COMPARATIVE BIOAVAILABILITY STUDIES OF PARACETAMOL BRANDS IN HUMANS

BY

TIJJANI MOHAMMED
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DEPARTMENT OF PHARMACEUTICAL AND MEDICINAL CHEMISTRY
FACULTY OF PHARMACEUTICAL SCIENCES,
AHMADU BELLO UNIVERSITY, ZARIA – NIGERIA.

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DECLARATION

I hereby declare that this thesis was successfully accomplished under the supervision of Dr. Magaji Garba and Dr. Ibrahim Yakassai.

It has not been presented in any previous application for higher degree. The work of other investigators are referred to and acknowledged accordingly.

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Tijjani Mohammed
Department of Pharmaceutical and Medicinal Chemistry,
Faculty of Pharmaceutical Sciences,
Ahmadu Bello University,
Zaria – Nigeria.

CERTIFICATION

DEDICATION

This work is dedicated to my father and mother as my mentors that brought me up

Mohammed Hauwabe and Falta Bundibe

ACKNOWLEDGEMENT

I remain grateful to the Almighty Allah, the most gracious; the most merciful for making this work a reality. My gratitude goes to my supervisors Dr, Garba Magaji, and Dr. Ibrahim Yakassai for their guidance and advice and also my thanks goes to Dr. Musa Abubakar for his advice.

ABSTRACT

The comparative bioavailabilities (bioequivalence) of five generic brands of paracetamol were compared in six (6) healthy male volunteers. The aim was to study whether the generic brands are bioequivalent to the standard brand.

The study was carried out following oral administration of 1g of each brand after wash-out period of two weeks. The concentration of paracetamol in the saliva samples were determined using UV – spectrophotometer. The pharmacokinetic parameters for bioavailability evaluation C_{max}, T_{max} and AUC were determined. The values of reference tablet panadol® was C_{max} 48.50 ± 21.82 µg/ml, T_{max} 1 ± 0.41 (hr), AUC 238.13 ± 12.4 µg/ml/hr, while test brand XA was C_{max}31.50 ± 16.83 µg/ml, T_{max} 0.5 ± 0.25 (hr), AUC 117.93 ± 8.10µg/ml/hr, XB C_{max}50.0±17.39µg/ml, T_{max}0.50 ±0.25 (hr), AUC 202.0 ± 12.56 µg/ml/hr, XC C_{max}42.0 ± 17.39µg/ml, T_{max}1 ± 0.41 (hr), AUC 192.63 ± 10.07µg/ml/hr, XD C_{max}50.0 ± 15.82µg/ml, T_{max}1±0.41 (hr), AUC 256.13 ±11.51µg/ml/hr and XE C_{max}5.3 ± 2.4 µg/ml, T_{max}3±1.07 (hr), AUC 27.03 ± 1.34 µg/ml/hr.

Reference ratio of three generic brands XB, XC and XD were bioequivalent to the standard brand because their limits lies within the bioequivalent range of 0.8 – 1.25 or 80% - 125% confidence limits with panadol® while the other two brands XA and XE were not within these bioequivalence range with panadol®.

TABLE OF CONTENTS

Declaration - - - - - - - - ii

Certification - - - - - - - - iii

Dedication - - - - - - - - iv

Acknowledge - - - - - - - - v

Abstract - - - - - - - - vi

Table of contents - - - - - - - vii

List of Table - - - - - - - - viii

List of figures - - - - - - - - ix

List of appendices - - - - - - - x

Abbreviation - - - - - - - - xi

CHAPTER ONE: INTRODUCTION

1.1 Bioavailability, Bioequivalence and Generic drugs - - - 1

1.2 Generic drugs - - - - - - - - - 2

1.3 Bioavailability - - - - - - - - 2

1.4 Absolute bioavailability - - - - - - 3

1.5 Comparative bioavailability - - - - - - 3

1.6 Factors affecting bioavailability - - - - - 4

1.7 Assessment of bioavailability - - - - - 6

1.8 Bioequivalence - - - - - - - 7

1.9 Assessment of Bioequivalence - - - - - 7

1.9.1 Pharmacokinetic criteria - - - - - - 8

1.9.2 Statistical criteria - - - - - - - 8

CHAPTER TWO: LITERATURE REVIEW

2.1 Paracetamol - - - - - - - - 11

2.1.1 History - - - - - - - - 11

2.1.2 Chemistry of paracetamol - - - - - - 11

2.1.3 Absorption and Bioavailability - - - - - 12

2.1.4 Distribution - - - - - - - - 13

2.1.5 Dosage form performance - - - - - - 13

2.1.5.1 Excepient and manufacturing variation - - - - 13

2.1.5.2 Risk for bioequivalence caused by excepient and manufacturing parameters - - - - - 16

2.1.5.3 Patients risks associated with Bioavailability - - - - 16

2.1.6 Comparative Bioavailability and plasma paracetamol profiles of panadol suppositories in children - - - - 16

CHAPTER THREE:MATERIALS AND METHODS

3.1 Materials - - - - - - - - 18

3.1.2 Glass wares - - - - - - - 19

3.1.3 Equipments - - - - - - - - 20

3.1.4 Reagents - - - - - - - - 20

3.2 In-vitro studies - - - - - - - - 20

3.2.1 Identification test - - - - - - - - 20

3.2.2 Assay - - - - - - - - 21

3.2.3 Disintegration test - - - - - - - - 21

3.2.4 Dissolution test - - - - - - - - 21

3.2.5 Preparation of standard samples - - - - - 22

3.2.5.1 Preparation of paracetamol solution - - - - 22
3.2.5.2 Preparation of dissolution medium - - - - - 22
3.3 In-vitro studies - - - - - - - 22
3.3.1 Protocols of study - - - - - - - 23
3.3.2 Analytical methods -- - - - - - - 23
3.3.3 Extraction methods - - - - - - - 23
3.3.4 Calibration curve - - - - - - - 24
3.3.5 Data handling - - - - - - - 26

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1 In-vitro studies - - - - - - - 27
4.1.1 Identification test - - - - - - - 27
4.1.2 Assay - - - - - - - - - 27
4.1.3 Disintegration time for paracetamol tablets - - - - 27
4.1.4 Dissolution profile for paracetamol tablets - - - - 28
4.2 In-vitro studies - - - - - - - 28
4.2.1 Calibration curve - - - - - - - 28
4.2.2 Pharmacokinetics - - - - - - - 31
4.3 Conclusion - - - - - - - - 44
References - - - - - - - - 46
Appendices - - - - - - - - xv

LIST OF TABLES

Table 3.3.4:	Concentration of paracetamol spiked into saliva samples from paracetamol stock solution for calibration curve.	25
Table 4.1.2:	Assay of paracetamol tablets using U.V spectrophotometric method of analysis.	27
Table 4.1.3:	Disintegration time for paracetamol tablets	27
Table 4.1.4:	In-vitro dissolution profile of paracetamol tablets	28
Table 4.2.1:	Data obtained for construction of calibration curve.	28
Table 4.2.2	Mean saliva concentration in 6 healthy volunteers following oral administration of 1g of panadol in fasting state. - - - - -	31
Table 4.2.3:	Mean saliva concentration in 6 healthy volunteers following oral administration of 1g of brand XA in fasting state. - - - - - -	33
Table 4.2.4	Mean saliva concentration in 6 healthy volunteers following oral administration of 1g of brand XB in fasting state. - - - - - -	35
Table 4.2.5	Mean saliva concentration in 6 healthy volunteers following oral administration of 1g of brand XC in fasting state. - - - - - -	37
Table 4.2.6	Mean saliva concentration in 6 healthy volunteers following oral administration of 1g of brand XD in fasting state. - - - - - -	39
Table 4.2.7	Mean saliva concentration in 6 healthy volunteers following oral administration of 1g of brand XE in fasting state. - - - - - -	41
Table 4.2.8	Pharmacokinetic parameters of paracetamol brands.-	43
Table 4.2.9	Bioavailability of paracetmaol brands - -	43

LIST OF FIGURES

Fig. 4.2.1:	Caliberation curve for analysis of paracetamol in saliva	30
Fig. 4.2.2:	Saliva concentration time curve for panadol as a standard	.32
Fig. 4.2.3:	Comparative bioavailability saliva concentration time curve for brand XA and panadol- - -	34
Fig. 4.2.4	Comparative bioavailability saliva concentration time curve for brand XB and panadol - -	36
Fig. 4.2.5	Comparative bioavailability saliva concentration time curve for brand XC and panadol- - -	38
Fig. 4.2.6	Comparative bioavailability saliva concentration time curve for brand XD and panadol- - -	40
Fig. 4.2.7	Comparative bioavailability saliva concentration time curve for brand XE and panadol- - -	42

LIST OF APPENDICES.

1. Data obtained for the construction of caliberation curve

2. Saliva concentration time curve in 6 healthy volunteers following oral administration of 1g of panadol in fasting state.

3. Saliva concentration time curve in 6 healthy volunteers following oral administration of 1g of brand XA in fasting state.

4. Saliva concentration time curve in 6 healthy volunteers following oral administration of 1g of brand XB in fasting state.

5. Saliva concentration time curve in 6 healthy volunteers following oral administration of 1g of brand XC in fasting state.

6. Saliva concentration time curve in 6 healthy volunteers following oral administration of 1g of brand XD in fasting state.

7 Saliva concentration time curve in 6 healthy volunteers following oral administration of 1g of brand XE in fasting state.

ABBREVIATIONS

AUC	=	Area under the curve
pH	=	Hydrogen ion concentration
B.P	=	British Pharmacopoeia
HCl	=	Hydrochloric acid
U.V	=	Ultra – violet
Hr.	=	Hour
min	=	Minute
ml	=	Milli-litre
μg	=	Micro-gram
g	=	Gram
^{0}C	=	Degree centigrade (Celsius)
rpm	=	Revolutions per minute
SEM	=	Standard Error of the Mean
μl	=	Microlitre
NaOH	=	Sodium hydroxide
et al	=	And others
Fig.	=	Figure
I.V	=	Intravenous
kg	=	Kilogram
nm	=	Nano-meter
λ	=	Wavelength
A.B.U	=	Ahmadu Bello University

APPENDIX 1

DATA OBTAINED FOR THE CONSTRUCTION OF CALIBRATION CURVE

ABSORBANCE								
Concentration of drug/ml of saliva	Experiment 1		Experiment 2		Experiment 3		Experiment 4	
	1	2	3	4	5	6	7	8
0.00 µg/ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
10.00 µg/ml	0.266	0.272	0.231	0.223	0.546	0.542	0.470	0.355
20.00 µg/ml	0.551	0.555	0.358	0.350	0.717	0.713	0.562	0.566
30.00 µg/ml	0.844	0.840	0.770	0.765	1.017	1.013	0.717	0.713
40.00 µg/ml	1.073	1.070	1.021	1.025	1.138	1.133	1.242	1.127
50.00 µg/ml	1.172	1.160	1.174	1.162	1.207	1.200	1.307	1.203

APPENDIX 2

SALIVA CONCENTRATION IN 6 HEALTHY VOLUNTEERS FOLLOWING ORAL ADMINISTRATION OF 1g OF PANADOL IN FASTING STATE.

Paracetamol Concentration (μg/ml)						
Time (hr)	Volunteer No					
	I	II	III	IV	V	VI
0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	69.47	32.3	39.27	50.22	38.35	46.39
1	57.13	61.12	0.82	60.63	62.84	48.46
2	70.33	69.37	39.72	6.24	11.51	39.68
3	49.01	28.07	36.71	38.63	34.21	41.46
4	9.86	66.78	61.57	37.04	44.04	5.69
5	33	17.5	37.32	36.07	4.71	53.11
6	62.26	25.91	13.48	3.58	5.96	3.07

APPENDIX 3

SALIVA CONCENTRATION IN 6 HEALTHY VOLUNTEERS FOLLOWING ORAL ADMINISTRATION OF 1g OF BRAND XA IN FASTING STATE

Paracetamol Concentration (μg/ml)						
Time (hr)	Volunteer No					
	I	II	III	IV	V	VI
0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	29.38	32.67	63.42	31.36	26.19	6.09
1	50.02	19.03	57.24	27.52	6.16	6.16
2	15.54	23.66	46.02	18.12	6.11	5.37
3	10.95	13.22	31.94	8.13	6.65	4.15
4	7.15	7.56	28.84	13.06	4.26	5.02
5	4.45	8.27	25.72	5.87	4.96	4.37
6	5.36	4.51	16.64	5.78	3.63	4.23

APPENDIX 4

SALIVA CONCENTRATION IN 6 HEALTHY VOLUNTEERS FOLLOWING ORAL ADMINISTRATION OF 1g OF BRAND XB IN FASTING STATE

Paracetamol Concentration (μg/ml)						
Time (hr)	Volunteer No					
	I	II	III	IV	V	VI
0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	63.86	41.36	78.16	42.61	49.96	23.55
1	26.39	63.11	69.82	66.61	51.54	17.61
2	32.14	35.51	69.81	29.76	33.28	18.40
3	21.84	26.00	40.70	35.58	24.05	16.79
4	14.84	26.13	42.78	26.23	22.29	6.11
5	13.28	16.48	36.37	26.82	22.79	4.28
6	13.05	11.34	18.48	21.75	21.65	3.7

APPENDIX 5

SALIVA CONCENTRATIO IN 6 HEALTHY VOLUNTEERS FOLLOWING ORAL ADMINISTRATION OF 1g OF BRAND XC IN FASTING STATE

Paracetamol Concentration (μg/ml)						
Time (hr)	Volunteer No					
	I	II	III	IV	V	VI
0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	33.22	41.46	42.46	33.18	37.18	37.5
1	43.00	56.58	38.28	69.81	26.43	18.05
2	35.8	10.2	40.25	52.43	22.17	43.14
3	14.75	18.80	35.81	52.0	41.22	32.64
4	8.38	16.05	75.15	38.31	9.32	11.85
5	33.04	36.24	29.35	7.78	10.13	6.47
6	25.56	29.00	35.12	5.55	6.64	6.12

APPENDIX 6

SALIVA CONCENTRATION IN 6 HEALTHY VOLUNTEERS FOLLOWING ORAL ADMINISTRATION OF 1g OF BRAND XD IN FASTING STATE

Paracetamol Concentration (μg/ml)						
Time (hr)	Volunteer No					
	I	II	III	IV	V	VI
0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	59.99	51.29	45.5	50.12	49.94	18.13
1	44.71	59.21	60.61	59.32	59.39	16.79
2	55.7	53.07	49.91	41.77	54.21	15.28
3	41.58	46.83	40.94	36.5	55.85	15.19
4	45.72	47.54	52.00	38.87	25.91	14.92
5	25.77	48.82	37.72	31.57	48.57	14.67
6	28.02	49.35	33.5	28.02	45.13	11.02

APPENDIX 7

SALIVA CONCENTRATION IN 6 HEALTHY VOLUNTEERS FOLLOWING ORAL ADMINISTRATION OF 1g OF BRAND XE IN FASTING STATE

Paracetamol Concentration (μg/ml)						
Time (hr)	Volunteer No					
	I	II	III	IV	V	VI
0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	0.68	4.28	2.89	6.12	-	-
1	-	-	4.5	-	-	-
2	-	-	1.7	7.74	-	-
3	-	-	4.32	6.28	-	-
4	6.62	0.99	3.04	-	-	-
5	-	9.06	2.34	3.41	-	-
6	1.93	0.17	0.48	3.41	-	-

CHAPTER ONE
INTRODUCTION

1.1 BIOAVAILABILITY, BIOEQUIVALENCE AND DRUG SELECTION

Bioavailability and bioequivalence of drug products, and drug product selection have emerged as critical issues in pharmacy and medicine during the last three decades concern about lowering health care cost has resulted in tremendous increase in the use of generic drug products, currently about one half of all prescriptions written are for drugs that can be substituted with a generic product (Miller S.W, Strom J.G 1990).

With the increasing availability and use of generic drug products, health care professionals are confronted with an ever-larger array of multi-source products from which they must select those that are therapeutically equivalent.

This phenomenal growth of the generic pharmaceutical industry and the abundance of multi-source products have prompted some questions among many health professionals and scientists regarding the equivalence of this products particularly those in certain critical therapeutic categories (Miller S.W, Stom J.G 1990), (Lamy, P. 1985), (Colaizzi. J, Lowenthal, D., 1986), (Foster, T.S 1991). Inherent in the currently accepted guidelines for product substitution is the assumption that a generic drug considered to be bioequivalent to a brand-name drug will elicit the same clinical effect. As straight forward as this statement regarding bioequivalence appears to be, it has generated a great deal of controversy among scientist and professionals in the health care field. Numerous papers in the literature indicate that there is concern that the current standard for approval of generic drugs may not always

assure therapeutic equivalence (Meyer, M., 1985), (Nuwer, M.R et al 1990). The availability of different formulations of the same drug substance given at the same strength and in the same dosage form poses a special challenge to health care professionals making this issue very relevant to pharmacist in all practice settings.

1.2 GENERIC DRUGS

Generic is the term used for products that contain the same medical ingredients as the brand name drugs but which are generally cheaper in price. More and more generic drugs are being used to fill prescriptions because generic drugs are as safe and effective as brand name drugs.

Over 80% of the approximately 10,000 prescription drugs available in 1990 were available from more than one source (the food and drug letter 1990)

1.3 BIOAVAILABILITY

Although the concept of bioavailability was initially introduced by (Oser et al in 1945), its problem has only recently been recognized and discussed, as a result of controversies involving chloramphenicol, digoxin and phenytoin (Hailman. K, 1984, Greenblatt D.J et al 1976, Bochner F et al 1972). A change in formulation caused decreased bioavailability of digoxin (Green Blatt D.J et al 1976), and phenytoin intoxication in Australia (Bochner et al 1972). In 1966 FDA found that of 4000 formulations available in USA, more than 300 were ineffective (Heilman K. 1984). The availability of over 45,000 formulations of 5,000 drugs in India (Scrip 1988), the recent interest in cheap generic formulations (Nightingale SL, Morrison JC 1987) and

availability of special long acting formulation (Lamy PP 1986) have made it imperative for the physician to consider and understand the influence of bioavailability on the therapeutic decision. Bioavailability is defined as the fraction of a dose reaching the systemic circulation as unchanged drug following administration by any route other than intravenous (Benet LZ. Et al 1984).

1.4 ABSOLUTE BIOAVAILABILITY

Absolute bioavailability, F is the fraction of an administered dose which externally reaches the systemic circulation and ranges from F=O (no drug absorption) to F=I (complete drug absorption). Since the total amount of drug reaching the systemic circulation is directly proportional to the area under the plasma drug concentration as a function of time curve (AUC), F is determined by comparing the respective AUCs of the test product and the same dose of drug administered intravenously. The intravenous route is the reference standard since the dose is by definition, completely available.

$$F = \frac{AUC_{er}}{AUC_{iv}}$$

Where AUCev and AUCiv are respectively, the area under the plasma concentration-time curve following the extravascular and intravenous administration of a given dose of drug (Michael C. Makoid 1999).

1.5 COMPARATIVE BIOAVAILABILITY

This refers to the availability of a drug product as compared to another dosage form product of the same drug given in the same dose.

These measurements determine the effects of formulation differences on drug absorption. The comparative bioavailability of product A compared to product B, both products containing the same dose of the same drug, is obtained by comparing their respective AUCs.

$$\text{Comparative bioavailability} = \frac{AUC_A}{AUC_B}$$

Where drug B is the reference standard, when the bioavailability of a generic product is considered, it is usually the comparative bioavailability that is referred to. A more general form of equation results from considering the possibility of different doses.

1.6 FACTORS AFFECTING BIOAVAILABILITY

Bioavailability of a drug may be affected by many factors, the most important of which are formulation and physiochemical characteristics (Spiker B. 1986). A change of excipient led to an outbreak of phenytoin toxicity due to increased bioavailability. Differences in bioavailability of carbamazepine brands are reported (Bhatia SC, et al. 1988) and a change of brand with good bioavailability to one with uncertain bioavailability can precipitate seizures in a controlled epileptic (Sachedo R.C, Belendiuck G. 1987).

In-vitro dissolution data may not always predict how the drug will behave in humans (Spiker B. 1986). Hence it is necessary to have comparative bioavailability of conventional as well as sustained release formulation e.g. the phylline (Hurwitz A. 1987). The rize and shape of tablets can influence esophageal transit. (Channer K.S, Virjee JP 1986).

Drug absorption can be influenced by a variety of gastro intestinal conditions (Benet LZ 1984). Rapid intestinal transit due to diarrhoea may inhibit drug absorption. Pregnancy has occurred after the use of oral contraceptives during a period of diarrhoea (Benet LZ 1984). Metochlopramide which accelerates gastric emptying has been shown to increase rate of absorption of aspirin, levodopa, lithium, and tetracycline (Benet LZ 1984). In contrast, propantheline decreases the absorption rate of many drugs (Benet LZ 1984). The changes in bioavailability after food may not be of clinical relevance when therapeutic effect is unaffected e.g. sulphadiazine (Ghosh SS et al 1988).

For drugs like chloroquine increase bioavailability with food can improve compliance by reducing gastric side-effects (Tulpule A, Krishnaswamy K. 1982). As food can also reduce or delay absorption (Bhatt AD, Vaidya AB, 1986) proper instructions regarding spacing of dose in relation to food are necessary for drugs like rifampicin, isoniazid. Temporal variations in drug absorption have been shown for Benzodiazepines, e.g. triazolam (Smith RB et al 1986). Bioavailability of nitroglycerine and propranolol is affected by hepatic first pass and is likely to increase liver dysfunction (Bhatt AD, Vaidya AB 1986). Giving nitroglycerine sublingually avoids the problems, however, the buccal absorption will be impaired if the mucosa is dry due to concomitant administration of imipramine or an anti-cholinergic. In addition to these factors, changing absorption, bioavailability, especially at a steady state will be affected by factors influencing distribution, metabolism and excretion (Vessell ES 1982).

Genetic factors, variations in vascular, cardio-renal hepatic or endocrine disorders can affect bioavailability and bioequivalence (Lamy PP. 1986).

1.7 ASSESSMENT OF BIOAVAILABILITY

Assessment of bioavailability from plasma concentration, time data usually involves determining the maximum (peak) plasma drug concentration the time at which maximum plasma drug concentration occurs (peak time), and the area under the plasma concentration, time curve (AUC). The plasma drug concentration increases with the extent of absorption, the peak is reached when the drug elimination rate equates absorption rate. Bioavailability determinations based on the peak plasma concentration can be misleading, because drug elimination begins as soon as drug enters the bloodstream.

The most widely used general index of absorption rate is peak time, the slower the absorption, the larger the peak time. However, peak time is often not a good statistical measure because it is a discrete value that depends on frequency of blood sampling and, in the case of relatively flat concentrations near the peak, an assay reproducibility (Merck H. Beers 2004).

AUC is the most reliable measure of bioavailability. It is directly proportional to the total amount of unchanged drug that reaches the systematic circulation. For an accurate measurement, blood must be sampled frequently over a long enough time to observe virtually complete drug elimination. Drug products may be considered bioequivalent in extent and rate of absorption if their plasma-level curves are essentially superimposed drug products that have similar AUC but differently shaped

plasma-level curves are equivalent in extent but differ in their absorption rate-time profiles (Merck H. Beers 2004).

1.8 BIOEQUIVALENCE

With the phenomenal increase in the availability of generic drugs in recent years, the issues of bioavailability and bioequivalence have received increasing attention. In order for a drug product to be interchangeable with the pioneer (innovator or brand name) product, it must be both pharmaceutically equivalent and bioequivalent to it. Pharmaceutical equivalents are drug products that contain identical active ingredients and are identical in strength or concentration, dosage form, and rout of administration (CFR 1991).

Bioequivalence is a comparison of the bioavailability of two or more drug products. Thus, two products or formulations containing the same active ingredients are bioequivalent if their rate and extent of absorption are the same. When a new formulation of an existing drug is developed, its bioavailability is generally evaluated relative to the standard formulation. For a generic drug to be considered bioequivalent to a pioneer product, there must be no statistical differences between their plasma concentration time profiles (Michael C. Makoid 2004).

1.9 ASSESSMENT OF BIOEQUIVALENCE

In order for different formulations of the same drug substance to be considered bioequivalent, they must be equivalent with respect to the rate and extent of drug absorption. Thus the two predominant issues involved in the assessment of bioequivalence are:

The pharmacokinetic parameters that best characterize the rate and extent of absorption and the most appropriate methods of statistical analysis of the data.

1.9.1 Pharmacokinetic criteria

With regard to the choice of the appropriate pharmacokinetic characteristics, Westlake suggests comparisons of the formulations should be made with respect to only those parameters of the blood level profile that possess some meaningful relation to the therapeutic effect of the drug (Westlake, W.J 1979).

Since the AUC is directly proportional to the amount of drug absorbed, this pharmacokinetic parameter is most commonly used to characterize the extent of absorption in single and multiple-dose studies. Although a brood array of methods exist for calculating absorption rates (e.g. moment analysis, deconvolution procedures and curve-fitting), the most commonly used parameters are peak concentration (Cmax) and time to peak concentration (Tmax).

Although these parameters have been observed to have significant variances and may be difficult to determine accurately, they remain the parameters generally requested as rate characteristics by most regulatory authorities for immediate-release products (Steinijars, V.W et al 1992).

1.9.2 Statistical criteria

After a bioequivalence study is conducted and the appropriate parameters are determined, the pharmacokinetic data must be examined according to a set of predetermined criteria to confirm or refute the bioequivalence of the test and reference formulation. That is one must determine whether the test and reference products differ within a predefined level of statistical significance. Since the statistical outcome of a

bioequivalence study is the primary basis of the decision for or against therapeutic equivalence of two products, it is critically important that the experimental data be analysed by an appropriate statistical test.

In the early 1970s, bioequivalence was usually determined only on the basis of mean data. Mean AUC and Cmax values for the generic product had to be within + 20% of those of the references (innovator) product (Dighe S.V and Adams W.P 1991). Although the 20% value was some what arbitrary, it was felt that for most drugs, a 20% change in the dose would not result in significant differences in the clinical response to drugs (Meyer M.C, 1991).

Westlake was the first to suggest the use of confidence intervals as a means of testing for bioequivalence (Westlake W.J 1972).

Recognizing that no two products will result in identical blood-level profile, and that there will be differences in mean values between products. Westlake pointed out that the critical issue was to determine how large these differences would be before doubts as to therapeutic equivalence arose (Dighe S.V and Adams W.P 1991, Westlake W.J 1988).

A test formulation was considered to be bioequivalent to a reference formulation.

$$\text{If } 0.8 < \frac{AUC_{test}}{AUC_{ref}} < 1.2 \ \textit{and} \ 0.8 < \frac{CP\max_{test}}{CP\max_{ref}} < 1.2 \ (\text{Re}\textit{scino A.}\ 1992)$$

By this process, if test and reference products were not bioequivalent (i.e. means differed by more than 20%), there was a 5% chances of concluding that they are bioequivalent.

Since this test requires that the 90% confidence interval of the difference between the means be within the range of 20%/+25%, it is more stringent than simply requiring the comparison of the test and reference products AUC and Cmax to be within the 80 to 125% range. If the mean response of the generic product in the study population is near 20% below or 25% above the innovator mean, one or both of the confidence limits will fall outside the acceptable range and the product will fail the bioequivalence test. Thus, the confidence interval requirement ensures that the difference in mean values for AUC and Cmax will actually be less than 20%/+25% (Madan P.L 1992).

CHAPTER TWO:

GENERAL LITERATURE REVIEW

2.1 PARACETAMOL

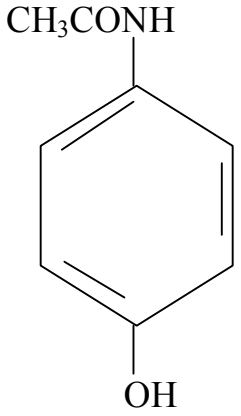
2.1.1 History

Paracetamol (acetaminophen) was discovered in Germany at the end of the 19th century, but was not widely used until midway through the 20th century. The toxicity of over the counter (OTC) analgesics was noticed in the 1960s and 1970s, but paracetamol was considered safe at normal dose. There were few, if any, reports of abuse involving paracetamol and the use of paracetamol steadily increased, replacing the more toxic analgesics available at the time (acetanilide and phenacetin) (Prescott, L. F., 1996). Consumption throughout the world has increased.

Paracetamol did not undergo the stringent toxicity testing prior to its introduction that now occurs during drug development. It was not until 1966 that hepatotoxicity due to paracetamol was first reported in humans (Thomson, JS, Prescott, LF, 1966) (David, DGD and Eastham, WN, 1966).

2.1.2 Chemistry of paracetamol

Paracetamol is 4 – acetamidophenol and may be represented by the following formula:



$C_8H_9N0_2$

M. W. 151.2

M. P. 169 – 172^{0}C

pKa 9.5

In some publications, it is described as 4 – hydroxyacetanilide or N-acetyl-p-aminophenol and in the US Pharmacopoeia it is known as acetaminophen.

Paracetamol is a white, odourless crystalline powder with a bitter taste, soluble in 70 parts of water (1 in 20 boiling water), 7 parts of alcohol (95%), 13 parts of chloroform, or 10 parts of methyl alcohol. It is also soluble in solutions of alkali hydroxides. It is insoluble in benzene and ether. A saturated aqueous solution has a pH of about 6 and is stable (half-life over 20 years) but stability decreases in acid or alkaline conditions, the paracetamol being slowly broken down into acetic acid and p-aminophenol (Fairbrother, J. E., 1974).

2.1.3 Absorption and bioavailability

The absolute bioavailability in the fasted state was reported in the range 62%-89% (Eandi M et al 1984). The incomplete absolute bioavailability is caused by a presystemic clearance of about 20% of an oral dose (Clements JA et al 1984). Peak plasma concentrations are reached within 0.17-1.2h post dosing (Zapater P et al 2004).

The oral absolute bioavailability was reported not to vary with the dose in the range between 5 and 20 mg/kg, (Clements JA et al 1984) but other authors reported AUC values and peak plasma concentrations to be dose-dependent at doses between 325 and 2000mg (Borin MT et al 1989). Food reduces the absorption of acetaminophen tablets by increasing Tmax and decreasing Cmax values (Rostami-Hodjegan A. et al 2002).

Food delays is primarily due to delays in gastric emptying (Williams M et al 2001). Although there are no direct published data on the absolute bioavailability in

the fed state, food does not affect the total amount of acetaminophen reaching the blood (Stillings M et al 2000).

2.1.4 Distribution

The apparent volume of distribution of acetaminophen is reported to be 0.69-1.36 L/kg (Vozeh S et al 1988, Zapater P et al 2004, Clemens JA, Prescott LF 1976).

Plasma protein binding is 20%-25% at usual therapeutic concentrations (Forest JA et al 1982, Moris ME, Levy G 1984). After over dosage, 20%-50% of the drug may be bound to proteins (Drug valuation monograph 1988). Binding to red blood cells is reported to be 10%-20% (Forest JA et al 1982). Acetaminophen crosses the placenta and is present in breast milk (Forest JA et al 1982), with an average milk/plasma concentration ratio of about 1.24 (Arama A et al 2001) of the acetaminophen present in breast milk, 85% is bound to milk proteins (Bailey DIV, Briggs JR 2004).

2.1.5 DOSAGE FORM PERFORMANCE

2.1.5.1 Excipient and Manufacturing Variations:

The comparative bioavailability of acetaminophen from solid dosage forms has been studied frequently. Most studies were carried out in humans, but two animal studies have been also reported in rabbits no significant differences in Cmax and AUC were found between rapidly disintegrating tablets and conventional tablets (Ishikawa T et al 2001). In dogs, no significant differences were found between two conventional tablets (Kalantzi et al 2005).

Studies in humans in general show similar results, while most studies report no differences in extent of absorption between drug products were sometimes found. In

one of the earliest relevant studies (Sotiropulus et al 1981) evaluated three tablets and one liquid acetaminophen product for their comparative bioavailability, reporting a bioavailability relative to the liquid dosage form of 82%, 87%, and 92% respectively. However, based on urinary excretion data, these differences were not statistically significant and only the amount excreted from O to 4h varied with the formulation. (Hekimoghu et al 1987) evaluated the bioavailability of three brands of acetaminophen tablets in comparison to a solution. Bioavailability of the brands relative to the solution were 98%, 95% and 99% respectively, with difference being not statistically significant. However, the amount excreted during the first hour varied among the formulations. (Walter-sack at al 1989) compared a solid and liquid oral dosage forms that did not show differences in the AUC and in Cmax. An evaluation of four brands 0-12h of acetaminophen tablets by (Hekimoglu et al 1991) did not display statistically significant differences in bioavailability, but differences in the urinary excretion during the first hours, reflecting differences in the rate of absorption were observed. (Retaco et al 1996) studied the bioavailability of too lot of paracetamol tablet and although the total amount excreted in urine was similar between the two formulations, differences were found during the early stages of the absorption process. (Dominguez et al 2000) using urinary excretion data, reported non significant differences in the rates and relative bioavailabilities ranging from 94% to 131% of three commercial formulations versus the innovators. (Bababola et al 2001) reported a study of two commercial brands versus the innovator. While the absorption rate of one brand, as indicated by Tmax was significantly shorter than those of the innovator, the extent of absorption as indicated by AUC was comparable among the three brands. (Sevilla-

Tirado et al 2003) compared three tablets, one effervescent tablets, and a powder sachet, and found that the extent of absorption, expressed as AUC, did not exhibit differences between formulation. However for the rate of absorption expressed as Cmax and Partial AUC values tablets had a rate of absorption as fast as the effervescent tablet, but the other tablet being the innovator, had a some what slower absorption rate (Sevilla-Tirado et al 2003). Of special interest are recently introduced acetaminophen products containing large amounts of sodium bicarbonate. Such dosage forms are claimed to have fast drug absorption. (Grattan et al 2000) compared the pharmacokinetics of one commercially available acetaminophen tablet and one soluble commercially available acetaminophen tablet with two development tablet formulations containing 400 mg sodium bicarbonate and the other containing 639mg sodium bicarbonate. The results demonstrated that addition of 639mg sodium bicarbonate increased the rate of absorption of acetaminophen relative to both the conventional tablets and the soluble tablets as indicated by a shorter Tmax and higher Cmax, where as the addition of 400mg sodium bicarbonate increased the absorption rate of acetaminophen relative to conventional acetaminophen tablets only. These findings were recently confirmed by (Kelly et al 2003) who compared an acetaminophen tablet containing 630mg sodium bicarbonate with a conventional tablet. The rate of absorption, indicated by $t_{50\%}$ and $t_{90\%}$, was about twice as fast compared to the conventional tablets, both in the fasted state and the fed state. It was suggested that a combination of faster disintegration and gastric emptying of the tablets containing sodium bicarbonate is responsible for the faster rate of absorption. The differences in gastric emptying were thought to be more pronounced in the fasted

state and the differences in disintegration more pronounced in the fed state (Kelly K et al 2003). The data of Grattan et al and Kelly et al are supported by earlier reports that effervescent tablets show faster absorption characteristics than conventional solid tablets (Sevilla et al 2003, Rygnestad et al 2000).

2.1.5.2 Risk for bioequivalence caused by excipients and manufacturing parameters:

Absorption rate, differences between brands and test formulations have been observed as in the case of acetaminophen of tablets containing high amounts of sodium bicarbonate. It was suggested that these differences were caused by differences in disintegration or gastric emptying rates. Although data in humans are lacking, data in rabbits suggest that high concentrations of osmotically active excipients such as manitol may have an impact on the Tmax of acetaminophen (Ishikawa et al 2001).

2.1.5.3 Patients risks associated with bioinequivolence:

When considering a drug substance, its therapeutic index also needs to be taken into account (CDER 2000, CPMP, 2001). The therapeutic indicators of acetaminophen are not critical and there is a wide difference between the usual therapeutic dose and toxic doses. So it can be assumed that acetaminophen is not a narrow therapeutic index drug.

2.1.6 Comparative bioavailability and plasma paracetamol profiles of panadol suppositories in children

Absorption of paracetamol following retal administration of panadol suppositories to post operative children is slower and reduced as compared to oral

therapy. The hard wax and liquid filled products have similar absorption characteristics. The usually quoted antipyretic therapeutic range for paracetamol is 10-20 mg/L, although 5 mg/L may be effective. A single retal dose of 25 mg/kg will obtain this lower concentration within 1 hour of administration and maintain it for 6 hours. When given in an appropriate dose for analgesia, maximum plasma paracetamol concentration would be available in the immediate post operative period if restal dose was given 2 hours before the planned end of the procedure (Coulthard et al 1998).

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Drugs:

Panadol(R)

Manufacturer – Glaxo Smithkline

Batch number – 074D

Manufacturing date – Dec 2004

Expiry date – Dec 2009

Strength – 500 mg

Generic Brands Paracetamol

1. Brand XA

Manufactuere – Archy Pharmaceuticals

Batch no – PT 6262

Manufacturing date – Oct 2006

Expiry date – Nov 2009

Strength – 500 mg

2. Brand XB

Manufacturer – Danapharmaceuticals

Batch no – PT 6260

Manufacturing date – May 2006

Expiry date – April 2006

Strength – 500 mg

3. Brand XC

Manufacturer – Emzor Pharmaceutical

Batch no – 5530G

Manufacturing date – Dec 2004

Expiry date – Dec 2009

Strength – 500 mg

4. Brand XD

Manufacturer – May & Baker Nig Plc

Batch no – IW310

Manufacturing data – March 2006

Expiry date – March 2011

Strength – 500 mg

5. Brand XE

Manufacturer – Vitabiotics Nig. Ltd

Batch no – T 39406

Manufacturing date – April 2006

Expiry date – March 2011

Strength – 500 mg

3.1.2 Grass Wares:

Extraction tubes – Pyrex England

Pippetes – 0.02ml, 0.1ml, 1ml, 2ml, 5ml

Measuring cylinders – 100ml, 1000ml

Volumetric flask – 10ml, 100ml, 200ml

Beakers – 250ml

Sample bottles

3.1.3 Equipments

Centrifuge Junior – Gallenkamp, England

Flask shaker – Gallenkamp England

Filter papers

Spectrophotometers

Electronic balance, Metler AE 240

Refrigerator – Premier Thermocool Nigeria

Disintegration rate study apparatus – Erweka England

Dissolution rate study apparatus – Erweka England

3.1.4 Reagents

Methanol – May and Baker England

Ethyl acetate – May and Baker England

Distilled water

Chewable Para film

Acetone – May and Baker England

Hydrochloric acid – May and Baker England

Potassium dichromate – May and Baker England

Sodium hydroxide – May and Baker England

3.2 IN-VITRO STUDIES

3.2.1 Identification Test (B.P 2002)

0.15g powdered paracetamol was extracted with 20ml of acetone, filter, the filtrate evaporated to dryness at 105 ^{0}.

0.1g of the residue was boiled with 1ml of hydrochloric acid for 3 minutes, 10ml of water added and cooled, no precipitate was produced. 0.05ml of 0.0167 potassium dichromate was added, a violet colour was produced slowly which does not turn red, melting point about 169^{0}c.

3.2.2 Assay (B.P 2002)

20 tablets was weighed and powdered. A quantity of the powder containing 0.15g paracetamol was added to 50 ml of 0.1m sodium hydroxide and this was diluted with 100ml of water, shakened for 15 minutes and sufficient water added to produce 200ml.

10ml of the filtrate was mixed, filtered and diluted to 100ml, with water and the absorbance of the resulting solution measured at the maximum at 257nm. The content of paracetamol was calculated taking 715 as the value of A (1% 1cm) at the maximum at 257nm.

3.2.3 Disintegration Test (B.P 2002)

The test was carried out using rigid basket rack assembling supporting six cylindrical glass tubes. One tablet was introduced into each tube and a disc added into each beaker containing specified liquid and the apparatus operated for the specified time. The assembly was removed from the liquid after the disintegration of all the six tablets.

3.2.4 Dissolution Test (B.P 2002)

The rotatory basket method as described by B.P 2002 was used.

One tablet of paracetamol was placed in the basket and placed in the round bottom flask containing 900ml of phosphate buffer (pH 5.8) at 37.5 ^{0}C + 0.5 ^{0}C.

The paddle was rotated at 50 revolutions per minute. A sample of 20ml of the dissolution medium withdrawn at 45 minutes from a point halfway between the basket wall and the side of the vessel. The filtrate was diluted with 0.1ml sodium hydroxide. The absorbance of the solution was measured, at the maximum at 257nm using 0.1ml sodium hydroxide in reference cell. The content of paracetamol in the medium was calculated taking 715 as the value of A (1%, 1cm) at the maximum at 257nm. The operation was repeated five times.

3.2.5 Preparation of Standard Samples

3.2.5.1 Preparation of Paracetamol Solution

A stock solution of pure paracetamol in methanol was prepared by dissolving 100mg paracetamol in 25ml of methanol.

3.2.5.2 Preparation of Dissolution medium

Phosphate buffer solution pH 5.8 prepared by dissolving 1.19g of disodium hydrogen orthophosphate dehydrate and 8.25g of potassium hydrogen orthophosphate in sufficient water to produce 1000 ml.

3.3 In-Vivo Studies

Glynn and Bastin (1973), have established a correlation between saliva and plasma concentrations of paracetamol after the ingestion of one tablet. Therefore the data which shall be obtained from salivary sampling in this study shall represent the in-vivo pharmacokinetics profile of paracetamol.

3.3.1 Protocols of study

Six healthy volunteers age between 20 to 30 years and weighing 40 to 70 kg were involved in the study. The volunteers were free from liver, kidney and respiratory diseases with normal laboratory values. They were non-smokers and non-alcohol consumers.

In the first phase, each volunteer was administered with 2x500mg panadol with 100ml of water to swallow after overnight fasting without taking breakfast. Saliva samples were taken at 0, 0.5, 1, 2, 3, 4, 5, 6, hours after the dose. Collected saliva samples were kept at - 4^0C until analysis.

The five brands of generic paracetamol were subjected to similar procedure as described above (Phase II) after a wash out period of two weeks involving the same volunteers.

3.3.2 Analytical methods

Analytical method was adopted and modified from (Garba M, et al, 1996) with λmax 285nm and ethyl acetate as solvent.

3.3.3 Extraction methods

2ml of saliva was placed in 10ml centrifuge tube using auto-pipette. 5ml of ethyl acetate was added. The centrifuge was stopped with plastic screw-caps and shaken vigorously for one minute with a rotamixer, and centrifuge for five minutes at 2500 revolutions per minute. The ethyl acetate layer was removed with Pasteur pipette and its absorbance measured at 285nm by a double beam U.V spectrophotometer.

The absorbance of the blank was subtracted from those of the samples containing paracetamol in order to obtain a set of absorbance reading from time zero (t_0) to time 6 hrs (t_6).

3.3.4 Calibration curve

A calibration curve of the concentration of paracetamol in human saliva was constructed. They were done by collecting sufficient quantity of blank saliva samples by chewing a piece of paraffin wax. 2ml of blank samples were distributed into each of the twelve ten milliliter (10ml) centrifuge tubes. Two of the twelve tubes were kept as blanks, and the remaining twelve were spiked with different concentrations of paracetamol in methanol from the stock solutions, using a micro litre Hamilton syringe. Each concentration was spiked in duplicate (as indicated in Table 3.3.4) and each duplicate was repeated four times on different days, resulting in 8 replicate data for each concentration of paracetamol and blanks. The stock solution of pure paracetamol in methanol was prepared by serial dilution of 10, 20, 30, 40 and 50µg/ml of paracetamol.

Two in-vivo studies were carried out for each phase and the mean of absorbance obtained were converted to the corresponding concentration from calibration curve.

TABLE 3.3.4:Concentrations of paracetamol spiked into saliva samples from paracetamol stock solution for calibration curve.

Sample serial number	Volume of stock solution added to saliva sample (µl)	Concentration of paracetamol per ml of saliva (µg)
1	0.00 µl	0.00 µg/ml
2	0.00 µl	0.00 µg/ml
3	5.00 µl	10.00 µg/ml
4	5.00 µl	10.00 µg/ml
5	10.00 µl	20.00 µg/ml
6	10.00 µl	20.00 µg/ml
7	15.00 µl	30.00 µg/ml
8	15.00 µl	30.00 µg/ml
9	20.00 µl	40.00 µg/ml
10	20.00 µl	40.00 µg/ml
11	25.00 µl	50.00 µg/ml
12	25.00 µl	50.00 µg/ml

In order to obtain absorbance readings for the various paracetamol, five milliliters (5ml) of ethyl acetate were added to each of the twelve saliva samples. The centrifuge tubes were then stopped with plastic screw caps and each was vigorously shaken for one minute (1min) with a rotamixer. All the tubes were then centrifuged for five minutes (5min) at 2500 revolutions per minute. The ethyl acetate layer was removed with Pasteur pipette and absorbance readings for each sample at 285nm wavelength were obtained from a double beam spectrophotometer. The absorbance of the blank samples were separated from those of the samples containing paracetamol in

order to obtain a set of absorbance readings correspond for 0.00 to 50.00 μg/ml concentrations of paracetamol in saliva to construct a calibration curve.

3.3.5 Data handling

Mean paracetamol concentration in the saliva samples were use to generate pharmacokinetic parameters i.e. Cmax, Tmax and AUC (trapezoidal rule) and values obtained were compared statistically using Student t-test were ($P < 0.05$) considered significant and bioavailability values for each generic brand was obtained by the ratio: $AUC_{generic}$: $AUC_{standard}$

CHAPTER FOUR

RESULTS

4.1 INVITRO STUDIES

4.1.1 Identification Test

The identification tests for the different brands of paracetamol were performed according to BP 2002.

a. A violet colour was formed by each brand

b. Melting point of the residue of each brand of drug, after drying at 105^0C was 168^0C.

4.1.2 Assay

The content of each brand of paracetamol was determined as shown in Table 4.1.2

Table 4.1.2: Assay of paracetamol tablets using U.V spectrophotometric method of analysis

Tablet Brand	percentage content	comment
Panadol®	99.9%	Satisfactory
Brand XA	58.5%	Fail
Brand XB	97.4%	Satisfactory
Brand XC	94.2%	Satisfactory
Brand XD	100.9%	Satisfactory
Brand XE	13.0%	Fail

The results are within the acceptable range of 95% and 105% (BP 2002) except brands XA and XE that were not within the acceptable range.

4.1.3 Disintegration time for paracetamol tablets

The official B.P 2002 for time limit for tablet disintegration is 15 minutes. The results obtained are within the time limit. The results are shown in Table. 4.1.3

Table 4.1.3

Tablets brand	Time (mins) Mean ± SEM	Comments
Panadol(R)	4.33 ± 0.42	satisfactory
Brand XA	2.97 ± 0.57	satisfactory
Brand XB	4.05 ± 0.74	satisfactory
Brand XC	3.38 ± 0.12	satisfactory
Brand XD	4.23 ± 0.53	satisfactory
Brand XE	2.61 ± 0.34	satisfactory

4.1.4 Dissolution profile for paracetamol tablets

In-vitro dissolution profile of tablet brands using B.P 2002 Rotating basket method.

Table 4.1.4

Tablet Brand	cumulative percentage released mean ± SEM
Panadol (R)	95.47 ± 1.30
Brand XA	56.75 ± 2.57
Brand XB	84.23 ± 1.61
Brand XC	76.09 ± 1.48
Brand XD	100.9 ± 0.50
Brand XE	9.80 ± 1.20

4.2 In – vivo studies

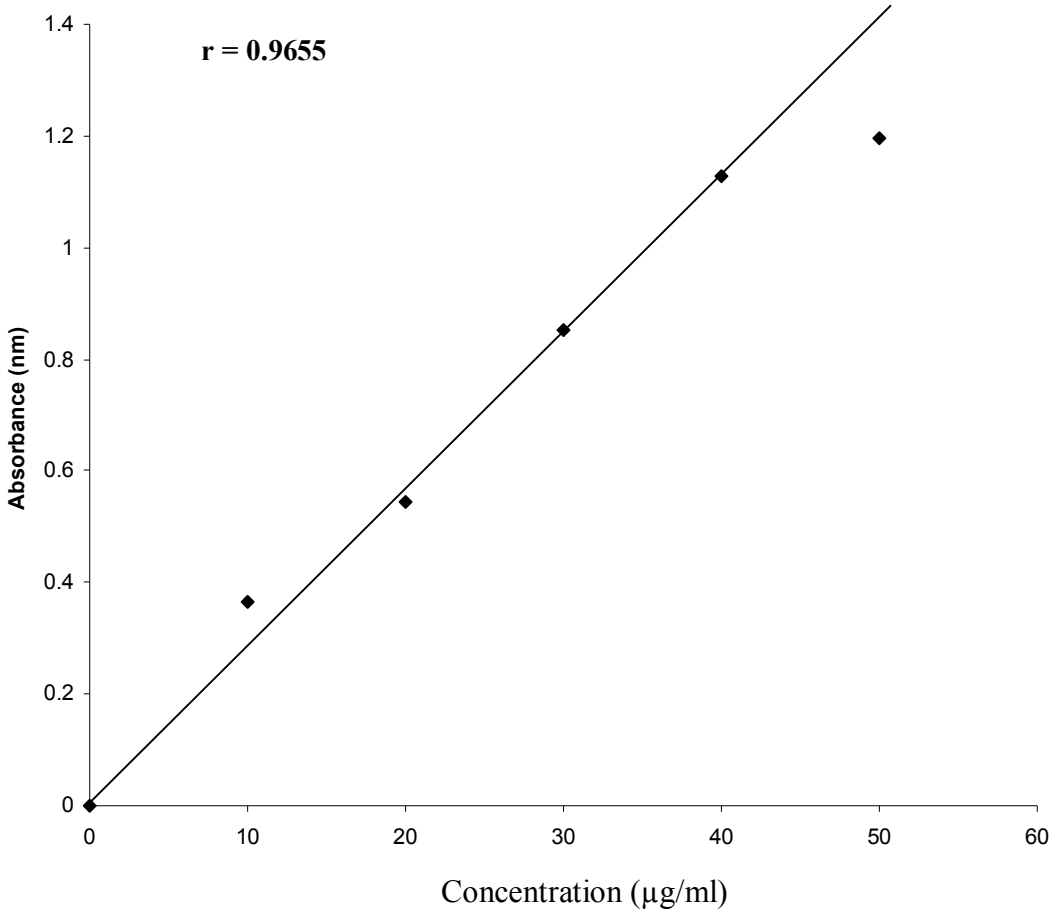
4.2.1 Calibration curve

Linear caliberation curve with good correlation coefficient (r = 0.9655) of paracetamol in ethyl-acetate using U.V. spectrophotometer (λmax = 285nm) is shown in figure 4.2.1.

Table 4.2.1: Table shows data obtained for the construction of the caliberation curve.

Concentration of drug/ml of saliva	Total mean X	Standard deviation (SD)
0.00 µg/ml	0.00	0.00
10.00 µg/ml	0.364	0.128
20.00 µg/ml	0.543	0.137
30.00 µg/ml	0.852	0.113
40.00 µg/ml	1.128	0.068
50.00 µg/ml	1.197	0.045

Fig. 4.2.1: Calibration curve for the analysis of paracetamol in saliva



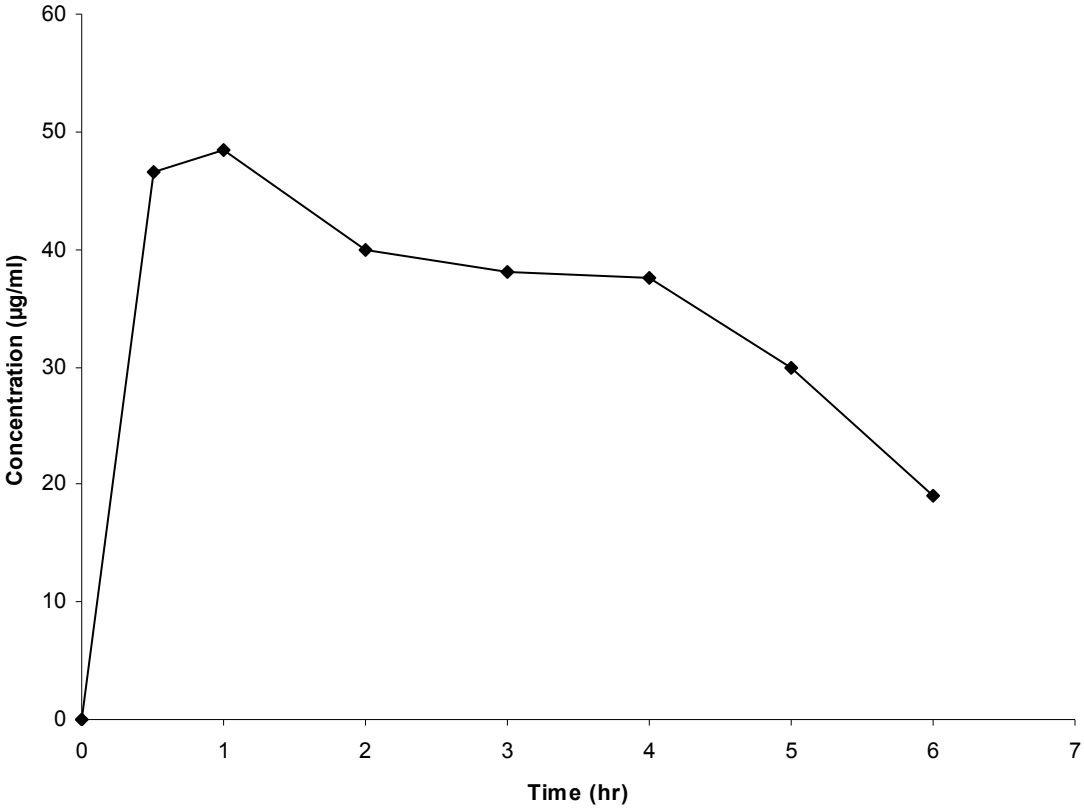
4.2.2 Pharmacokinetics

Tables 4.2.2 to 4.2.7 and Figures 4.2.2 to 4.2.7 show the mean saliva levels and pharmacokinetics parameters of paracetamol brands after a single oral dose of 1g of each brand.

Table 4.2.2: Mean saliva concentration in 6 healthy volunteers following oral administration of 1g of panadol in fasting state.

Time (hr)	Paracetamol concentration μg/ml MEAN + SEM n = 6
0.00	0.00
0.5	46.50 ± 11.97
1	48.50 ± 8.91
2	40.00 ± 10.18
3	38.00 ± 2.63
4	37.50 ± 9.51
5	30.00 ± 6.3
6	19.00 ± 8.52

Fig. 4.2.2

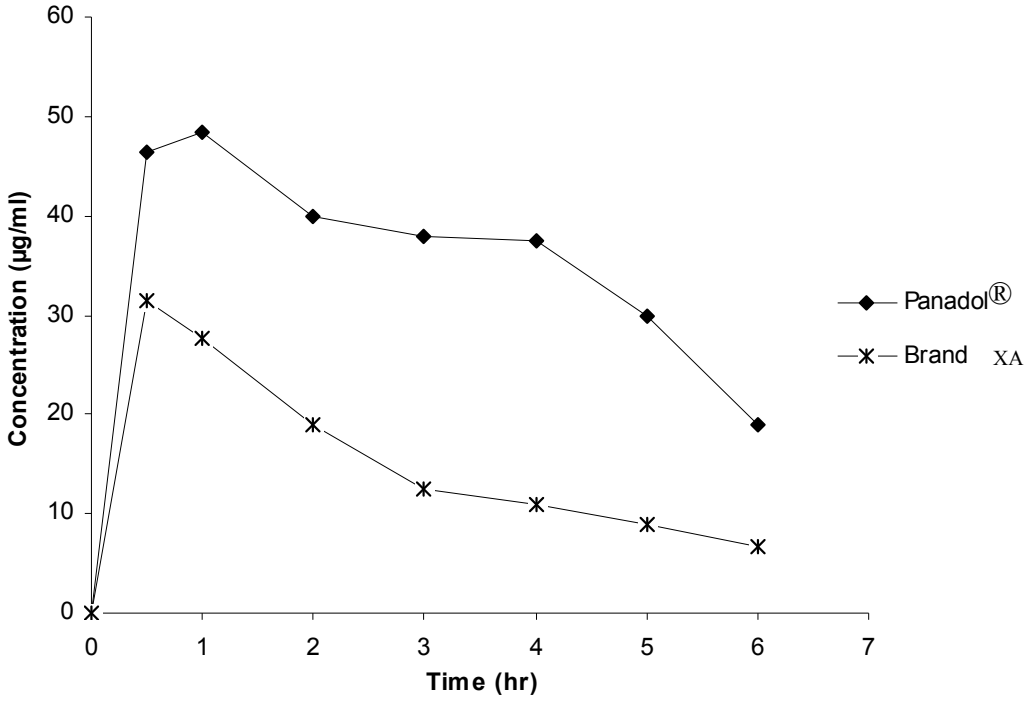


Saliva concentration time curve for panadol®

Table 4.2.3 Mean saliva concentration in 6 healthy volunteers following oral administration of 1g of Brand XA in fasting state.

Time (hr)	Paracetamol concentration (μg/ml) MEAN + SEM n = 6
0.00	0.00
0.5	31.50± 6.87
1	27.70± 8.12
2	19.00 ± 5.57
3	12.50 ± 3.74
4	11.00 ± 3.46
5	9.00 ± 3.11
6	6.70 ± 1.84

Fig. 4.2.3

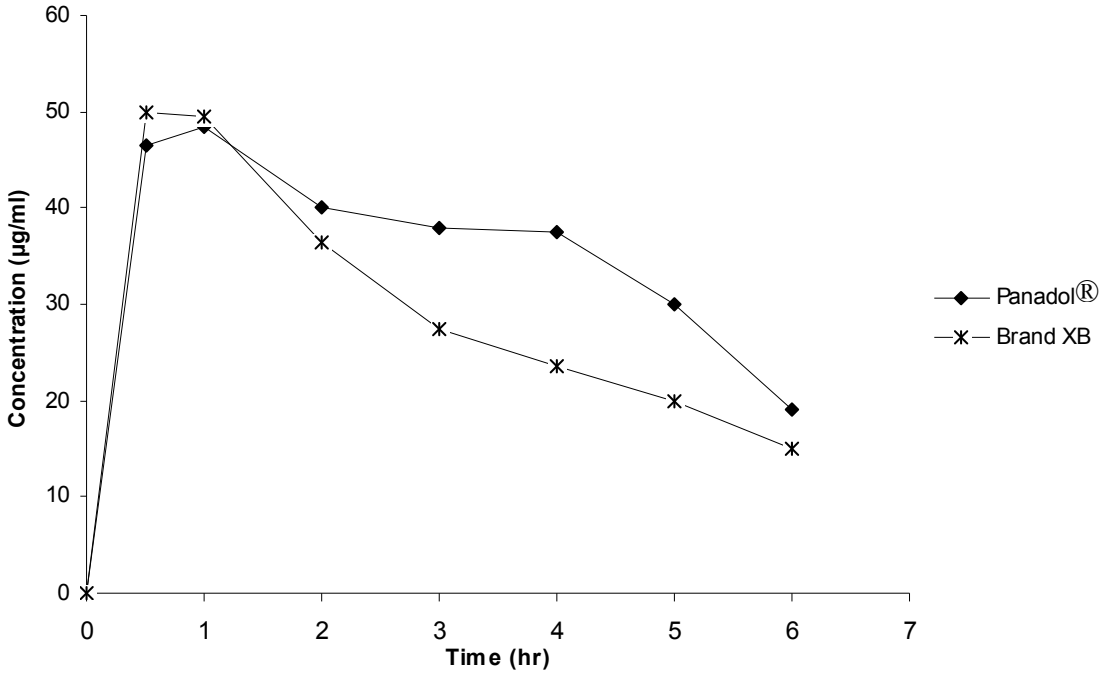


Comparative bioavailability saliva concentration time curve for brand XA and panadol®

Table 4.2.4 Mean saliva concentrations in 6 healthy volunteers following oral administration of Brand XB in fasting state.

Time (hr)	Paracetamol concentration µg/ml Mean ± SEM n = 6
0.5	50.00 ± 7.10
1	49.50 ± 8.24
2	36.50 ± 6.11
3	27.50 ± 3.34
4	23.50 ± 4.61
5	20.00 ± 2.62
6	15.00 ± 4.17

Fig. 4.2.4

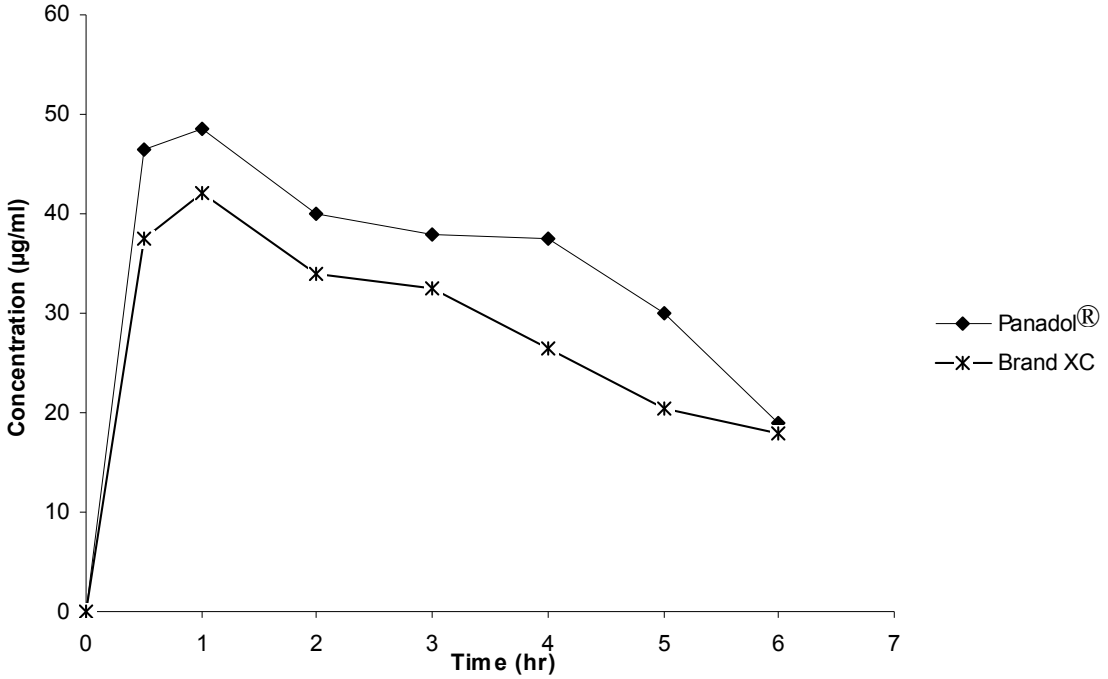


Comparative bioavailability saliva concentration time curve for brand XB and panadol®

Table 4.2.5 Mean saliva concentration in 6 healthy volunteers following oral administration of 1g of Brand XC in fasting state.

Time (hr)	Paracetamol concentration (μg/ml) MEAN ± SEM n = 6
0.00	0.00
0.5	37.50 ± 1.47
1	42.00 ± 7.1
2	34.00 ± 5.7
3	32.50 ± 5.19
4	26.50 ± 9.79
5	20.5 ± 5.14
6.	18.00 ± 5.00

Fig. 4.2.5

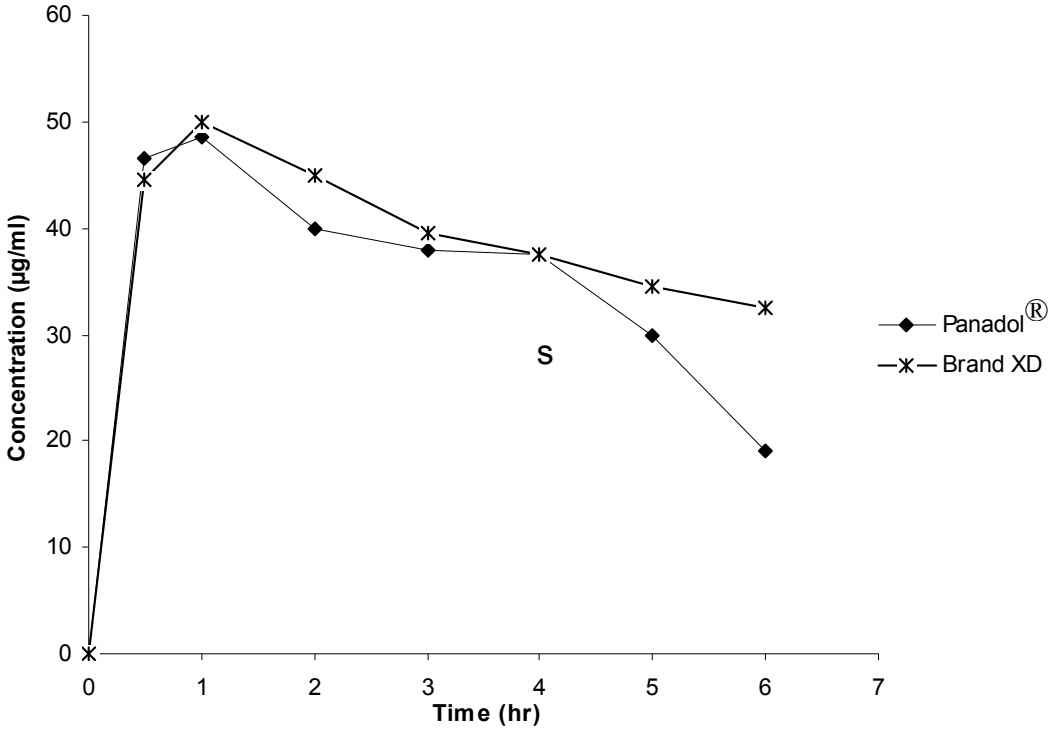


Comparative bioavailability saliva concentration time curve for brand XC and panadol®

Table 4.2.6 Mean saliva concentration in 6 healthy volunteers following oral administration of 1g of Brand XD in fasting state.

Time (hr)	Paracetamol concentration (μg/ml). MEAN ± SEM n = 6
0.00	0.00
0.5	44.50 ± 5.36
1	50.00 ± 6.46
2	45.00 ± 5.73
3	39.50 ± 50.08
4	37.50 ± 5.34
5	34.50 ± 4.98
6	32.50 ± 5.13

Fig. 4.2.6

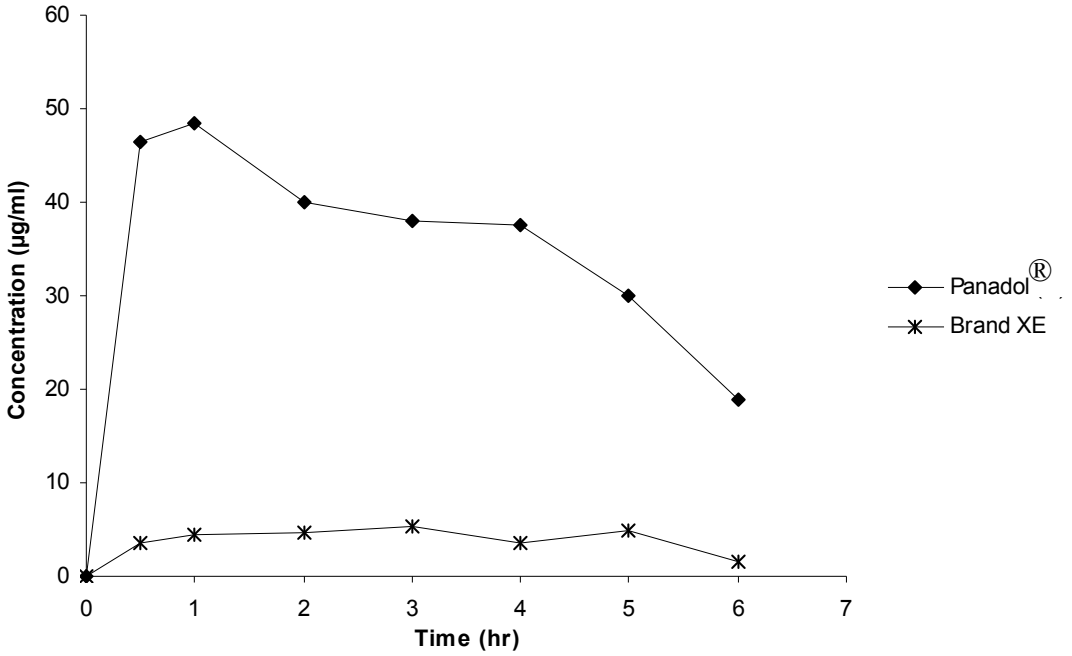


Comparative bioavailability saliva concentration time curve for brand XD and panadol®

Table 4.2.7. Mean saliva concentration in 6 healthy volunteers following oral administration of 1g of Brand XE in fasting state.

Time (hr)	Paracetamol concentration (µg/ml) MEAN ± SEM n = 6
0	0
0.5	3.5 ± 0.99
1	4.5 ± 0
2	4.72 ± 2.14
3	5.3 ± 0.98
4	3.55 ± 1.34
5	5.0 ± 1.71
6	1.5 ± 0.64

Fig. 4.2.7



Comparative bioavailability saliva concentration time curve for brand XE and panadol®

4.2.8 Pharmacokinetic parameters of paracetamol brand

Table 4.2.8

Tablet brand	C_{max} (µg/ml)	T_{max} (hr)	AUC
Panadol	48.50	1	238.13
Brand XA	31.50	0.5	117.93
Brand XB	50.00	0.5	202.00
Brand XC	42.0	1.0	192.63
Brand XD	50.0	1.0	256.13
Brand XE	5.30	3	27.03

Table 4.2.9: Bioavailability of paracetamol brand

Table brand	**Bioavailability (µg/ml/hr)**
Brand XA	0.5
Brand XB	0.85
Brand XC	0.81
Brand XD	1.08
Brand XE	0.11

For the bioequivalent brands whose limits lie within 0.8 to 1.25 or 80% to 125% confidence limits there is clear indication from the comparative bioavailability saliva concentration time curve and correlation coefficient that the pharmacokinetics parameters of the different paracetamol brands are linearly related.

Sotiropoulus et al. (1981) reported bioavailability of 82%, 87%, and 92%, after evaluation of three tablets in comparison to one liquid acetaminophen for their comparative bioavailability. Hekimoglu et al. (1987) reported bioavailability of 98%, 95% and 99% after evaluation of three brands of acetaminophen in comparison to one liquid acetaminophen with differences not statistically significant. Walter- Sack et al. (1989) compared a solid and a liquid oral dosage forms that did not show differences in the $AUC_{0\text{-}12h}$ and in C_{max}.

Dominquez et al. (2000) reported nonsignificant differences in the rates and relative bioavailability ranging from 94% to 121 % of three commercial formulations versus the innovator.

4. 3 CONCLUSION

All the brands of paracetamol tablets and the standard powder used in this study passed the official tests of identification, disintegration, assay and dissolution according to B.P 2002 except brand XA and XE that failed the test. The method of extraction and analysis adopted showed a good correlation between standard concentration and the estimated concentration obtained from the calibration curve which conform with already established value.

All the generic brands, except brand XA and XE are bioequivalent to panadol, with confidence interval of the area ratio falling within 0.8-1.25 or 80% to 125% confidence limits.

Thus all the generic brands except XA and XE will release their active ingredients into the blood stream at virtually the same speed and in virtually the same

amount as panadol and also the generic versions will produce virtually the same levels of drugs in the blood overtime thereby ensuring their therapeutic efficacy. Brand XA and XE have low bioavailability as a result their therapeutic efficacy will not be felt.

4.4 RECOMMENDATION

According to this finding I therefore recommend brand XB, XC and XD to be alternative to the innovator brand panadol®.

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