4.4.3 Content of amoxicillin released over all time points in all the simulated physiological pHs.....65

4.4.4 Analysis of variance of % mean content released of amoxicillin in all simulated physiological pHs over all time points.....66

**CHAPTER FIVE**

**5.0**

**Discussion.....68**

**5.1 Quality Control Studies.....68**

<b>5.2</b>	<b>Analytical</b>	
<b>Methods.....</b>		<b>69</b>
<b>5.3</b>	<b><i>In vitro</i></b>	
<b>Bioequivalence.....</b>		<b>70</b>
<b>CHAPTER SIX</b>		
<b>6.0 Summary, Conclusion and Recommendations.....</b>		<b>74</b>
<b>6.1 Summary.....</b>		<b>74</b>
<b>6.2</b>		
<b>Conclusion.....</b>		<b>75</b>
<b>6.3 Recommendations.....</b>		<b>75</b>
References.....		77
Appendices.....		86

## List of Figures

Fig. 2.1: Amoxicillin Chemical Structure.....	9
Fig. 2.2: 6-Aminopenicillanic acid showing sites of attack by bacterial enzymes.....	14
Fig. 4.1: Superimposed spectra of reference (BP, 2009) and standard amoxicillin Powder.....	38
Fig. 4.2: Superimposed spectra of reference (BP, 2009) and brand A amoxicillin Capsule.....	39
Fig. 4.3: Superimposed spectra of reference (BP, 2009) and brand B amoxicillin Capsule.....	40
Fig. 4.4: Superimposed spectra of reference (BP, 2009) and brand C amoxicillin Capsule.....	41
Fig. 4.5: Superimposed spectra of reference (BP, 2009) and brand D amoxicillin Capsule.....	42
Fig. 4.6: Superimposed spectra of reference (BP, 2009) and brand E amoxicillin Capsule.....	43
Fig. 4.7: Superimposed spectra of reference (BP, 2009) and brand F amoxicillin Capsule.....	44
Fig. 4.8: Scanned $\lambda_{max}$ . of amoxicillin standard powder in pH 1.2 and 4.5.....	48
Fig. 4.9: Scanned $\lambda_{max}$ . of amoxicillin standard powder in pH 6.8 and 7.4.....	49
Fig. 4.10: Calibration Curve of amoxicillin in pH 1.2.....	52
Fig. 4.11: Calibration Curve of amoxicillin in pH 4.5.....	53
Fig. 4.12: Calibration Curve of amoxicillin in pH 6.8.....	54
Fig. 4.13: Calibration Curve of Amoxicillin in pH 7.4.....	55

Fig 4.14: Dissolution profile of amoxicillin in (SGF) pH 1.2.....	57
Fig 4.15: Dissolution profile of amoxicillin in (SPM) pH 4.5.....	58
Fig 4.16: Dissolution profile of amoxicillin in (SIF) pH 6.8.....	59
Fig 4.17: Dissolution profile of amoxicillin in (SBF) pH 7.4.....	60

**List of Tables**

Table 4.1: NAFDAC requirement inspection of the selected samples.....	37
Table 4.2: Uniformity of weight of the amoxicillin capsules brands.....	45
Table 4.3: Assay of the amoxicillin capsules brands.....	46
Table 4.4: Disintegration time test of the amoxicillin capsules brands.....	47
Table 4.5:(Intra and inter-day) Precision of 40 µg/ml amoxicillin solution.....	50
Table 4.6: Accuracy and percentage recovery in all the media.....	51
Table 4.7: Summary of the calibration curves parameters of the developed methods.....	56
Table 4.8: $f_1$ , $f_2$ and % D.E. of amoxicillin in pH 1.2.....	61
Table 4.9: $f_1$ , $f_2$ and % D.E. of amoxicillin in pH 4.5.....	62
Table 4.10: $f_1$ , $f_2$ and %D.E. of amoxicillin in pH 6.8.....	63

Table 4.11: $f_1$ , $f_2$ and % D.E. of amoxicillin in pH 7.4.....	64
Table 4.12: Content of amoxicillin released for analysis of variance (ANOVA).....	65
Table 4.13: Result for analysis of variance of % mean release of amoxicillin (two tailed).....	66
Table 4.14: Dunnett multiple comparison test of % mean released of amoxicillin (two tailed).....	67

## List of Appendices

Appendix I: Label information of six brands of amoxicillin capsules (500mg) .....	86
Appendix II: Uniformity of weight (g) raw data.....	86
Appendix III: Table of average titre value of Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> standardization with KIO <sub>3</sub> .....	87
Appendix IV: Table of average titre values of Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> in assay of amoxicillin .....	87
Appendix V: Sample calculations of amoxicillin assay using iodometry titration .....	87
Appendix VI: Disintegration test raw data .....	89
Appendix VII: Scanned 40µg/ml amoxicillin in simulated physiological media (pH 1.2 and 4.5) .....	90
Appendix VIII: Scanned 40µg/ml amoxicillin in simulated physiological media (pH 6.8 and 7.4) .....	91
Appendix IX: Linearity study absorbance for simulated physiological media (pH 1.2, 4.5, 6.8 and 7.4) .....	92
Appendix X: Calibration curve of amoxicillin in simulated physiological medium (pH 1.2) .....	92
Appendix XI: Calibration curve of amoxicillin in simulated physiological medium (pH 4.5).....	93
Appendix XII: Calibration curve of amoxicillin in simulated physiological medium (pH 6.8) .....	93
Appendix XIII: Calibration curve of amoxicillin in simulated physiological medium (pH 7.4).....	94
Appendix XIV: Dissolution test (% mean content dissolved in pH 1.2) raw data.....	94
Appendix XV: Dissolution test (% mean content dissolved at pH 4.5) raw data .....	95

Appendix XVI: Dissolution test (% mean content dissolved at pH 6.8) raw data .....	95
Appendix XVII: Dissolution test (% mean content dissolved at pH 7.4) raw data .....	96
Appendix XVIII: Melting point determination .....	96
Appendix XIX: Preparation of 1N NaOH (200ml) .....	96
Appendix XX: Preparation of 1.2N HCl (300ml) .....	97
Appendix XXI: Preparation of simulated gastric fluid (SGF) pH 1.2 .....	97
Appendix XXII: Preparation of 0.1N Iodine (250 ml) .....	97
Appendix XXIII: Preparation of 0.1N sodium thiosulphate (500 ml) .....	97
Appendix XXIV: Standardization of sodium thiosulphate solution .....	97
Appendix XXV: Preparation of starch iodide paste (50 ml) .....	98
Appendix XXVI: Preparation of simulated physiological media (pH 4.5, 6.8 and 7.4) .....	98
Appendix XXVII: Reference IR spectrum of amoxicillin .....	99
Appendix XXVIII: IR spectrum of amoxicillin standard powder .....	99
Appendix XXIX: IR spectrum of sample A amoxicillin .....	100
Appendix XXX: IR spectrum of sample B amoxicillin .....	100
Appendix XXXI: IR spectrum of sample C amoxicillin .....	101

Appendix	XXXII:	IR	spectrum	of	sample	D	amoxicillin
.....							101
Appendix	XXXIII:	IR	spectrum	of	sample	E	amoxicillin
.....							102
Appendix	XXXIV:	IR	spectrum	of	sample	F	amoxicillin
.....							102

## Abbreviations

AUC	Area under the curve.
BA	Bioavailability.
BCS	Biopharmaceutics Classification System.
BE	Bioequivalence.
BP	British Pharmacopoeia.
cm	Centimetre.
CV	Coefficient of variation.
DE	Dissolution efficiency.
EMA	European Medicine Agency.
$f_1$	Difference factor.
$f_2$	Similarity factor.
FDA	Food drug Administration.
g	gram.
GMP	Good Manufacturing Practice.
ICH	International Conference on Harmonization.
IR	Infrared
L	Litre.
ml	Millilitre.
mm	Millimetre.
NAFDAC	National Agency for food drug administration and control.
nm	Nanometre.
°C	Degree Celsius.

PPM	Part per million.
PR	Percentage recovery.
Rpm	Revolution per minute.
RSD	Relative Standard Deviation.
SD	Standard Deviation.
USP	United State Pharmacopoeia.
UV	Ultraviolet.
WHO	World Health Organization.
μg	Microgram.
μl	Microlitre.
μm	Micrometre

## CHAPTER ONE

### 1.1 INTRODUCTION

Bioequivalence evaluation using *in vivo* pharmacokinetic parameters are often assumed to be the gold standard to established product bioequivalence (BE) of immediate release solid oral dosage forms (Polli *et al.*, 2008). However, *in vitro* studies are sometimes better than *in vivo* studies in assessing BE of immediate release solid oral dosage forms due to the fact that *in vitro* studies serve as the better method that lead to reduce costs, directly assess product performance, offers benefits in terms of ethical considerations (Polli *et al.*, 2008). *In vitro* studies directly assess product performance than do conventional human pharmacokinetic BE studies, since *in vitro* studies focus on comparative drug absorption from the two products (Arlene *et al.*, 2014). Also *in vivo* BE testing suffers from complications due to its indirect approach (Polli *et al.*, 2008). Regarding ethical considerations, *in vitro* studies better embrace the principle “No unnecessary human testing should be performed” and can result in faster product development (Polli *et al.*, 2008). Dissolution of solid oral dosage is preceded by disintegration prior to being absorbed into blood circulation to be made bioavailable at the site (s) of a drug action (Kassaye and Genete, 2013). Therefore, drug filled in a capsule shell is released rapidly as the capsule shell disintegrates; essential step for immediate release oral dosage forms because the rate of disintegration affects the dissolution and subsequently the therapeutic efficacy of the medicine (Kassaye and Genete, 2013). Dissolution is the main *in vitro* method used in quality control and of recent to determine bioequivalence between certain drug products (Arlene *et al.*, 2014). Hence, dissolution procedure has played many roles including its contribution in drug development, quality assurance, and investigation of

similarity between the different brands of the same active pharmaceutical ingredients (APIs) formulation (Arlene *et al.*, 2014).

Amidon *et al.*, (1995) proposed the biopharmaceutics drug classification system (BCS) as a schematic scientific framework for correlating *in vitro* drug product dissolution with *in vivo* bioavailability based on the belief that drug dissolution and gastrointestinal permeability are the major parameters controlling rate and extent of drug absorption. The BCS classified drug products into four classes according to their aqueous solubility and intestinal permeability; Class I: HIGH solubility / High permeability, Class II: LOW solubility / High permeability, Class III: HIGH solubility / LOW permeability, and Class IV: LOW solubility / LOW permeability (WHO, 2005). Furthermore, BCS is used for biowaiver of *in vivo* studies, which means that *in vivo* bioavailability and/or bioequivalence studies may be waived (not considered necessary for product approval) (FDA, 2015b). Instead of conducting expensive and time consuming *in vivo* studies, a dissolution test could be adopted as the substitute for the decision as to whether the two pharmaceutical products are equivalent (Ferraz *et al.*, 2007). The rate and extent of drug absorption from the gastrointestinal (GI) tract are very complex and are affected by various factors; including physicochemical factors (e.g. pKa, solubility, stability, diffusivity, lipophilicity, polar-nonpolar surface area, presence of hydrogen bonding, particle size, and crystal form), physiological factors (e.g., GI pH, GI blood flow, gastric emptying, small intestinal transit time, colonic transit time, and absorption mechanisms), and factors related to the dosage form (e.g., tablet, capsule, solution, suspension, emulsion, and gel) (Dahan and Amidon, 2008 ; Yu *et al.*, 1996). Despite these complexities, the work of Amidon *et al.*, (1995) revealed that the fundamental events controlling oral drug absorption are the permeability of the drug through the GI membrane

and the solubility/dissolution of the drug dose in the GI environment (Dahan *et al.*, 2009). These key parameters are characterized in the Biopharmaceutics Classification System (BCS) by three dimensionless numbers:

- I. Absorption number ( $A_n$ ),
- II. Dissolution number ( $D_n$ ) and
- III. Dose number ( $D_o$ ).

These numbers take into account both physicochemical and physiological parameters and are fundamental to the oral absorption process (Lobenberg and Amidon, 2000; Martinz and Amidon, 2002). Class I drugs exhibit a high absorption number and a high dissolution number. Therefore, the rate limiting step is drug dissolution and if dissolution is very rapid then gastric emptying rate becomes the rate determining step (Amidon *et al.*, 1995). Hence, rate of absorption is higher than rate of excretion e.g. Amoxicillin, Metoprolol, Diltiazem (Amidon *et al.*, 1995). Class II drugs have a high absorption number but a low dissolution number (Amidon *et al.*, 1995). *In vivo* drug dissolution is then a rate limiting step for absorption except at a very high dose number (Amidon *et al.*, 1995). The absorption for class II drugs is usually slower than class I and occurs over a longer period of time e.g. Mefenamic acid, Nifedipine (Amidon *et al.*, 1995). Class III drugs, permeability is rate limiting step for drug absorption (Cheng, *et al.*, 2004). These drugs exhibit a high variation in the rate and extent of drug absorption (Jantratid *et al.*, 2006). Since the dissolution is rapid, the variation is attributable to alteration of physiology and membrane permeability rather than the dosage form factors. e.g. Cimetidine, Neomycin B, Captopril (Amidon *et al.*, 1995). Class IV drugs exhibit a lot of problems for effective oral administration (Amidon *et al.*, 1995). Fortunately, extreme examples of class IV compounds are the exception rather than the rule and are rarely

developed and reach the market. Nevertheless, a number of class IV drugs do exist. e.g. Taxol, Griseofulvin.

Amoxicillin has been classified as the BCS class I which is highly soluble and highly permeable active pharmaceutical ingredients (API) and to release  $\geq 85$  % or more of their drug in 30 min (rapid release) or 15 min (very rapid release) (WHO, 2006b). BCS guidelines are approved by USFDA, WHO, and EMEA (European Medicines Agency) (Lipka and Amidon, 1999; FDA, 1995a). BCS class I drug products, should met these conditions to support *in-vivo* biowaiver:

- The drug substance is highly soluble
- The drug substance is highly permeable
- The drug product (test and reference) is rapidly dissolving, and
- The product does not contain any excipients that will affect the rate or extent of absorption of the drug.
- While class III drug products should have;
- The drug substance is highly soluble
- The drug product (test and reference) is very rapidly dissolving and
- The test product formulation is qualitatively the same and quantitatively very similar, e.g., falls within scale-up and post-approval changes (SUPAC) immediate release level 1 and 2 changes, in composition to the reference.

The recommended methods for determining solubility, permeability, and *in vitro* dissolution are;

The solubility class boundary is based on the highest strength of an immediate release drug product that is the subject of a biowaiver request (WHO, 2005). A drug substance is considered highly soluble when the highest strength is soluble in 250 ml or less at  $37 \pm 0.5$  °C of aqueous media over the pH range of 1-6.8 (Krämer *et al*, 2005). The 250 ml volume was obtained from BE study protocols that recommend administration of a drug product to fasting human volunteers with a glass (about 8 ounces) of water (FDA, 2015b).

The permeability class is based indirectly on the extent of absorption (fraction of dose absorbed, not systemic BA) of a drug substance in humans, and directly on measurements of the rate of mass transfer across human intestinal membrane (Krämer *et al*, 2005). Alternatively, other systems capable of predicting the extent of drug absorption in humans can be used (e.g., in situ animal, *in vitro* epithelial cell culture methods). A drug substance is considered to be highly permeable when the extent of absorption in humans is determined to be 85 percent or more of an administered dose based on a mass balance determination (along with evidence showing stability of the drug in the GI tract) or in comparison to an intravenous reference dose (Krämer *et al*, 2005).

An immediate release drug product is considered rapidly dissolving when 85 percent or more of the labeled amount of the drug substance dissolves within 30 minutes, using United States Pharmacopeia (USP) Apparatus I at 100 rpm or Apparatus II at 50 or 75 rpm when appropriately justified in a volume of 900 ml or less in each of the following media: (1) 0.1 N HCl or simulated gastric fluid without enzymes; (2) a pH 4.5 buffer; and (3) a pH 6.8 buffer or simulated intestinal fluid without enzymes (FDA, 2015b). Moreover, formulations of the same active ingredient can lead to different therapeutic effects in terms of their dissolution profile (Jinginger *et al*, 1998; Vanitasgar *et al*, 2012). In effect, poor dissolution of active

ingredient can result in low bioavailability which may lead to therapeutic ineffectiveness initially and subsequently lead to spread of resistance (Benouda *et al.*, 2009; Chadli *et al.*, 2005). Defects in the formulation or manufacturing process may be responsible for the development of generic drugs of poor quality (Ghorab *et al.*, 2012). An *in vitro in vivo* correlation (IVIVC) has been defined by the Food and Drug Administration (FDA) as a predictive mathematical model describing the relationship between an *in vitro* property of a dosage form and an *in vivo* response (Sakore and Chakraborty, 2011). Generally, the *in vitro* property is the rate or extent of drug dissolution or release while the *in vivo* response is the plasma drug concentration or amount of drug absorbed (FDA, 1995b). The purpose of IVIVC is to use drug dissolution results from two or more products to predict similarity or dissimilarity of expected plasma drug concentration (Sakore and Chakraborty, 2011). Before considering relating *in vitro* results to *in vivo*, it is imperative to know how to establish similarity or dissimilarity of *in vivo* response i.e. plasma drug concentration parameters. An important method of establishing similarity or dissimilarity of plasma drug concentrations profile is commonly known as bioequivalence testing (Sakore and Chakraborty, 2011). There are guidances and standards set for establishing bioequivalence between drug profiles and products (FDA, 1995a). For the IVIVC perception, dissolution is proposed to be a surrogate of drug bioavailability. Thus, dissolution standard may be necessary for the *in vivo* waiver (Amidon *et al.*, 1995). So far, The FDA has implemented the BCS system to allow waiver of *in vivo* BA/BE testing of immediate release solid dosage forms for class I, high-solubility, high permeability drugs (Blume and Schug, 1999). As for class III (high-solubility low-permeability) drugs, as long as the drug product does not contain agents and/or excipients

that may modify intestinal membrane permeability, *in vitro* dissolution test can ensure BE (Cheng *et al.*,2004).

## **1.2Statement of the Research Problem**

More than 25 % of drugs in the developing countries are said to be counterfeited or substandard, 50% of which are said to be beta-lactams(Kelesidis and Falagas, 2015). Amoxicillin is one of the most prescribed beta-lactam penicillin drugs with numerous generics available, this has been accompanied with diverse problems of which the most serious is the prevalence of substandard generics and fake drug products. Consequently, healthcare providers and patients are mostly concerned when selecting one brand from among several generic brands of the same drug during the treatment regime (Almeri *et al.*, 2012).*In vivo* BE studies are costly and time consuming involving the use of human volunteers (Polli *et al.*, 2008).

## **1.3Justification**

Amoxicillin is one of the first line drug in Nigeria as contained in the standard treatment guideline of the Federal Ministry of Health in the treatment of upper respiratory tract infections, tonsillitis, typhoid, as well as other indications.Owing to this, there is availability of numerous generics in the market leading to concern on selection among generics available to interchange with the innovator brand(STG, 2008).Hence, the need to evaluate the quality and bioequivalence of the numerous generics available. BCS as better alternative to *in vivo*

studies, in terms of cost, time utilization and avoidance of unnecessary use of human volunteers can be used to evaluate bioequivalence of various generics against innovator brand.

## **1.4 Research Hypothesis**

### **1.4.1 Null hypothesis**

There is no statistically significant difference in the pharmaceutical, chemical, dissolution profile and *in vitro* bioequivalence between the generic and innovator brand of Amoxicillin capsules available in Dutse, Jigawa State, Nigeria.

### **1.4.2 Alternate hypothesis**

There is statistically significant difference in the pharmaceutical, chemical, dissolution profile and *in vitro* bioequivalence between the generics and innovator brand of Amoxicillin capsules available in Dutse, Jigawa State, Nigeria.

## **1.5 Aim and Objectives of the Study**

### **1.5.1 Aim**

The aim of this study is to evaluate the bioequivalence of six brands of amoxicillin using *in vitro* dissolution profile as surrogate to *in vivo* bioequivalence studies.

### **1.5.2 Objectives**

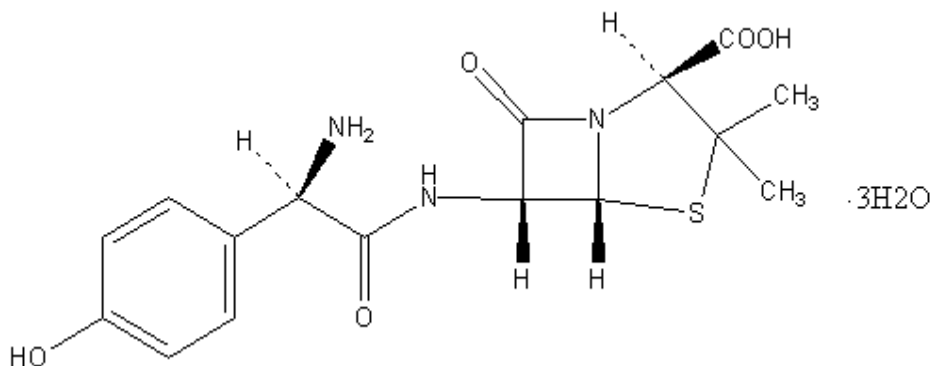
The objectives of the study are to;

- randomly select six different brands of amoxicillin using systematic random sampling.
- conduct quality control studies on amoxicillin capsules sampled (BP and USP, 2009).

- develop and validate UV methods for determination of amoxicillin in simulated physiological media pH 1.2, 4.5, 6.8 and 7.4
- determine the dissolution profiles of the individual brands in each of the simulated physiological media.
- evaluate the bioequivalence of the six brands using difference factor ( $f_1$ ), similarity factor ( $f_2$ ) and dissolution efficiency (D.E.).

## CHAPTER TWO

### 2.0 LITERATURE REVIEW



**Fig. 2.1: Amoxicillin Chemical Structure**

Chemical name: (2*S*,5*R*,6*R*)-6-[[*(2R)*-2-Amino-2-(4-hydroxyphenyl) acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid trihydrate.

## 2.1 Physicochemical Properties of Amoxicillin

Molecular Formula:  $C_{16}H_{19}N_3O_5S \cdot 3H_2O$

Molecular Weight: 419.4g/mol

Route of administration: oral or intravenous

Appearance: White or almost white, crystalline powder.

Solubility: Slightly soluble in water, very slightly soluble in 96 % ethanol, practically insoluble in fatty oils. It dissolves in dilute acids and dilute solutions of alkali hydroxides.

Specific optical rotation:  $+290^\circ$  to  $+315^\circ$ , determined in a 0.2 per cent w/v solution in carbon dioxide-free water.

Melting point: 192-196°C

Storage: store in tight closed container, away from light.

### Pharmacokinetics Properties;

Absorption: Rapid and almost complete

Protein binding: 20-30%

Metabolism: 40%

Excretion: 60% excreted unchanged

Half-life: 1.5-2 hours

## 2.2 Analytical Methods of Amoxicillin

### 2.2.1 Compendial methods of analysis

#### 2.2.1.1 Identification test of amoxicillin

The British Pharmacopeia recommended infrared absorption spectrophotometry and Melting point determination for identification of pure Amoxicillin drug.

#### 2.2.1.2 Assay test of pure amoxicillin and dosage form

BP (2009), recommend liquid chromatography method for assay of amoxicillin in pure and dosage form using the following chromatographic conditions; inject 50  $\mu$ l of each of these solutions. solution (1) add 80 ml of mobile phase A to a quantity of the mixed capsule contents containing the equivalent of 0.15 g of amoxicillin and shake for 15 minutes. Mix with the aid of ultrasound for 1 minute, add sufficient mobile phase A to produce 100 ml, mix and filter. Solution (2) dilute 1 volume of solution (1) to 100 volumes

with mobile phase A. Solution (3) contains 0.0004% w/v of cefadroxil and 0.003% w/v of amoxicillin trihydrate in mobile phase A.

The chromatographic procedure may be carried out using;

(a) a stainless steel column (25 cm × 4.6 mm) packed with octadecylsilyl silica gel for chromatography (5 μm) (Hypersil 5 ODS is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute the changing proportions of mobile phases A and B described below and (c) a detection wavelength of 254 nm.

Mobile phase A consist of 1 volume of acetonitrile and 99 volumes of a pH 5.0 buffer solution prepared as follows, to 250 ml of 0.2M potassium dihydrogen orthophosphate 2M sodium hydroxide was added until the pH reaches 5.0 and the volume made up with water to produce 1000ml.

Mobile phase B consist of 20 volumes of acetonitrile and 80 volumes of the pH 5.0 buffer solution.

Equilibrate the column with a mobile phase ratio A: B of 92:8. The test is not valid unless, in the chromatogram obtained with solution (3), the resolution factor between the peaks due to amoxicillin and cefadroxil is at least 2.0. If necessary, adjust the composition of the mobile phase. Inject solutions (1) and (2) and start the elution isocratically with the chosen mobile phase. Immediately after elution of the amoxicillin peak start a linear gradient elution to reach a mobile phase ratio A: B of 1:100 over a period of 25 minutes. Continue the chromatography with mobile phase B for 15 minutes then equilibrate the column for 15 minutes with the mobile phase chosen originally. Inject mobile phase A and use the same elution gradient to obtain a blank. In the chromatogram obtained with solution (1) the area of any secondary peak is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%).

## 2.2.2 Reported methods of analysis from literature

### 2.2.2.1 Ultraviolet spectroscopy

Literature survey showed that, several Ultraviolet spectroscopy methods were developed for assaying amoxicillin in drug substances, pharmaceutical formulations and biological fluids. Ultraviolet derivative method can also be used to assay amoxicillin in urine. Rojanarata *et al.*, (2010) reported a green bienzymatic UV-spectrophotometric method based on two enzymatic reactions in which, d-4-hydroxy phenyl glycine side chain of amoxicillin was selectively cleaved off by penicillin acylase and subsequently, reacted with 2-oxoglutarate, by the catalysis of d-phenyl glycine aminotransferase, to yield 4-hydroxy benzoyl formate and absorbance measured at 335 nm. While, Nagaralli *et al.*, (2002) reported a sensitive spectrophotometric method for amoxicillin based on the measurement of absorbance of tris(o-phenanthroline) iron(II) [method A] and tris(bipyridyl) iron(II) [method B] complexes at 510 and at 522 nm, respectively.

### 2.2.2.2 Colorimetric methods

Amin *et al.*, (1994) reported a selective colorimetric method based on the reaction of amoxicillin with 4-nitrophenol (I), 2,4-dinitrophenol (II), 3,5-dinitrobenzoic acid (III) or 3,5-dinitrosalicylic acid (IV) in alkaline medium. The method is selective for the determination of amoxicillin in the presence of its degradation products, other antibiotics and different amines that are normally encountered in dosage forms.

### 2.2.2.3 High performance liquid chromatography methods

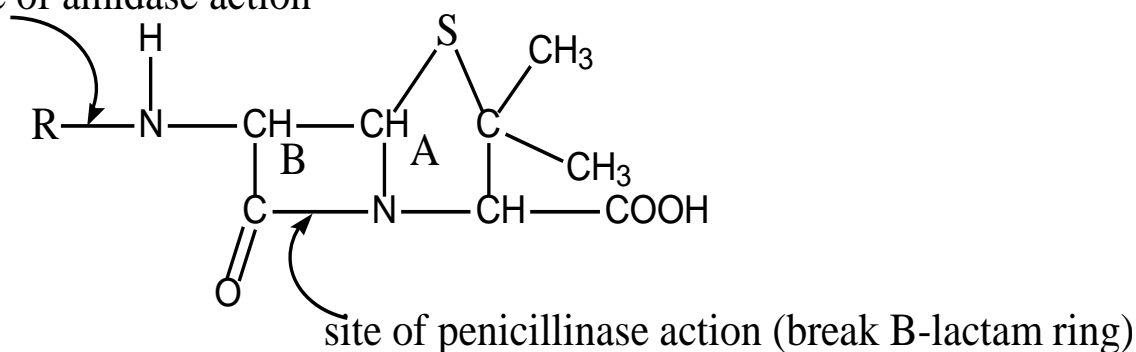
Torres *et al.*, (2010) developed an accurate and sensitive reversed-phase high-performance liquid chromatography–diode array–fluorescence (RP-HPLC –DAD–FLD) for the quantitative determination of amoxicillin along with 10 other antibiotics and their main metabolites in urine has been using, diode array (DAD) and fluorescence (FLD) detectors for analysis. The separation of the analyzed compounds was conducted by means of a C18 (150mm×4.6mm I.D. particle size 5μm) analytical column with C18

(4mm×4mm, particle size 5µm) guard column. Analyzed drugs were determined within 34 minutes using formic acid 0.1% in water and acetonitrile in gradient elution mode as mobile phase.

### 2.3 Structure Activity Relationship

All penicillins have the basic structure of a thiazolidine ring (A) attached to a β-lactam ring (B) that carries a secondary amino group (RNH-) substituents R can be attached to the amino group (Daniel and Lisa, 2012). Structural integrity of the 6-aminopenicillanic acid nucleus (rings A and B) is essential for the biologic activity of these compounds and chemical substituent attached to the nucleus can influence the stability of penicillin as well as spectrum of activity, substitution on R-group of the primary amine with electron withdrawing group decreases the electron density on the side chain carbonyl and protect these penicillins, in part, from acid degradation this property has clinical implication, because these compound survive passage through stomach better and many can be given orally for systemic purpose whereas hydrolysis of the β-lactam ring by bacterial β-lactamases yields penicilloic acid, which is deficient in antibacterial activity. (Lester *et al.*, 2008 and Daniel and Lisa 2012).

site of amidase action



### 6-Aminopenicillanic acid

**Fig. 2.2: 6-Aminopenicillanic acid showing sites of attack by bacterial enzymes**

#### 2.4 Pharmacokinetics

Amoxicillin is resistant to inactivation by gastric acid, it is rapidly and almost completely absorbed when given orally (Sean *et al.*, 2009). Presence of food in the stomach does not reduce the total amount absorbed (Daniel and Lisa, 2012). Peak plasma amoxicillin concentrations of about 4-8 micrograms/ml, after 500 mg dose (Daniel and Lisa, 2012). With traceable amounts present for up to 8 hours, doubling the dose can double the concentration (Sean *et al.*, 2009). Concentrations of amoxicillin after intramuscular injection are similar to those achieved with oral doses, plasma protein binding is about 20–30% and plasma half-lives of 1 to 1.5 hours (Daniel and Lisa, 2012). Amoxicillin is widely distributed at varying concentrations in body tissues and fluids (Sean *et al.*, 2009). It crosses the placenta; small amounts are distributed into breast milk. Little amoxicillin passes into the CSF unless the meninges are inflamed (Sean *et al.*, 2009).

#### 2.5 Metabolism

Amoxicillin is metabolized to some degree to penicilloic acid which is excreted in the urine. About 60% of an oral dose of amoxicillin is excreted unchanged in the urine in 6 hours by glomerular filtration and tubular secretion (Laurence *et al.*, 2008). Urinary concentrations above 300 micrograms/ml have been reported after a dose of 250 mg (Lester *et al.*, 2008). Probenecid reduces renal excretion. Amoxicillin is removed by haemodialysis. High concentrations have been reported in bile some may be excreted in the faeces (Sean *et al.*, 2009).

#### 2.6 Mechanism of Action of Amoxicillin

Amoxicillin, acts in similar way with other  $\beta$ -lactam antibiotics, through the inhibition of the biochemical transpeptidases reaction of bacterial cellwall synthesis (Daniel and Lisa, 2012). The cell wall is a rigid outer layer distinct to bacterial species (Laurence *et al.*, 2008). It completely surrounds the cytoplasmic membrane it retains cell shape and integrity, and prevents cell lysis from high osmotic pressure (Laurence *et al.*, 2008). The cell wall is composed of a complex, cross-linked polymer of polysaccharides and polypeptides, peptidoglycan (also known as murein or mucopeptide) (Laurence *et al.*, 2008). The polysaccharide contains alternating amino sugars, *N*-acetylglucosamine and *N*-acetylmuramic acid (Daniel and Lisa, 2012). A fiveaminoacid peptide is linked to the *N*-acetylmuramic acid sugar (Daniel and Lisa, 2012). This peptide terminates in D-alanyl-D-alanine (Laurence *et al.*, 2008). Penicillin binding protein (PBP, an enzyme) removes the terminal alanine in the process of making a cross-link with a nearby peptide (Laurence, *et al.*, 2008). Crosslinks give the cell wall its structural rigidity (Daniel and Lisa, 2012). Beta-lactam antibiotics, structural analogs of the natural D-Ala-D-Ala substrate, covalently bind to the active site of PBPs (Laurence *et al.*, 2008). This inhibits the transpeptidation reaction, halting peptidoglycan synthesis, and the cell dies (Laurence *et al.*, 2008). The exact mechanism of cell death is not completely understood, but autolysins and disruption of cell wall morphogenesis are involved (Laurence *et al.*, 2008; Daniel and Lisa, 2012). Beta-lactam antibiotics kill bacterial cells only when they are actively growing and synthesizing cell wall (Daniel and Lisa, 2012).

### **2.7 Therapeutic Indications**

The aminopenicillins, amoxicillin has wider spectrums of activity, with good oral absorption 250 to 500 mg of amoxicillin given three times daily, is comparable to the same amount of ampicillin given four times daily (Laurence *et al.*, 2008). Amoxicillin is given orally to treat urinary tract infections, sinusitis, otitis, and lower respiratory tract infections, Pneumonia, meningitis, and typhoid fever, amoxicillin, is also available in combination with one of several  $\beta$ -lactamase inhibitors clavulanic acid, sulbactam, or tazobactam. addition of a  $\beta$ -lactamase inhibitor extends its activity to include  $\beta$ -lactamase-producing strains of *S aureus* as well as some  $\beta$ -lactamase-producing gram-negative bacteria (Daniel and Lisa, 2012; Laurence *et al.*, 2008).

### **2.8 Adverse Effects**

The penicillins are generally well tolerated regrettably, this encourages their misuse and/or incorrect use (Daniel and Lisa, 2012). Most of the serious adverse effects are due to hypersensitivity (Sean *et al.*, 2009). All Penicillins are cross sensitizing and cross-reacting (Lester *et al.*, 2008). The antigenic factors are degradation products of penicillins, mainly penicilloic acid and products of alkaline hydrolysis bound to host protein. 5–8 % of people reported to have experienced adverse effects due to penicillins (Lester *et al.*, 2008). Less than 1% of persons who previously received penicillin without incident will have an allergic reaction when given penicillin (Daniel and Lisa, 2012). Because of the potential for anaphylaxis, however, penicillin should be administered with caution or a substitute drug given if the person has a history of serious penicillin allergy (Laurence *et al.*, 2008). Allergic reactions include anaphylactic shock (very rare—0.05 %), serum sickness-type reactions (now rare—urticaria, fever, joint swelling, angioneurotic edema, intense pruritus, and respiratory compromise occurring 7–12 days' post exposure) and a variety of skin rashes (Daniel and Lisa, 2012). Oral lesions, fever, interstitial nephritis (an autoimmune reaction to a penicillin-protein complex), eosinophilia, hemolytic anemia and other hematologic disturbances, and vasculitis may also occur. Most patients allergic to penicillins can be treated with alternative drugs, in patients with renal failure, penicillin in high doses can cause seizures (Lester *et al.*, 2008). Nafcillin is associated with neutropenia; oxacillin can cause hepatitis; and methicillin causes interstitial nephritis (and is no longer used for this reason) (Laurence *et al.*, 2008). Large doses of penicillins given orally may lead to gastrointestinal upset, particularly nausea, vomiting, and diarrhea (Daniel and Lisa, 2012). Ampicillin has been associated with pseudomembranous colitis (Laurence *et al.*, 2008). Secondary infections such as vaginal candidiasis may occur (Sean *et al.*,

2009). Ampicillin and amoxicillin can cause skin rashes that are not allergic in nature. These rashes frequently occur when aminopenicillins are inappropriately prescribed for a viral illness (Laurence *et al.*, 2008; Daniel and Lisa, 2012).

### **2.9 Some Reported Literatures on *In vitro* Dissolution Studies**

Extensive literature survey revealed Dissolution profiles studies of amoxicillin capsules containing 500 mg and other essential medicines using both compendial and developed methods. The United States Pharmacopoeia procedure (USP, 2009), was used by (Kassaye and Genete, 2013) for evaluation of dissolution profile data and compared using two different statistical methods: the fit factors ( $f_1$  &  $f_2$ ) and the dissolution efficiency (D.E.) model, most generic brands of amoxicillin capsules (62.5% of the tested brands) were found not to be interchangeable with the innovator brand.

Arlene *et al.*, (2014) compared the dissolution profile of Amoxicillin, Metronidazole and Zidovudine in simulated gastric pH 1.2 (SGF), simulated Intestinal pH 4.5 and 6.8 (SIF) media using HPLC. The fit factor ( $f_2$ ) was found to be below 50 % in most of them. Similarly, spectrophotometric evaluation of Amoxicillin dissolution profile at 272 nm, was reported by (Benmoussa *et al.*, 2012). Tanjinatus *et al.*, (2011) conducted an *invitro* bioequivalence study on ten generic Atorvastatin tablets from different manufacturers. Fit factor, result show similarity between the generics and innovator brand and can be used interchangeably. In the same vain Parvinet *et al.*, (2012) reported their study on eight brands of Metformin tablet subjected to various tests to evaluate bioequivalence. All brands except C achieved 80% of the drug dissolution within (30 min.) specified for immediate release oral dosage form. Similar studies were reported by Ngwuluka *et al.*, (2009), Ashraful Islam *et al.*, (2011), Panchagnula *et al.*, (2007), Olubukola *et al.*, (2012), Raheela *et al.*, (2011), Chandrasekaran *et al.*, (2011) and Ocheke *et al.*, (2012).

### **2.10 Relationship of Dissolution test with Bioavailability.**

Although oral route of drug administration for solid dosage form has been the main route of drug administration for almost a century (Aristides and Panos, 2006). Nevertheless, it was only 50 or so years ago that scientists recognized the significance of dissolution processes in the physiological availability of drugs, for now, the study of the dissolution process has been developing since the end of the 19th century by physical chemists (Aristides and Panos, 2006). Therefore, most of the major research in the field was not related to drugs at all, and the basic laws for the description of the dissolution process were already available when interest in drug dissolution started to emerge. Edwards in 1951 was the first to appreciate that following the oral administration of solid dosage forms, if the absorption process of drug from the gastrointestinal tract is rapid, then the rate of dissolution of that drug can be the step which controls its appearance in the body. In fact, he postulated that the dissolution of an aspirin tablet in the stomach and intestine would be the rate process governing the absorption of aspirin into the blood stream (Edwards, 1951). However, Nelson in 1957 unambiguously relates the blood level of orally administered theophylline salts to their *in vitro* dissolution rates. He used a non-disintegrating drug pellet, (mounted on a glass slide so that only the upper face was exposed), placed at the bottom of a 600 ml beaker in such a manner that it could not rotate when the dissolution medium was agitated at 500 rpm. In mid 1960s to early 1970s a number of studies establishing the effect of dissolution on the bioavailability of a variety of drugs were reported in the literature. Two reports were published in 1963 and 1964 drawing attention to the lack of full clinical effect for two brands of tolbutamide marketed in Canada (Campagna *et al.*, 1963; Levy *et al.*, 1964). These tablets were shown to have long disintegration times as well as slow dissolution characteristics (Levy, 1964). Besides, a slight change in formulation of an experimental tolbutamide preparation was shown to produce significantly lower blood levels and hypoglycemic effect (Varley, 1968). Martin *et al.*, (1968) reported significant differences in the bioavailability between different brands of sodium diphenylhydantoin, chloramphenicol and sulfisoxazole. MacLeod *et al.*, (1972) reported greater than 20% difference in peak concentration and area under the serum concentration time curve for three ampicillin products. In late sixties it was realized that differences in

product formulation could lead to significant differences in rate of onset, intensity and duration of drug response (Aristides and Panos, 2006). At that time the term “bioavailability” was invented to describe either the extent to which a particular drug is utilized pharmacologically or, more strictly, the fraction of dose reaching the general circulation (Aristides and Panos, 2006). The most vivid bioavailability examples have been with digoxin in the U.K. and the USA in 1971 and phenytoin in Australia and New Zealand in 1968. In the former case, different formulations of digoxin yielded up to sevenfold differences in serum digoxin levels (Lindenbaum *et al.*, 1971). These observations prompted the FDA in collaboration with the late John Wagner to carry detailed dissolution studies on 44 lots from 32 manufacturers of 0.25 mg digoxin tablets available in the 1972 North American market-place (Skelly, 1988). The studies revealed remarkable differences in the dissolution profiles of the digoxin products and confirmed the opinion that either lot-to-lot or amongst brands bio in equivalence originates from differences in dissolution rates. Additional dissolution studies conducted in other laboratories confirmed these findings (Fraser *et al.*, 1972). Phenytoin toxicity occurred in an enormous number of patients when the manufacturer replaced the excipient calcium sulphate with lactose in immediate release phenytoin tablets (Tyrer *et al.*, 1970). Initially, the lower extent of absorption of phenytoin in the presence of calcium sulphate was attributed to the formation of an insoluble calcium-phenytoin salt (Bochner *et al.*, 1972). However, Chapron *et al.*, (1979) found no effect when they studied the influence of calcium on bioavailability of phenytoin administering calcium gluconate before, with and after a single dose of 300 mg of phenytoin. These results indicated that the higher hydrophilicity of lactose compared to calcium sulphate, promoted the dissolution rate of phenytoin resulting in higher bioavailability and consequently higher concentrations of phenytoin in plasma, exceeding its narrow therapeutic range of 10–20 µg/ml. Loss of seizure control occurred in a patient on phenytoin was related to altered dissolution characteristics caused by the physical changes of phenytoin capsules (Cloyd *et al.*, 1980).

### **2.11 Advantages of *In vitro* Dissolution to Bioequivalence**

*In vitro* studies are sometimes better than conventional human pharmacokinetic *in vivo* studies in evaluating BE for immediate release solid oral dosage forms due to: reduce costs, directly assess product performance, and removal of ethical considerations (Polli *et al.*, 2008). *In vitro* test is recommended for BCS Class I drugs with rapid dissolution ( $\geq 85\%$  in 30 min or less in pH 1.2, 4.5 and 6.8 media) and Class III drugs with very rapid dissolution ( $\geq 85\%$  in 15 min or less in pH 1.2, 4.5 and 6.8 media). Therefore, *in vitro* studies achieve reduced costs by avoiding *in vivo* studies where BE is self evident (WHO, 2005b). Cook and Bockbrader (2002) studied the prospective cost savings using BCS based bio waiver for Class I drugs, in place of *in vivo* BE testing, they considered the number of BE studies done by the pharmaceutical industry between January 1998 and May 2001 and presumed 25% of BE studies are for Class I drugs (Cook and Bockbrader, 2002). They predictably estimated in 2002 that “there is the potential to save one quarter the annual expenses on bioequivalence studies, \$22 to \$38 million dollars/year.” This is direct costs of testing and pointed out further indirect savings can occur if BE studies are rate limiting to drug regulatory submission (e.g. avoid lost sales of over one million dollars per day if product leads to sales of \$400 million per year) and if opportunity costs are factored in (e.g. resources not deployed to running *in-vivo* studies can be diverted to bring other drugs to market faster) (Cook and Bockbrader, 2002). Though several tens of millions of dollars saving each year, can be viewed as minimal impact even if wholly transferred to patients, it would seem that this level of direct savings is preferred over no level of direct saving, thus BE is self evident for Class I drugs. (FDA, 2000; EMEA, 2001). Furthermore, the main regulatory concern about BE is safeguarding patients against approval of products that are not BE (FDA, 2008).

Direct product performance evaluation: *In vitro* Studies Centered on Drug Absorption which composed of the processes of drug release from the dosage form (i.e. dissolution) and drug permeation from the

gastrointestinal milieu while the pharmacokinetic metrics C<sub>max</sub> and AUC which are the most common methods used in assessing BE, neither the definition of bioequivalence nor bioequivalence requirement references C<sub>max</sub> or AUC, or even refer to pharmacokinetic plasma profiles (CFR 21 FDA, 2003). In fact, neither definition necessarily requires *in vivo* studies rather, C<sub>max</sub> and AUC are commonly used as metrics for the rate and extent of drug absorption (CFR 21 FDA 2003). The definitions of bioavailability and bioequivalent drug products as well as the conditions under which products are considered bioequivalent, feature drug absorption rather than pharmacokinetic plasma profiles (CFR 21 FDA, 2008). Furthermore, *in vitro* studies are considered as better BE method than *in vivo* studies, it embraces the principle “No unnecessary human testing should be performed” and can result in faster development. The US, 21 CFR 320.25(a) codifies the universal belief that “No unnecessary human testing should be performed” (US 21 CFR). Excitingly, 21 CFR 320.25(a) reads “The basic principle in an *in vivo* bioavailability study is that no unnecessary human research should be done.” This statement may at first appear contradictory by backing minimal human research, at same times consider an *in vivo* study necessary. However, the scope of 21 CFR 320.25 is the guidelines for conducting an *in vivo* bioavailability study, so this statement is simply advocating aspects like using the fewest number of human subjects when human testing is conducted. However, it is fascinating that 21 CFR 320 explicitly make no general preference against unnecessary human research or the preference for *in vivo* testing when *in vitro* testing is sufficient (FDA, 2015b). Rather, recent FDA guidance indicates that *in vitro* studies are less preferable than pharmacokinetic studies, and even less preferable than pharmacodynamics studies and clinical studies (FDA, 2015a). In spite of this recent guidance, FDA’s granting of BCS-based Biowaivers for Class I drugs whose immediate release formulations demonstrate rapid dissolution implies that *in vitro* studies are not less preferred in practice than pharmacodynamics studies and clinical studies (FDA, 2014). *In vivo* BE testing is generally safe, where the majority of ADRs are mild (Huic *et al.*, 1996). In particular, BE studies after drug has been approved as safe and effective can be expected to be generally safe. In addition to this conventional *in vivo* BE testing is single dose, limiting drug exposure (Polli *et al.*, 2008). However, ADRs have occurred in BE testing. Aripiprazole for treatment of schizophrenia and bipolar I disorder. The reference listed drug (RLD) for aripiprazole is now 5 mg tablet and not the 30 mg strength (FDA, 2008). The 30 mg strength caused ADRs in healthy volunteers, such that the lowest strength rather than highest strength is now used in BE testing of aripiprazole. Clozapine also exemplifies that serious ADRs can occur in BE testing. The FDA guidance on clozapine BE testing reads “In the 1996 guidance, the Agency recommended that doses of clozapine tablets be administered to healthy subjects, because a high number of healthy subjects experienced serious adverse effects such as hypotension, bradycardia, syncope, and asystole during clozapine bioequivalence studies, FDA is recommending that studies not be conducted using healthy subjects. In addition, a single dose study using a 12.5 mg dose is no longer recommended. Instead, this guidance recommends a multiple-dose bioequivalence study conducted in patients using the highest dosage strengths (e.g. 100 mg tablets) (FDA, 2017).

### **2.12 Post Market Assessment**

Post market analysis or checking encompasses all actions embarked to get more data and evidence about a product once it had been given marketing approval and made obtainable for public consumption (Ngwuluka *et al.*, 2009). The data and information thus obtained can be engaged for enhancement of product quality and development of standards (Ngwuluka *et al.*, 2009). Regulatory agencies depend on restricted evidence acquired in the course of clinical trials and to some extent scientific literature as guides to permitting marketing approval of medicines for public usage. It is therefore vital to carry out post marketing approval investigation of approved medicines in order to effectively evaluate the quality, therapeutic efficacy and safety of medicines for the larger public. Post market monitoring should not to be a one time off activity (Garcia, 2006). However, it should be a constant activity all over the life of a drug product post market surveillance activities of a drug product have been categorized to include; review of

products condition of approved study evaluation and investigation of reported drug complains; inspection of manufacturer's processes and procedures (Ngwuluka *et al.*, 2009).

### **2.13 Multisource (Generic) Pharmaceutical Products**

Multisource Pharmaceutical products are intended to be pharmaceutically equivalent or pharmaceutical alternatives that are bioequivalent and hence are therapeutically equivalent and interchangeable (WHO, 2005). The marketing of multisource drug products approved by local drug regulatory agencies in developing countries, with the intention of improving access to health care delivery commodities for all through competitive pricing, generic drugs substitution has been advocated by WHO with aim of maximizing population health subject to a constraint budgetary allocation (Almeriet *et al.*, 2012). This as a whole can result in the overall improvement of healthcare delivery system (Simeon, 2011). Generic drugs represent 47% of all prescription dispensed in 1999, 61% in 2006 and 69% in 2008 in US (Frank, 2007; Almeriet *et al.*, 2012). Generics drugs approval in US accounted for an average savings of 77% of the product cost within one year (Kozlowski *et al.*, 2011). In the same vain generics substitution in UK was highly successful and accounts for 83% (Kamerow, 2011). This rise has ensued because any drug product that are considered bioequivalent must be equal in quality (active ingredient, strength, purity, content uniformity, disintegration and dissolution rates) (Adegbolagun *et al.*, 2007). Nevertheless, this has been attended by a multiplicity of problems of which the most serious is the wide spread distribution of substandard generics, fake drug products, consequently health care providers are usually concerned when selecting one drug among several bioequivalent during treatment regime (Adegbolagun *et al.*, 2007; Almeriet *et al.*, 2011).

### **2.14 Fake and Counterfeit Drugs**

Substandard/counterfeit antimicrobial drugs are a growing global problem, the most common substandard/counterfeit antimicrobials consist of beta-lactams (among antibiotics) and chloroquine and artemisinin derivatives (among anti malaria's) (Kelasidis and Falagas, 2015). However, the most common type of substandard/counterfeit antimicrobial drugs have a reduced amount of the active drug, and the majority of them are manufactured in Southeast Asia and Africa (Kelasidis and Falagas, 2015). Counterfeit antimicrobial drugs may cause increased mortality and morbidity and pose a danger to patients (IOM., 2013). A counterfeit medicine is one that has been deliberately and fraudulently produced and/or mislabeled with respect to identity and/or source to make it appear to be a genuine product. Counterfeit products include drugs with no active ingredient, drugs that are super potent, and drugs with dangerous impurities (WHO, 2006a). The fatal consequences of counterfeit drugs are well expected to be a fundamental challenge to the reliability of public health systems around the globe, as well as a direct danger to our individual health and welfare (Attaran *et al.*, 2012). According to the World Health Organization (WHO), up to 10% of the drugs worldwide may be counterfeits (Pincock 2003; Gibson 2004). 50% of them involved antimicrobial drugs, and 78% were from developing countries. Furthermore, 59% of cases with available information on the quality of drugs were fraudulent, and only 7% had the standard concentration of the active drug (WHO 1999a; 1999b and 2000). However, reporting of counterfeit drugs within WHO is <15% (WHO, 2014). Drug product quality study conducted by WHO in developing countries of Africa found that 7.6% of major antibiotic formulations contained no active ingredient, whereas 17.8% of antibiotics and 13% of antiparasitic products were substandard by WHO standards but not necessarily counterfeit (Reidenberg and Conner, 2001). However, well designed studies to define the problem are deficient, and this has led to significant variability in the estimates of counterfeit drugs among other developing countries (Olori, 1996; Chakravarty *et al.*, 2001; Fackler, 2002). According to the U.S. Food and Drug Administration (FDA), up to 25% of all medicines in developing countries and 10% of drugs globally have low quality (Rudolf and Bernstein, 2004). The

Pharmaceutical Security Institute data indicate an increase in the reports of fake drugs of more than 10 fold within 2002 to 2012 (Pharmaceutical Security Institute, 2014.). The data reflect the regulatory oversight in countries where the counterfeit antimicrobials have been studied (Institute of Medicine, 2013.). Since pharmaceutical companies and regulatory authorities have not published most of their data on counterfeit antibiotics (Attaran *et al.*, 2012; Institute of Medicine, 2013.), published prevalence studies may provide useful epidemiology data (Institute of Medicine, 2013.). Nevertheless, it is clear that counterfeit pharmaceuticals remain one of the world's fastest growing industries (Stimson Centre, 2011). Recent trends suggest a massive increase in counterfeit drug sales to over \$70 billion globally in 2010 (Stimson Centre, 2011). This represents an increase of more than 90 percent from 2005(WHO, 2006a). Although the counterfeiting of, and trafficking in, all manner of products is on the rise globally including currency, documents, software, and electronics no other spurious product has the ability to hurt or even murder its end user as do illegal pharmaceuticals (Stimson Centre, 2011). Additionally, most other counterfeits are not quite as profitable as counterfeit drugs. In the United States, for example, negligent production at a Massachusetts compounding pharmacy sickened more than 600 people, killing 44, from September 2012 to January 2013 (Institute of Medicine 2013). The vast majority of problems, however, occur in developing countries where underpowered and unsafe medicines frequently compromise treatment of deadly diseases and accelerate drug resistance, affecting millions (Institute of Medicine, 2013). It is difficult to measure the public health burden of falsified and substandard drugs, the number of deaths they cause, or the amount of time and money wasted using them. But a network of security divisions at 25 major pharmaceutical companies found that falsified or substandard drugs were sold in at least 124 countries in 2011 (Institute of Medicine, 2013).

**CHAPTER THREE**  
**3.0 MATERIALS AND METHODS**  
**3.1 Materials**

**3.1.1 Drugs**

Six Brands of Amoxicillin capsules (500mg)  
Standard powder of Amoxicillin obtained from Emzor Pharmaceutical Ltd.

**3.1.2 Glass wares and accessories**

2×250ml Extraction tubes (Pyrex England)  
2×100ml measuring cylinders (Pyrex England)  
2×250ml Conical flasks (Pyrex England)  
2×100ml Conical flasks (Pyrex England)  
2×50ml beakers (Pyrex England)  
2×25ml beakers (Pyrex England)  
6×10ml Test tubes (Pyrex England)  
6×10ml Centrifuge tubes (Pyrex England)  
2×25ml, 2×50ml and 2×100ml volumetric flasks (Pyrex England)  
20× Filter papers

**3.1.3 Equipment and instruments**

Analytical weighing balance (Mettler Analytical Balance Phillip Harris., England)  
Dissolution test Machine (Tianjin Guoming Medicinal Equipment co. LTD., China)  
Euweka Disintegration Time Test apparatus (Type ZT3, GmbH, Germany)  
pH meter (Fisher Scientific, Singapore)  
UV double beam spectrophotometer model (MNF, Helios Zeta, Thermo Scientific England)  
Water bath, (model BJE 750A Gallen Kamp, England)  
Gallen Kamp Hot air oven (Philip Harries Ltd, England)  
Infrared spectrophotometer (Model. Cary 630, Agilent Technology Germany)  
Thermometer (Mc Donald Scientific International, England)

**3.1.4 Reagents**

Purified distilled water to be used for preparation of the solutions.  
Concentrated HCl (BPH Chemical, England)  
Sodium hydroxide pellets (BPH Chemical, England)  
Iodine crystal (BPH Chemical, England)  
Ethanol 96% (BPH Chemical, England)  
Sodium thiosulphate (BPH Chemical, England)  
Monobasic potassium phosphate (BPH Chemical, England)  
Sodium acetate (BPH Chemical, England)  
Zinc chloride (BPH Chemical England)  
Starch powder (BPH Chemical England)  
Potassium iodide (BPH Chemical England)  
Potassium iodate (BPH Chemical England)

## 3.2 Methods

### 3.2.1 Sample survey, purchase and coding of amoxicillin capsule

Sample survey of available amoxicillin brands within Dutse town was conducted in two hospital pharmacies, three community pharmacies and ten patent medicine vendor shops. Twenty-one (21) brands of amoxicillin capsule were found to be available, six brands were randomly selected using systematic random sampling using the formula  $N/n$  where  $N$  is the population size and  $n$  is sample size from the pool of the surveyed brands and purchased from different retail Pharmacies, Hospitals and drug outlets within Dutse Metropolis. The samples were coded as A, B, C, D, E and F. Code A was used for the innovator brand (Appendix I).

### 3.2.2 Physical inspection of the samples

Minimum NAFDAC label requirements such as Manufacturer's addresses, batch number, Manufacturing and Expiry dates were examined and recorded.

### 3.2.3 Identification test of pure amoxicillin powder

#### 3.2.3.1 Infrared spectrophotometry

This was carried out according to BP 2009, IR spectrum obtained from multi user laboratory chemistry department ABU. Zaria.

#### 3.2.3.2 Melting point determination

Small quantity of the standard Amoxicillin powder was filled into capillary tube by tapping and placed into the melting point determination machine and examined until the powder melt and temperature recorded. The procedure was repeated two more times to get average melting point (appendix XVIII).

### 3.2.4 Quality control of amoxicillin capsules

#### 3.2.4.1 Identification test

This was carried out by emptying two capsules of amoxicillin from each brand into 20 ml of 96% ethanol and filtered, the filtrate collected was then evaporated to dryness. The recovered powder was identified using Fourier Transform Infrared Absorption Spectrophotometre. The obtained IR spectrum was then compared with the standard IR spectrum (BP, 2009).

#### 3.2.4.2 Assay of amoxicillin content

The assay test was conducted using iodometric titration described by USP 2009. 0.01g of the standard amoxicillin powder was weighed into a beaker; 100ml of water was added to produce 100 µg/ml standard solution. Two millilitre (2 ml) of the solution was pipetted into a conical flask and 2 ml of a 1N sodium hydroxide solution was added and mixed by swirling. The solution was left standing for 15 minutes in the dark. The assay brands were extracted by dissolving the content of capsule in 100 ml beaker with distilled water, an amount equivalent to 0.01 g of amoxicillin was pipetted and transferred into conical flask. The procedure was repeated as done for standard powder solution. To each of the conical flasks, 2.0 ml of a 1.2 N hydrochloric acid and 10.0 ml of 0.1N iodine solution was added and allowed to stand for 15 minutes. The resulting solution was titrated with 0.1N sodium thiosulphate. As the endpoint approached one (1) drop of starch iodide paste was added and the titration continued to the discharge of blue colour. The titration was repeated for all the remaining flasks.

1 ml of 0.1 N  $\text{Na}_2\text{S}_2\text{O}_3$  is equivalent to 0.0699 g of  $\text{C}_{16}\text{H}_{19}\text{N}_3\text{O}_5\text{S}\cdot 3\text{H}_2\text{O}$

#### 3.2.4.3 Weight uniformity determination

The content uniformity test was performed according to the British Pharmacopeia, 2009. Twenty capsules each were randomly selected from each brand and were weighed individually using analytical weighing balance. Mean weight of each brand was calculated and their percentage mean deviation statistically analyzed.

#### 3.2.4.4 Disintegration test

Six (6) Capsules from each brand were employed for the test in distilled water heated to  $37\text{ }^{\circ}\text{C} \pm 0.5$  using disintegration apparatus. The time taken for each capsule to break up and pass completely through the sieve was recorded (BP, 2009).

### 3.2.5 UV spectrophotometric methods development

#### 3.2.5.1 Preparation of standard stock solutions

Stock solutions (100  $\mu\text{g/ml}$ ) were prepared by dissolving 10 mg of amoxicillin trihydrate standard powder in 100 ml of each of the prepared simulated physiological media (pH 1.2, 4.5, 6.8 and 7.4).

#### 3.2.5.2 Determination of wavelength of maximum absorption

Solutions (40  $\mu\text{g/ml}$ ) were prepared from each of the stock solutions and then scanned at 400-200 nm in order to obtain the wavelength of maximum absorption in each media.

#### 3.2.5.3 Construction of calibration curve

A six points calibration curve of amoxicillin in each of the media was constructed by preparing solutions of concentration range 10-60  $\mu\text{g/ml}$  by serial dilution of each standard stock solution (100  $\mu\text{g/ml}$ ) of the different simulated physiological media.

### 3.2.6 Validation of the developed methods

Each of the developed methods was validated for linearity, precision, accuracy and percentage recovery in accordance with ICH guideline.

#### 3.2.6.1 Linearity

This was established by least square methods using Microsoft excel 2016.

#### 3.2.6.2 Precision

This was conducted by determining both intra-day and inter-day precision as follow;

##### *Intra-day (within the day precision):*

This was done by determining the absorbance of a 40  $\mu\text{g/ml}$  solution of amoxicillin in each of the simulated physiological media six times at an hour interval within the same day.

##### *Inter-day (between the day precision):*

It was essentially carried out by determining the absorbance of a 40  $\mu\text{g/ml}$  solution of amoxicillin in each of the simulated physiological media daily for three consecutive days.

#### 3.2.6.3 Percentage recovery

Five millilitre (5ml) of a 10  $\mu\text{g/ml}$  solution of amoxicillin was quantitatively measured and transferred into four labeled 10ml test tubes. Test tubes B, C, and D were spiked with 1.3 ml, 1.5ml and 1.7ml of the standard stock solution to obtain 18, 20 and 22  $\mu\text{g/ml}$  respectively while test tube A was left unspiked. Absorbance was taken in triplicates and the mean % recovery was calculated

$$\% \text{ recovery} = \frac{\text{Conc. B} - \text{Conc. A}}{\text{Conc. A}} \times 100$$

This was done for solutions of amoxicillin in each of the simulated physiological media.

### 3.2.7 Determination of *in vitro* dissolution profiles of the samples

#### 3.2.7.1 Preparations simulated physiological media (pH 1.2, 4.5, 6.8 and 7.4)

Simulated gastric fluid SGF (pH 1.2) was prepared using concentrated hydrochloric acid while simulated physiological medium (pH 4.5), simulated intestinal fluid SIF (pH 6.8) and simulated blood (pH 7.4) were

prepared using potassium monobasic phosphate and the pH was adjusted with 0.1N sodium hydroxide or 0.1 N HCl as the case may be.

### 3.2.7.2 Procedure for dissolution

Dissolution testing were carried out in four different simulated physiological pH 1.2(SGF),pH 4.5(SPM),pH 6.8(SIF) and pH 7.4 (SB) using USP apparatus 1 (basket) dissolution apparatus. The basket speed was maintained at 100 revolutions per minute (rpm), and 900ml of each dissolution medium was used to test all the samples. The medium was preheated to  $37 \pm 0.5$  °C, thereafter, one capsule was placed in the dissolution basket and the machine was operated. 2ml sample was then withdrawn and was replaced by equal volume of the medium at the time intervals of 5, 15, 25, 35, 45 and 55 minutes. One (1 ml) of the aliquot solution was quantitatively taken in to 10 ml beaker and diluted to volume with the dissolution medium and the absorbance was measured by UV-vis spectrophotometer using validated working  $\lambda_{max}$ .

### 3.2.8 Determination of *in vitro* bioequivalence

Dissolution values obtained from the simulated physiological media were converted to concentrations and percentage content released from calibration graph of each media and statistically analyzed for *in vitro* bioequivalence using difference factor ( $f_1$ ), similarity factor ( $f_2$ ), Dissolution efficiency (% D.E.) and analysis of variance (ANOVA), using microsoft excel 2016, SPSS 20.

$$f_1 = \left\{ \frac{\left[ \sum_{t=1}^n |R_t - T_t| \right]}{\left[ \sum_{t=1}^n R_t \right]} \right\} \times 100$$

$$f_2 = 50 \cdot \log \left\{ \left[ 1 + \frac{1}{n} \sum_{t=1}^n (R_t - T_t)^2 \right]^{0.5} \times 100 \right\}$$

Fit factors ( $f_1$  and  $f_2$ ) indices are defined as follows:

$R_t$  = percentage content dissolved for the reference brand at time point  $t$

$T_t$  = Percentage content dissolved for the test brand.

$n$  = is the number of time points.

For each brand, the analysis was carried on the mean values.

Difference factor ( $f_1$ ) is the average % difference over all time points in the amount of test brand dissolved as compared to the reference brand. The ( $f_1$ ) value is 0 when the test and the reference profiles are identical and increases proportionally with the dissimilarity between the two profiles.

Similarity factor ( $f_2$ ) value is between 0 and 100. The value is 100 when the test and the reference profiles are identical and approaches zero as the dissimilarity increases.

Dissolution efficiency (% D.E.) is the area under the dissolution curve between time points  $t_1$  and  $t_2$  expressed as a percentage of the curve at maximum dissolution,  $y_{100}$ , over the same time period allows for the comparison of several formulations simultaneously and can be theoretically related to the mean plasma concentration-time curve. It is given by the formula;

$$DE = \frac{\int_{t_1}^{t_2} y \cdot dt}{y_{100} \times (t_2 - t_1)} \times 100$$

The integral of the numerator, i.e. the area under the curve is calculated by a model independent method, the trapezoidal rule. The area under the curve is the sum of all the trapeziums defined by:

$$AUC = \sum_{i=1}^{i=n} \frac{(t_i - t_{i-1}) (y_{i-1} + y_i)}{2}$$

Where  $y_{100}$  is the percentage of the dissolved product,  $t_i$  is the  $i^{\text{th}}$  time point,  $y_i$  is the percentage of dissolved product at time  $t_i$ .

## CHAPTER FOUR

### 4.0 Results

#### 4.1 Labeling Characteristics Result

All the brands were found to passed the NAFDAC requirement for labeling (table 4.1).

**Table 4.1: NAFDAC requirement inspection of the selected samples**

<b>Code</b>	<b>Batch</b>	<b>NAFDAC</b>	<b>Country</b>	<b>Date</b>	<b>Date of</b>
<b>Number</b>	<b>of origin</b>	<b>of Mng.</b>	<b>Expiry</b>		
Sample A	150211	04-2481	India	Feb.,2015	Jan., 2020
Sample B	15116	04-2898	India	Jan. 2015	Dec., 2019
Sample C	CM85	A4-3776	Nigeria	May.,2016	May, 2019
Sample D	Y018	A4-8982	Nigeria	June, 2016	May, 2019

Sample E	6001	A4-0701	Nigeria	Jan., 2016	Dec., 2019
Sample F	09	04-7635	Nigeria	March, 2016	Feb., 2019

## 4.2 Quality Control Studies

### 4.2.1 Identification test of amoxicillin standard powder

The melting point of the standard amoxicillin powder recorded was 193-195<sup>0</sup>C which agrees with the B.P 2009 specification. The identity of the powder was further confirmed with the IR spectrum obtained. The infrared IR spectrum was found to be superimposable with the reference (BP, 2009) IR spectrum of amoxicillin (figure 4.1).

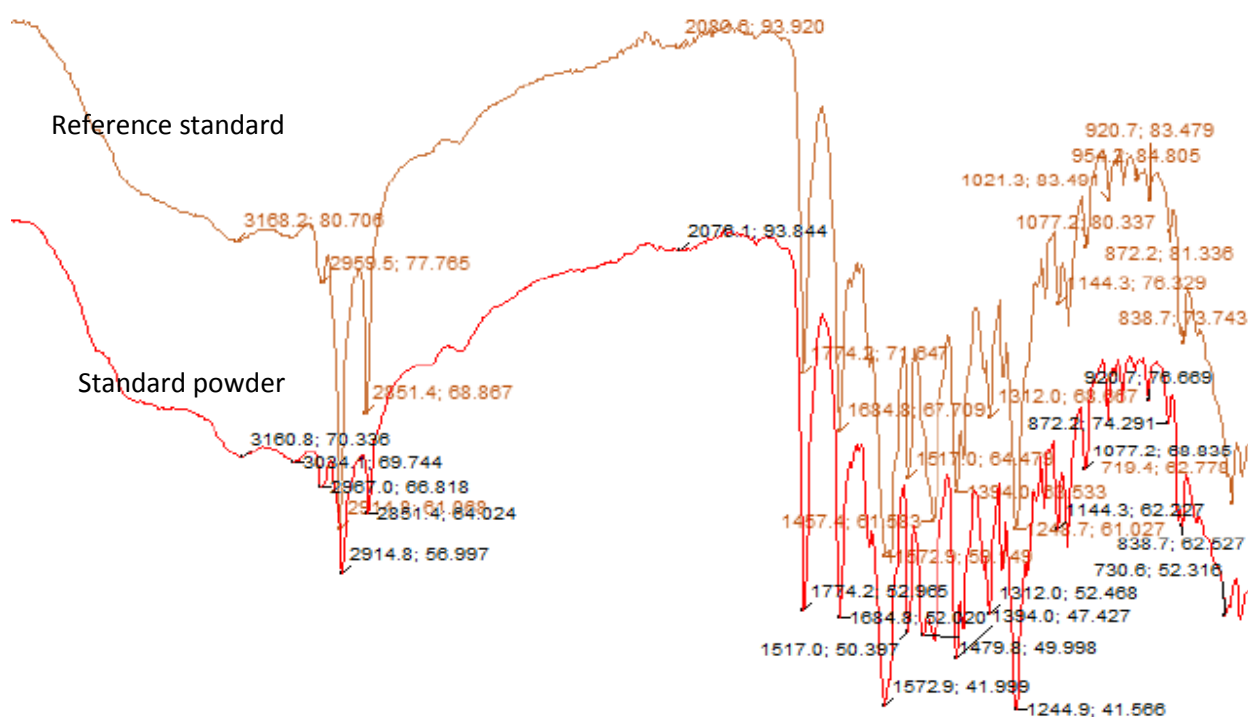


Fig. 4.1: Superimposed spectra of reference (BP, 2009) and standard amoxicillin powder

#### 4.2.2 Identification test of Amoxicillin Capsules

All the brands passed the identification test using IR spectrophotometry as their individual spectrum was found to be superimposable at the finger print region with the reference spectrum of amoxicillin (figures 4.2 to 4.7).

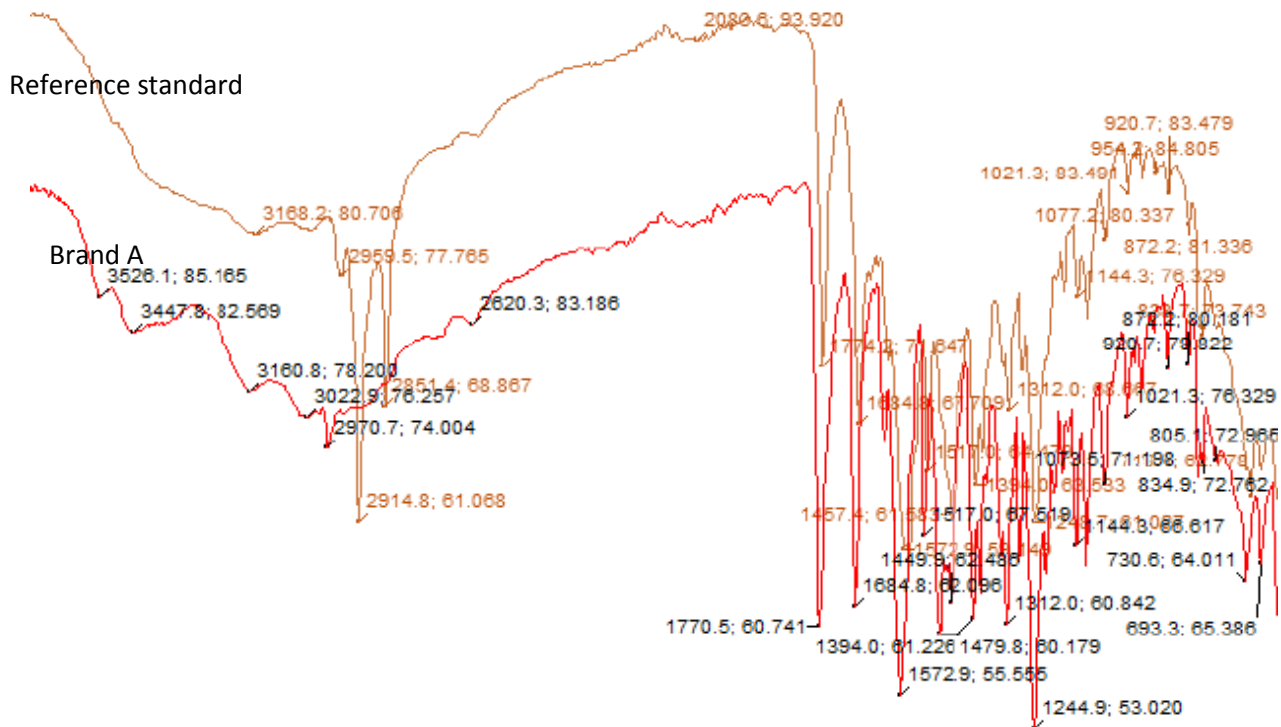
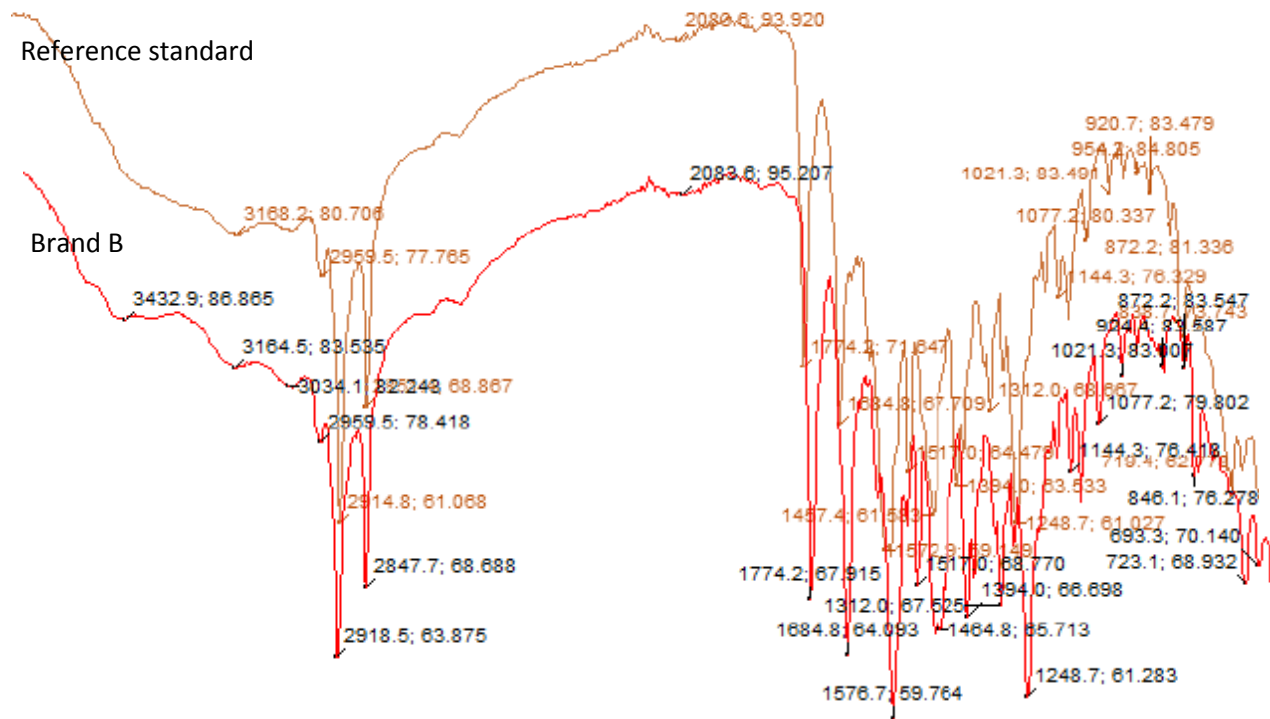


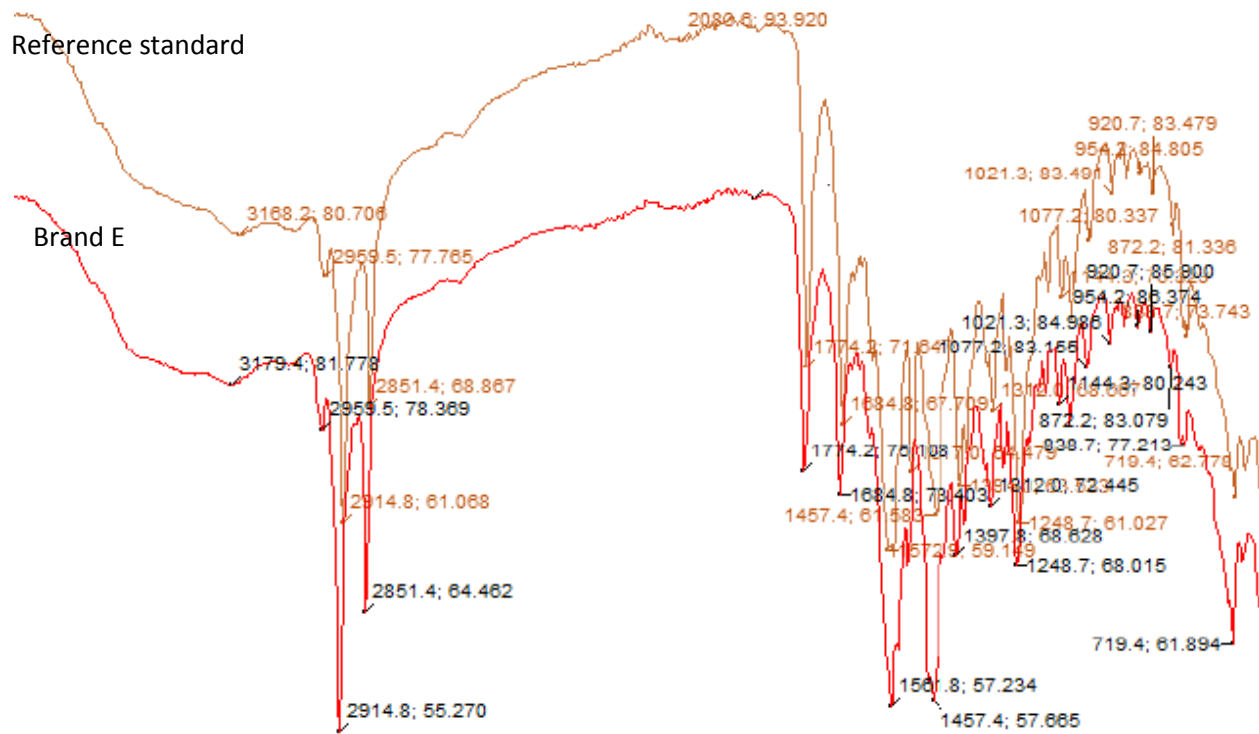
Fig. 4.2: Superimposed spectra of reference(BP, 2009) and brand A amoxicillin capsule



**Fig. 4.3: Superimposed spectra of reference (BP, 2009) and brand B amoxicillin capsule**







**Fig. 4.6: Superimposed spectra of reference(BP, 2009) and brand E amoxicillin capsule**



3	Brand C	699	0.0174		1.34	Passed
4	Brand D	432	0.0199	1.64		Passed
5	Brand E	699	0.0259	1.92		Passed
6	Brand F	620	0.0391		3.04	Passed

*% mean deviation <7.5 for tablets and capsules >334 mg (BP, 2009).*

**Table 4.3: Assay of the amoxicillin capsules brands**

S/N <sup>o</sup>	Brand	Quantity assayed	Quantity obtained	% Content	Remarks
<b>(g)</b>					
1	Brand A	0.67	0.548	109.60	Passed
2	Brand B	0.680	0.551	110.20	Passed
3	Brand C	0.670	0.490	97.96	Passed
4	Brand D	0.430	0.222	44.32*	Failed
5	Brand E	0.690	0.493	98.54	Passed
6	Brand F	0.630	0.318	63.56*	Failed

\* Stands for test failed by the brand.

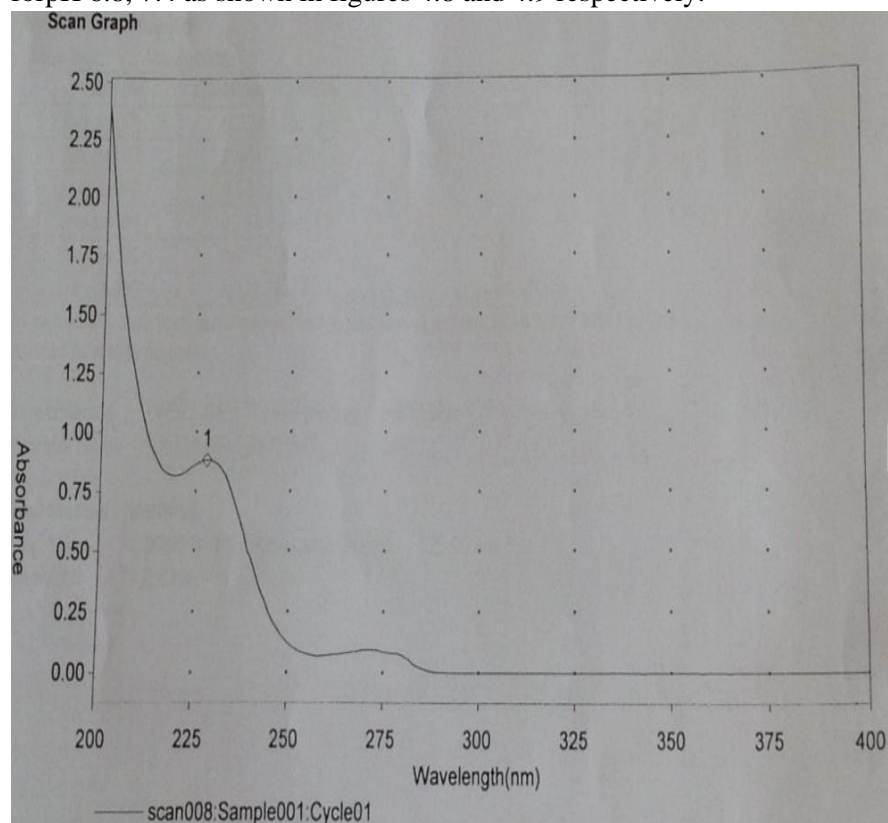
**Table 4.4: Disintegration time test of the amoxicillin capsules brands**

<b>Brands code</b>						
<b>A</b>	<b>B</b>	<b>C</b>	<b>D</b>	<b>E</b>	<b>F</b>	<b>Capsule</b>
<b>Disintegration time (min)</b>						
1	4.50	4.40	4.60	6.10	5.50	5.40
2	6.10	5.20	4.90	6.20	5.8	6.00
3	7.21	5.20	5.10	6.30	6.00	6.10
4	8.12	5.40	5.20	7.00	6.80	6.30
5	8.10	6.30	5.20	7.10	7.10	6.79
6	8.20	7.00	5.30	7.20	7.81	7.00

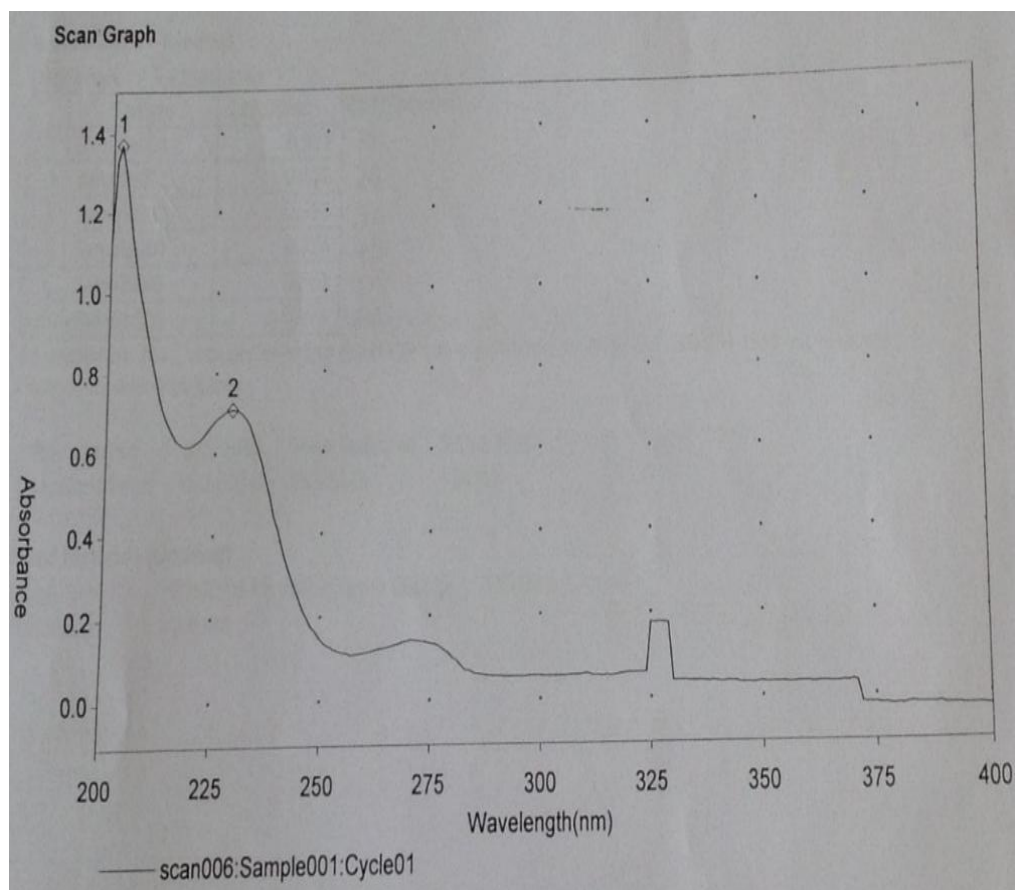
### 4.3 Analytical Methods

#### 4.3.1 Wavelength of maximum absorption

The wavelength of maximum absorption ( $\lambda_{max}$ ) was found to be 229 nm for pH 1.2, 4.5 and 228 nm for pH 6.8, 7.4 as shown in figures 4.8 and 4.9 respectively.



**Fig. 4.8: Scanned  $\lambda_{max}$  of amoxicillin standard powder in pH 1.2 and 4.5**



**Fig. 4.9: Scanned  $\lambda_{max}$ . of amoxicillin standard powder in pH 6.8 and 7.4**

#### **4.3.2 Validation parameters of the developed methods**

The result for linearity, precision and accuracy (tables 4.5 to 4.6).

##### *4.3.2.1.(Intra and inter-day precision):*

The co-efficient of variation (CV) was <15% in all the Simulated Physiological fluids; Thus precise.

**Table 4.5: Precision (Intra and inter-day) of the developed methods**

<u>Absorbance</u>	<b>pH 1.2</b>	<b>pH 4.5</b>	<b>pH 6.8</b>	<b>pH 7.4</b>
Intra-day				

---

Absorbance					
(Mean ±SD)	0.732 ±0.015	0.752 ±0.026	0.806 ± 0.020	0.759±0.005	
CV	2.05%	3.46%	2.48%	0.66%	
Inter-day					
Absorbance					
(Mean ± SD)	0.732 ±0.067	0.757±0.068	0.791 ± 0.046	0.721±0.057	
CV	9.15%	9.00%	5.82%	0.66%	

---

**Table 4.6: Accuracy and percentage recovery in all the media**

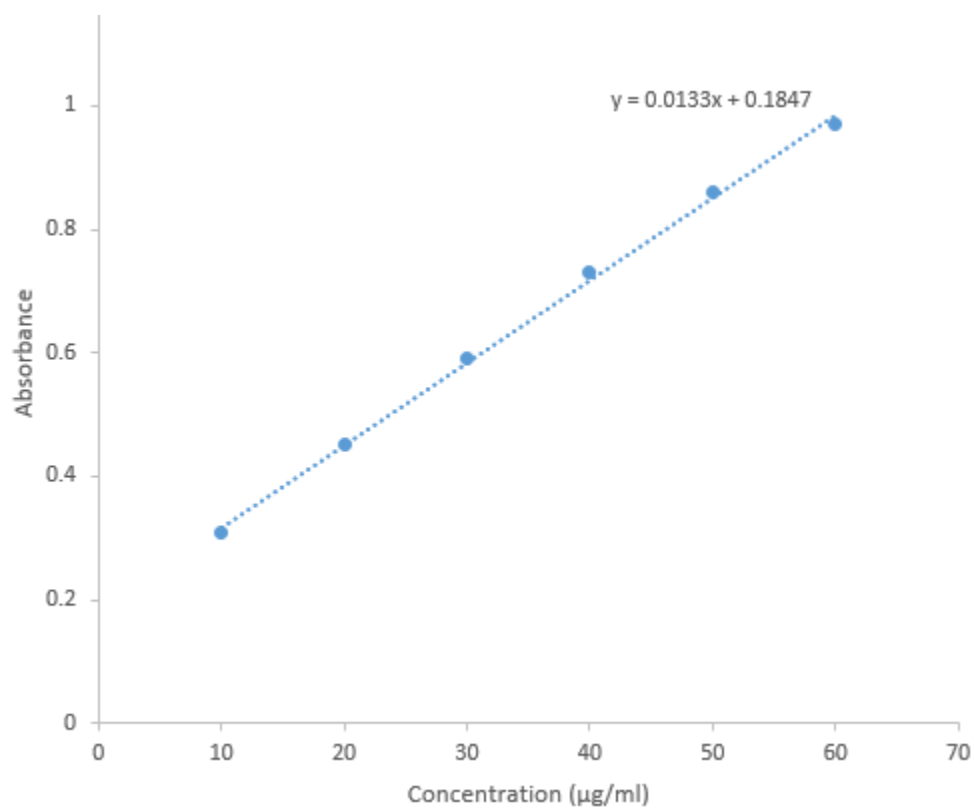
**S/No:**

		<b>pH 1.2</b>			<b>pH 4.5</b>			<b>pH 6.8</b>			<b>pH 7.4</b>		
1	Amount added	8	10	12	8	10	12	8	10	12	8	10	12
	(µg/ml) n=3												
2	Amount found	7.8	9.9	11.8	7.92	9.8	11.9	7.8	9.9	12.1	7.9	9.9	11.8
	(µg/ml) n=3												
3	Percentage recovery (%)	98	99	98	99	98	99	98	99	101	99	99	98

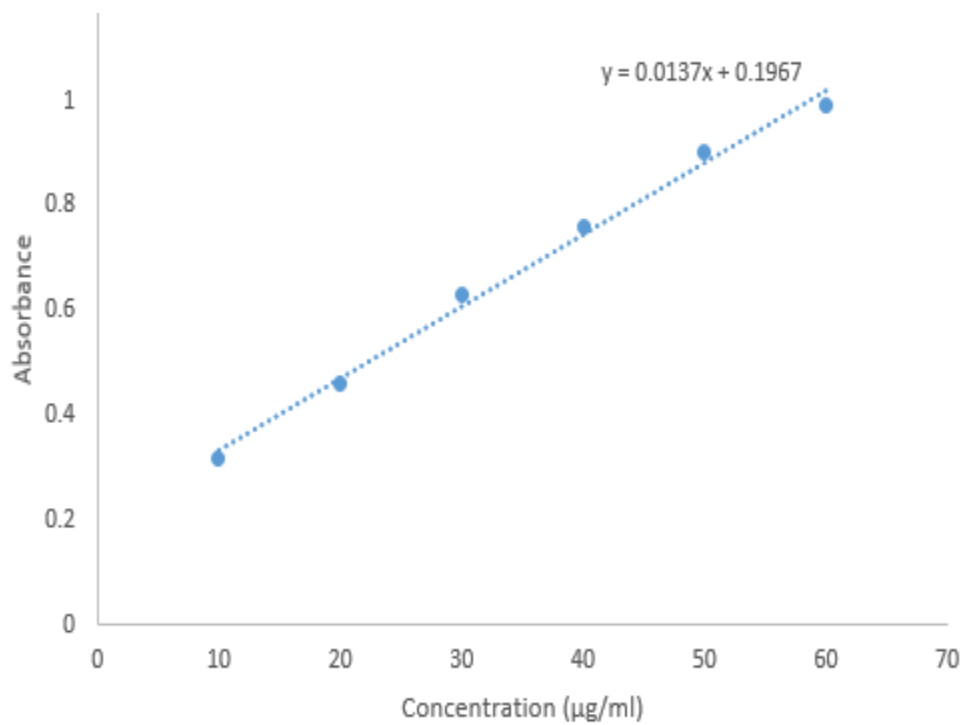
---

#### 4.3.2.4 Linearity of the methods:

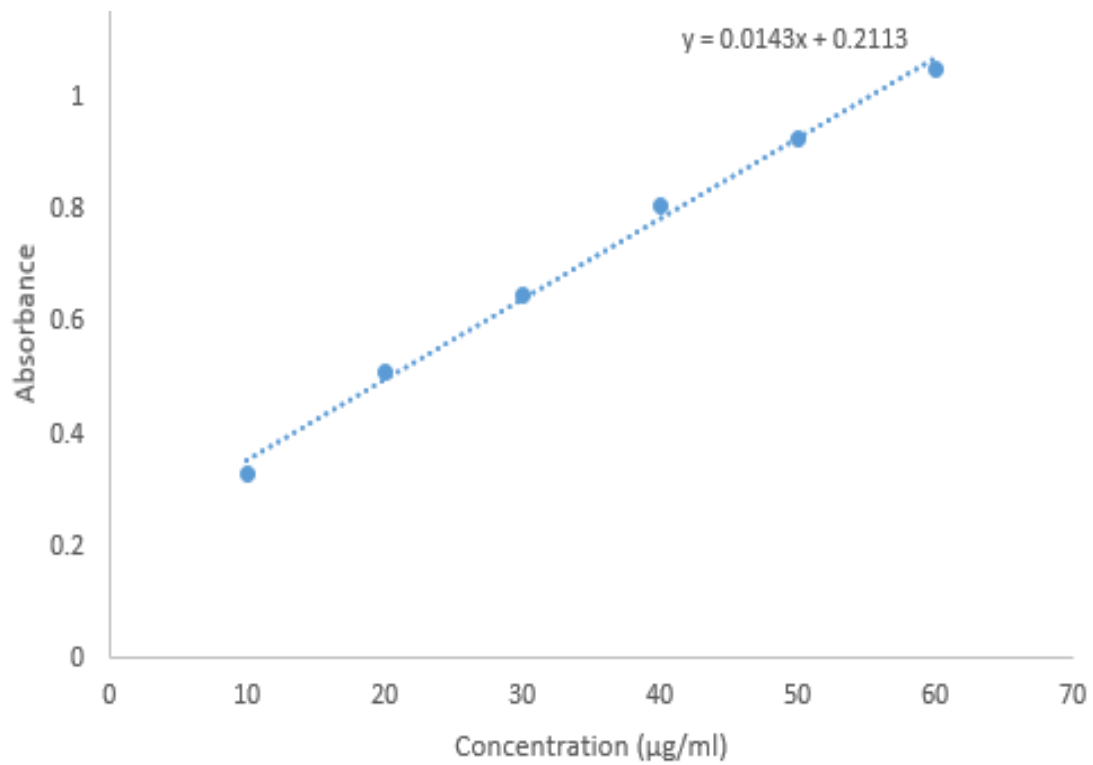
The methods were found to obey Beer-lambert's law within the concentration range of 10-60  $\mu\text{g/ml}$  in all the simulated physiological pH as their correlation coefficients were close to unity (figures 4.10 to 4.13).



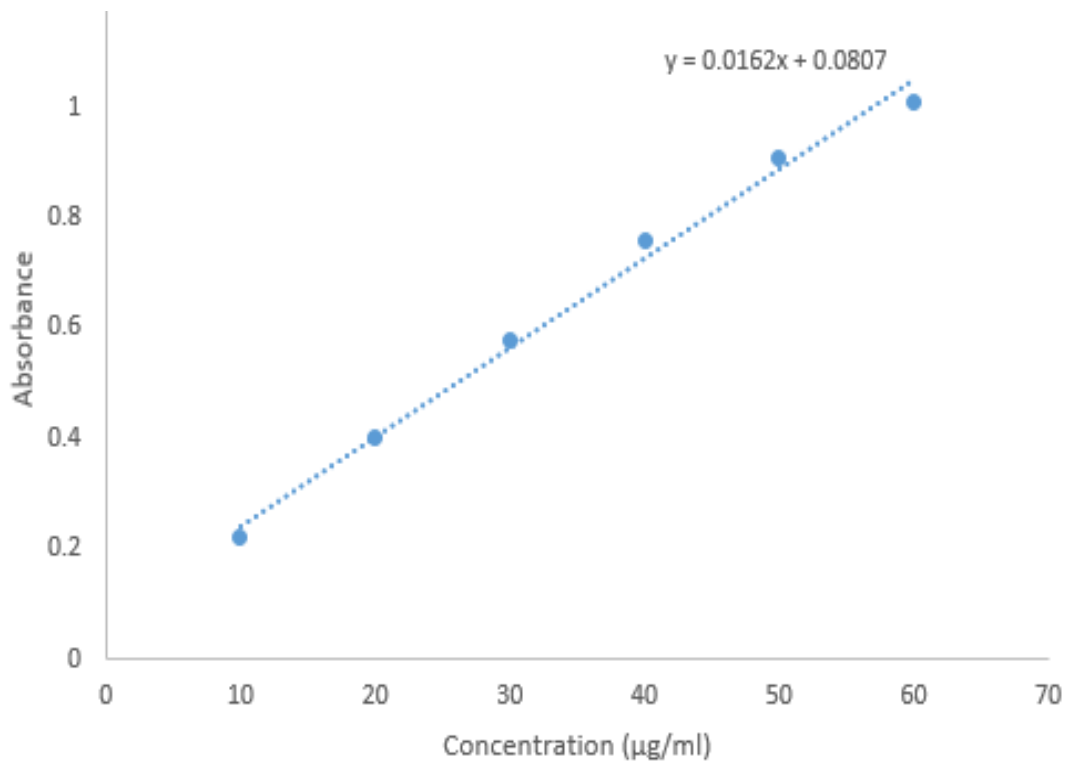
**Fig. 4.10: Calibration curve of amoxicillin in simulated physiological (pH 1.2)**



**Fig. 4.11: Calibration curve of amoxicillin in simulated physiological (pH 4.5)**



**Fig. 4.12: Calibration curve of amoxicillin in simulated physiological (pH 6.8)**



**Fig. 4.13: Calibration curve of amoxicillin in simulated physiological (pH 7.4)**

#### **4.3.3. Calibration parameters**

The summary of the calibration parameter obtained from the methods developed for each of the simulated physiological media (table 4.7).

**Table 4.7: Summary of the calibration curves parameters of the developed methods**

<b>Parameter</b>		
<b>pH 1.2</b>	<b>pH 4.5</b>	<b>pH 7.4</b>

---

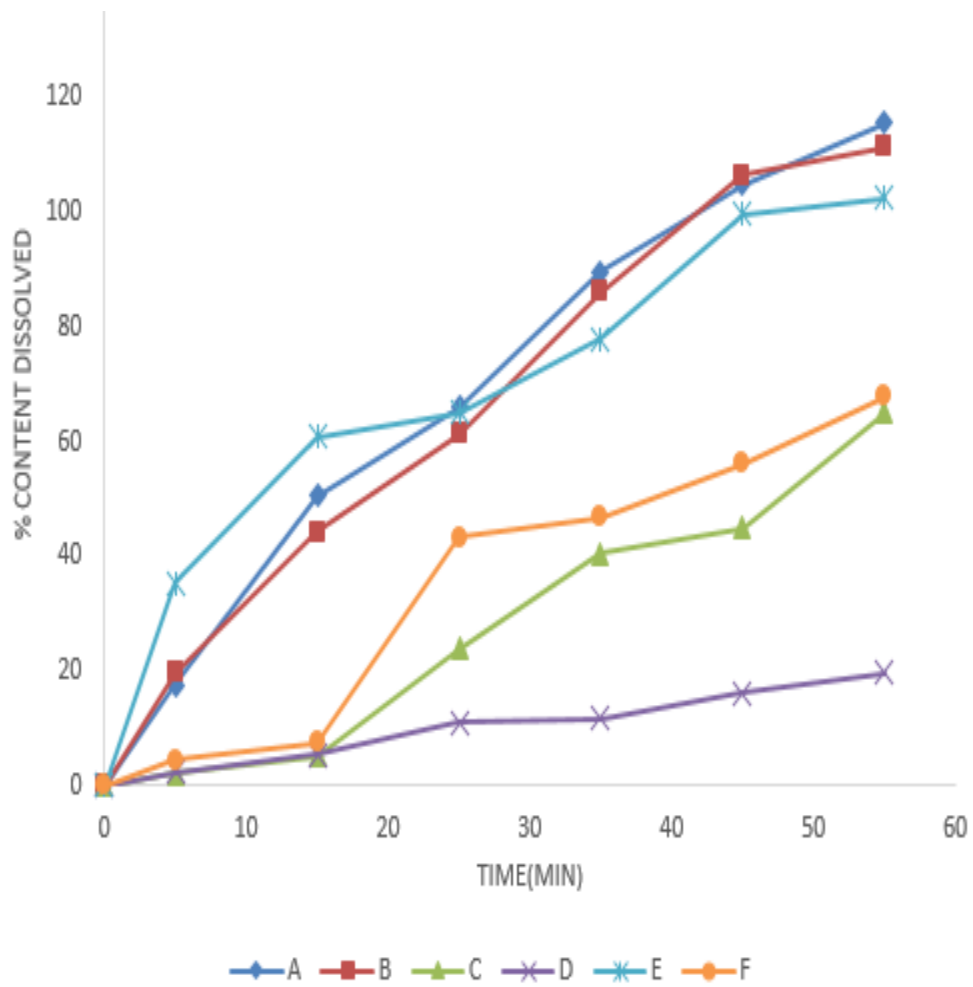
1 $\lambda$ max	229nm	229 nm	228nm	228 nm
2 Calibration curve Range	10-60 $\mu$ g/ml	10-60 $\mu$ g/ml	10-60 $\mu$ g/ml	10-60 $\mu$ g/ml
3 Regression equation	$y=0.0133x+0.1847$	$0.0137x+0.1967$	$0.0143x+0.2113$	$0.0162x+0.0807$
4 Coefficient of correlation (r)	0.9990	0.9970	0.9970	0.996
5 Intercept	0.1847	0.1967	0.2113	0.0807

---

#### **4.4 *In vitro* Bioequivalence Prediction Analysis**

##### **4.4.1 Dissolution profile of amoxicillin in simulated physiological media**

The dissolution profile (s) of amoxicillin in each of the four simulated physiological media (figures 4.14 to 4.17).



**Fig 4.14: Dissolution profile of amoxicillin in simulated physiological pH 1.2**

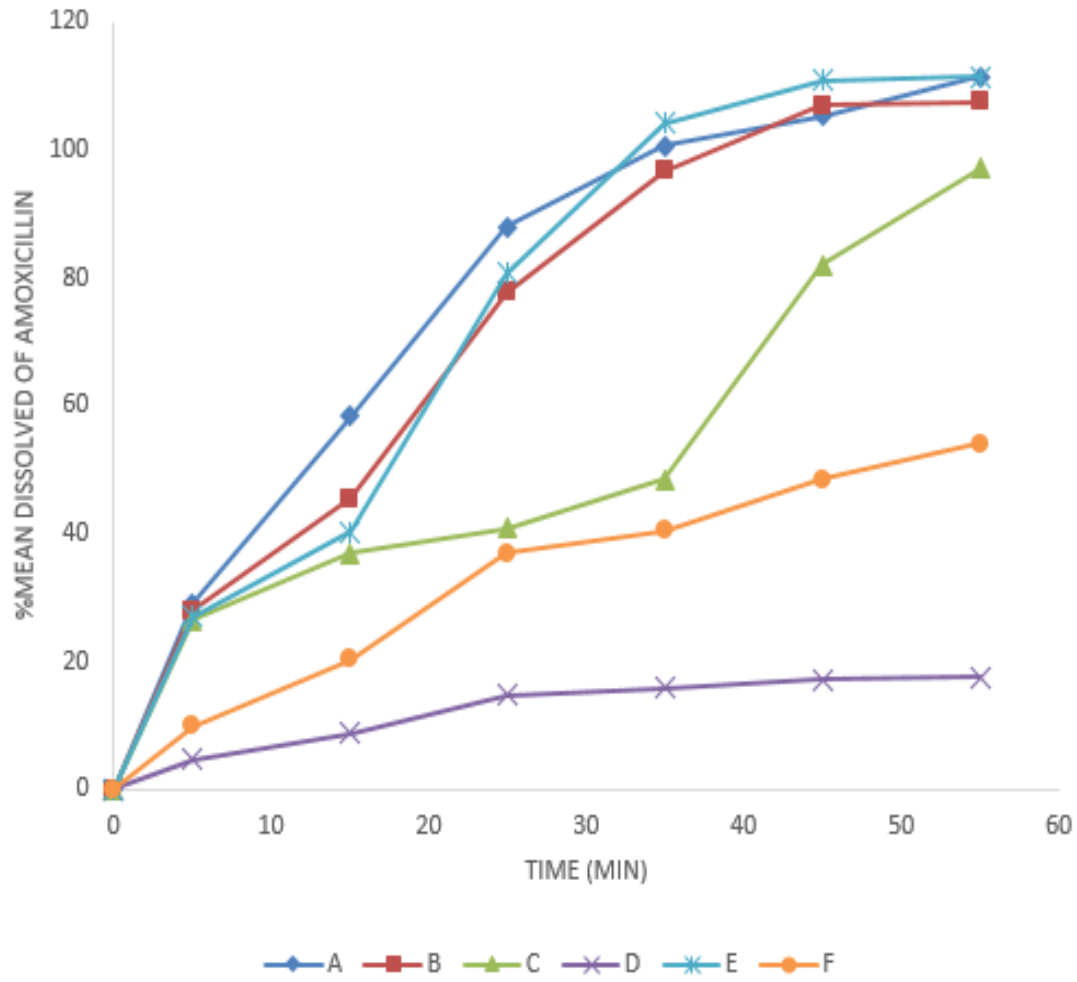
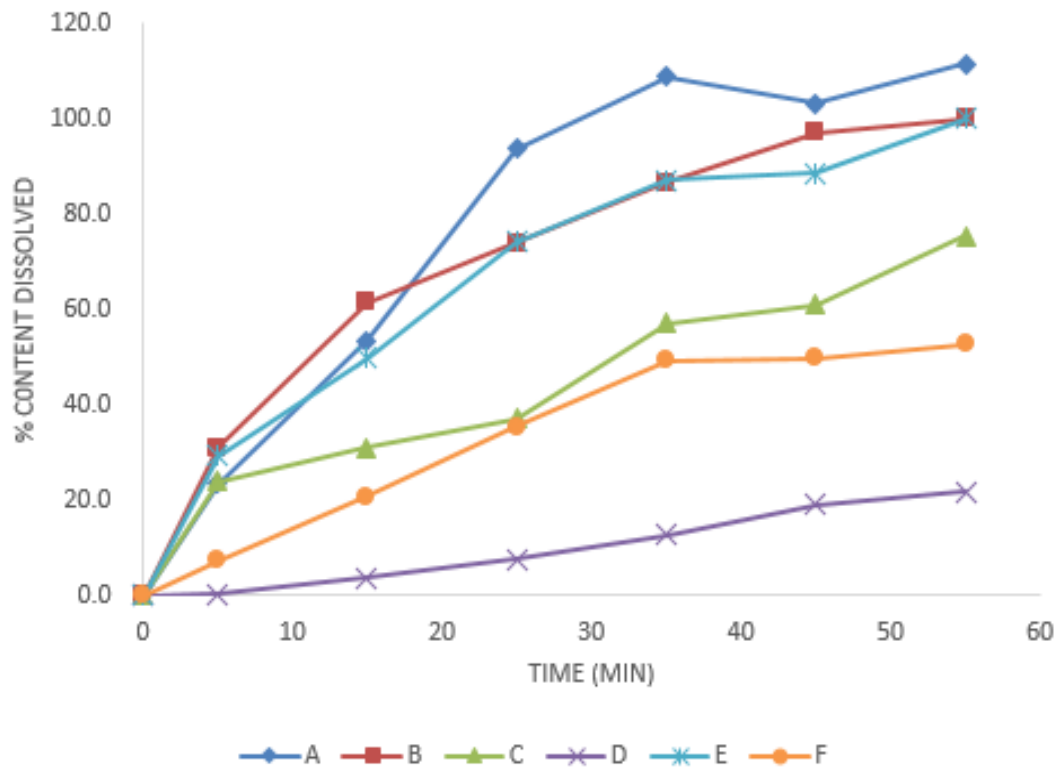
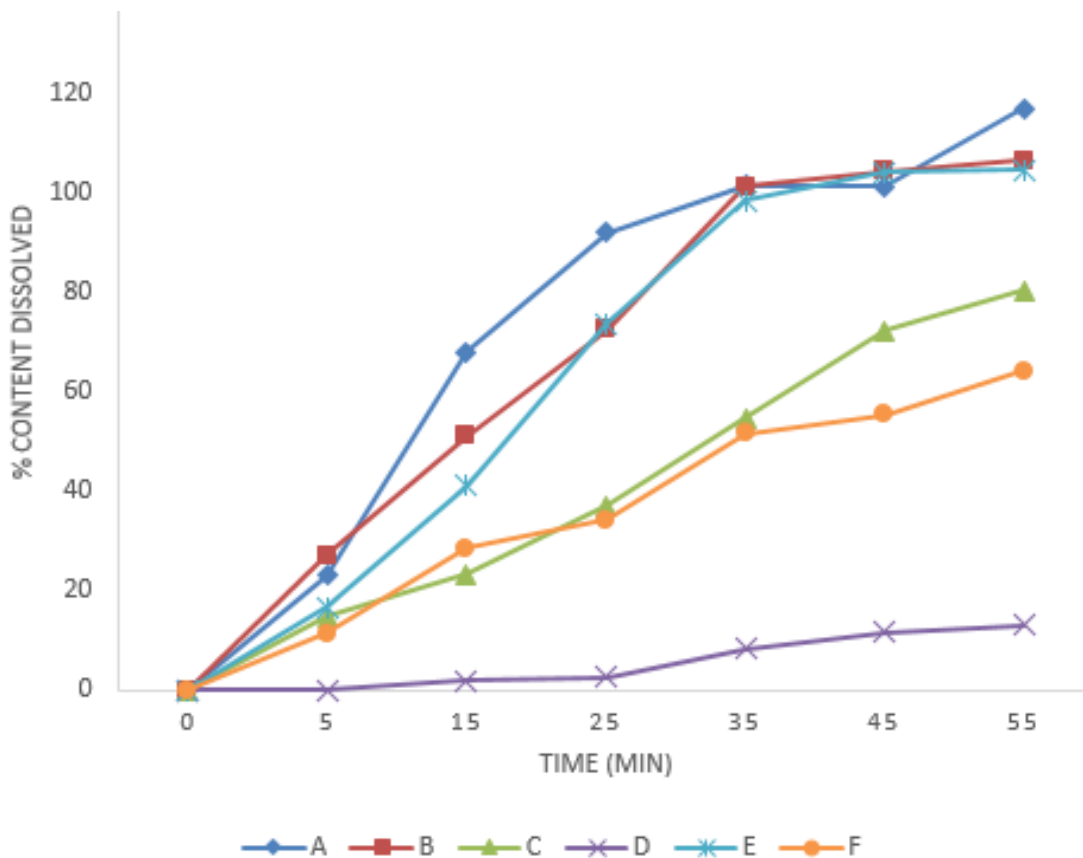


Fig 4.15: Dissolution profile of amoxicillin in simulated physiological pH 4.5



**Fig 4.16: Dissolution profile of amoxicillin in simulated physiological pH 6.8**



**Fig 4.17: Dissolution profile of amoxicillin in simulated physiological pH 7.4**

#### 4.4.2 Difference and similarity factors ( $f_1$ & $f_2$ ) and dissolution efficiency (% D.E.) of amoxicillin

Difference factor ( $f_1$ ), similarity factor ( $f_2$ ) and dissolution efficiency (% D.E.) as predictors of *in vitro* bioequivalence of the samples studied are presented in tables 4.8 to 4.11. A sample is said to be bioequivalent to the innovator (A) when the result of the fit factors is within the acceptable limits.

**Table 4.8:  $f_1$ ,  $f_2$  and % D.E. of amoxicillin in pH 1.2**

Code $f_1 f_2$	Mean D.E. (%) $\pm$ S.D.	% D.E.

A		0.00	100	80.63±5.6	0.00
B	3.35	69.00	75.63±4.7	4.00	
C	59.11	17.00	65.45±7.9	15.18	
D	84.97	8.12	55.24±6.7	25.39	
E	0.70	47.36	70.93±3.5	9.70	
F	34.66	28.20	67.68±7.6	12.95	

*Acceptable limit of  $f_1$  is  $\leq 15$ , while  $f_2$  is  $\geq 50$  and % D.E. is  $\pm 10\%$*

**Table 4.9:  $f_1$ ,  $f_2$  and % D.E. of amoxicillin in pH 4.5**

Code	$f_1$	$f_2$	Mean D.E (%)±S.D.	% D.E.	
A		0.00	100.00	75.35±4.5	0.00
B	6.12	57.15	82.23±7.2	-6.88	
C	32.64	24.72	71.61±3.9	3.74	
D	84.00	6.77	57.69±8.5	17.54	
E	3.74	53.43	76.23±4.5	-0.88	
F	57.33	15.37	70.40±7.4	4.95	

*Acceptable limit of  $f_1$  is  $\leq 15$ , while  $f_2$  is  $\geq 50$  and % D.E. is  $\pm 10\%$*

**Table 4.10:  $f_1$ ,  $f_2$  and %D.E. of amoxicillin in pH 6.8**

Code	$f_1$	$f_2$	Mean D.E (%) $\pm$ S.D.	% D.E.	
A		0.00	100.00	77.66 $\pm$ 3.4	0.00
B	8.834	2.67	72.47 $\pm$ 3.8	2.05	
C	42.122	0.09	70.24 $\pm$ 6.7	18.66	
D	86.775	0.95	34.36 $\pm$ 7.9	43.30	
E	13.104	2.12	69.32 $\pm$ 2.5	8.34	
F	56.371	5.40	46.57 $\pm$ 5.6	31.09	

Acceptable limit of  $f_1$  is  $\leq 15$ , while  $f_2$  is  $\geq 50$  and % D.E. is  $\pm 10\%$

**Table 4.11:  $f_1$ ,  $f_2$  and % D.E. of amoxicillin in pH 7.4**

Code	$f_1$	$f_2$	Mean D.E (%) $\pm$ S.D.	% D.E.
------	-------	-------	-------------------------	--------

A	0.00	100.00	73.21±3.3	0.00
B	7.94 46.76	71.16±2.52.05		
C	48.7820.09	54.55±5.618.66		
D	92.414.31	25.55±4,3	47.66	
E	12.7741.85	81.22±3.7	-8.01	
F	49.4017. 98	43.11±7.2	30.10	

Acceptable limit of  $f_1$  is  $\leq 15$ , while  $f_2$  is  $\geq 50$  and % D.E. is  $\pm 10\%$

#### 4.4.3 Content of amoxicillin released over all time points in all the simulated physiological pHs

The content of amoxicillin released over all time points (5-55min.) in all the four simulated physiological pHs were used for analysis of variance (ANOVA) among the brands tested (table 4.12).

**Table 4.12: Content of amoxicillin released for analysis of variance (ANOVA)**

Code	% mean release ±S.D.
A	80.58±6.64
B	75.22±6.17
C	45.09±5.11
D	10.39±1.35
E	74.31±6.13
F	40.34±4.75

**4.4.4 Analysis of variance of % mean content released of amoxicillin in all simulated physiological pHs over all time points**

The result of analysis of variance of % mean content released of amoxicillin over all time points in the four simulated physiological pHs showing significance among the brands at ( $p < 0.05$ ), (table 4.13).

**Table 4.13: Result for analysis of variance of % mean released of Amoxicillin (two tailed)**

Time (min).		Sum of squares	Df	Mean square	F-value	Sig.
5-55	Between group	89602.167	5	17920.433	26.322	.000
	Within group	93954.167	138	680.827		
	Total	183556.334	143			

*Df= degree of freedom*

*Critical region for the test statistics F is 2.21*

**4.4.7.1 Dunnett test of ANOVA of amoxicillin in simulated physiological pHs:**

The result of dunnett multiple comparison test of analysis of variance of % mean content released of amoxicillin in four simulated physiological pHs, indicating statistically significant different with the innovator brand at ( $p < 0.05$ )(table 4.14).

**Table 4.14: Dunnett multiple comparison test of % mean released of amoxicillin (two tailed)**

Time (min)	Pair Comparison	% Mean Difference	significance

5-55	A vs B	-5.35750	.928
	A vs C	-35.48542*	.000
	A vs D	-70.18708*	.000
	A vs E	-6.26750	.874
	A vs F	-40.13542*	.000

\* mean difference is significant at  $p < 0.05$

## CHAPTER FIVE

### 5.0 DISCUSSION

#### 5.1 Quality Control Studies

All the selected brands used in this study were found to comply with NAFDAC requirements and were within the acceptable shelf lives (table 4.1). Thus, they are presumed to be genuine of good quality since they are registered by NAFDAC.

The quality control studies conducted on selected brands indicated that, in the uniformity of weight test all the brands passed the test as their percentage mean deviation was not more than 7.5 % specified by (BP, 2009). The percentage weight deviation was in the range of 0.485-3.040% (table 4.2). In addition, both the standard amoxicillin powder and the selected brands passed both BP 2009 and USP 2009 identification test, as their IR spectra were superimposable with the standard reference amoxicillin spectrum (figures 4.1 to 4.7). This is an indication that, the selected samples contained the active pharmaceutical ingredient (API). Also, assay test results showed that all the samples (except D and F) were within the specified limit set by USP, 2009 (90-120%) of amoxicillin content (table 4.3). Therefore, brands D and F may be substandard based on the assay test result. All the brands complied with official specification for disintegration test, as all disintegrated in less than 15 minutes (table 4.4) which conforms with specification of British Pharmacopoeia (BP, 2009) for uncoated tablets and hard gelatin capsules. Drug filled in a capsule shell released rapidly as the shell disintegrates; a rate limiting step for immediate release oral solid dosage form because the rate of disintegration affects dissolution and subsequently the therapeutic efficacy of the drug (Kassaye and Genete, 2013). This clearly indicated that all the brands would have their active ingredients released into the system for absorption. Kassaye and Genete (2013) conducted similar study on nine brands of amoxicillin and reported that all the tested brands complied with official requirement for disintegration. Likewise, Benmoussa *et al.*, (2012) carried out similar study on three brands of amoxicillin tablets and all were found to pass the test. The use of generic drugs and substitution from multisource has been advocated by the World Health Organization

(WHO) which prescribed that all persons have the “right to health” and should have access to essential medicines, defined as medicines that satisfy the priority health care needs of a population (WHO, 2010). The main goal of multisource substitution is to ensure that drugs are made available, accessible and affordable to all without compromising the quality and therapeutic efficacy of the branded drug (Arlene *et al.*, 2014). However, routine quality evaluation of the generics to ensure interchangeability with branded should be carried out to guarantee the choice of best and affordable brand thus, achieving better pharmacological outcome compared with the branded.

## 5.2 Analytical Methods

Each of the methods developed for the determination of amoxicillin in the four dissolution media have a very good linear relationship between absorbance (Y) and concentration (C) (table 4.7). Both the intra-day and inter-day precisions of the developed methods (table 4.5) were within the acceptable limit of 15 % recovery confirming that the developed methods showed good repeatability. The percentage recoveries were within the acceptable range of (98-102%)(table 4.6). Hence, the developed methods were simple, cheap, precise, accurate and reproducible. Therefore, can be used for *in vitro* bioequivalence analysis of amoxicillin.

## 5.3 *In vitro* Bioequivalence

Dissolution of solid oral dosage form determine its absorption into systemic circulation and serves as predictor of *in vivo* bioavailability (Kassaye and Genete, 2013). It is therefore, employed to assess the bioequivalence between the branded drug and generic counterpart, it also provides vital information on batch-batch difference in the release of active ingredients between same brands. Thus, utilized as substitute to *in vivo* performance of the drug (Polli *et al.*, 2008). For any brand to be considered *in vitro* bioequivalent with the innovator, US FDA, WHO and EMEA require that the dissolution profile should be similar in at least three simulated physiological media (pH 1.2, 4.5, 6.8 and/or 7.4) (WHO, 2010). In addition, generic brand is considered *in vitro* equivalent with its branded counterpart when the similarity factor ( $f_2$ ) is  $\geq 50$  and the difference factor ( $f_1$ ) is 0-15, so also dissolution efficiency of  $\pm 10$  %.

The dissimilarity factor ( $f_1$ ) for brands B and E were within the acceptable range in each of the four media (tables 4.8 to 4.11), while brands C, D and F values were above the acceptable limit of (tables 4.8 to 4.11). Hence, from the  $f_1$  results brands B and E achieved similar dissolution with A, therefore, B and E are considered bioequivalent with A. While, brands C, D and F were not similar with brand A as such they are not considered bioequivalent with A. Kassaye and Genete (2013) reported similar study with 62.5 % of the samples failed  $f_1$ . Similarly, Ngwuluka *et al.*, (2009) reported a study of ciprofloxacin in which 50 % were not bioequivalent.

The similarity factor ( $f_2$ ) for brand B were within the acceptable limit in simulated physiological media (1.2 and 4.5) (tables 4.8 and 4.9), but fails in simulated physiological media (pH 6.8 and 7.4) (tables 4.10 and 4.11) while, brands C, D and F failed to achieved the acceptable limit in each of the simulated physiological media (tables 4.8 to 4.11). Similarly, brand E achieved the acceptable limit in only one simulated physiological medium (pH 4.5) (table 4.9) and failed in the three simulated physiological media (tables 4.8, 4.10 and 4.11). Therefore, from the  $f_2$  results brand B passed the requirement in only two simulated media (pH 1.2 and 4.5) (tables 4.8 and 4.9) while, in simulated media (pH 6.8 and 7.4) (tables 4.10 and 4.11) the values were close to the acceptable limit thus, can be considered similar with brand A

since, its  $f_1$  values were within normal range in each of the simulated physiological media. Arlene et al., (2014) reported similar study of zidovudine similarity in only simulated media (pH 1.2 and 4.5). Some Class I pharmaceutical formulations may possess the same active ingredient and amount of drug but may show significant differences to in vitro equivalence requirements. nevertheless, the dissolution process is suitable to detect these variations (Arlene et al., 2014). Likewise, brand E achieved similar dissolution with A in simulated physiological (pH 4.5) only while in (pH 1.2, 6.8 and 7.4) the  $f_2$  values were less than the acceptable limit of  $\geq 50$ , thus E was similar with A in one simulated physiological media only, hence, is not considered bioequivalent with A based on  $f_2$  approach. Whereas, brands C, D and F were not similar with brand A in each of the simulated physiological media (pH 1.2, 4.5, 6.8 and 7.4) (tables 4.8 to 4.11) their  $f_2$  values were far below the acceptable limit. Therefore, Brand C, D and F were not bioequivalent with innovator brand A according to  $f_2$  bioequivalence. Kassaye and Genete (2013) study reported 37.5 % of the test samples to be bioequivalent with innovator based on  $f_2$  result. Similarly, Arlene *et al.*, (2014) reported that none of the four tested generic amoxicillin was bioequivalent with branded based on  $f_2$  in each of the media used.

The dissolution efficiency (% D.E.) model was also used in comparing the release profile of tested brands. The mean D.E. values obtained were compared by measuring the difference between the mean D.E. of the innovator and that of the test brands. If the differences of the mean dissolution efficiencies are within acceptable limits of ( $\pm 10\%$ ), it can be concluded that the reference and test dissolution profiles are similar and can be used interchangeably. Consequently, in the dissolution efficiency (% D.E.) model approach, brand B and E achieved the acceptable limit in each of the simulated physiological media (pH 1.2, 4.5, 6.8 and 7.4) (tables 4.8 to 4.11). While, brand C achieved the acceptable limit in simulated media (pH 4.5) (table 4.9) and failed in the remaining three simulated physiological media (pH 1.2, 6.8 and 7.4) (tables 4.8, 4.10 and 4.11). Moreover, brand D failed the D.E. prediction in each of the simulated physiological media (tables 4.8 to 4.11) but brand F achieved the acceptable limit in two simulated physiological media (pH 1.2 and 4.5) (tables 4.8 and 4.9) and failed in (pH 6.8 and 7.4) (tables 4.10 and 4.11). From the result above brands B and E were similar with A in each of the simulated physiological media. Hence, B and E are considered bioequivalent with brand A. Accordingly, brand C was not similar with brand A in all the simulated physiological media, except in (pH 4.5) where its % D.E. values was within the acceptable limit. The % D.E. values of brands C, D and F were found to be higher than the acceptable limit (tables 4.8 to 4.11). Therefore, brands C, D and F were not similar with brand A. Based on dissolution efficiency bioequivalent prediction. This further confirmed the results obtained from fit factor ( $f_1$  and  $f_2$ ) calculations which shows that only brand B and E fall within the appropriate limit as compared to the innovator while the rest of the brands (C, D and F) were higher than the specified range. Hence, they are not interchangeable with innovator brand A.  $f_1$  and  $f_2$  values are very simple to calculate, but the calculation for D.E. is more complex but easy to comprehend the result (Anderson *et al.*, 1999). Fit factors comparison ( $f_1$  and  $f_2$ ) do not reveal the intra batch disparity due to the fact that calculations need to be made on the mean (Kassaye and Genete, 2013). It is also said not to be sensitive to the shapes of dissolution profiles and does not put into consideration uneven spacing between sampling time points (Khan, 1975). Even though  $f_2$  is somewhat closely interrelated with D.E. it is more difficult to infer  $f_2$  than D.E. data without reference to dissolution data or curves, as it relates to differences between curves, and because of its nonlinear behaviour (Anderson *et al.*, 1999).

However, the dissolution profiles were further subjected to analysis of variance (Anova) to find if the significant difference observed in  $f_1$ ,  $f_2$  and D.E. analysis among the brands will be supported through anova and dunnett multiple comparison test investigation. The anova test indicated difference in the

release profile at ( $p < 0.05$ ) (table 4.13) while, dunnett multiple comparison confirmed that the dissolution profiles of brands C, D and F were significantly different with innovator brand A at ( $p < 0.05$ )(table 4.14).

In this study, all the comparative methods have proven the similarity of the dissolution profiles and bioequivalence of brand B and E with the innovator A. About 60% of the generic products are not interchangeable with the innovator product. The two brands B, and E can be used as alternative to each other as well as with the innovator brand A. However, brands C, D and F cannot be used as alternative to the innovator brand A.

## CHAPTER SIX

### 6.0 Summary, Conclusion and Recommendations.

#### 6.1 Summary

This study was designed to evaluate the dissolution profiles and bioequivalence of six selected samples of amoxicillin capsules with a view to determine the interchangeability between the generics and innovator brand. It is however, aimed to first assess the samples for compliance with NAFDAC requirements for product registration in country. It also intends to carry out quality control studies, develop and validate four UV spectrophotometry methods for bioequivalence studies following successful conduct of dissolution testing of the selected samples in each of the developed method.

All the tested brands of the 500mg Amoxicillin were within the appropriate shelf life and meet the NAFDAC requirements of registration number, address of the manufacturer, date of manufacture and expiry etc.

The result of quality control tests carried out confirmed brands to pass identification, uniformity of weight test, assay test and disintegration time test (except brand D and F) which failed assay test.

The developed and validated UV-spectrophotometric methods for analysis of Amoxicillin have good precision, accuracy and high percentage recovery thus can be successfully used for *in vitro* bioequivalence study of Amoxicillin in simulated physiological media. Dissolution profile data was generated using the developed methods and were used for the calculations of difference factor, similarity factor and % dissolution efficiency.

Bioequivalence among the tested brands of amoxicillin capsules was predicted using the data obtained from the dissolution testing. The two major statistical approaches fit factor comparison  $f_1, f_2$  and Dissolution efficiency model (%D.E.) were determined. About 40% of the tested brands were found to be

*in vitro* bioequivalent with innovator brand A whereas 60% were not bioequivalent with the innovator brand A.

## **6.2 Conclusion**

The present study was carried out with successful development and validation of four UV spectrophotometric methods for determination of amoxicillin. The developed methods were utilized in the bioequivalence evaluation of the six selected samples of amoxicillin after carrying routine quality control studies on the samples. About 60 % of the samples were not bioequivalent with the innovator thus, may not be used interchangeably while, 40 % of the samples were bioequivalent with the innovator, therefore, they may be used interchangeably. The findings of the study show potential for counterfeiting of antibiotics among the generics brands studied.

## **6.3 Recommendations**

Based on the findings of this research, two brands (B and E) of amoxicillin capsules may be used interchangeably with innovator brand A.

There is need for collaborations between NAFDAC and our educational research institutions, so that academic researches are practically utilized for the benefit of the general population.

It is also imperative for the drug regulatory agency (NAFDAC) at national level to put more stringent measures of quality assurance of the drugs before given them marketing authorization.

Further studies should be carried out using higher precision techniques such as HPLC methods.

*In vitro in vivo* correlation study should be conducted to ascertain the interchangeability between the innovator and the various brands.

There is urgent need to continuously undertake dissolution testing of BCS class I drugs e.g. Amoxicillin

Post market monitoring through *in vitro* bioequivalent testing, *in vivo* bioequivalent studies as well as other established method of quality assurance are greatly recommended.

## REFERENCE

- Adegbolagun OA., Olalade OA., and Osumah SE., (2007). Comparative evaluation of biopharmaceutical and chemical equivalence of some commercially available brands of Ciprofloxacin hydrochloride tablets. *Tropical Journal of Pharmaceutical Research*. 6(3):737-45.
- Al meri MN., Whittacker, C. Tucker, A. Yaqoob, and M. Johnston, A. (2011). A Survey to determine the views of renal transplant patients on generic substitution in the UK. *Transplant International*. 24(8):770-779.
- Al meri MN., Nanda N., KG., Anil, K. David, P., Arthur, T. and Atholl, J. (2012). The difference between branded and generic medicines using solid dosage forms: In vitro dissolution testing. *Journal of pharmaceutical sciences*. 2:1-8.
- Amidon, G. L., Lennernäs, H. Shah V.P. and Crison, J.R. (1995). A theoretical basis for a biopharmaceutical drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. *Pharmaceutical Research*, 12(3):413-420.
- Amin, AS., El-Ansary, AL., and Issa, YM., (1994). Colorimetric determination of amoxicillin in pure form and in pharmaceutical preparations. *Talanta*. 41(5): 691-694.
- Anderson NH, Bauer M, Boussac N, Khan- Malek R, Munden P, and Sardaro S., (1999). An evaluation of fit factors and dissolution efficiency for the comparison of in vitro dissolution profiles. *Journal of Pharmaceutical and Biomedical Analysis* 17: 811 – 822.
- Aristides, D. and Panos, M. (2006). A century of dissolution research: From Noyes and Whitney to the Biopharmaceutics Classification System *International Journal of Pharmaceutics* 14;321(1-2): 1–11.
- Arlene, V. S. Jieyu, Z. and Raimar, L. (2014). Investigating the Dissolution Profiles of Amoxicillin, Metronidazole and Zidovudine Formulations used in Trinidad and Tobago, West Indies. *American Association of Pharmaceutical Scientists* 15(5): 1060-1069.
- Ashraful Islam, S.M. Sharmi, I. Mohammad, S. and Irin, D. (2011). Comparative in vitro dissolution study of Aceclofenac marketed tablets in two different dissolution media by validated analytical method, *Journal of Applied Pharmaceutical Sciences*, 1(9):87-92.
- Attaran, A. Barry, D. Basheer, S. Bate, R. Benton, D. Chauvin, J. Garrett, L. Kickbusch, I. Kohler, J.C. Midha, K. Newton, P.N. Nishtar, S. Orhii, P. and McKee, M. (2012). How to achieve international action on falsified and substandard medicines. *British Medical Journal*. 345:e7381. doi: <https://doi.org/10.1136/bmj.e7381>.
- Benouda, A. Sibile, S. Ziane, Y. Elouennass, M. Dahani, K. and Hassani, A. (2009). Place of *Streptococcus pyogenes* in the throat infections and overview of its susceptibility to antibiotics. *Pathology Biology* 57(1): 76-80.

- Benmoussa, A. El Harti, J. Lamsaouri, J. Cherrah, Y. and Taoufik, J. (2013). Comparison of the dissolution of reference dispersible tablets of amoxicillin versus two generics manufactured in Morocco. *International Journal of Pharmacy and Pharmaceutical Sciences* 5(1):49-51.
- Blume, H.H. and Schug, B.S. (1999). The biopharmaceutics classification system (BCS): class III drugs—better candidates for BA/BE waiver? *European Journal of Pharmaceutical Sciences* 9(2):117-121.
- Bochner, F. Hooper, W.D., Tyrer, J.H., and Eadie, M.J., (1972). Factors involved in an outbreak of phenytoin intoxication. *Journal of Neurological Sciences*. 16(4): 481–487.
- British Pharmacopoeia (2009). Volume I, II & III Monographs: Medicinal and Pharmaceutical substance Amoxicillin. Her majesty stationary office, London. PP 361-7978.
- Campagna, F.A., Cureton, G., Mirigian, R.A., and Nelson, E., (1963). Inactive prednisone tablets USP XVI. *Journal of Pharmaceutical Sciences* 52(1): 605–606.
- Chadli, M., Rtabi, N., Alkandry, S., Koek, J., Achour, L., Buisson, Y., and Baaj, A., (2005). Incidence of surgical wound infections a prospective study in the Rabat Mohamed-V military hospital, Morocco. *Med Mal Infect* 35(4): 218-222.
- Chakravarty, S. Unnithan, S. and Ram, A. (2001). Deadly doses. *India Today*, 29 January 2001, p 58–61.
- Chandrasekaran, A.R. Chen, Y. H. Alex, Chin, Y. Lim, W. and Low, S. (2011). Post market *in-vitro* equivalency evaluation of Paracetamol tablets in Kedah, Malaysia, *International Journal of Pharmaceutical Science and Nanotechnology*, 4(2):1403-1407.
- Chapron, D.J., Kramer, P.A., Mariano, S.L., and Hohnadel, D.C., (1979). Effect of calcium and antacids on phenytoin bioavailability. *Archives of Neurology* 36(7): 436–438.
- Cheng, C. L. Yu, L. X. Lee, H. L. Yang, C. Y. Lue, C. S. and Chou, C. H. (2004). Bio waiver extension potential to BCS Class III high solubility– low permeability drugs: bridging evidence for metformin immediate- release tablet. *European Journal of Pharmaceutical Science*. 22(4):297-304.
- Cloyd, J.C., Gummit, R.J., and Lesar, T.S., (1980). Reduced seizure control due to spoiled phenytoin capsules. *Annals of Neurology*. 7(2): 191–193.
- Cook, J.A. and Bockbrader, H.N. (2002). An industrial implementation of the Biopharmaceutics Classification System. *Dissolution Technologies*.
- Dahan, A. and Amidon, G.L. (2008). Gastrointestinal dissolution and absorption of class II drugs. In: Van de Waterbeemd and H, Testa B, editors. *Drug bioavailability: estimation of solubility, permeability, absorption and bioavailability*. Weinheim: *Wiley-VCH*; p. 33– 51.
- Dahan, A. Miller, J.M., and Amidon, G.L., (2009). Prediction of Solubility and Permeability Class Membership: Provisional BCS Classification of the World's Top Oral Drugs. *Journal of American Association Pharmaceutical Scientists*. 11(4):740-746.

Daniel, D. H., and Lisa, G.W. (2012). Beta-Lactam & Other Cell Wall- & Membrane-Active Antibiotics. In: Katzung Basic & Clinical Pharmacology. *The McGraw-Hill Companies, Inc.* pp 490-497.

Edwards, L.J., (1951). The dissolution and diffusion of aspirin in aqueous media. *Transaction Faraday Society.* 47(1): 1191–1210.

EMA (2001). Committee for Proprietary Medicinal Products. Note for Guidance on the Investigation of Bioavailability and bioequivalence  
<http://www.emea.europa.eu/pdfs/human/ewp/140198en.pdf> (accessed January 9, 2017).

Fackler, M. (2002). China's fake drugs kill thousands. San Francisco Examiner, *Associated Press.* 31 July 2002.

FDA (1995a). Guidance for industry immediate release solid oral dosage forms scale-up and postapproval changes: chemistry, manufacturing, and controls, *in vitro* dissolution testing, and *in vivo* bioequivalence documentation. (retrieved February 6, 2017).

FDA (1995b). Guidance for industry immediate release solid oral dosage forms scale-up and post approval changes: chemistry, manufacturing, and controls, *in vitro* dissolution testing, and *in vivo* bioequivalence documentation. (retrieved November 17, 2016).

FDA (2000). Waiver of *in vivo* bioavailability and bioequivalence studies for immediate-release solid oral dosage forms based on a biopharmaceutics classification system, Guidance for Industry; U. S. Government Printing Office: Washington, D.C.

CFR 21/FDA (2003). Food and drugs containing a codification of documents of general applicability and future effect office of the federal register national archives and records administration. U.S. government printing office Washington.

FDA (2008). Approved Drug Products with Therapeutic Equivalence Evaluations, 28th edition. <http://www.fda.gov/cder/orange/obannual.pdf> (accessed November 9, 2016).

FDA (2015a). Dissolution testing and specification criteria for immediate-release solid oral dosage forms containing biopharmaceutics classification system class 1 and 3 drugs guidance for industry.

<http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm>.

FDA (2015b). Guidance for Industry, Waiver of *In-Vivo* Bioavailability and Bioequivalence Studies for Immediate-Release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System.

FDA (2017). Approved drug products with therapeutic equivalence evaluations 37<sup>th</sup> edition the products in this list have been approved under section 505 of the federal food, drug, and cosmetic act. *Washington.*

Ferraz, HG., Carpentieri, LN., and Watanabe, SP., (2007). Dissolution Profile Evaluation of Solid Pharmaceutical Forms Containing Chloramphenicol Marketed in Brazil. *Brazilian archives of biology and technology* 50(1): 57 –65.

Frank, RG. (2007). The ongoing regulations of generic drugs. *New England Journal of Medicine.* 357(20):1993-1996.

- Fraser, E.J., Leach, R.H., and Poston, J.W., (1972). Bioavailability of digoxin. *Lancet* 2, 541.
- Garcia J (2006) Post-Market Surveillance: A Product Regulator's View. Australian Government, Department of Health Therapeutic Goods Administration  
[http://asiahwpa.org/upload/id213/Workshop%20IV:%20Ensuring%20the%20Safety%20of%20Marketed%20Medical%20Devices%20%20Vigilance%20Reporting%20System%20\(Session%201\).pdf](http://asiahwpa.org/upload/id213/Workshop%20IV:%20Ensuring%20the%20Safety%20of%20Marketed%20Medical%20Devices%20%20Vigilance%20Reporting%20System%20(Session%201).pdf) (Accessed 20th October, 2016).
- Ghorab, M. Khafagy, E. Kamel, M. and Gad, S. (2012). Formulation, characterization and comparative in vitro in vivo evaluation of sustained release theophylline tablets. *International Journal of Pharmacy and Pharmaceutical Science*.4 (3): 721-728.
- Gibson, L. (2004). Drug regulators study global treaty to tackle counterfeit drugs. *British Medical Journal* 328(1);486. <http://dx.doi.org/10.1136/bmj.328.7438.486>.
- Huic, M. Vrhovac, B. Macolic-Sarinic, V. Francetic, I. Bakran, I. and Giljanovic, S. (1996). How safe are bioequivalence studies in healthy volunteers? *Therapie* 51:410–413.
- ICH (1996). Technical Requirements for the Registration of Pharmaceuticals for Human Use, Validation of analytical procedures: Methodology, ICH-Q2B, *Geneva Switzerland*.
- Institute of Medicine (2013). Countering the problem of falsified and substandard drugs. *Washington, DC*.  
<http://www.iom.edu/Reports/2013/Countering-the-Problem-of-Falsified-and> (accessed May 14, 2016).
- Jantratid, E. Prakongpan, S. Amidon, G. L. and Dressman, J. (2006). Feasibility of bio waiver extension to biopharmaceutics classification system class III drug products: cimetidine. *Clinical Pharmacokinetics*.45(4):385–99.
- Jinginger, H. E. Gundert-Remy, U. Moller, H. Pabst, E. and Steinijans, V. (1998). Studies on bioavailability and bioequivalence. *Directive APV. STP Pharma* 4 (6): 503-508.
- Kamerow, D. (2011). The pros and cons of generic drugs. *British Medical Journal* 343:4584.
- Kassaye, L. and Genete, G. (2013). Evaluation and comparison of in-vitro dissolution profiles for different brands of amoxicillin capsules. *African Health Sciences*.13 (2): 369-379.
- Kelesidis, T. and Falagas, ME., (2015). Substandard/counterfeit antimicrobial drugs. *Journal American Society for Microbiology* 28(2):443-464 doi:10.1128/CMR.00072-14.
- Khan, K.A., (1975). The concept of dissolution efficiency. *Journal of Pharmacy and Pharmacology*. 27:48-9.
- Kozlowski, S. Janet, W. Karen, M. and Rachel, BS. (2011). Developing the Nation's biosimilars program. *New England Journal of Medicine* 365 (20):385-8.
- Krämer J, Grady LT, and Gajendran J., (2005). Historical development of dissolution testing. In: Dressman, J. Krämer, J. (ed.). *Pharmaceutical Dissolution Testing*. Taylor and Francis Group, LLC; 2005. pp 1 – 37.

- Laurence, L. B., Keith, L. P., Donald, K. B., and Iain L.O. B., (ed.) (2008). Penicillins, Cephalosporins and other B-lactam antibiotics in: Goodman & Gilman's Manual of Pharmacology and Therapeutics. The McGraw-Hill Companies, Inc. New York, U.S.A. pp 730-738.
- Lester, A. M., Thomas L.L., and Elmer, J.G., (2008). Antibiotics and antimicrobial agents. In: Foye's principles of medicinal chemistry. Lippincott Williams & Wilkins, a Wolters Kluwer business, Baltimore, USA. Pp 1047-1055.
- Levy, G. Hall, N.A. and Nelson, E. (1964). Studies on inactive prednisone tablets USP XVI. *American Journal of Hospital Pharmacists*. 21, 402.
- Lindenbaum, J. Mellow, M.H., Blackstone, M.O., and Butler, Jr. V.P., (1971). Variation in biologic availability of digoxin from four preparations. *The New England Journal of Medicine*. 285(1) 1344–1347.
- Lipka, E. and Amidon, G.L. (1999). Setting bioequivalence requirements for drug development based on preclinical data: optimizing oral drug delivery systems. *Journal of Controlled Release*, 62(1-2) 41–49.
- Lobenberg, R. and Amidon, G.L. (2000). Modern bioavailability, bioequivalence and biopharmaceutics classification system. New scientific approaches to international regulatory standards. *European Journal of Pharmaceutics and Biopharmaceutics*. 50(1):3-12.
- MacLeod, C. Rabin, H. Ruedy, J. Caron, M. Zarowny, D. and Davies, R. (1972). Comparative bioavailability of three brands of ampicillin. *Canadian Medical Association Journal*. 107(3): 203–209.
- Martin, C.M. Rubin, M. O'Malley, W.G. Garagusi, V.F. and McCauley, C.E. (1968). Brand, generic drugs differ in man. *Journal of the American Medical Association* 205 (1): 23.
- Martinez, M.N. and Amidon, G.L. (2002). A mechanistic approach to understanding the factors affecting drug absorption: a review of fundamentals. *Journal of Clinical Pharmacology*. 42(6):620–643.
- Nagaralli, B.S., Seetharamappa, J. and Melwanki, M.B., (2002). Sensitive spectrophotometric methods for the determination of amoxicillin, ciprofloxacin and piroxicam in pure and pharmaceutical formulations. *Journal of Pharmacy and Biomedical Analysis*. 29:859-864.
- Nelson, E. (1957). Solution rate of theophylline salts and effects from oral administration. *Journal of the American Pharmaceutical Association*. 46(10): 607–614.
- Ngwuluka, N.C. Lawal, K. Olorunfemi, P.O. and Ocheke, N.A. (2009). Post-market *in-vitro* bioequivalence study of six brands of Ciprofloxacin tablets in Jos, Nigeria, *Scientific Research and Essay*, 4 (4); 298-305.
- Ocheke, N.A. Ngwuluka, N.C. Agbowuro, A.A. and Obodozie, O.O. (2012). Dissolution Profiles of Twelve Brands of Sulphadoxine Pyrimethamine in the Nigerian market, *Dissolution Technology*, 19(1):59-64.
- Olori, T. (1996). Nigeria-Health: bogus drugs—a national headache. *Inter Press Service News agency*, 5 December 1996.

Olubukola, O. Oyetunde, Fola, T. Moshood, O. A. and Bolajoko, A. A. (2012). *In-vitro* equivalence studies of generic Metformin Hydrochloride tablets and Propranolol Hydrochloride tablets under biowaiver conditions in Lagos State, Nigeria, *Dissolution Technology*; 51-55.

Panchagnula, R. Singh, R. and Ashokraj, Y. (2007). *In-vitro* evaluation of modified release formulations of Nifedipine from Indian market, *Indian Journal of Pharmaceutical Sciences*, 69(4):556.

Parvin, Z.M., Peyman, N.M., Saeed, G. M. N. and Hadi, V. (2012). *In-vitro* bioequivalence study of 8 brands of Metformin tablets in Iran market, *Journal of Applied Pharmacy Science*, 2(8):194-197.

Pharmaceutical Security Institute (2014). Counterfeit situation. <http://www.psi-inc.org/incidentTrends.cfm>. (accessed 24<sup>th</sup> August, 2016).

Pincock, S. (2003). WHO tries to tackle problem of counterfeit medicines in Asia. *British Medical Journal*, 327:1126.

Polli, J.E. Bertil, S. A. and Lawrence, X. Yu (2008). *In-vitro* Studies Sometimes Better than Conventional *In-Vivo* Studies in Assessing Bioequivalence of Immediate-Release Oral Solid Dosage Forms. *Journal of American Association of Pharmaceutical Scientist*. 10 (2):289-299.

Raheela, B. Shahnaz, G. Syed, B. Shyum, N. and Shoukat, M. (2011). Pharmaceutical evaluation of different brands of levofloxacin tablets (250 mg) available in local market of Karachi (Pakistan), *International Journal of Current Pharmaceutical Research*, 3(1):15-22.

Reidenberg, M.M. and Conner, B.A. (2001). Counterfeit and substandard drugs. *Clinical Pharmacology Therapeutics*. 69(4):189–193.

Rojanarata, T. Opanasopit, P. Ngawhirunpat, T. Saehuan, C. Wiyakrutta, S. and Meevootisom, V. (2010) A simple, sensitive and green bienzymatic UV-spectrophotometric assay of amoxicillin formulations. *Enzyme Microbial Technology* 46: 292-296.

Rudolf, P.M. and Bernstein, I.B. (2004). Counterfeit drugs. *New England Journal of Medicine* 350: 1384–1386. <http://dx.doi.org/10.1056/NEJMp038231>.

Sakore, S. and Chakraborty, B. (2011). In Vitro–In Vivo Correlation (IVIVC): A Strategic Tool in Drug development. *Journal of Bioequivalence and bioavailability*. 3(1):1-12. doi:10.4172/jbb.S3-001.

Sean, C S. (36).(2009). Antibacterial in Martindale the complete drug reference. *1 Lambeth High Street, London SE1 7JN, UK: China by Everbest Printing Co. Ltd.* pp 158-205.

Simoens, S. (2011). Generic and therapeutic substitution: Ethic meets health economics. *International Journal of clinical pharmacology*. 33(3): 469-470.

Skelly, J.P., (1988). Bioavailability of sustained release dosage forms—relationship with *in-vitro* dissolution. In: Yacobi, A., Holperin-Walega, E. (Eds.), *Oral Sustained Release Formulations*. Pergamon, New York, p. 57.

Standard treatment guidelines (2008) Federal Ministry of Health, Abuja, Nigeria. Pp 79-91.

Stimson Centre (2011). Counterfeit drugs and national security. *Washington, DC*.  
[www.stimson.org](http://www.stimson.org).(retrieved May 14, 2016).

Tanjinatus. S. O. Ishrat, N. and Ashraf Islam, S.M. (2011). Comparative *in-vitro* Bioequivalence Analysis of Some Generic Tablets of Atorvastatin, a BCS Class II Compound, *Bangladesh Pharmaceutical Journal*.14(1): 61-65.

Torres, RF., Consentino, MO., Lopez, MAB., and Mochon, MC., (2010). Simultaneous determination of 11 antibiotics and their main metabolites from four different groups by reversed-phase highperformance liquid chromatography–diode array–fluorescence (HPLC–DAD–FLD) in human urine samples. *Talanta*. 81:871-880.

Tyrer, J.H., Eadie, M.J., Sutherland, J.M., and Hooper, W.D., (1970). Outbreak of anticonvulsant intoxication in an Australian city. *British Medical Journal*. 4(5730): 271–273.

United State Pharmacopoeia (2009). USP Monographs development: antibiotic Amoxicillin pp 162-1402.

Vanitasagar, S. Srinivas, C. Subhashini, N.J.P. and Mallesh, K. (2012); Solid dispersion-a comparative study on the dissolution rate of aceclofenac. *International Journal of Pharmacy and Pharmaceutical Sciences* 4: 274-278.

Varley, A. B. (1968). The generic in equivalence of drugs. *Journal of the American Medical Association* 206(1): 1745–1748.

World Health Organization (2005) Proposal to waive in vivo bioequivalence requirements for the WHO model list of essential medicines immediate release, solid oral dosage forms.  
[http://www.who.int/medicines/services/expertcommittees/pharmprep/QAS04\\_109Rev](http://www.who.int/medicines/services/expertcommittees/pharmprep/QAS04_109Rev).

World Health Organization (2006a). “Combating counterfeit drugs: A concept paper for effective international cooperation,” p.3.

World Health Organization (2006). World Health Organization Expert Committee on specifications for pharmaceutical preparations, 40th report. Available from: [http://apps.who.int/prequal/info\\_general/documents/TRS937/WHO\\_TRS\\_937\\_eng.pdf#page=359](http://apps.who.int/prequal/info_general/documents/TRS937/WHO_TRS_937_eng.pdf#page=359). (accessed 4<sup>th</sup> May, 2016).

World Health Organization (2010). Medicines: essential medicines. Available from: <http://www.who.int/mediacentre/factsheets/fs325/en/>. (accessed 28<sup>th</sup> September, 2016).

World Health Organization. (1999 a). Summary of WHO counterfeit drug database as of April 1999, unpublished paper of the WHO Division of Drug Management and Policies. *Geneva, Switzerland*.

World Health Organization. (1999). Counterfeit and sub-standard drugs in Myanmar and Vietnam. *Geneva, Switzerland*.

World Health Organization. (2000). World Health Organization counterfeit drug reports: 1999- October 2000.  
[www.who.int/medicines/services/counterfeit/overview/en/1](http://www.who.int/medicines/services/counterfeit/overview/en/1). Accessed 10 October 2016.

World Health Organization. (2014).[http://www.who.int/medicines/areas/quality\\_safety/quality\\_assurance/QualityAssurancePharmVol2.pdf?ua\\_1](http://www.who.int/medicines/areas/quality_safety/quality_assurance/QualityAssurancePharmVol2.pdf?ua_1). Accessed 16 January 2017.

Yu, L. X. Lipka, E. Crison, J. R. and Amidon, G. L. (1996). Transport approaches to the biopharmaceutical design of oral drug delivery systems: prediction of intestinal absorption. *Advance Drug Delivery Review*.19(3):359-376.

**Appendices:**

Appendix I: Label information of six brands of Amoxicillin Capsules (500mg)

---

<b>Code</b>	<b>Batch</b>	<b>NAFDAC</b>	<b>Country</b>	<b>Date</b>	<b>Date</b>
<b>of origin</b>	<b>of Mng.</b>		<b>of expiry</b>		

---

Sample A	150211	04-2481	India	Feb.,2015	Jan., 2020
Sample B	15116	04-2898	India	Jan. 2015	Dec., 2019
Sample C	CM85	A4-3776	Nigeria	May.,2016	May, 2019
Sample D	Y018	A4-8982	Nigeria	June, 2016	May, 2019
Sample E	6001	A4-0701	Nigeria	Jan., 2016	Dec., 2019
Sample F	09	04-7635	Nigeria	March, 2016	Feb., 2019

All the six brands complied with the NAFDAC requirement as shown above.

Appendix II: Uniformity of weight (g) raw data

Brand A	Brand B	Brand C	Brand D	Brand E	Brand F
0.68	0.67	0.63	0.44	0.72	0.64
0.67	0.68	0.69	0.41	0.71	0.61
0.68	0.68	0.67	0.43	0.72	0.62
0.67	0.67	0.66	0.47	0.65	0.63
0.68	0.68	0.68	0.43	0.70	0.69
0.70	0.70	0.69	0.45	0.71	0.57
0.68	0.68	0.67	0.45	0.70	0.63
0.68	0.68	0.66	0.42	0.70	0.60
0.69	0.68	0.68	0.41	0.65	0.55
0.68 0.67	0.66	0.41	0.72	0.64	

Appendix III: Table of average titre value of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> standardization with KIO<sub>3</sub>

S/No.	Reagent	Titre value (ml)
1.	Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub>	13.00
2.	KIO <sub>3</sub>	12.10

Appendix IV: Table of average titre values of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> in assay of amoxicillin

Code	Average Titre (ml)
A	18.80
B	18.90
C	16.80
D	7.60
E	16.90
F	10.90

**Appendix V: Sample calculations of amoxicillin assay using iodometry titration**



$$1\text{N of KIO}_3 = 6\text{ N of Na}_2\text{S}_2\text{O}_3$$

$$214\text{ g of KIO}_3 \text{ in } 1000\text{ml} = 6\text{ N Na}_2\text{S}_2\text{O}_3$$

$$3.5666\text{ g of KIO}_3 \text{ in } 1000\text{ml} = 0.1\text{ N Na}_2\text{S}_2\text{O}_3$$

$$1.7833\text{ g of KIO}_3 \text{ in } 500\text{ml} = 0.1\text{N Na}_2\text{S}_2\text{O}_3$$

$$\text{Factor of KIO}_3 = \frac{\text{Actual weight}}{\text{Nominal weight}} = \frac{0.8}{1.7833} = 0.4486$$

$$\text{Factor of KIO}_3 (\text{F1}) = 0.4486$$

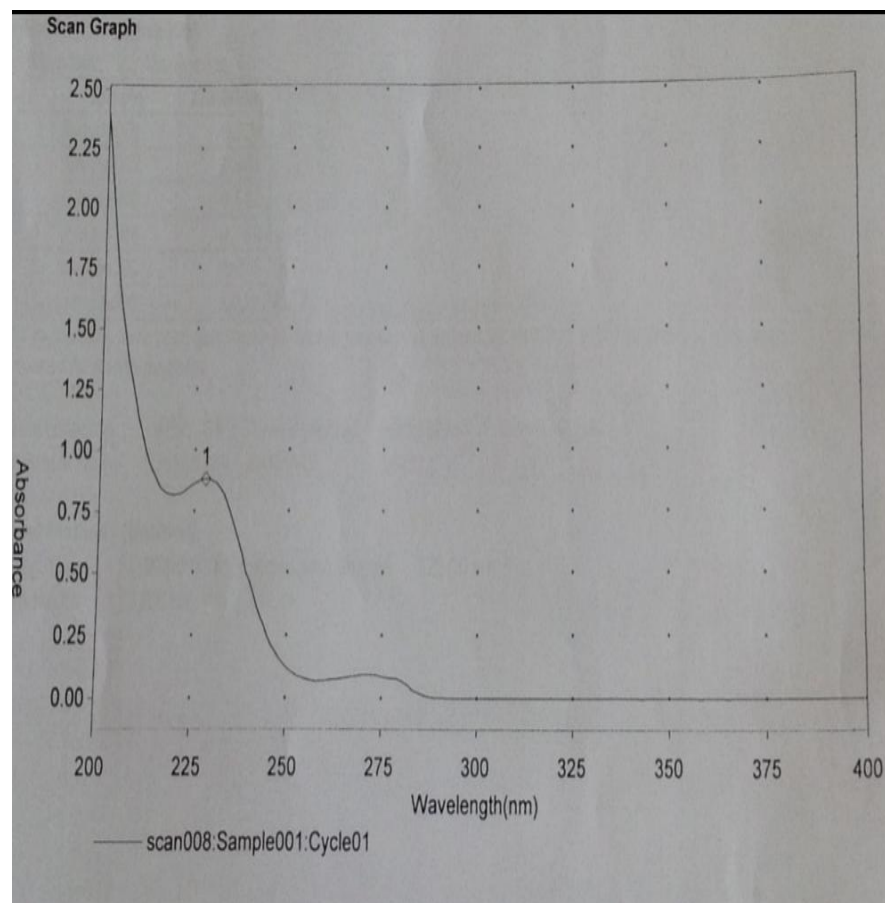
$$\text{Factor of Na}_2\text{S}_2\text{O}_3 (\text{F2}) = \frac{\text{F1V1}}{\text{V2}}$$

Volume of  $\text{KIO}_3$  (V1) = 11.90 ml  
 Volume of  $\text{Na}_2\text{S}_2\text{O}_3$  (V2) = 12.80 ml  
 Factor of  $\text{Na}_2\text{S}_2\text{O}_3$  (F2) =  $\frac{0.4486 \times 11.90}{12.80}$   
 = 0.4171  
 0.0699 g of  $\text{C}_{16}\text{H}_{19}\text{N}_3\text{O}_5\text{S} \cdot 3\text{H}_2\text{O}$  = 1 ml of 0.1 N  $\text{Na}_2\text{S}_2\text{O}_3$   
 Actual volume of  $\text{Na}_2\text{S}_2\text{O}_3$  = Factor of  $\text{Na}_2\text{S}_2\text{O}_3$  × Average titre  
 For brand A =  $0.4171 \times 18.80$   
 = 7.84 ml  
 7.84 ml of 0.1N  $\text{Na}_2\text{S}_2\text{O}_3$  =  $7.84 \times 0.0699$   
 = 0.5480 g  
 Strength of the capsule = 0.5 g  
 % Content of Amoxicillin =  $\frac{0.5480}{0.5} \times 100$   
 = 109.6 %  
 All other calculations were done in the same manner.

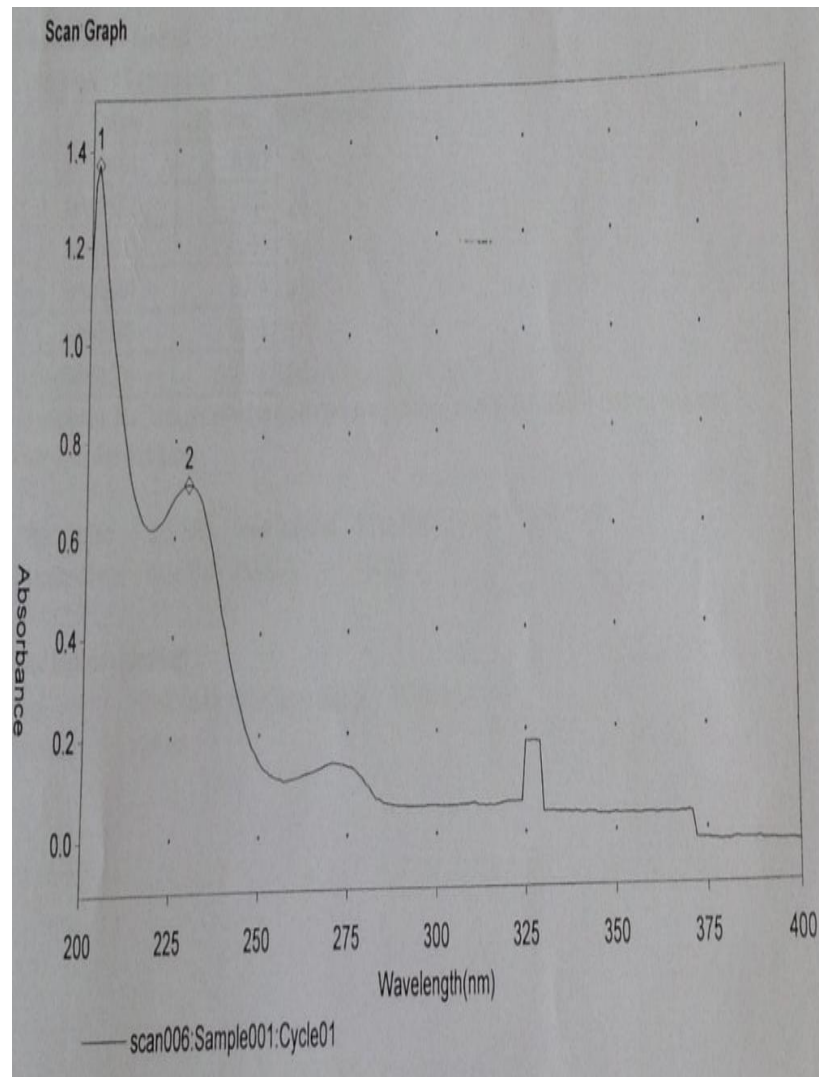
Appendix VI: Disintegration test raw data

Brand A	Brand B	Brand C	Brand D	Brand E	Brand F
4.50	4.40	4.60	6.10	5.50	5.40
6.10	5.20	4.90	6.20	5.80	6.00
7.21	5.20	5.10	6.30	6.00	6.10
8.12	5.40	5.20	7.00	6.80	6.30
8.10	6.30	5.20	7.10	7.10	6.79
8.20	7.00	5.30	7.20	7.81	7.00

Appendix VII: Scanned 40 $\mu$ g/ml amoxicillin in simulated physiological media  
(pH 1.2 and 4.5)



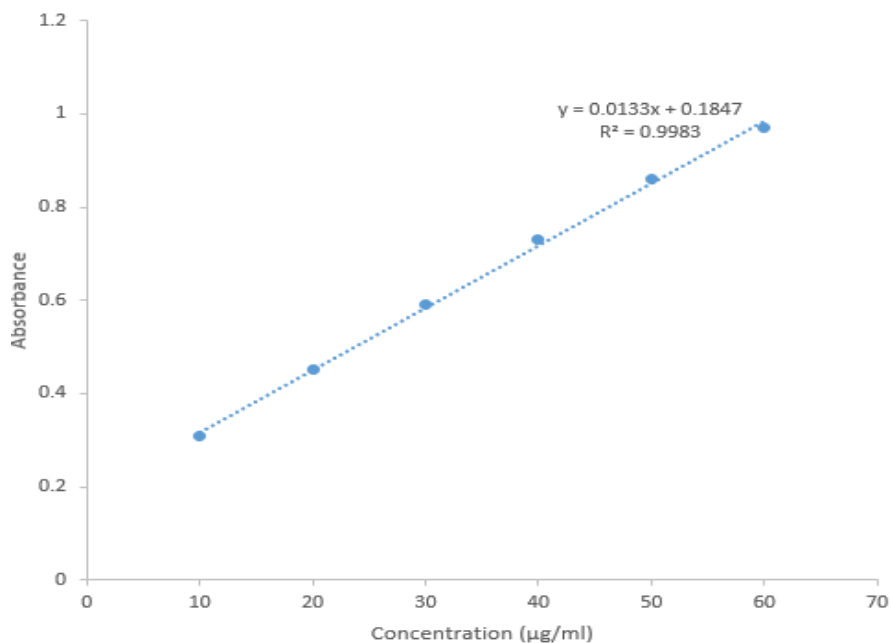
Appendix VIII: Scanned 40 $\mu$ g/ml amoxicillin in simulated physiological media (pH 6.8 and 7.4)



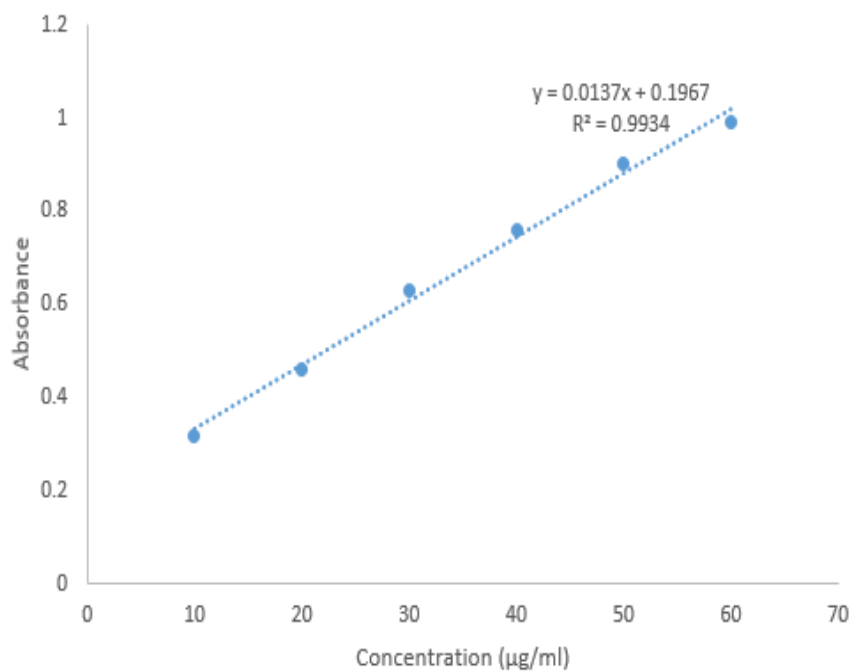
Appendix IX: Linearity study absorbance for simulated physiological media (pH 1.2, 4.5, 6.8 and 7.4)

S/Nº	Conc. (µg/ml)	Absorbance			
		pH 1.2	pH 4.5	pH 6.8	pH 7.4
1	10	0.310	0.321	0.333	0.221
2	20	0.451	0.461	0.512	0.401
3	30	0.585	0.626	0.645	0.576
4	40	0.726	0.765	0.812	0.757
5	50	0.861	0.901	0.927	0.905
6	60	0.967	0.992	1.046	1.013

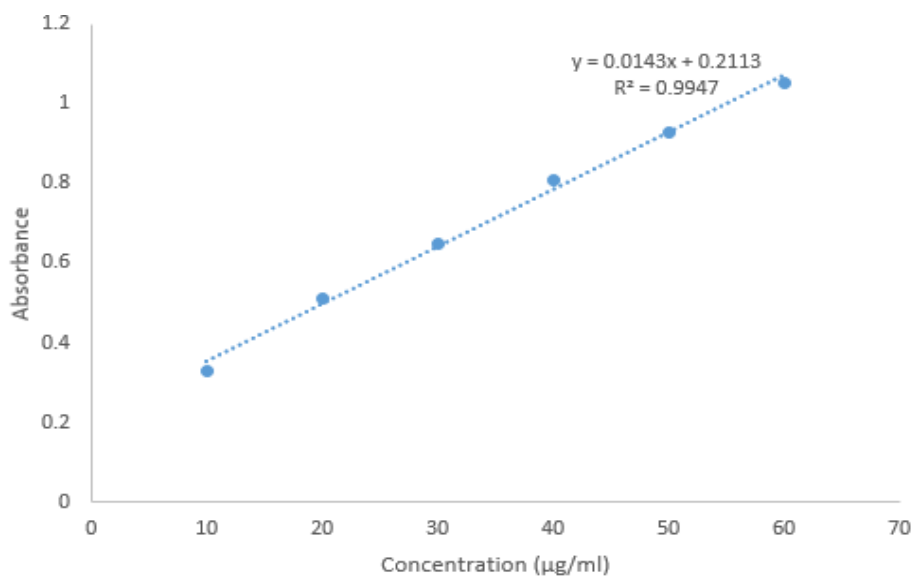
Appendix X: Calibration curve of amoxicillin in simulated physiological medium (pH 1.2)



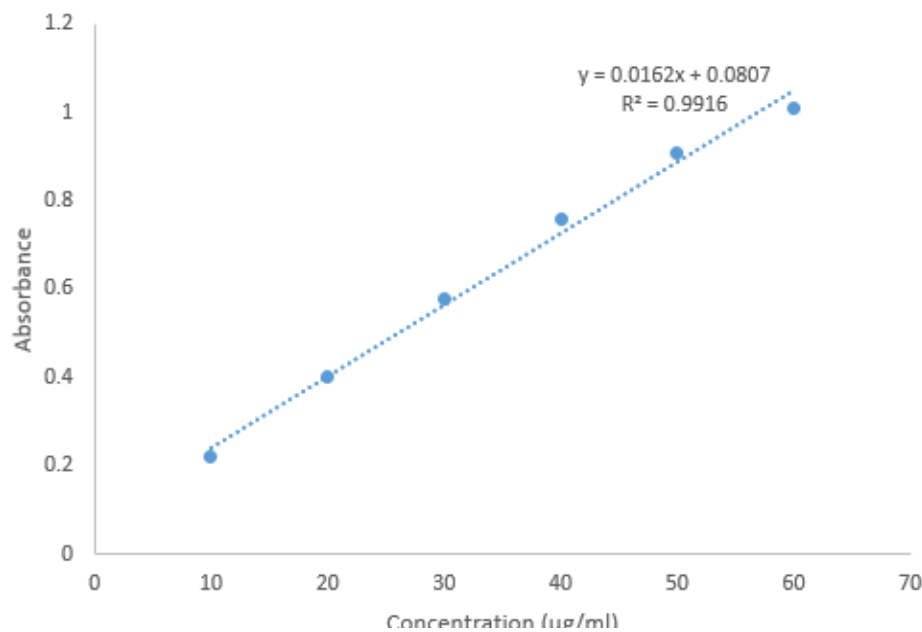
Appendix XI: Calibration curve of amoxicillin in simulated physiological medium  
(pH 4.5)



Appendix XII: Calibration curve of amoxicillin in simulated physiological medium (pH 6.8)



Appendix XIII: Calibration curve of amoxicillin in simulated physiological medium (pH 7.4)



Appendix XIV: Dissolution test (% mean content dissolved in pH 1.2) raw data

Time(min)	Brand A	Brand B	Brand C	Brand D	Brand E	Brand F
05	17.65±2.1	50.54±3.2	2.08±0.9	2.42±1.0	35.31±2.1	4.50±2.1
15	50.54±4.5	44.31±3.8	5.19±2.9	5.54±1.3	60.92±3.2	7.62±3.2
25	66.12±3.2	61.27±2.1	23.88±3.4	11.08±0.9	65.08±3.2	43.27±4.3
35	89.65±3.4	86.19±4.3	40.50±2.1	11.77±3.2	77.88±5.1	46.92±2.7
45	104.88±2.7	106.62±3.2	45.00±5.1	16.27± 2.1	99.69±2.9	56.19±4.3
55	108.62±3.2	110.46±3.4	65.08±3.2	19.73±3.2	102.46±2.1	67.92±5.1

Appendix XV: Dissolution test (% mean content dissolved at pH 4.5) raw data

Time(min)	Brand A	Brand B	Brand C	Brand D	Brand E	Brand F
05	28.08±3.2	28.11±1.2	26.45±2.9	4.71±1.8	27.00±2.1	9.97±2.1
15	58.43±5.5	45.55±3.5	36.97±4.9	8.86±2.3	40.29±3.2	20.35±3.2
25	88.06±4.2	77.82±2.7	40.85±3.4	14.82±2.9	80.72±3.2	36.97±4.3
35	100.66±6.4	96.78±7.3	48.60±2.1	15.92±3.2	104.12±5.1	40.43±2.7
45	106.89±2.7	106.89±7.2	82.11±5.4	17.17± 2.3	107.91±2.9	48.46±4.3
55	107.58±3.4	107.58±3.6	97.06±4.2	17.58±3.2	108.46±2.1	54.14±5.1

Appendix XVI: Dissolution test (% mean content dissolved at pH 6.8) raw data

Time(min)	Brand A	Brand B	Brand C	Brand D	Brand E	Brand F
05	23.40±3.2	31.11±1.2	24.04±1.8	0.39±0.1	29.31±1.5	7.46±2.4
15	53.36±2.5	61.46±3.1	30.99±2.9	3.37±1.1	49.76±3.1	20.70±1.9
25	93.60±2.3	73.93±2.7	37.03±3.5	7.59±2.0	74.19±5.2	35.49±2.7
35	103.11±3.4	86.53±4.9	57.21±5.1	12.73±2.2	87.17±4.1	49.24±2.9
45	108.77±2.9	96.94±6.2	60.94±5.1	19.16± 2.7	88.46±3.9	49.76±4.1
55	109.34±4.2	100.03±4.6	75.47±3.8	21.73±3.1	100.03±2.7	52.71±3.3

Appendix XVII: Dissolution test (% mean content dissolved at pH 7.4) raw data

Time(min)	Brand A	Brand B	Brand C	Brand D	Brand E	Brand F
-----------	---------	---------	---------	---------	---------	---------

05	23.06±2.1	27.11±3.2	14.74±0.9	0.11±0.01	16.76±2.1	11.36±2.1
15	68.06±4.5	51.08±3.8	23.29±2.9	2.03±1.0	41.19±3.2	28.58±3.2
25	91.91±3.2	72.45±2.1	37.24±3.4	2.59±0.9	73.69±3.2	38.25±4.3
35	101.36±3.4	101.36±4.3	54.79±2.1	8.44±1.2	98.33±5.1	51.64±2.7
45	101.48±2.7	104.40±3.2	72.23±5.1	11.81± 2.1	104.06±2.9	55.46±4.3
55	108.43±3.2	107.54±3.4	80.44±3.2	13.16±2.2	104.63±2.1	64.19±5.1

### Appendix XVIII: Melting point determination

Sample	Melting point (°C)		Average	Remark
	1 <sup>st</sup>	2 <sup>nd</sup>		
Standard powder	193.0	195.0	194.0	pass

### Appendix XIX: Preparation of 1N NaOH (200ml)

8g of NaOH was weighed and dissolved into 200ml of distilled water contained in 500ml volumetric flask to produce 200ml of 1M NaOH solution as follows:

$$V=200\text{ml} \text{ (.2) Conc.} = 1\text{M, Mol} = 1 \times 0.2 \text{ Mol} = 0.2$$

$$\text{Mass} = \text{Mol} * \text{Molar Mass} = 0.2 * 40 = 8\text{g}$$

### Appendix XX: Preparation of 1.2N HCl (300ml)

30ml of the Concentrated HCl solution was measured and made up to 300ml with distilled water to produce 300ml of 1.2 N HCl solution as follows:

$$C_1 = 12 \text{ N}, V_1 = ? \quad C_2 = 1.2 \text{ N}, V_2 = 300 \text{ ml}$$

$$12 \times V_1 = 1.2 \times 300 / 12$$

$$V_1 = 30 \text{ ml}$$

#### **Appendix XXI: Preparation of simulated gastric fluid (SGF) pH 1.2**

8.3 ml of the conc. HCl was measured and transferred into 1000 mL beaker containing 250 ml of distilled water and the volume made up with distilled water as follows:

$$C_1 = 12 \text{ N}, V_1 = ?, \quad C_2 = 0.1 \text{ N}, V_2 = 1000 \text{ ml}$$

$$V_1 = \frac{0.1 * 1000}{12} = 8.3 \text{ ml}$$

#### **Appendix XXII: Preparation of 0.1N Iodine (250ml)**

5.076 g of iodine and 9.0g of potassium iodide were weighed and dissolved in 100ml of water contained in 500ml beaker, 3 drops of conc. HCl was added and volume made up to 500ml.

#### **Appendix XXIII: Preparation of 0.1N sodium thiosulphate (500ml)**

12.5 g of sodium thiosulphate and 0.1g of sodium bicarbonate were weighed and dissolved in 100 ml of water contained in 500 ml volumetric flask, the volume was made up to 500 ml mark with water.

#### **Appendix XXIV: Standardization of sodium thiosulphate solution**

0.8g of dried potassium iodate was accurately weighed into a 500ml volumetric flask, dissolved in water and made up to volume. 25.0ml of this solution was pipetted into a conical flask. 2g of KI and 5ml of 2N HCl were added. The liberated iodine was titrated with 0.1N sodium thiosulphate solution with constant stirring. The liquid mixture was diluted to about 200ml with water when the colour had become pale yellow. 2ml of starch mucilage was subsequently added and titration continued until the colour changed from blue to colourless. The titration was repeated to obtain three replicate results.

#### **Appendix XXV: Preparation of starch iodide paste (50 ml)**

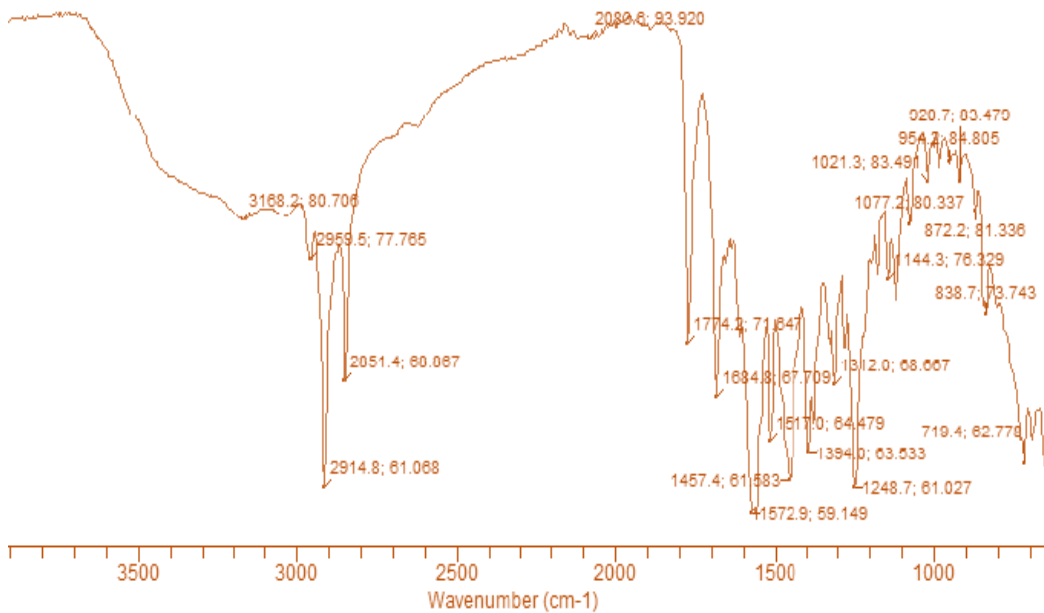
50 ml of water was boiled in a 100 ml beaker then 2.5 ml solution of 0.38g of potassium iodide and 5 ml solution of 1 g zinc chloride were added while the solution was boiling a

smooth suspension of 2.5 g soluble starch dissolved in 15 ml of cold water was added while stirring. The boiling continued for 2 minutes and the solution allowed to cool and stored in a well closed container in cool place before used.

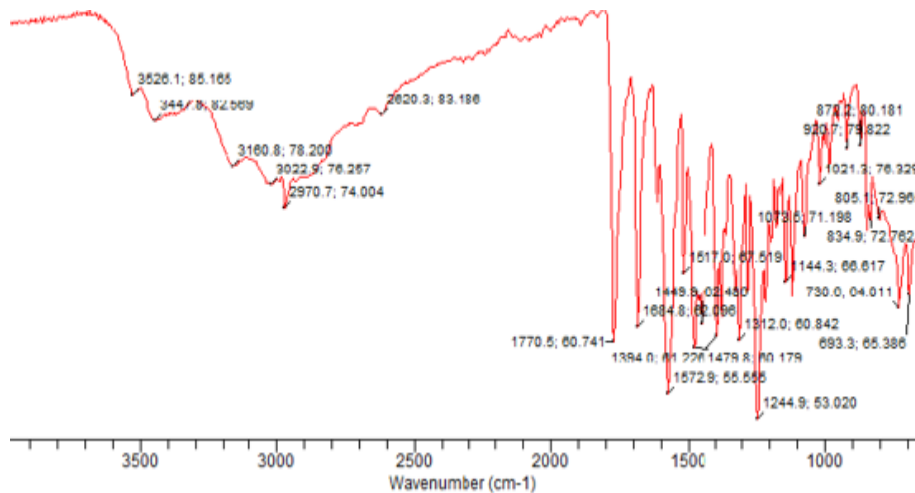
**Appendix XXVI: Preparation of simulated physiological media (pH 4.5, 6.8 and 7.4)**

6.8 g of Monobasic potassium phosphate was weighed and dissolved in 1000ml beaker containing 250ml then 77 ml of 0.2N sodium hydroxide was added. The solution was made up to with distilled water to 1000 ml. The pH was adjusted to 6.8 using 0.2N NaOH or HCl as the case may be

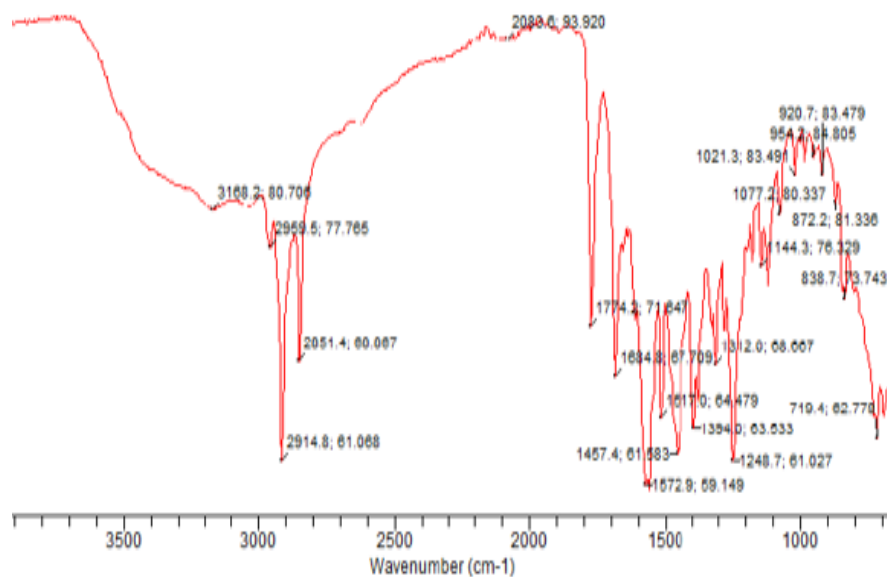
**Appendix XXVII: Reference IR spectrum of amoxicillin**



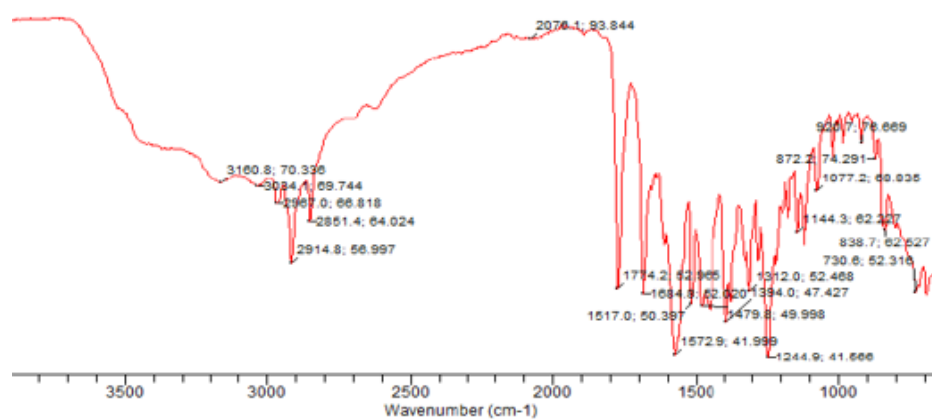
**Appendix XXVIII: IR spectrum of amoxicillin standard powder**



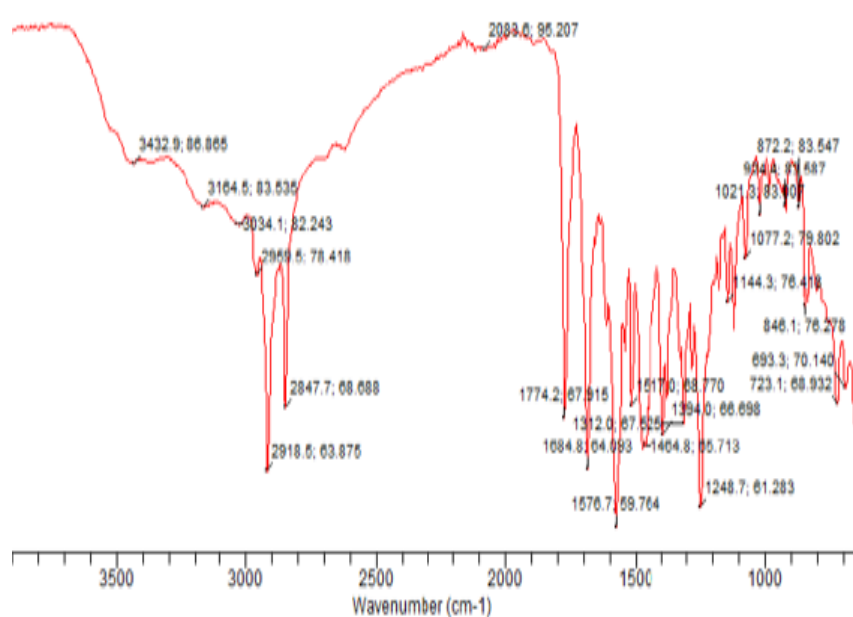
**Appendix XXIX: IR spectrum of sample A amoxicillin**



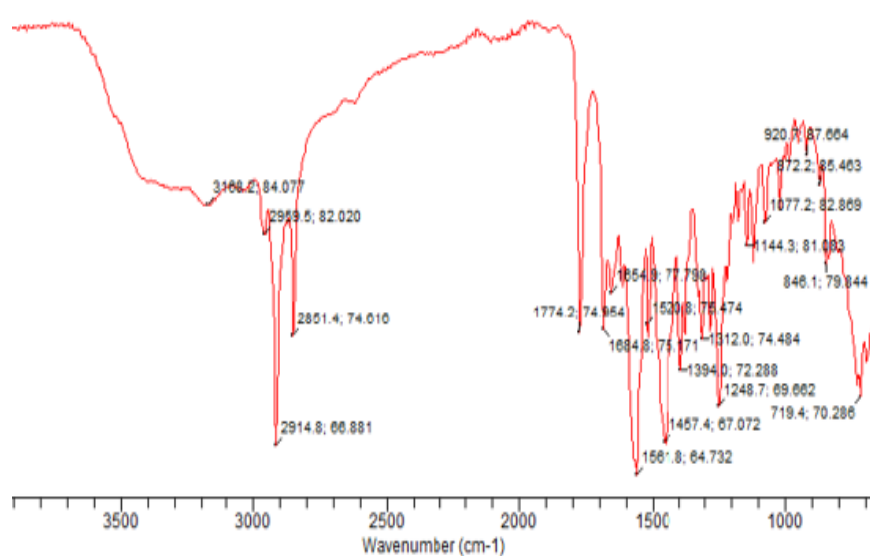
**Appendix XXX: IR spectrum of sample B amoxicillin**



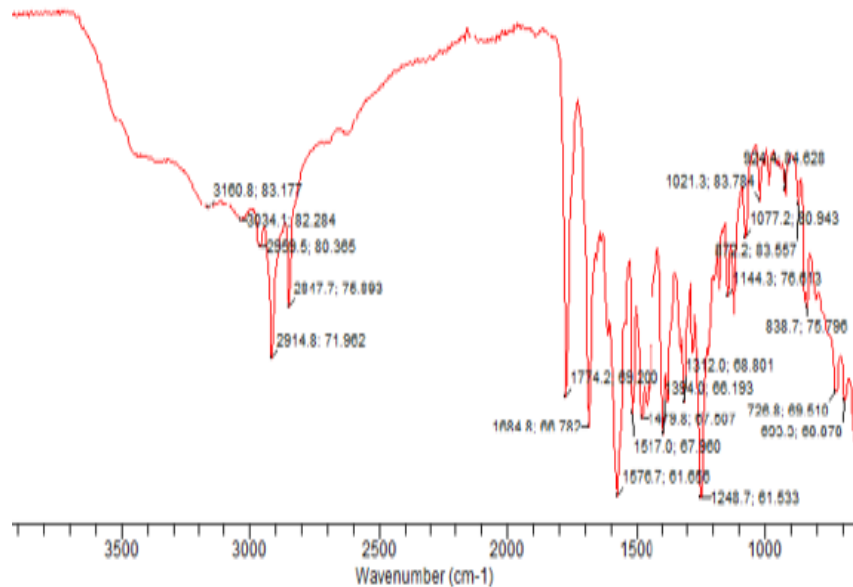
**Appendix XXXI: IR spectrum of sample C amoxicillin**



**Appendix XXXII: IR spectrum of sample D amoxicillin**



**Appendix XXXIII: IR spectrum of sample E amoxicillin**



**Appendix XXXIV: IR spectrum of sample F amoxicillin**

