EFFECT OF TAMARINDUS INDICA AND SODIUM/POTASSIUM HAUSA SALT ON THE BIOAVAILABILITY OF ASPIRIN IN MAN

BY

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

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DECLARATION

I hereby declare that the work reported in this thesis was carried out by me under the supervision of Dr. Abdullahi Mustapha, and Dr. Ibrahim Abdu-Aguye. It has not been presented in any previous application for higher degree. The work of other investigators are acknowledged and referred to accordingly.

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CERTIFICATION

This thesis entitled "THE EFFECT OF TAMARINDUS INDICA AND SODIUM/POTASSIUM HAUSA SALT ON THE BIOAVAILABILITY OF ASPIRIN" by YAKASAI IBRAHIM ADAMU meets the regulations governing the award of the degree of MASTER OF SCIENCE (PHARMACEUTICAL CHEMISTRY) of Ahmadu Bello University, and is approved for its contribution to science, knowledge and literary presentation.

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DEDICATION

Dedicated to my late father, Mallam Adamu Yakasai and my wife Hajiya Fatima Ibrahim.

ABSTRACT

Aspirin and salicylic acid powders and aspirin tablets identification tests were carried out following B.P. (1980) specifications. The results obtained were in conformity with the official book specifications under identification tests for aspirin and salicylic acid.

Volumetric method of chemical assay B.P. (1988) were employed to determine the total drug content for aspirin and salicylic acid powders and aspirin tablets. The results compared favourably with the stated amount in the B.P. (1988).

Dissolution rate profile for the two brands of aspirin tablets (Polfa and Vitalink) in 0.1M HC1 was investigated by means of B.P. (1988) rotating basket method. Total drug release of the active ingredient for the two brands was obtained in less than 45 minutes.

Disintegration time test was carried out on the two brands of aspirin tablets. There was no considerable variation in their disintegration time. The times taken for the two brands to disintegrate was less than 5 minutes. They all passed the official requirement of 15 minutes for tablet disintegration.

In-vivo bioavailability studies were carried out in six healthy human volunteers under four conditions. Each volunteer ingested 600mg aspirin only; then with starch; with Tamarindus indica extract (Tsamiya) and

also with sodium/potassium Hausa salt extract (Kanwa) all in the form of Hausa food drink Kunu.

A simple, rapid and accurate method for the determination of plasma and saliva aspirin and salicylic acid levels by multiple component spectrophotometry method was employed. Also employed in the analysis was HPLC method. Because of the reliability, accuracy and sensitivity of HPLC, it was used as a standard in comparing the results got from U.V. spectrophotometry method.

Pharmacokinetic parameters for plasma and saliva aspirin and salicylic acid samples were generated by computer application.

Statistical analysis of the results showed, however, that concurrent ingestion of aspirin with T. indica extract gave rise to a significant increase ($P<0.01$) in the amount of drug absorbed compared with the fasting state. Also concurrent administration of the drug with sodium/potassium Hausa salt extract gave rise to a significant decrease in the amount of drug absorbed ($P<0.05$) compared with the fasting state.

Hence, it can be concluded that the bioavailability of aspirin is enhanced by T. indica and diminished by sodium/potassium salt in man.

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TABLE OF CONTENTS

CONTENTS PAGE

Title page.....i
Declaration.....ii
Certification.....iii
Dedication.....iv
Abstract.....v
Table of contents.....vii
List of tables.....viii
List of figures.....ix
Acknowledgement.....vi
List of appendices.....x
Abbreviations.....xi

CHAPTER ONE: INTRODUCTION

1.1 Bioavailability.....1
1.1.1 Assessment of bioavailability.....3
1.1.2 Initiation of bioavailability studies.....6
1.1.3 Influence of food on bioavailability of drugs.....7
1.1.4 Pharmacokinetics.....11

CHAPTER TWO: GENERAL LITERATURE REVIEW ON THE HAUSA FOOD MATERIALS AND THE DRUG (ACETYLSALICYLIC ACID)

2.1 Tamarindus indica (Linn)..... 14
2.2 Sodium/potassium Hausa salt..... 16
2.3 Elensine coracana (L) (Millet)..... 18
2.4 Acetylsalicylic acid (Aspirin)..... 19
2.5 Assay methods of Aspirin..... 25
2.6 High-Performance Liquid Chromatography..... 26
2.7 Standardisation of the food materials..... 27
2.8 Aims and Objectives of the study..... 29

CONTENTS PAGE

CHAPTER THREE: MATERIALS AND METHODS

3.1.2 Drugs..... 31
3.1.2 Equipment..... 31
3.1.3 Solvents/Reagents..... 32
3.2.0 Methods..... 33
3.2.1 Identification tests..... 33
3.2.2 Aspirin powder..... 33
3.2.3 Aspirin tablets..... 33
3.3.0 Solvent system..... 33
3.4.0 Assay of aspirin powder B.P.(1988)..... 34
3.5.0 Assay of salicylic acid powder B.P.(1988)..... 34
3.6.0 Assay of aspirin tablets B.P. (1988)..... 35
3.7.1 Dissolution rate test..... 35
3.7.2 Calibration graph..... 37
3.7.3 Disintegration test..... 37
3.8.0 In-vivo absorption studies..... 38
3.8.1 Simultaneous determination of aspirin and salicylic acid in plasma and saliva using multiple component spectrophotometric method of analysis..... 38
3.8.2 Calibration graphs (Beer's law plots)..... 39
3.8.3 Extraction procedure (U.V.)..... 41
3.8.4 Simultaneous determination of aspirin and salicylic acid in plasma and saliva using High-Performance Liquid Chromatography..... 42
3.8.5 Preparation of standard solutions and calibration graphs..... 42
3.8.6 Extraction procedure for HPLC..... 43
3.8.7 Study conditions..... 44

CHAPTER FOUR: RESULTS

4.1.0 Identification tests B.P. (1988)..... 47
4.1.1 Aspirin powder..... 47
4.1.2 Aspirin tablets..... 47
4.2.0 Solvent system..... 47
4.3.0 Chemical assay B.P. (1988) of aspirin and salicylic acid powers and tablets..... 50
4.4.1 Dissolution rate tests..... 50

CONTENTS PAGE

5.1.0 Identification tests B.P. (1988).....90
5.2.0 Content of active ingredients.....90
5.3.1 Dissolution rate studies.....90
5.3.2 Disintegration times.....92
5.4.0 In-vivo bioavailability studies.....93
5.5.0 Conclusion.....99

REFERENCES.....100

APPENDICES.....106

LIST OF TABLES

TABLE		PAGE
1	Percentage content of the active ingredient in each drug from the chemical assay (B.P. 1988)	51
2	Dissolution rate profile of Polfa and Vitalink aspirin tablets in 0.1M hydrochloric acid	51
3	Disintegration times of Polfa and Vitalink aspirin tablets in water maintained at 37 ± 0.5°C	53
4	Chromatographic parameters for aspirin, salicylic acid and paracetamol (internal standard) under different conditions using u-Bonda-Pak C_{18} radial-pak catridge (Waters Associates)	48
5	Effect of pH on the capacity factor (R) and resolution (d) for aspirin, salicylic acid and paracetamol (internal standard) using solvent system Methanol : 5% Acetic acid (25:75) and u-Bonda-Pak C_{18} radial-pak catridge (Waters Associates)	49
6	Mean plasma concentrations of aspirin (ASA) and salicylic acid (SA) in six healthy volunteers following oral ingestion of 600mg aspirin under different conditions	79
7	Mean saliva concentrations (ug/ml) of aspirin (ASA) and salicylic acid (SA) in six healthy volunteers following oral administration of 600mg aspirin under different conditions	80
8	Extraction recoveries for aspirin (ASA) and salicylic acid (SA) in plasma and saliva using U.V. spectrophotometer	81
9	Extraction recoveries for aspirin (ASA), salicylic acid (SA) and paracetamol (internal standard) in plasma using HPLC	82
10	Pharmacokinetic parameters of salicylic acid derived from the mean plasma concentration data after a single oral dose of 600mg aspirin	83

TABLE — PAGE

11 Pharmacokinetic parameters of aspirin derived from the mean plasma concentration data after a single 600mg oral dose under 4 conditions..... 84

12 Pharmacokinetic parameters of aspirin derived from the mean saliva concentration data after a single 600mg oral dose under 4 conditions..... 85

13 Comparison between HPLC and U.V. spectrophotometric plasma concentrations of aspirin and salicylic acid pharmacokinetic parameters after a single 600mg oral dose of aspirin..... 86

14 Comparison between HPLC and U.V. spectrophotometric plasma concentrations of aspirin and salicylic acid pharmacokinetic parameters after concurrent administration of 600mg aspirin and 200ml food drink containing T. indica extract..... 87

15 Relationships between salivary and plasma concentrations of salicylic acid..... 88

LIST OF FIGURES

FIGURE		PAGE
1	Plasma drug concentration-time curve after oral administration	4
2	Calibration graph for standard aspirin in 0.1M HCl at 276nm	52
3	Calibration graph for standard aspirin in 0.1M HCl after extraction at 276nm	55
4	Calibration graph for standard aspirin in 0.1M HCl after extraction at 231nm	56
5	Calibration graph for standard salicylic acid in 0.1M HCl after extraction at 276nm	57
6	Calibration graph for standard salicylic acid in 0.1M HCl after extraction at 231nm	58
7	Calibration graph for the plasma concentration of standard aspirin at 276nm after extraction pH 3.9	59
8	Calibration graph for the plasma concentration of standard aspirin at 231nm after extraction pH 3.9	60
9	Calibration graph for the plasma concentration of standard salicylic at 276nm after extraction pH 3.9	61
10	Calibration graph for the plasma concentration of standard salicylic acid at 231nm after extraction pH 3.9	62
11	Calibration graph for the salivary concentration of standard aspirin after extraction at 231nm and pH 3.9	63
12	Calibration graph for the salivary concentration of standard aspirin after extraction at 276nm and pH 3.9	64

FIGURE PAGE

13 Calibration graph for the salivary concentration of standard salicylic acid after extraction at 276nm and pH 3.9.....65

14 Calibration graph for the salivary concentration of standard salicylic acid after extraction at 231nm and pH 3.9.....66

15 Calibration graph for plasma aspirin assay using HPLC with the moble phase Methanol : 5% Acetic acid (25:75) pH 3.45 67

16 Calibration graph for plasma salicylic acid assay using HPLC with the mobile phase Methanol : 5% Acetic acid (25:75) pH 3.5, 0.01 aufs.....68

17 HPLC chromatogram of aspirin, salicylic acid and paracetamol.....69

18 Mean plasma concentrations of aspirin and salicylic acid (◭) following oral ingestion of 600mg aspirin after overnight fast.....71

19 Mean salivary concentrations of aspirin (○) and salicylic acid (◭) following oral ingestion of 600mg aspirin after overnight fast.....71

20 Mean plasma concentrations of aspirin (○) and salicylic acid (◭) following oral ingestion of 600mg aspirin and starch after overnight fast.....72

21 Mean salivary concentrations of aspirin (○) and salicylic acid (◭) following oral ingestion of 600mg aspirin and starch after overnight fast.....73

22 Mean plasma concentrations of aspirin (○) and salicylic acid (◭) following oral ingestion of 600mg aspirin and T. indica extract (Tsamiya) after overnight fast.....74

FIGURE PAGE

23 Mean salivary concentrations of aspirin (⊙) and salicylic acid (◅) following oral ingestion of 600mg aspirin and T. indica extract (Tsamiya) after overnight fast..... 75

24 Mean plasma concentrations of aspirin (⊙) and salicylic acid (◅) following oral ingestion of 600mg aspirin and sodium/potassium extract (Kanwa) after overnight fast..... 77

25 Mean salivary concentrations of aspirin (⊙) and salicylic acid (◅) following oral ingestion of 600mg aspirin and sodium/potassium extract (Kanwa) after overnight fast..... 78

26 Mean concentrations of salicylic acid in saliva (⊙) and plasma (◅) following oral ingestion of 600mg aspirin after an overnight fast..... 89

LIST OF APPENDICES

APPENDIX		PAGE
1	Apparatus for the disintegration test of tablets	105
2	Aspirin plasma absorption and salivary secretion studies of individual volunteer following oral ingestion of 600mg aspirin under four conditions	107
3	Comparison between the values of plasma concentrations (ug/ml) for aspirin (ASA) and salicylic acid (SA) in subject Ss using HPLC and U.V. spectrophotometric methods of analysis following oral ingestion of 600mg aspirin	119

ABBREVIATIONS

B.P.	=	British Pharmacopoeia
^{o}C	=	Degree centigrade (Celsius)
g	=	Gram
ug	=	Microgramme
mg	=	Milligramme
ml	=	Millilitre
min	=	Minute
hr	=	Hour
HCl	=	Hydrochloric Acid
NaOH	=	Sodium Hydroxide
$NaNO_2$	=	Sodium Nitrite
G.L.C.	=	Gas Liquid Chromatography
H.P.L.C.	=	High Performance Liquid Chromatography
U.S.P.	=	United States Pharmacopoeia
pH	=	Hydrogen-ion concentration
U.V.	=	Ultraviolet

CHAPTER ONE

INTRODUCTION

1.1 BIOAVAILABILITY

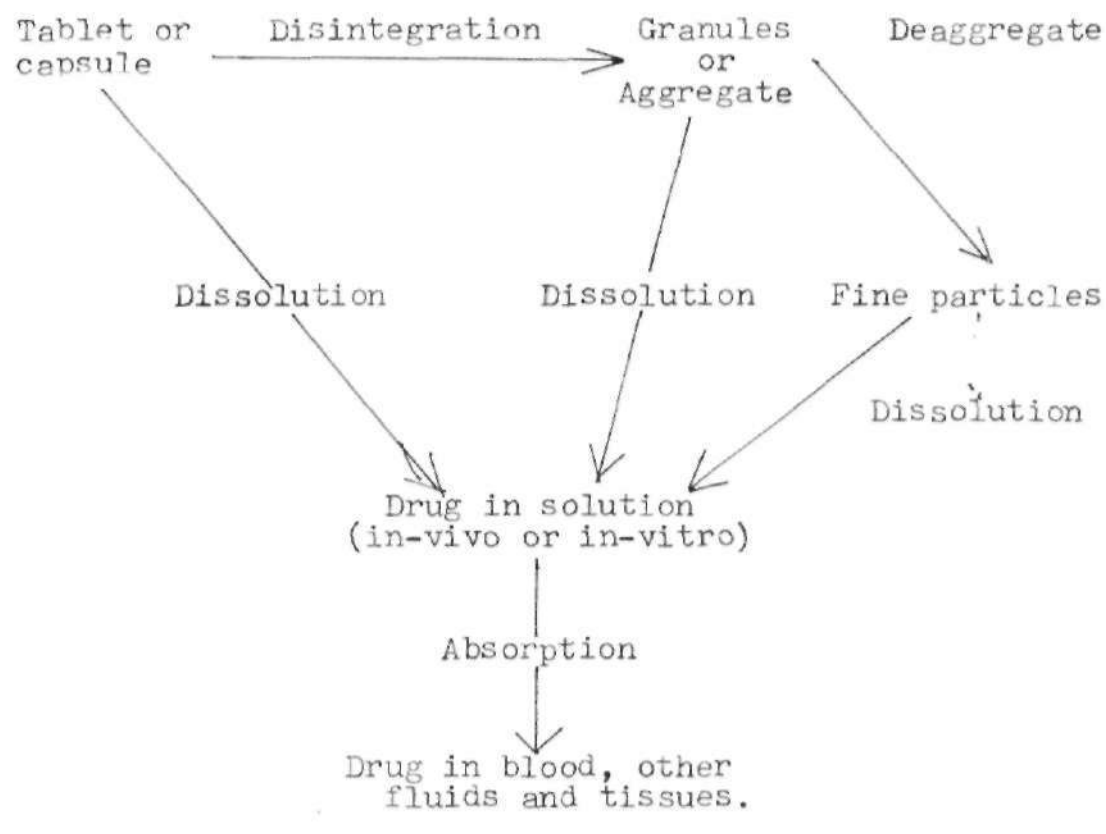
The term bioavailability has been used to describe the number of concepts associated with the effect of administration of a drug preparation, including the extent of absorption of the drug into the blood stream, the availability of the drug at the receptor sites, and the therapeutic effectiveness of the preparation. The term is used here to describe the biological bioavailability of a drug from a preparation, this availability being quantified in terms of the amount and rate of appearance of the drug in an appropriate body fluid, such as saliva, blood or urine, after the administration of the preparation. Such measurements may be used as a means of comparing the bioavailability of a drug under varied conditions of administration or in alternative formulations.

The therapeutic action of a drug is directly related to the concentration of the drug available at the site of action, and this concentration is in turn dependent on the passage of the drug across biological membrane. The rate and extent of absorption of a drug by the body is influenced by a number of biological and physiological factors, but this may also be affected by

AHMADU BELLO

the rate at which the drug dissolves in the body fluid at or before the site of absorption. Frequently, this dissolution is the rate-limiting factor in the absorption process. The dissolution rate is often dependent on the physiochemical characteristics of the drug itself, such as solubility, particle size, polymorphism and solvation, and on other materials present in the formulation as well as the manufacturing process used in the preparation of dosage form.

Scheme 1: Process undergone by capsule or tablet when exposed to fluid in-vivo and in-vitro (Wagner, 1971).



1.1.1 Assessment of Bioavailability

It is necessary to have some means whereby drug formulations can be assessed in terms of the extent to which they make drug available to the body. Ideally, this assessment would involve measurement of the amount of drug available at the site of action at suitable periods of time after administration. In practice, this is not generally possible because either the pricise site of action is not known or in a few cases where it can be identified, it is impracticable to determine the drug concentration attained. However, it is possible to determine the drug concentrations in a selected body fluids over a period of time after administration of the preparation and hence obtain an indication of the amount and rate of absorption of the drug from the preparation. The body fluid employed in this study is plasma. Other fluid such as serum, urine and saliva are also routinely employed in drug measurement.

Plasma Concentration/Time Curve

When plasma is the body fluid employed for drug measurement, serial samples of blood are withdrawn from the subject and are examined for drug content at specific periods of time after administration of the preparation. If the drug concentrations are plotted against time, a curve is obtained which usually shows a fairly steep

to a peak concentration over a long period of time.

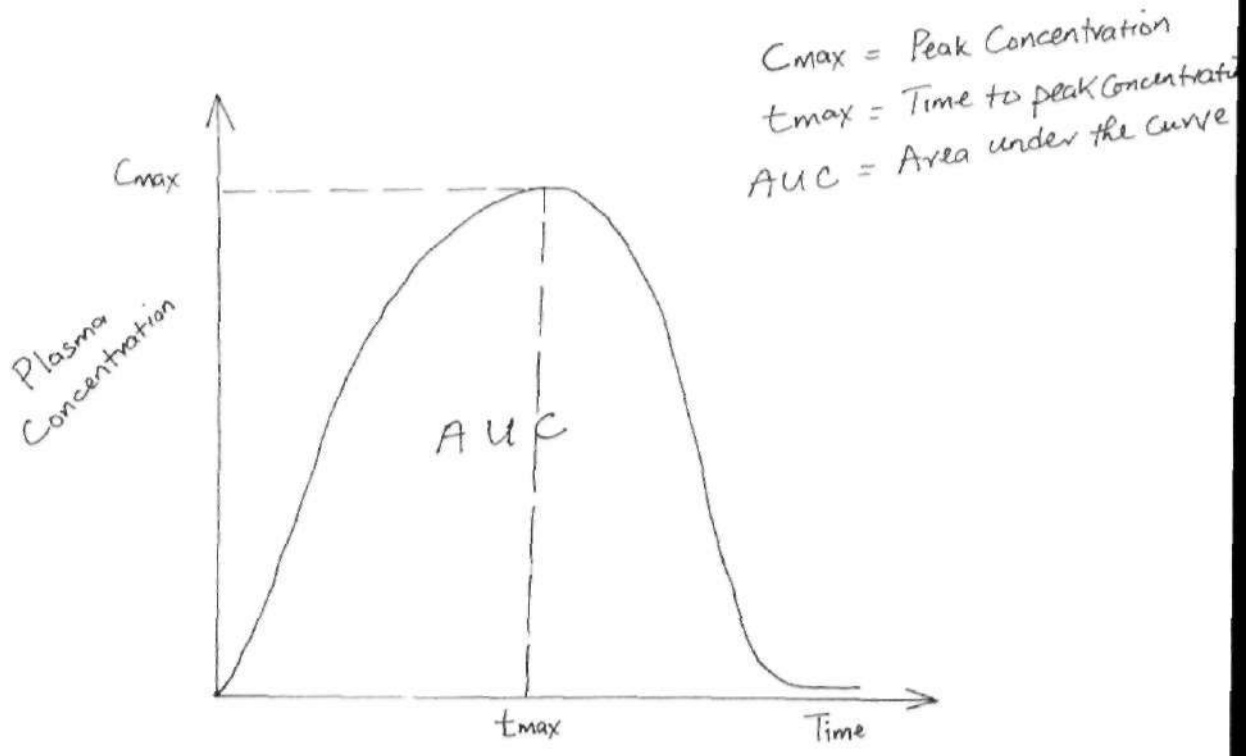


Figure 1: Plasma Concentration/Time Curve.

The shape of the curve is dependent on a number of events in the body including absorption, distribution and elimination (metabolism and excretion). Elimination of the drug begins as soon as it appears in the blood. There are three parameters which can be derived from the plasma concentration/time curve - the peak concentration, the time to peak concentration and the area under the curve.

The peak concentration represents the highest concentration of the drug achieved in the plasma. It is reached when the rate of appearance of the drug in the plasma equals the rate of its removal by distribution,

metabolism and excretion. The time to peak concentration is related to the peak of absorption and can be used to assess that rate. The area under the curve is related to the total amount of drug absorbed from the dose. The values obtained from these parameters may or may not be related to the therapeutic activity, and if there is a relationship, it may not be linear.

Urinary Excretion

Measurement of drug and/or its metabolites in the urine can also be used in comparative bioavailability studies. This has the advantage that samples are more easily obtained and it is less unpleasant for the subject than the method involving blood samples. Also, the drug concentration in the urine is often greater than in blood, thus permitting less sensitive analytical methods for determination. However, urinary excretion measurement is usually applicable to those drugs of which a significant amount is excreted unchanged or as metabolites in the urine. The method is useful also if it has been shown that the urinary excretion of the drug is proportional to the plasma concentrations. The parameters in this regard are the cummulative amount excreted and the rate at which the excretion takes place.

Salivary Secretion

The secretion of drugs and other foreign compounds in saliva is well known. The salivary secretions of weak acids such as salicylates (1), sulfonamides (2) and barbiturates (3, 4) has been investigated in some details. It was found that the concentrations of several sulfonamides in human saliva were proportional to the concentration of the unbound drug in the plasma (5). These observations indicated that measurement of concentrations of drugs in consecutive samples of saliva may be a useful technique in investigating kinetics of absorption and elimination of drugs, although difficulties may arise if the plasma binding characteristics of the drug varied significantly during the time course of the experiment.

1.1.2 Initiation of Bioavailability Studies

Generally, bioavailability studies may need to be initiated to detect potentialities of drug formulations for clinical failure or toxic reactions. Clinical failures often result from sub-therapeutic blood levels of drugs due to slow rate or low extent of absorption from their conventional dosage formulation. On the other hand, toxic reactions manifests when blood levels exceed certain tolerable limits due to enhanced rate and extent of absorption. Either clinical failures or toxic reactions may result for certain drugs when:-

(a) a number of different formulations are available from several manufacturers;

(b) the therapeutic dose of the drug is close to its toxic dose;

(c) the drug substance is relatively insoluble, or may become insoluble in gastro-intestinal fluids;

(d) the drug has been shown to be completely absorbed from the gastrointestinal tract;

(e) the absorption of the drug substance from the gastrointestinal tract is affected by the kind of food concomittantly taken.

1.1.3 Influence of Food on Bioavailability of Drugs

The three major physiological function that controls gastrointestinal drug absorption have been found to be blood flow rate, gastric motility and emptying rate. It was shown that there is general increased splanchnic blood and intestinal lymph flow rate after eating and this is accompanied by increased cardiac output (6). The inhibition of stomach emptying rate has also been found to be greater the higher the osmolarity of the solute entering the duodenum from the stomach. Similarly, the gastric emptying was delaved by ingestion of hot meals, solutions of high concentration, viscosity, by fat and to a lesser extent by

protein and carbohydrates. Solid diets were shown to almost double stomach emptying time compared to liquid meals in ratss (7). Thus, mechanical inhibition of drug passage from the stomach into the intestinal would have varying effects on drug absorption depending on the solubility, stability and the lipophilic character of the dissolved molecules. In the upper small intestine, however, foods have been shown to have stimulatory effect on intestinal motility, and thus increased motility may accelerate dissolution of solid drug particles and also decrease the diffusion path of drug molecules to the intestinal mucosa. Thus intestinal motility may, on the other hand, increase the rate of transit time through the intestine.

Food ingestion, particularly fat, apart from its influence on spanchnic blood flow and gastrointestinal motility, also stimulates the secretion of bile flow. Bile salts are surface active and can increase the dissolution of poorly soluble drugs, and hence promote absorption. Food may also influence drug absorption more directly because of adsorption of drug on to food components.

Considerable interest has been shown as to how concurrent use of traditional food materials and pharmaceuticals can affect the bioavailability of the drugs. Because many plant constituents are pharmacologically active, one needs to be alert to the same type

of interactions that occur among different purified or synthesised pharmaceuticals. However, this interaction could be in form of antagonism - where one drug diminishes the activity of another; additive - where the combined action of two drugs is summed; potentiation - when the effect is greater than additive; and synergistic - when one drug markedly increases the action of another.

For example, tannic acid is a compound which binds some classes of pharmaceuticals into insoluble complexes which makes them unavailable for action ( ). It is a constituent of many plants and has an easily detected astringency. Thus, tannin containing Hausa plants are used for costive and other astringent action (e.g. barks of Acacia, Arabica, Adansonia digitata) and this also limits the availability of some orally ingested pharmaceuticals used in the treatment of gastrointestinal disorders. Tannins interfere as well with the absorption of iron, which becomes significant when tannin-containing plants are consumed with ferrous sulphate preparations (8).

The antagonistic effects also of calcium on tetracycline absorption are likely to be realised, since particularly the leafy parts of many Hausa medicines are rich sources of calcium (e.g. Abrus precatorins, Hibiscus sabdariffa, Momordica charantia) (8).

Drug efficacy may also be compromised because of the high calcium content of milk, a popular vehicle for the administration of Hausa medicine.

Other antagonistic effects are revealed in the reduced activity of some penicillins when combined with fruit juices. Especially trouble-some are the fruits of citrus and capsicum which figure prominently in the medicine for fever and gastrointestinal disorders, and even more conspicous in diet. Similarly, the pectins that occur in the fruits of Adansonia digitata, Psidium guajava, Tamarindus indica (9,10) and many other plants - reduce the activity of some antibiotics.

Potentiation of pharmaceutical effects for several analgesic - antipyretic is observed on consumption of plants of the Cruciferae family. This is particularly significant for the combinations that include the ubiquitous headache tablets APC (which contain phenacetin) and leaves of Lepidium sativum (11), the popular Hausa medicine and food, LAPSUR. Similarly, ascorbic acid in large amounts enhances iron absorption, so that one could anticipate this potentiating effect when Hausa plants high in Vitamin C (e.g. fruits of Adansonia digitata, Capsicum spp, Citrus spp (12,13) are consumed with ferrous sulphate preparations.

And finally, both synergistic and antagonistic interactions might occur in the presence of KANWA, an alkaline (sodium/potassium) Hausa salt that is commonly added to medicines and to all but a few foods. Alkalinity diminishes the absorption of the antibiotic tetracyclines, but it also increases the solubility of oral sulphonamides and has potentiating effects on many other synthetic drugs (14).

1.1.4 Pharmacokinetics

Pharmacokinetics is the study of the rate processes associated with absorption, distribution, metabolism and excretion of a drug, i.e quantitating drug and or metabolite(s) levels in body fluids, tissues and excreta at any point in time from the moment of administration until elimination from the body is complete (15). The primary standard of a pharmacokinetic study is based upon the data obtained following intravenous administration. Since the entire dose administered is placed directly into the blood stream following intravenous administration, the pharmacokinetic parameters obtained are unaffected by all the potential rate-limiting and metabolic factors associated with drug absorption through other routes.

In pharmacokinetics, attempts are made to describe a biological event(s) in mathetical terms, usually by developing mathetical models to represent the body as a

system of compartments, even though these compartments often have no apparent physiologic or anatomic reality. The one-compartment model, the simplest model, dipicts the body as a single homogenous unit. This model is particularly useful for the pharmacokinetic analysis of blood, plasma or serum concentration, salivary and urinary excretion data for drugs which rapidly distribute between plasma and other body fluids and tissues upon entry into the systemic circulation.

To assume the body behaves as a one-compartment model does not necessarily mean that the drug concentrations in all body tissues at any given time are the same. However, a one-compartment model does assume that any changes that occur in the plasma quantitatively reflects changes occurring in tissue drug levels. A second criterion for utilising a one-compartment model is that drug elimination occurs from the body in a first-order fashion (i.e the rate of elimination of drug from the body at any time is proportional to the amount of drug in the body at that time). The proportionality constant relating the rate and amount is the first-order elimination rate constant which has units of reciprocal time (for example min^{-1}, hr^{-1}) (16).

Half-life is one of the most useful pharmacokinetic parameters obtained following drug administration. The half-life of a drug is simply the time required to reduce the post-absorption concentration of a drug

in the blood stream by fifty percent (17,18). This parameter is calculated by dividing 0.693 by the calculated slope of each exponential phase of multi-phasic blood level curve. Another pharmacokinetic parameter in frequent use is the area under the curve of blood level versus time. The method of calculating the area are discussed by various authors (19;20). Many authors have used area analysis to develop a pharmacokinetic pathway and to obtain the rate constants associated with the proposed pharmacokinetic model. Total clearance is the sum of individual clearances of the drug by the various organs and tissues. The volume of distribution (Vd) is inversely proportional to blood level, i.e. the larger the volume of distribution the more extensively the drug distributes from the blood stream into the various tissues, organs, and binding sites of the body. There are various ways of calculating Vd depending upon the pharmacokinetic profile of the drug (21).

CHAPTER TWO

GENERAL LITERATURE REVIEW ON THE HAUSA FOOD MATERIALS AND THE DRUG (ACETYLSALICYLIC ACID)

2.1 TAMARINDUS INDICA (LINN)

The plant Tamarindus indica is found in the Caesalpinnaceae plant family. It is a large tree with paripinnate leaves commonly found in an open country and savannah forest. It grows in the Northern and Southern parts of Nigeria and some other West African countries like Senegal, Sierra Leone, Ghana, etc. In addition to these West African countries, it is also found to grow spontaneously in Sudan and India.

The plant is commonly called 'Tsamiya' in Hausa language, 'Ajagbon' in Yoruba, 'Icheteu-Oyibo' in Ibo and 'Darra' in Nupe languages. The plant formsa dense symmetrical crown with branches all round. It has an abundant leaf-fall and usually almost no under-growth beneath it. In damp weathers, the leaves have some corrosive action on the fabrics on which they fall and because of this, natives of India object to sleeping under a Tamarind tree, attributing to it a harmful acid exhalation. The tree is a suitable ornament and grows in most soils (2).

FRUIT

The Tamarind fruits are about 5-15cm long. They have a brittle epicarp, a pulpy mesocarp through which run from the stalk about 5-9 branched fibre and then a leathery endocarp. The balls of the pulp are pressed together with the seeds to form cakes and it is these cakes that are sold in the market. These balls in form of cakes may be used without any special preparation or the pulp may be boiled, concentrated into a paste and then dried in the sun before use.

The pulp occurs as a reddish-brown moist sticky mass in which the yellowish-brown fibres mentioned above are readily seen. The pulp has a pleasant and fruity odour, tastes sweet and acidic (sour). The fruit has laxative properties and is used as food seasoner in various ways such as:- boiling the pulp with cereal pap and drinking for constipation or prepared as a drink to which onions are added. A pleasantly acidic drink is made by a cold infusion of the pulp with sugar or honey and or with water and then left to mature for several days. The pulp itself is also pleasant to suck to relief thirst on a journey. The pulp contains free organic acid (about 10% tartaric, citric and malic acids) and their salts (8% potassium hydrogen tartarate), a little nicotinic acid and about 30-40% invert sugar (2).

The fruit extract could be taken alone or mixed with other ingredients like lime-juice or honey as a laxative and as a refringerant drink for patients with fever and dysentry. The fruit extract is also believed to be a preventive of fevers and sun-stroke. However, the best form for internal use is the boiled and evaporated pulp which when mixed with water in which ir is partly soluble is widely used as a specific for fevers especially in Sudan.

In conclusion, the fruit is a very important source of laxatives or saline purgatives. It can be used both locally and in the hospitals where the constituents can be extracted and used in the pure forms, especially as the plant grows almost all round. It would be a good idea to include the plant Tamarindus indica as an official source of producing laxatives due to its ready availability and its laxative action causes no irritation.

2.2 SODIUM/POTASSIUM HAUSA SALT (KANWA)

Sodium/potassium salt, known as KANWA in Hausa, is found deposited in the ground of semi-arid to arid regions. It is found mostly in the extreme north of Nigeria and some West African countries like Niger, Mali and Upper Volta and other areas where desert features predominate. They are obtained from the ground in the form of red salt and white salt but the

concentration of the salt (potash) is more with the red salt than the white one judging from the taste of the extract of each. Normally the white one is commonly used in food preparations while the red one is used in medicine.

Eventhough no much literature work was done on the food material, it is believed to have the following medicinal uses according to the information given by a traditional herbalist in Kano. (54) The medicinal uses include:-

(a) It is used as a laxative and antacid and for treatment of gastrointestinal disorders.

(b) For patients having pile, the drug is used to relieve the pain and irritation associated with the pile.

(c) A pleasantly alkaline drink is made by cold infusion of the material with sugar added and then added to a hot pap and stirred until it is the right consistency and is given to women who have just been put to bed in order to restore the blood they lost during labour.

(d) The material is also added to stews and soups in order to increase palatability or to mask sour test associated with the soup or stew.

2.3 PENNISETUM TYPHOIDEUM, Rich (MILLET)

Also known as African millet, ragi (India), Wimbi (Swahili), bulo (Uganda), telebum (Sudan), gero (Nigeria).

Millet is an important staple food in parts of East, Central and West Africa and India. It is the principal cereal grain in Northern Nigeria. The grain is ground into a flour and made into a stiff mush or porridge by adding to boiling water and stirred during heating until it is the right consistency. It is eaten with other foods when available, such as sweet potatoes, peas, beans, groundnuts and occasionally meat. The porridge is somewhat gritty and the use of mucilaginous pot herbs such as Corchorus olitorius L. help it to slip down.

Beer is made from fresh or stored grain, which is germinated dried, ground and mixed with water. Yeast is added, usually as cold beer and the mixture fermented for three days. Ground germinated sorghum may sometimes be added. The beer will not keep for more than 5 days before going sour.

Chemical composition: It was found that the average composition of the grain is as follows:- Water 13.0%, Protein 8%. Fat 1.3%, Carbohydrate 72%, Fibre 3%, Ash 2.7%. The protein content shows considerable variations ranging from 6-11%. The grain also is a rich source of

calcium, containing 0.33%, compared with 0.01 - 0.06% in many other cereals. It is also rich in phosphorous and iron (22).

2.4 ACETYLSALICYLIC ACID (ASPIRIN)

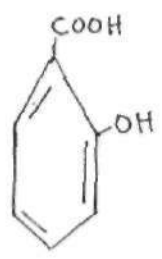
History and Source

Willow bark (Salix alba) the antipyretic property of which was known to the ancients, yields a bitter glycoside called salicin, first discovered by Levoux in 1827. On hydrolysis, salicin liberates glucose and salicylic alcohol (saligenin). Piria, in 1938, made salicylic acid from salicin. Six years later, salicylic acid was prepared from oil of gaultheria (oil of wintergreen) by Cahours. The synthetic manufacture of this acid from phenol was accomplished in 1860 by Kolbe and Lautemann. Sodium salicylate was first used as an antipyretic and for rheumatic fever by Buss in 1875, and in the following year its value in rheumatic fever and was discovered independently by Stricker and MacLagan. In 1879, See observed that salicylates increased the urinary excretion of uric acid, and this property was utilised in the treatment of gout by Campbell in 1879. Phenylsalicylate was introduced into medicine in 1886 by Nencki, and aspirin (acetylsalicylic acid) in 1899 by Dreser. The synthetic

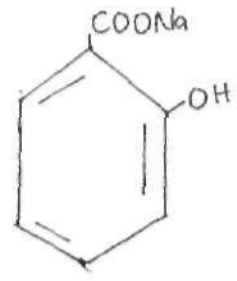
salicylates soon completely displaced the more expensive compounds obtained from natural sources.

Chemistry:

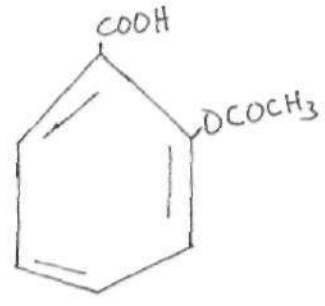
Salicylic acid (orthohydroxybenzoic acid) is so irritating that it can only be used externally, and, therefore, various derivatives of this acid have been synthesised for systemic use. These comprise two large classes, namely, esters of salicylic acid obtained by substitution in the carboxyl group, and salicylate esters of organic acids in which the carboxyl group of salicylic acid is retained and substitution is made in the OH group. For example, aspirin is an ester of acetic acid. In addition, there are salts of salicylic acid. The chemical relationships can be seen clearly from the structural formulas shown below:-



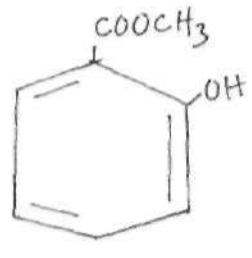
Salicylic acid



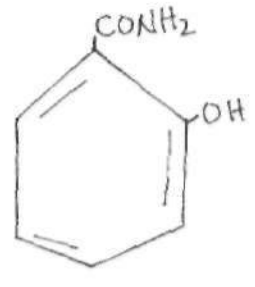
Sodium salicylate



Aspirin



Methyl-salicylate



Salicylamide

All the above compounds are referred to as "salicylates" or the "salicyl" drugs.

Structure-Activity Relationship

Salicylates act by virtue of their salicylic acid content. Substitutions on the carboxyl or hydroxyl groups serve only to change the potency or toxicity of the compound. The ortho position of the OH is an important feature for the action of salicylates. Benzoic acid, C_6H_5COOH, shares many of the actions of salicylic acid but as much weaker. Salicylamide has pharmacological properties similar to those of aspirin but is more potent with regard to central depression (23).

The efficacy of the different salicylate depends in part on the amount of salicylic anion liberated in the body. For example, the salicylic acid equivalents of the commonly used drugs in this group are within 10% of each other, and yet the therapeutic potency of aspirin and methylsalicylate is about 50% greater than that of sodium salicylate; this difference also holds true for their toxicities.

Physical Properties of Aspirin:

Aspirin powder is colourless or white crystals or white crystalline powder, odourless, soluble in

1 in 300 of water, 1 in 5 to 7 of alcohol, 1 in 17 of chloroform and 1 in 10 to 20 of ether.

Absorption and Fate

Absorption of non-ionised aspirin occurs in the stomach. Acetylsalicylates and salicylates are also readily absorbed from the intestine. Hydrolysis to salicylic acid occurs rapidly in the intestine and in circulation. Salicylates are extensively bound to plasma protein, aspirin to a lesser extent. Aspirin and salicylates are rapidly distributed to all body tissues; they appear in the milk and across the placenta. The rate of excretion of aspirin varies with pH of the urine, increasing as the pH rises and being greatest at pH 7.5 and above. Aspirin is excreted as salicylic acid and as glucoronide conjugated and as salicyluric acid, and gentisic acid (24).

The clinical importance of aspirin as a weak analgesic has led to numerous studies concerning the absorption of this drug in man. Several of these studies confirmed that, although aspirin is known to be rapidly hydrolysed to salicylic acid in vivo, intact drug is absorbed and blood levels of aspirin can be demonstrated up to 1 hr. following an oral dose of the drug. Using a specific assay, Rowland and Riegelman examined the kinetics of distribution and metabolism of aspirin following intravenous administration as its

N-methylglutamine salt (4). The decay was biexponential with half-lives of 4 and 15 min. for the initial and final phases, respectively. A two-compartment model was the minimum that could be proposed which fitted both the observed aspirin and resulting salicylic acid plasma levels data.

Saliva and blood concentrations of salicylic acid showed good correlation in 3 subjects given aspirin 650mg. Significant amount of salicylic acid appeared in the saliva 30 mins after administration (1). Measurement of salivary concentrations of salicylic acid would be of value in the evaluation of aspirin preparations.

In a study of 104 Caucasian subjects (50 male, 54 females), aspirin-esterase activity of the serum was significantly higher in males than in females. As aspirin-esterase activity in the serum was considered to be responsible for about 20% of the metabolism of aspirin, this could explain the higher incidence of side effects in women than in men (5).

Adverse Effects of Aspirin

The most common adverse effects occurring with therapeutic doses of aspirin are gastrointestinal disturbances such as nausea, dyspepsia and vomitting. Irritation of the gastric mucosa with erosion, ulceration, haematemesis and melaena may occur; slight

blood loss may occur in about 70% of patients with most aspirin preparations, whether buffered, soluble or plain. Some persons especially asthmatics, exhibit notable sensitivity to aspirin which may provoke various reactions including urticaria and other skin eruptions, angioneurotic oedema, rhinitis, and severe, even fatal, paroxysmal bronchospasm and dypsnoea. Aspirin increases the bleeding time, decreases platelet adhesiveness, and in large doses, may cause hypothrombinaemia (25).

Treatment of Adverse Effects

In acute salicylate overdosage, the stomach should be emptied by inducing emesis or by aspiration and lavage patients with mild intoxication should be encouraged to drink plenty of fluids. In patients with more severe intoxication, forced alkaline diuresis may be required and should be continued until the plasma concentration of salicylates is less than 350 ug/ml in adults, then i.v. fluids can be stopped and the patient encouraged to take fluid by mouth. Plasma electrolytes especially potassium, and the acid-base balance should be monitored regularly. Acidaemia should be corrected by infusion of sodium bicarbonate solution before starting forced diuresis.

2.5 ASSAY METHODS OF ASPIRIN

Various methods have been reported for quantifying aspirin and its metabolites in different biologic media. These include the standard colorimetric and fluorimetric methods and the more modern analysis by gas-liquid chromatography and high-performance liquid chromatography (HPLC). These methods have employed partition chromatography (26), (27), gas-liquid chromatography (28), liquid chromatography (29), high-performance liquid chromatophy (30), spectrophotometric (31), colorimetric (32) and fluorometric (33) procedures.

This project however reports a simple, rapid and accurate method for the direct determination of aspirin and salicylic acid by multiple component spectrophotometric analysis. The method involved the determination of the absorption spectra of the two compounds (aspirin and salicylic acid), which led to the selection of two analytical wavelengths in which absorption by the compounds is maximal at these wavelengths, with the absorptivity for one compound being greater than the other at one wavelength and less at the other.

The next step in the analysis was the making of Beer's law plots, using solution of the pure substance for each compound at each wavelength. This gave four Beer's law plots from which were calculated four

absorptivities (slope = ab, where a = absorptivity and b = internal path length of the sample cell) which may be symbolised a_1^{ASA}, a_1^{SA}, a_2^{ASA}, a_2^{SA}, with the superscrift indicating the compound and superscrift the wavelength.

Now any solution containing aspirin and salicylic acid can be analysed. The absorbance of the solution is measured at the two wavelengths. It will be assumed that the absorbance of a mixture is equal to the sum of the absorbances of the components of the mixture. Therefore, if A_1 and A_2 are the absorbances of the mixture at the two wavelength, then;

$$A_1 = A_1^{ASA} + A_1^{SA}$$

$$A_2 = A_2^{ASA} + A_2^{SA}$$

$$A_1 = a_1^{ASA} + bc^{ASA} + a_1^{SA} + bc^{SA}$$

$$A_2 = a_2^{ASA} + bc^{ASA} + a_2^{SA} + bc^{SA}$$

From the above equations, the concentrations of the components of the solution mixture can be determined.

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

Another method employed in this study for the direct determination of aspirin and its metabolites was high-performance liquid chromatography. The growth in the reliability and versatility of the method has

been reflected in the large number of papers published in this area in the past 3-4 years.

Of many HPLC methods which analyse various combinations of ASA and its metabolites in different biological fluids, majority of the methods employed are time-consuming solvent extraction and nitrogen-evaporation process (34 - 35). Others which analyse plasma following deproteinisation detect gentisic acid, salicyluric acid and salicylic acid and not aspirin. Only the method employed here (35) has measured aspirin and its metabolites in plasma without solvent extraction (35). The method improved separation and reduced sample analysis time to less than 10 minutes.

2.7 STANDARDISATION OF THE FOOD MATERIALS

Tamarindus indica (Tsamiya)

Tamarind pulp was extracted with distilled water by boiling in a water bath.

A measured amount of about 50gm of dried pulp was placed in a conical flask of a suitable size and 500ml of distilled water added enough to cover the pulp inside the flask. The conical flask was then placed on a water-bath containing boiling water and allowed to boil for about 45-50 minutes. The water extract which was light-brown in colour, pH 3.5, sour in taste

and acidic to litmus paper was then decanted from the flask and filtered through a sieve.

Then 50gm of ground millet was obtained and made into a stiff mush or porridge by adding to 500ml boiling water and stirred during heating until it was the right consistency. Then the tamarind extract was added to the mixture and stirred until the tamarind was evenly distributed in the mixture. pH of the mixture was 5.1.

After the preparation, each volunteer took about 200ml of the food drink prior to the study.

Sodium/Potassium Hausa Salt (Kanwa)

The salt was extracted with distilled water by cold infusion. A measured amount of about 10g of dried salt was placed in a conical flask and 100ml of water was added and left for about 15 to 30 minutes. The water extract was decanted from the flask.

Then 50gm of ground millet was obtained and made into a stiff mush or porridge by adding to 500ml boiling water and stirred during heating until it was the right consistency. Then the salt extract, which turned litmus paper blue, pH 9.5 was added to the mixture and stirred until the salt was evenly distributed in the mixture. pH of the mixture was 8.9.

After the preparation, each volunteer took about 200ml of the food drink prior to the study.

AIMS AND OBJECTIVES OF THE STUDY

The aim of the work is to study the effect, if any, of the concurrent administration of Tamarindus indica or sodium/potassium Hausa salt and aspirin on the bioavailability of aspirin. The reason for the selection of these two food materials is that they differ in both physical and chemical properties. Tamarindus indica is purely acidic, while the sodium/potassium salt is purely alkaline in nature. It is believed that the interaction of any of these two food materials with aspirin (which is acidic in nature), would have a great effect on the pharmacokinetics of the drug.

In general terms, according to the pH partition theory, the absorbability of drugs is favoured when they are present in unchanged form at the absorption site. Weakly acidic drugs will exist in the stomach in the undissociated form, and hence their absorption will be favoured from this acidic environment. Weakly basic drugs will exist at the same site as cations, hence charged and poorly absorbed.

It would then be assumed that if aspirin is concurrently administered with T. indica preparation, it would enhance its absorption because more of the drug would be in an uncharged form and hence be absorbed leading to increased activity of the drug.

On the other hand, if the drug is co-administered with sodium/potassium salt, more of the drug would exist at the site of absorption as cations, hence charged and poorly absorbed.

In this study, the food materials were used in the form of traditional Hausa food drink called KUNU. The one containing T. indica extract is called KUNUN TSAMIYA; while that containing sodium/potassium salt is called KUNUN KANWA in Hausa. The reason for choosing these two food materials is that they are commonly added to medicines and to all but a few foods especially among Hausas. And also among the analgesics commonly taken by Hausas and Fulanis, aspirin is among. Hence this study would enable us to know the effect if any on the concurrent administration of these two food materials with aspirin.

CHAPTER THREE

3.1.0 MATERIALS AND METHODS

3.1.1 DRUGS

1. Standard Aspirin Powder. Production Series No. P7205, manufacturers M & B Lab. Chemicals.
2. Standard Salicylic acid powder. Production Series No. 70529, manufacturers M & B Lab. Chemicals.
3. Polfa Aspirin tablets with batch No. 50790, manufacturers Polfa (Nig) Limited.
4. Vitalink Aspirin tablets batch No. 397, manufacturers Vitalink Pharm. Industries.
5. Standard Paracetamol powder as internal standard.

3.1.2 EQUIPMENT

- waters 204 HPLC Model equipped with 441 Model U.V. detector fitted with 254nm filter, Model U6K septum-less injector and SE 120 model recorder was used. The column used for the chromatographic separation was a u-Bondapak radial Pak catridge, Polyethylene 15cm x 8.0mm I.D. packed with 10 um reversed phase C_{18}-support, Waters Part No. 85721, (Waters Associates, Inc., Milford).

- Gallenkamp melting point apparatus.
- SP8-100 Ultraviolet Spectrophotometer (Pye-Unicam Ltd, York Street, Cambridge, England CB1 2PX).
- Camag. U.V. lamp type 2900.
- Erweka dissolution apparatus and accessories, Erweka Appatebau-G.M.B.H. 6056. Heusenstamm k.r., Offenbach-Main.
- Erweka disintegration apparatus and accessories, Erweka Appatebau-G.M.B.H. 6056 Heusenstamm Kr, Offenbach-Main.
- Autovortex-Whirlmixer (Gallenkamp, England).
- Mechanical shaker (Gallenkamp, England).
- Centrifuge (Gallenkamp, England).
- pH Meter (PW 2418, Philips, England).
- Vacuum pump and compressor, RBS, Edwards, Britain.
- Analytical balance, Type H35 AR Mettler Gallenkamp.
- Quickfit Conical centrifuge tubes 15ml.
- 100 ul Syringe (Hamilton Reno, Nevada).

3.1.3 SOLVENTS/REAGENTS

- Hydrochloric acid - Analar grade (BDH Chemicals, Poole, England).
- Sodium hydroxide pellets (BDH Chemicals, Poole, England).
- Chloroform - Analar grade (BDH Chemicals, Ltd).
- Methanol - GPR (Hoplein & William, Chadwell Heath, Essex, England).
- Phenolphthalein and methyl red indicators.

3.2 METHODS

3.2.1 Identification Tests (B.P. 1988)

3.2.2 Aspirin Powder (B.P. 1988)

0.2g of aspirin powder was dissolved in 4ml 2M sodium hydroxide and the mixture was heated to boiling for 3 minutes and cooled. 5ml of 1M H_2SO_4 was added and the precipitate formed was filtered. The melting point of the precipitate after washing and drying was determined.

3.2.3 Aspirin Tablets

A quantity of the powdered tablets containing 50g of aspirin was dispersed in 10ml of warm water. 0.5ml of iron(III) chloride test solution was added to the mixture. The colour was noted.

3.3 SOLVENT SYSTEM

In developing a suitable mobile phase for the analysis of aspirin and its metabolite(s) (salicylic acid) using the high-performance liquid chromatography with paracetamol as the internal standard, some solvent combinations were tried. The constituents of the mobile phase were premixed, filtered under vacuum and ultrasonicated before use. The pH of the mobile phase was also noted.

The mobile phases tried consisted of either methanol and water or methanol and 5% acetic acid or acetonitrile and acetic acid in different ratios.

The effect of pH on the capacity factor of the mobile phases was also investigated to obtain the best separation of the drugs and internal standard (see results).

3.4 ASSAY OF ASPIRIN POWDER (B.P. 1988)

1gm of aspirin powder was dissolved in 10ml of ethanol (96%), then 50ml of 0.5M NaOH V.S. was added and the flask stoppered and allowed to stand for 1 hr. 0.2ml of dilute phenolphthalein solution was added and titrated with 0.5M HCl V.S. The operation was repeated without the substance being examined and the volume of 0.5M HCl V.S. used was also noted. The difference between the titrations represented the amount of 0.5M NaOH V.S. required. Each millilitre of 0.5M NaOH is equivalent to 0.045 of aspirin.

3.5 ASSAY OF SALICYLIC ACID POWDER (B.P. 1988)

0.12g of salicylic acid powder was dissolved in 30ml ethanol (96%). 20ml of water was added and titrated with 0.1M NaOH V.S.; using phenol red solution as indicator, until a reddish-violet colour was obtained. Each millilitre of 0.1M NaOH is equivalent to 0.01381g of salicylic acid.

3.6 ASSAY OF ASPIRIN TABLETS (B.P. 1988)

20 tablets (each of Polfa and Vitalink brands) were weighed and powdered. To a quantity of the powder containing 0.5g of aspirin was added 30ml of 0.5M NaOH V.S. The mixture was boiled gently for 10 minutes and then the excess alkali was titrated with 0.5M HCl V.S. using phenol red solution as indicator. The operation was repeated without the substance being examined. The difference between the titrations represented the amount of 0.5M NaOH V.S. required. Each millilitre of 0.5M NaOH V.S. is equivalent to 0.0450g of aspirin.

3.7.1 Dissolution Rate Test

The USP XIX basket method was described for the dissolution rate test. The apparatus consisted of a cylindrical, flat bottom glass vessel with an internal diameter of 100mm, a flagged upper rim, and a capacity of 1000ml; a variable speed motor; a cylindrical stainless steel basket assembly made of woven wire cloth, with a normal aperture size of 425 um; joined down one side with a narrow welded seam and with a flanged rim at each end. The variable speed motor causes the basket to rotate in the vessel. A speed of 100 revolutions per minute was used. The motor drives the basket by means of a shaft which is approximately 6mm in diameter and about 300mm long

in such a way that the basket revolves smoothly and without perceptible wobble.

The shaft passes through the vessel and opening in the lid of the vessel and is positioned so that its axis is not more than 2mm from the vertical axis of the vessel. It carries at its lower end a stainless steel disc of 25mm in diameter in which there is a 2mm vent.

The vessel was securely clamped in a water bath in such a way that displacement vibration from other equipment including water circulating device is minimised. The water bath was set to maintain the temperature of the dissolution medium in the glass at $37 \pm 0.5^{\circ}C$.

Procedure

One litre of 0.1M HCl, as the dissolution medium, was introduced into the vessel and immersed in a constant temperature water bath, maintained at $37 \pm 0.5^{\circ}C$. For each test, one tablet was placed in the basket. When the required temperature was attained, the apparatus was assembled and the distance between the bottom of the basket and the internal surface of the vessel adjusted to between 18 to 22mm. The motor was started at a rotational speed of 100 revolutions per minute. A sample of 10ml of the dissolution medium was withdrawn

at 45 minutes. Sample was withdrawn from a point half way between the basket wall and the wall of the vessel, level with the mid-point of the side of the basket to ensure that the normal pattern of flow of the dissolution medium was not disturbed.

The dissolution run was performed five times. Samples of the dissolved drugs were filtered, diluted to 100ml and assayed by reference to a standard calibration curve for aspirin. The absorbance of each diluted sample was measured at a maximum wavelength of 276nm.

3.7.2. Calibration Graph

A stock solution of standard aspirin of 1mg/ml was prepared in 0.1M HCl out of which the absorbance of each of a series of concentrations ranging from 1 to 50 ug/ml was measured. The absorbance was determined at 276nm using 0.1M HCl as blank. The graph of the absorbance versus concentration was plotted.

3.7.3 Disintegration Test

Apparatus:

As described in B.P. 1988. See appendix I.

Procedure

Five tablets each of Polfa and Vitalink brand of aspirin were placed in the tubes of the basket; a disc was added to each tube. The assembly was suspended in the beaker containing distilled water maintained at 37 $\pm$ 0.5^{o}C and the apparatus was operated for 15 minutes.

3.8 INVIVO ABSORPTION STUDIES

3.8.1 Simultaneous Determination of Aspirin and Salicylic Acid in Plasma and Saliva Using Multiple Component Spectrophotometric Method of Analysis

This was done by determining the absorption spectra of the two pure compounds (standard aspirin and salicylic acid solutions). This led to the selection of two analytical wavelengths in which absorption by the compounds is maximal at these wavelengths, with the absorptivity for one compound being greater than the other at one wavelength and less at the other.

The next step in the analysis was the making of Beer's law plots, using solutions of the pure substances for each compound at each wavelength. This gave four Beer's law plots from which are calculated for absorptivities (Slope = ab, where a = absorptivity, and b = internal path length of the sample cell) which may be symbolished a_1^{ASA}, a_2^{SA}, a_1^{SA}, a_2^{ASA} with the

superscrift indicating the compound and subscrift the wavelength.

Any solution containing aspirin and salicylic acid can be analysed by measuring the absorbance of the solution at the two wavelengths. Assuming that the absorbance of a mixture is equal to the sum of the absorbances of the components of the mixture, it follows that if A_1 and A_2 are the absorbances of the mixture at the two wavelengths, then;

$$A_1 = A_1^{ASA} + A_1^{SA}$$

$$A_2 = A_2^{ASA} + A_2^{SA}$$

$$A_1 = a_1^{ASA}\ bc^{ASA} + a_1^{SA}\ bc^{SA}$$

$$A_2 = a_2^{ASA}\ bc^{ASA} + a_2^{SA}\ bc^{SA}$$

From the above equations, the concentrations of the components of the solution mixture can be analysed. However, this method does not discriminate other metabolites of aspirin which might interfere with the absorbance of the two solution mixture.

3.8.2 Calibration Graphs (Beer's law plots)

The standard solutions were prepared in water, plasma and saliva as follows:-

(i) Water (0.1M HCl)

For each of the salicylates (ASA and SA), 50mg were accurately weighed and dissolved in 100ml methanol to yield the stock standard solution (500 ug/ml). This stock solution was further diluted to give working standards ranging from 1 to 30 ug/ml in 0.1M HCl. Each of these concentrations were run in the U.V. spectrophotometer and the absorbances noted at 276nm and 231nm respectively. A graph of concentration against absorbance at each wavelength was plotted.

(ii) Plasma

1ml of the working standard solutions ranging from 1 to 30 ug/ml were added to 1ml plasma. Each of these concentrations after extraction were run in the U.V. spectrophotometer and the absorbances noted at 276nm and 231nm respectively. A graph of concentration against absorbance at each wavelength was plotted.

(iii) Saliva

Similarly, 1ml of the working standard solutions were added to 1ml saliva to provide standards ranging from 1 to 30 ug/ml of the drugs in the saliva. Each of these concentrations after extraction were run in the U.V. spectrophotometer and the absorbances noted noted at 276nm and 231nm respectively. A graph of concentration against absorbace at each wavelength was plotted.

3.8.3 Extraction Procedure (U.V)

The extraction procedure for both saliva and plasma is the same. Saliva (1ml) spiked with 1ml working standards was mixed with a 2ml aliquot of 0.05M HCl in a glass-stoppered tube. After the addition of 10ml ethylacetate, the drugs were extracted by shaking the tubes gently for 15 minutes on a mechanical shaker, followed by centrifugation for 15 minutes at 5000g. Exactly 5ml of the supernatant were transferred into a glass tube which was placed in a water bath at 40°C and the solvent was evaporated under a gentle stream of oxygen-free nitrogen. The residue was reconstituted in 5ml methanol. The absorbance of the clear solution was measured at 231nm and 276nm respectively for each of the solutions. A graph of absorbance against concentration was then plotted for each wavelength. The levels of aspirin and salicylic acid were derived from these graphs.

The above extraction was repeated with 10 and 20 ug/ml of aspirin and salicylic acid without plasma or saliva and percentage extraction recoveries of aspirin and salicylic acid at 231nm and 276nm calculated using the relationship:

$$\frac{\text{Amount recovered after plasma extraction}}{\text{Amount recovered after extraction in 0.1M HCl}} \times \frac{100}{1}$$

3.8.4 Simultaneous Determination of Aspirin and Salicylic Acid in Plasma and Saliva Using High-Performance Liquid Chromatography

The chromatographic conditions for separation of the drug and its metabolites were set as follows: mobile phase, methanol : 5% acetic acid (25:75), pH 3.45, flow rate 1.5ml/min, recorder chart speed, 0.5cm/min; detection wavelength 280nm; injection volume, 15ul; sensitivity 0.02 aufs. Under the described chromatographic conditions, the mean retention times were 3.0, 5.0 and 7.0 minutes for paracetamol (internal standard), salicylic acid and aspirin respectively.

3.8.5 Preparation of Standard Solutions and Calibration Graphs

Working solutions of appropriate concentrations were prepared every week by dilutions of the stock solutions with methanol.

Plasma calibration graph was prepared by spiking blank plasma sample (200 ul) with working standards ranging from 1 to 120 ug/ml for salicylic acid; 1 to 80 ug/ml for aspirin and 20 ug/ml for paracetamol as internal standard. The samples were processed as described below in the extraction procedure for HPLC.

Standard graphs were constructed by plotting the peak height ratio of aspirin to the internal standard against the drug concentration in each

standard; and also peak ratio of salicylic acid to the internal standard against the drug concentration in each standard. The level of drug in an unknown sample (plasma) was derived from these graphs.

3.8.6 Extraction Procedure for HPLC

Aliquots of plasma (200 ul) were mixed with 20 ul of a 30% perchloric acid solution containing the internal standard paracetamol (30 ug/ml) in 1.5ml polypropylene Eppendorf micro-test tubes; followed by a pinch of sodium chloride salt and 200 ul of methanol. The mixture was then vortexed for 2 mins and centrifuged for 4 mins. A 20 ul sample of the clear supernatant was injected into the chromatogram.

The above extraction procedure was repeated with 40, 60, 80 ug/ml aspirin and salicylic acid concentrations and 20, 40 and 60 ug/ml of paracetamol without plasma and percentage extraction recoveries of aspirin, salicylic acid and paracetamol calculated using the relationship:

$$\frac{\text{Peak height ratio of plasma aspirin extraction}}{\text{Peak height ratio of aspirin extraction}} \times \frac{100}{1}$$

3.8.7 STUDY CONDITIONS

(a) Fasting State (Drug alone)

Six normal healthy adult volunteers (all males) of 18 to 24 years old took part in the study. The protocol of the study was explained to each of them and an informed concent was obtained. No drugs including alcohol was allowed a week to the study.

On the day of each study, an indwelling cannula with a heparin lock was inserted into the antecubical vein of each volunteer. Basal measurements were performed after a minimal period of stabilisation of 15 mins on a couch. Each volunteer was given two tablets containing 600mg of aspirin with 100ml of water after overnight fasting. They remained on the couch and continued to fast for a period of 6h. Blood samples (5ml) were withdrawn prior to the dose and at 0.5, 1, 2, 3, 4, 5 and 6h after the dose. The samples were collected in chilled heparinised tubes, centrifuged for 10 mins and immediately kept at -20^{o}C until analysis. Salivary samples (5ml) were also collected after stimulation with a rubber band at the same times as the plasma was collected. The saliva samples were collected in chilled plastic tubes, centrifuged for 10 mins and stored at -20^{o}C until assayed.

(b) Drug with Starch

After two weeks of wash out period, the same volunteers each took two tablets containing 600mg aspirin together with 200ml starchy portion of the food drink. Blood and saliva samples were taken at 0.5, 1, 2, 3, 4, 5 and 6 hr intervals respectively as stated above.

(c) Drug with Tamarindus indica

A wash out period of two weeks was allowed before the same volunteers took each two tablets of 600mg aspirin together with 200ml of the food drink containing T. indica extract (Tsamiya) after overnight fasting. Blood and saliva samples were then taken at 0.5, 1, 2, 3, 4, 5 and 6 hr. intervals.

(d) Drug with Sodium/Potassium Salt

After another two weeks of wash out period, the same volunteers each took two tablets containing 600mg aspirin together with 200ml of the food drink containing sodium/potassium salt extract (KANWA) and the blood and saliva samples were taken at 0.5, 1, 2, 3, 4, 5 and 6 hr intervals.

3.9.8 Results

The results of the various studies were carefully recorded. Tables were drawn where appropriate as were graphical representations. The data were statistically analysed where appropriate. Student's paired t-test was used. Probability values greater than 0.05 was considered to be statistically significant.

CHAPTER FOUR

RESULTS

4.1 IDENTIFICATION TESTS (B.P. 1988)

4.1.1 Aspirin Powder

The melting point of the dried precipitate was 159° which was within the range of 156° to 161° provided by B.P. 1988.

4.1.2 Aspirin Tablets

A violet red colour was produced indicating that aspirin was present in all the brands of the tablets used.

4.2 SOLVENT SYSTEM

The solvent combination tried in the development of the mobile phase used for this work are given in Table 4. From the table, it can be seen that the solvent system - methanol : 5% acetic acid (25:75) with pH 3.45 gave the highest resolution or capacity factor which is a measure of chromatographic separation. The time for analysis for the solvent system was less than 10 min . Hence, this solvent system was chosen for the work since it has the highest value of R, better peaks symmetry and no baseline depression as observed in the

48

Table 4: Chromatographic parameters for aspirin, salicylic acid and paracetamol (internal standard) under different conditions using u-Bonda-Pak C_{18} radial-pak catridge (Waters Associates).

Solvent Combinations and Ratio	ph	Flow Rate (ml/min)	Retention Times (Min)			Capacity Factor
			ASA	SA	PCM	
Methanol : H_2O (40:60)	3.2	1.5	11.1	8.4	7.0	1.14
Methanol : H_2O (10:90)	3.4	1.0	9.2	7.0	6.1	2.0
Methanol : 5% Acetic Acid (25:75)	3.0	1.0	9.0	5.1	3.0	2.13
Methanol : H_2O (40:60)	4.5	1.0	12.2	11.0	9.7	1.50
Methanol : 5% Acetic Acid (25:75)	3.0	1.8	12.0	10.2	8.0	1.33
Methanol : 5% Acetic Acid (25:75)	3.45	1.8	7.0	5.0	3.0	2.75
Acetonitrile : 5% Acetic Acid (30:70)	3.0	1.0	11.2	9.0	7.4	1.25

Table 5: Effect of pH on the capacity factor (R) and resolution (d) for aspirin, salicylic acid and paracetamol (internal standard) using solvent system methanol : 5% Acetic acid (25:75) and u-Bonda-Pak C_{18} radial-Pak catridge (Waters Associates)

pH of Mobile Phase	Capacity Factor (R)	Resolution (d)
3.0	2.13	1.62
3.2	2.48	1.71
3.4	2.74	2.43
3.6	2.21	1.92
3.8	1.50	1.87
4.0	1.33	1.20
4.2	1.24	1.31
4.4	1.13	1.02

chromatogram using other solvent combinations. Table 5 and Figure 18 show the effect of pH on the value of R (capacity factor). The result indicated that pH 3.45 was found to be most suitable for the solvent system. This is also within tolerable range for the life span of the column used (pH 3.4 - 6.5).

4.3 Chemical Assay (B.P. 1988) of Aspirin and Salicylic Acid Powders and Tablets

The results obtained in all the preparations investigated contained between 99.15 and 101.0 percent of the active ingredient. Thus, the aspirin and salicylic acid powders and the different brands of aspirin tablets employed in this study have complied with the B.P. 1988 specification of drug content (95.0 - 105.0%). Table 1 shows the results obtained from all the assays.

4.4.1 Dissolution Rate Tests

Linear calibration graph with good correlation coefficient (r = 0.99989) of aspirin in 0.1M hydrochloric acid using U.V. spectrophotometer is shown in Figure 2. The dissolution rate profile of the two brands of aspirin tablets in 0.1M hydrochloric acid is shown in Table 2.

From the results obtained, over 70% of the active ingredient was released from all the brands in 45 mins.

Table 1: Percentage content of the active ingredient in each drug from the chemical assay (BP 1988)

Drug	% Drug Content
Aspirin powder	99.90
Salicylic acid powder	104.50
Vitalink aspirin tablets	100.30
Polfa aspirin tablets	99.45

Table 2: Dissolution rate profile of Polfa and Vitalink aspirin tablets in 0.1M hydrochloric acid.

Drug	% of Drug Released at 45 minutes
Polfa aspirin tablets	100.0
Vitalink aspirin tablets	84.4

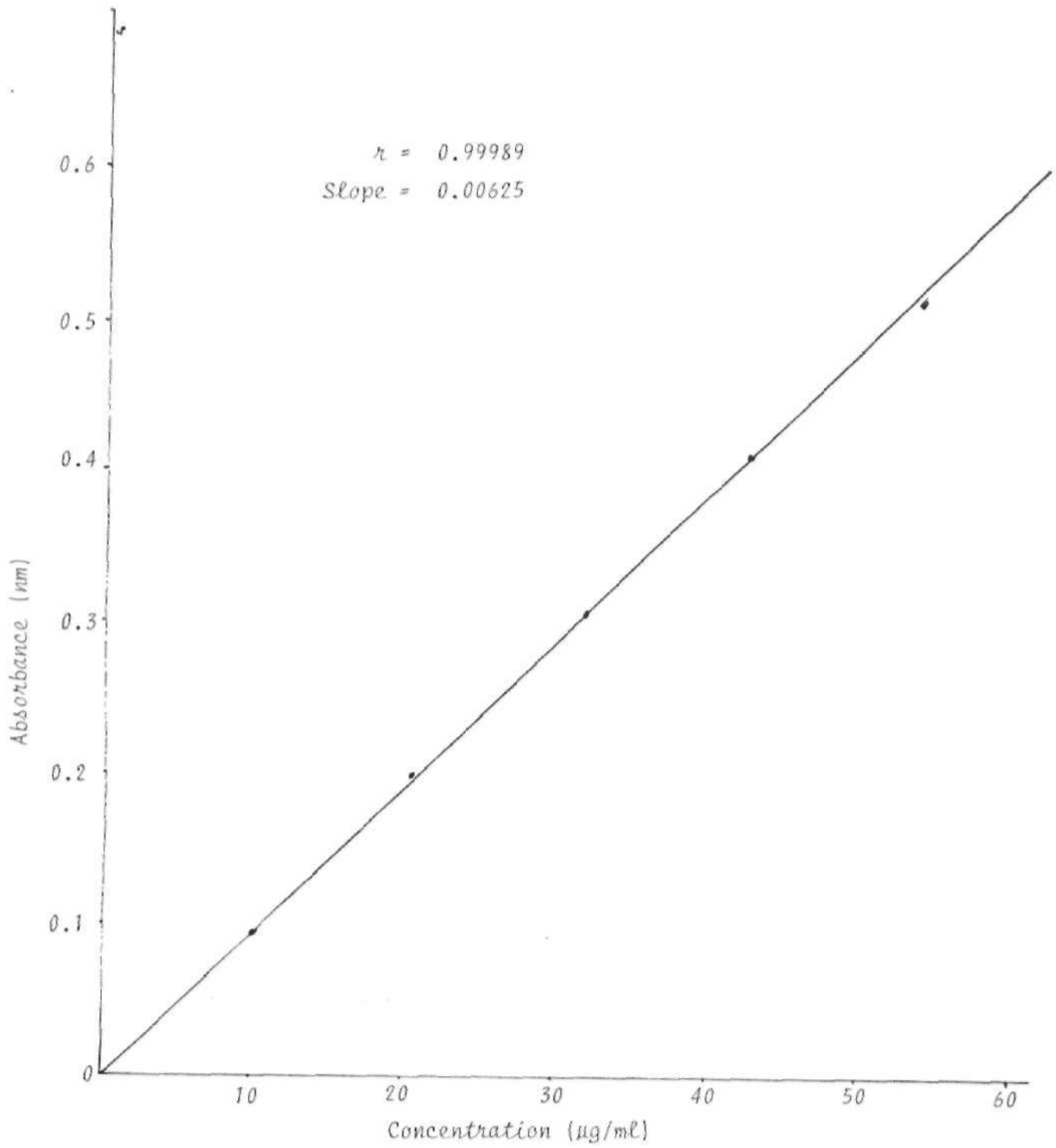


Fig. 2: Calibration graph for standard aspirin in 0.1M HCℓ at 276nm.

Table 3: Disintegration times of Polfa and Vitalink aspirin tablets in water maintained at 37 ± 0.5°C.

Tablet No.	Disintegration Times (Min)	
	Polfa Tablets	Vitalink Tablets
1	3.0	2.5
2	2.5	3.0
3	2.5	3.0
4	3.0	3.5
5	3.5	3.5
6	3.0	3.5
Mean ± S.D.	2.92 ± 0.42	3.17 + 0.98

S.D. = Standard Deviation

4.4.2 Disintegration Time Test

The results obtained for the disintegration of the two brands of aspirin tablets in water maintained at 37 ± 0.5°C are shown in Table 3.

4.5 INVIVO ABSORPTION STUDIES

Linear calibration graphs with good correlation coefficient using U.V. spectrophotometry (r = 0.9998) and 0.9948); and HPLC (r = 0.9975 and 0.99748) for both aspirin and salicylic acid in plasma and saliva are shown in Figures 3 to 14 and 15 to 16 respectively. Figure 17 shows HPLC chromatogram of the extracted plasma sample at the time of maximum concentration which gave peaks corresponding to retention times of reference aspirin (7.0), salicylic acid (5.0) and paracetamol (3.0) (internal standard). These peaks were absent from the blank plasma extract which was collected before the ingestion of aspirin tablets.

Drug Alone

Following oral administration of 600mg aspirin tablets, mean peak plasma concentrations (C_{max}) ranged from 40 to 65 ug/ml for salicylic acid and 10 to 15 ug/ml for aspirin respectively - Figure 18. While the mean salivary concentrations ranged from 10 to 14 ug/ml

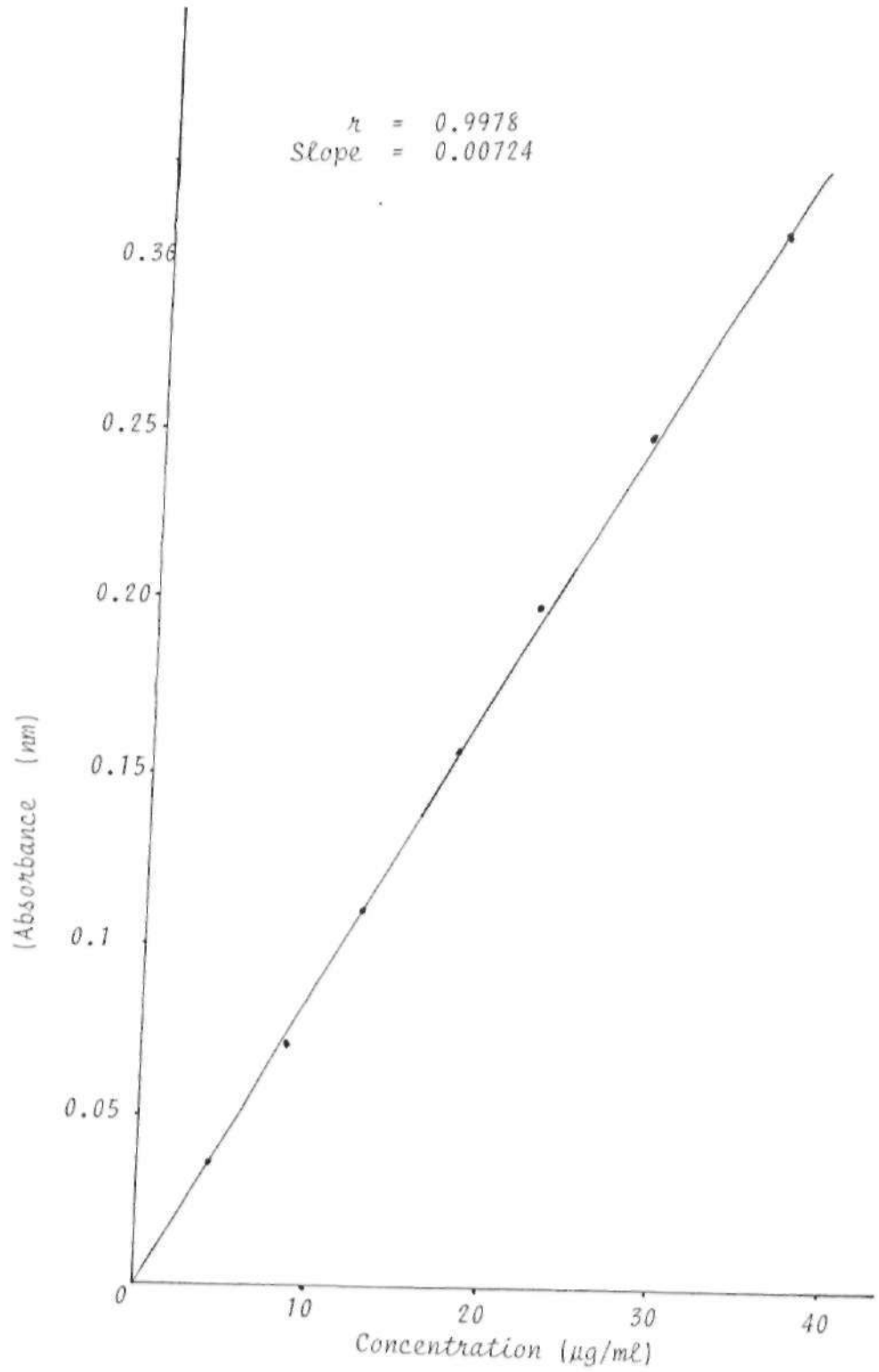


Fig. 3: Calibration graph for standard aspirin in 0.1M HCℓ after extraction at 276nm.

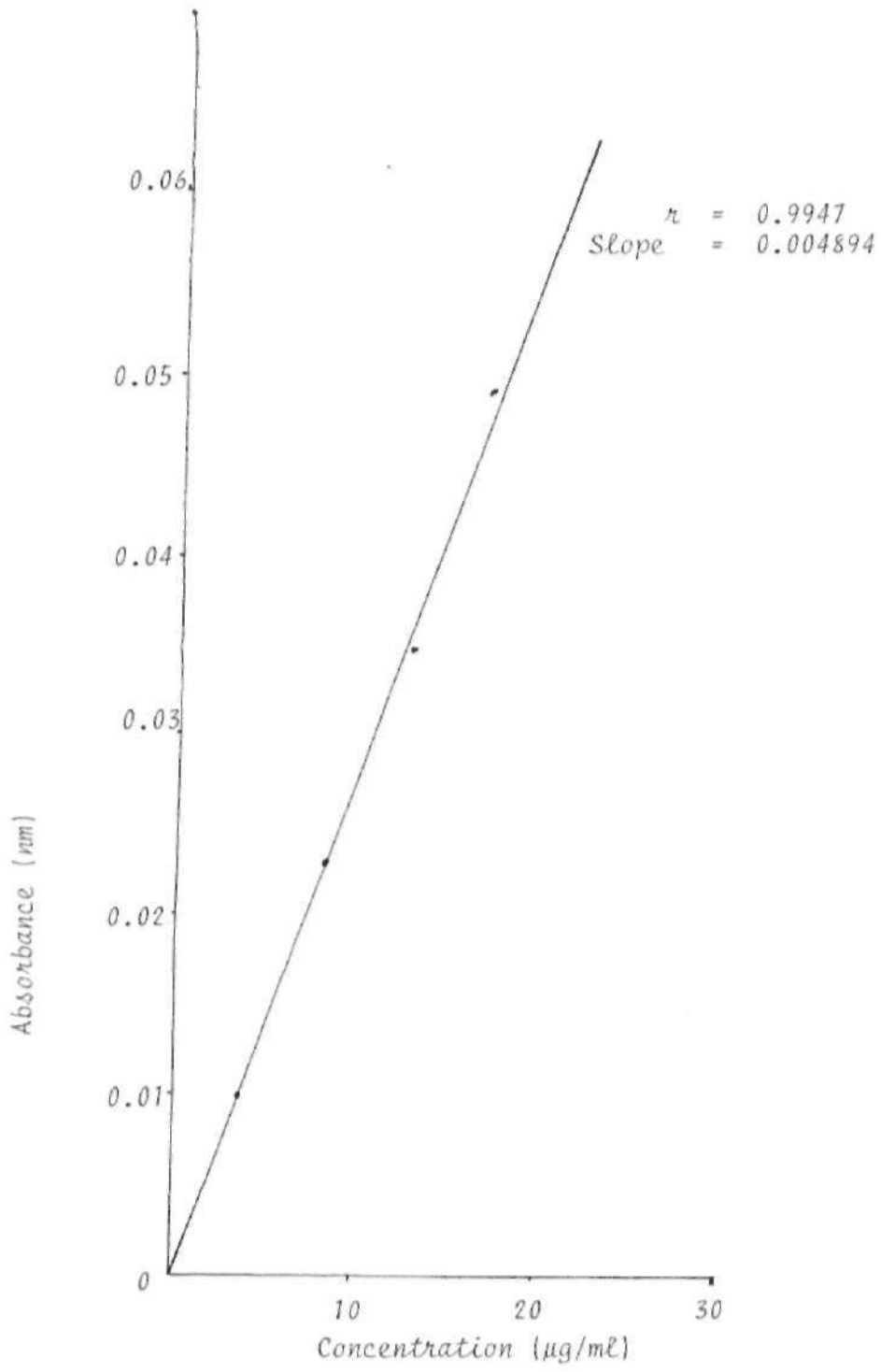


Fig. 4: Calibration graph for standard aspirin in 0.1M HCℓ after extraction at 231nm.

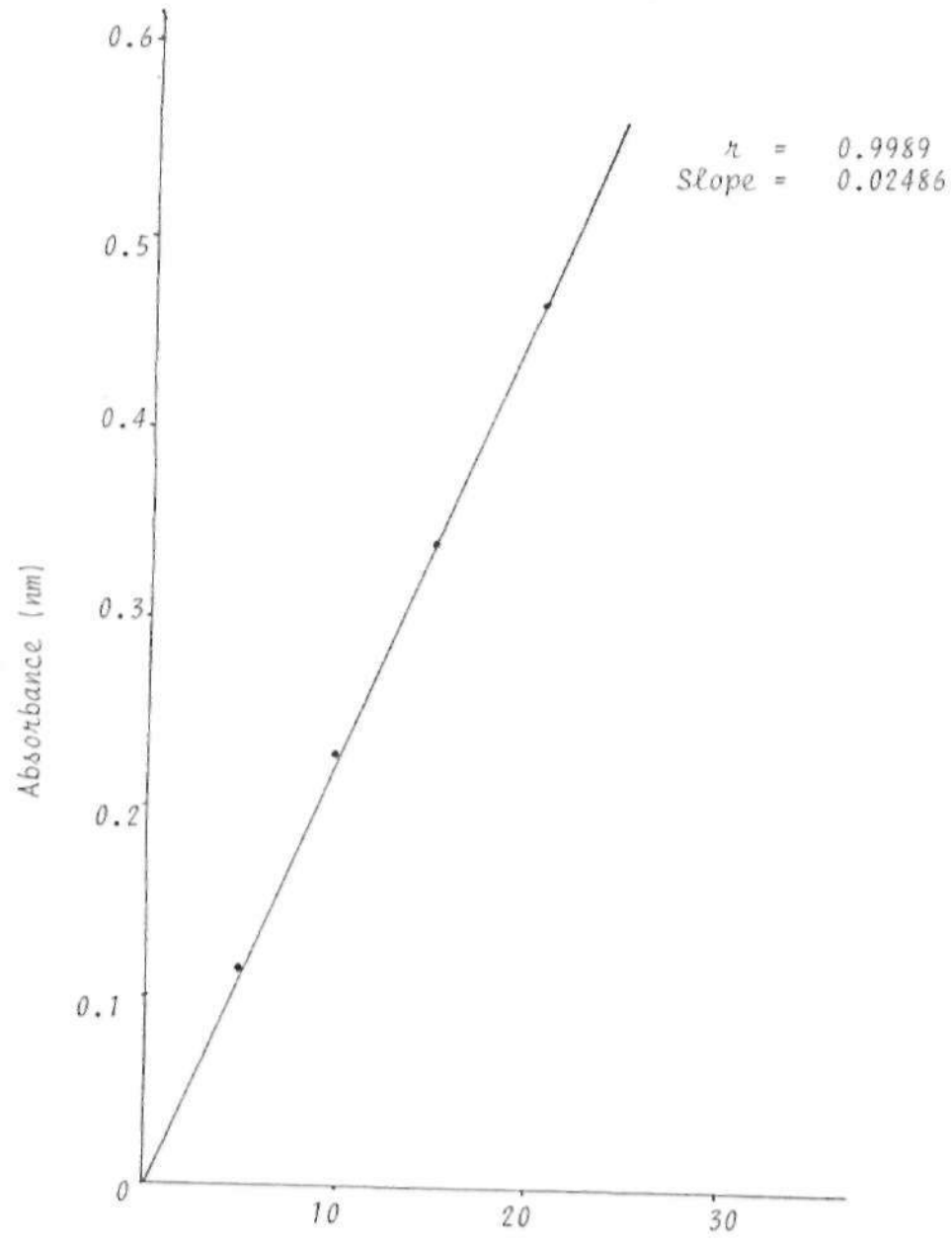


Fig. 5: Calibration graph for standard salicylic acid in 0.1M HCl after extraction at 276nm.

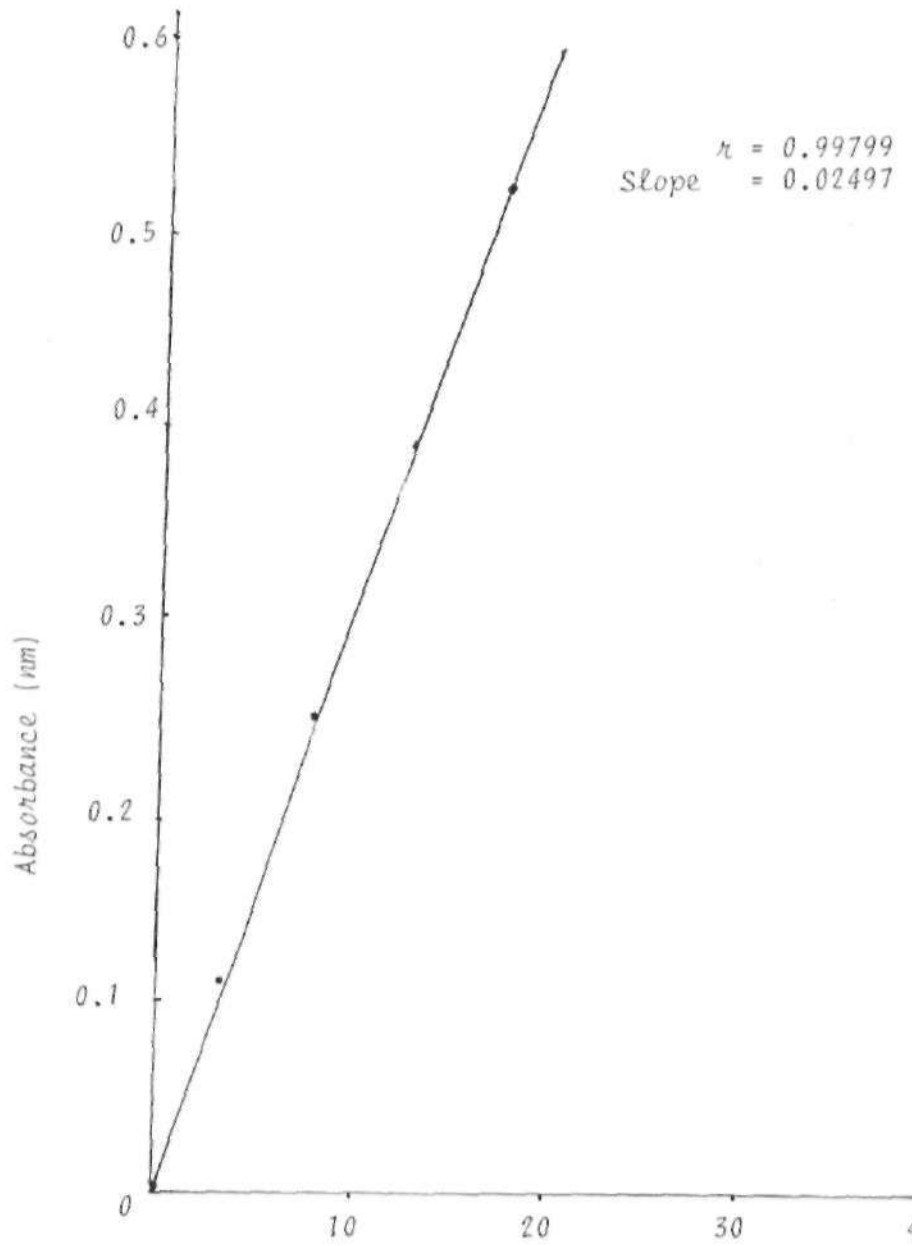


Fig. 6: Calibration graph for standard salicylic acid in 0.1M HCl after extraction at 231nm.

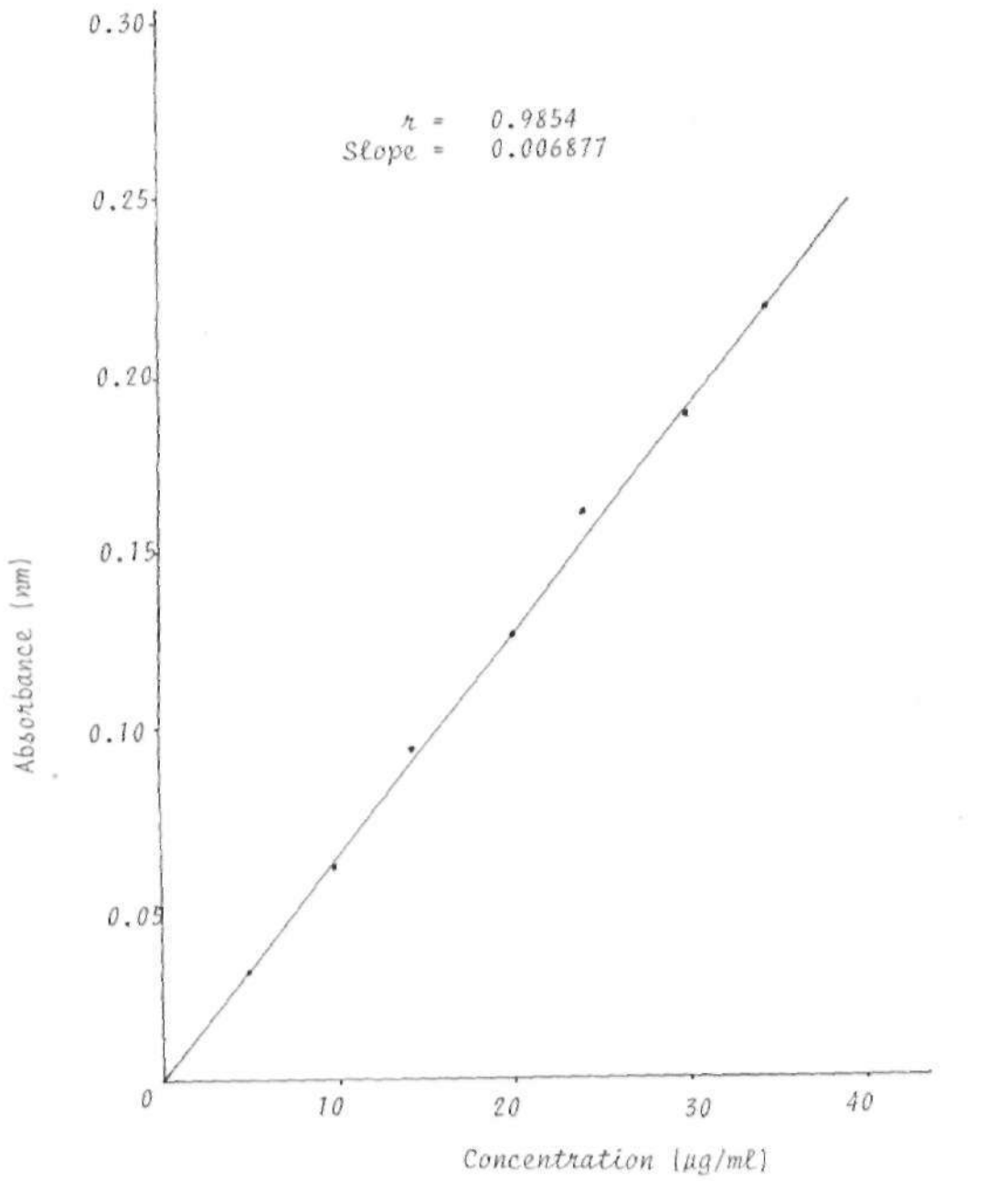


Fig.78: Calibration graph for the plasma concentration of standard aspirin at nm after extraction pH 3.9.

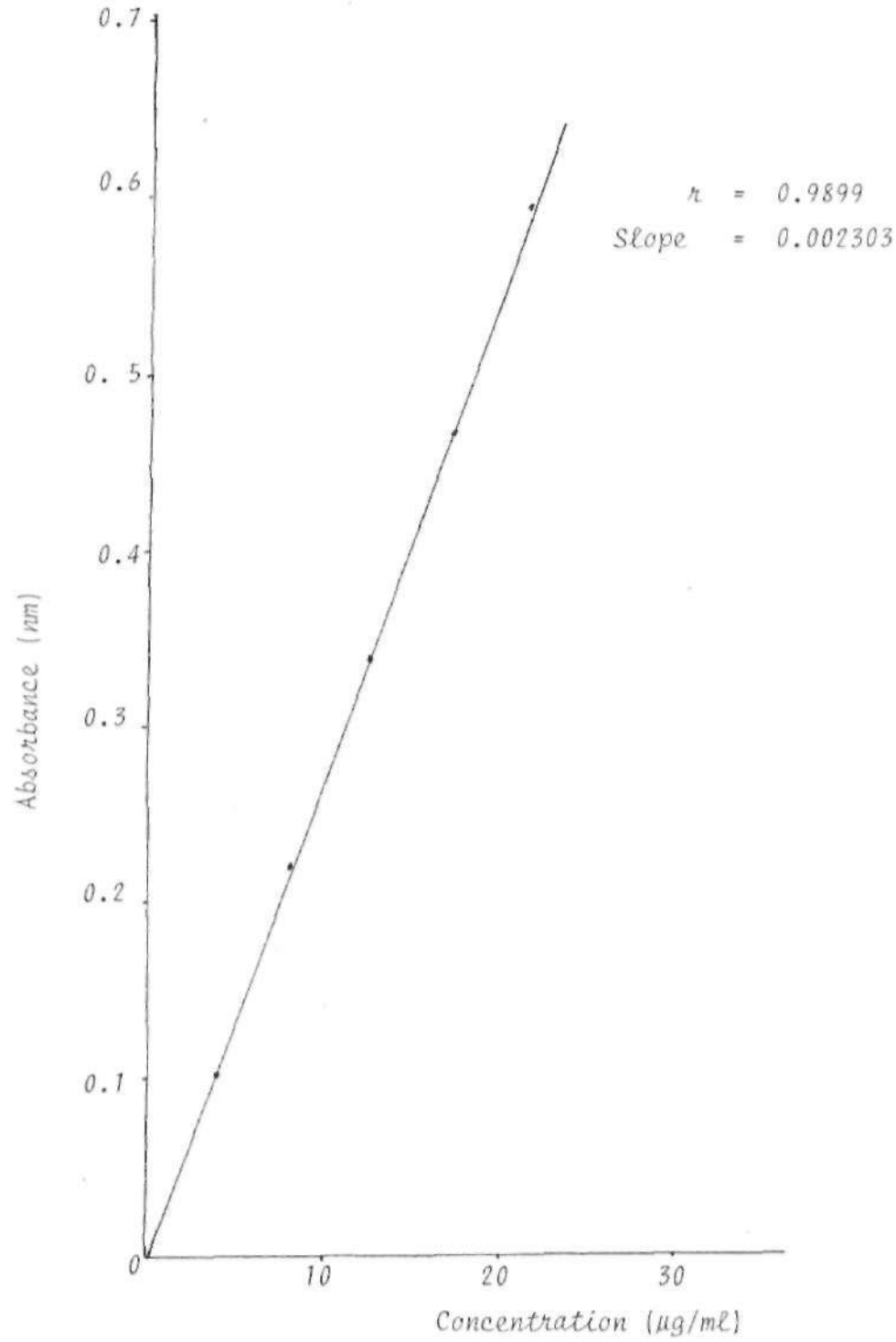


Fig. 8: Calibration graph for the plasma concentration of standard aspirin at 231nm after extraction pH 3.9.

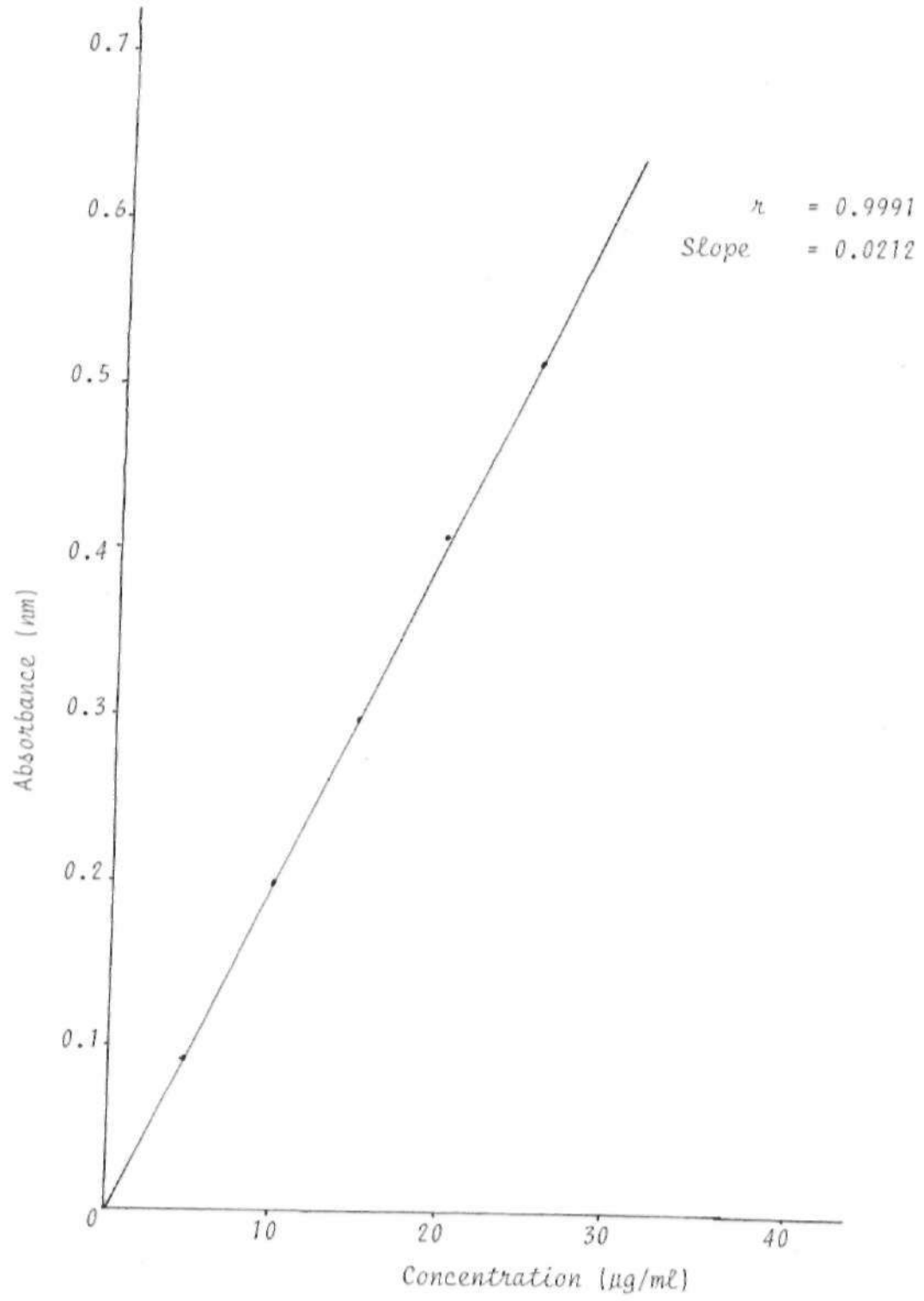


Fig. 9: Calibration graph for the plasma concentration of standard salicylic acid at 276nm after extraction pH 3.9

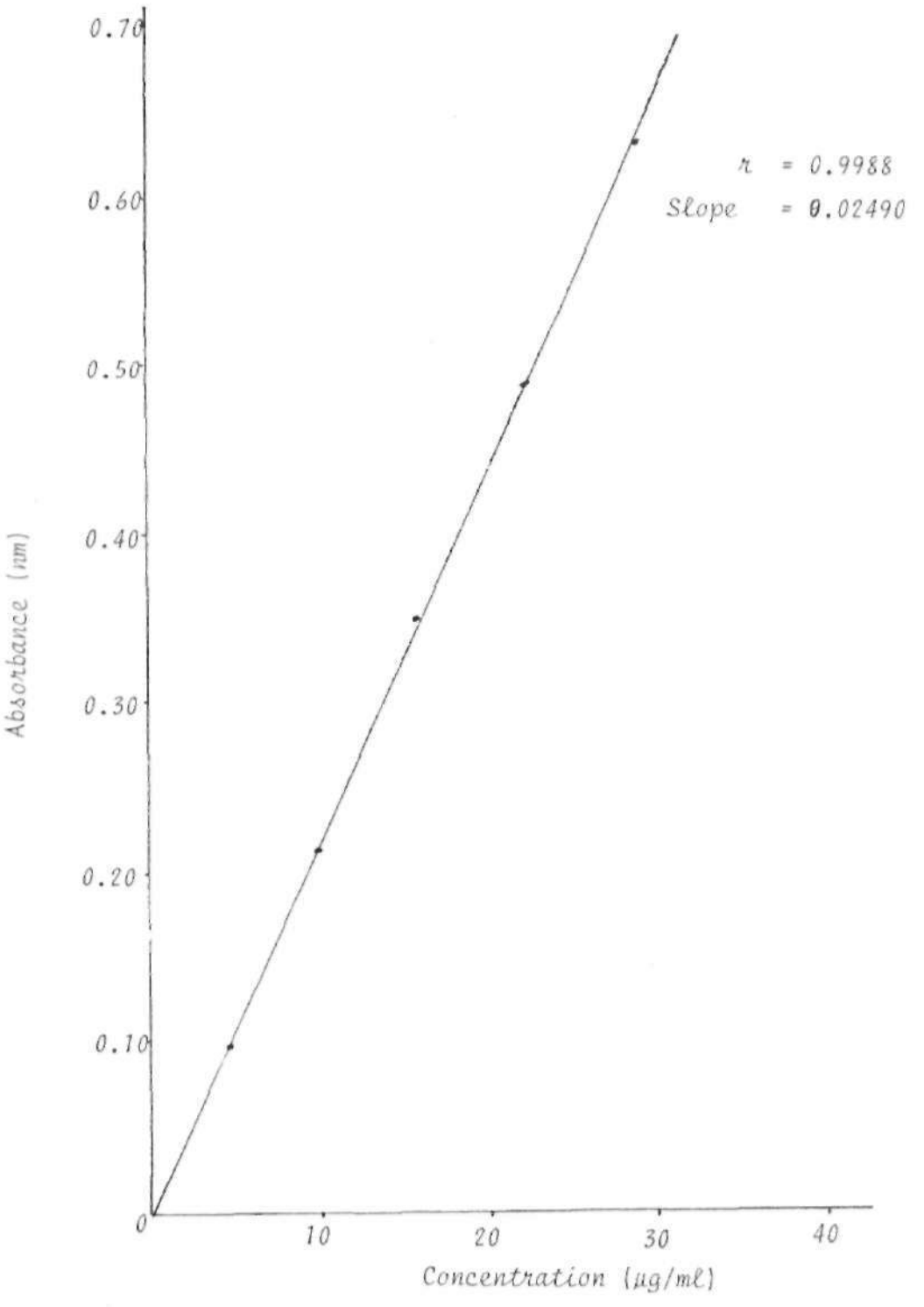


Fig. 10: Calibration graph for the plasma concentration of standard salicylic acid at 231nm after extraction pH 3.9

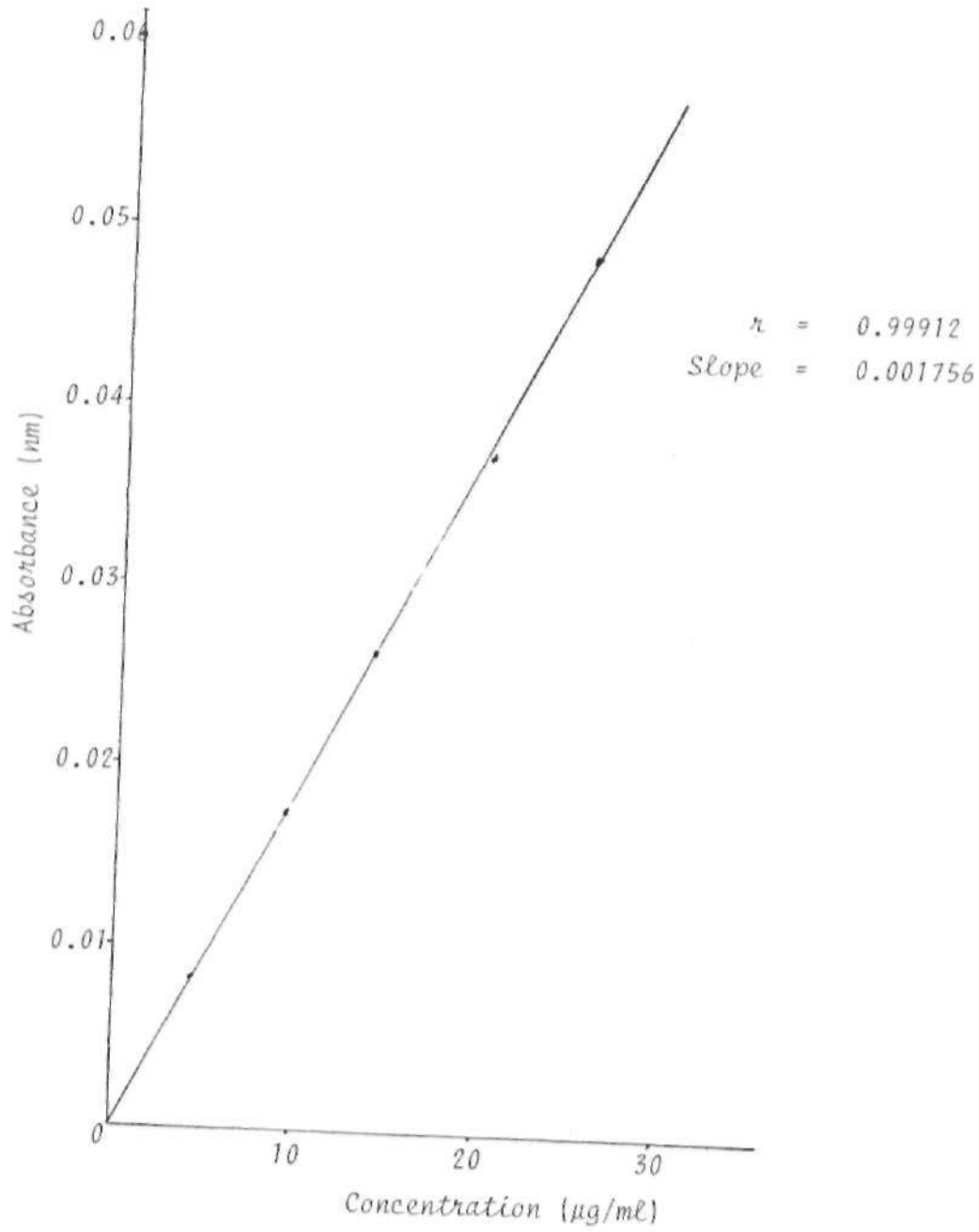


Fig. 11: Calibration graph for the salivary concentration of standard aspirin after extraction at 231nm and pH 3.9.

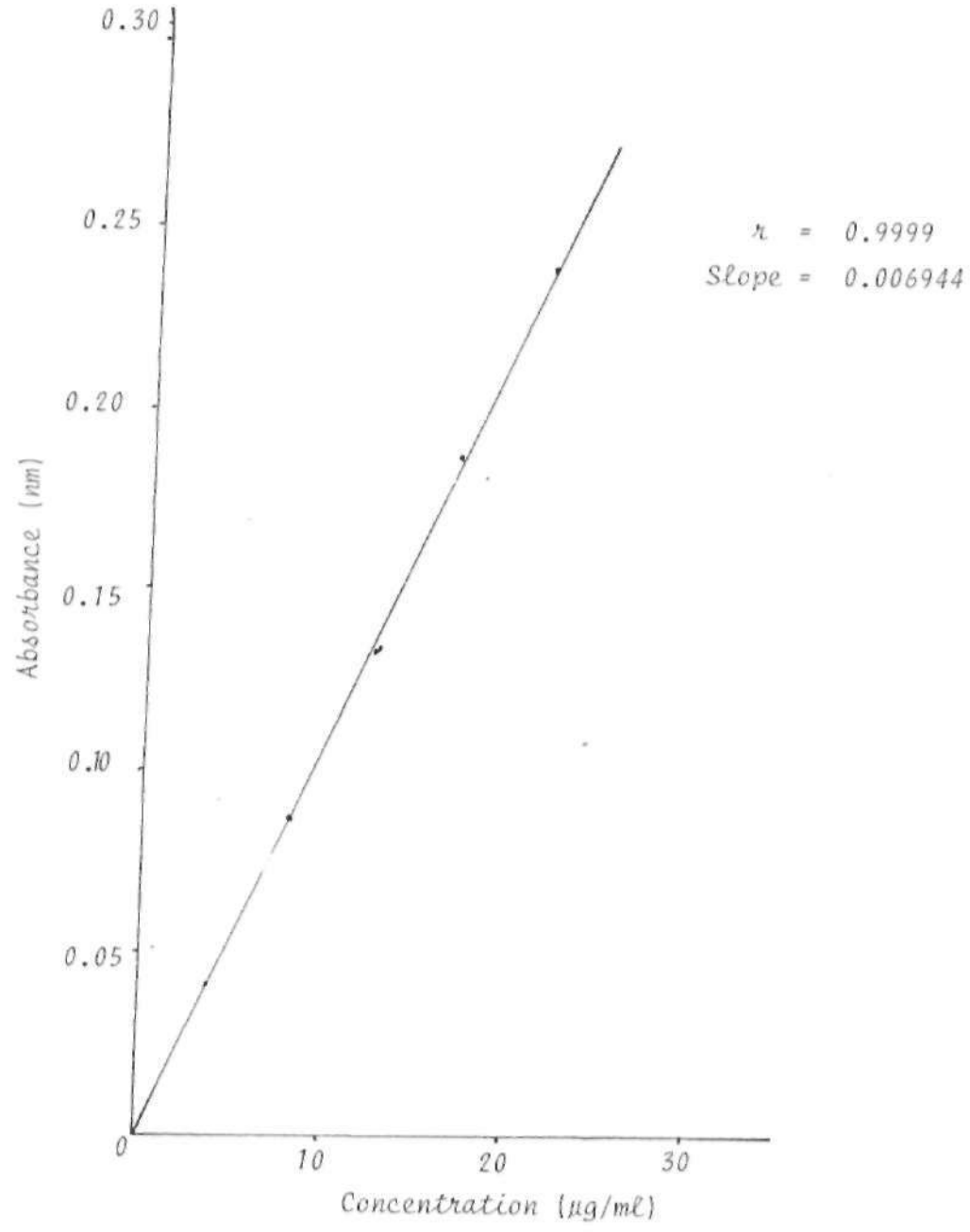


Fig.12: Calibration graph for the salivary concentration of standard aspirin after extraction at 276nm and pH. 3.9 .

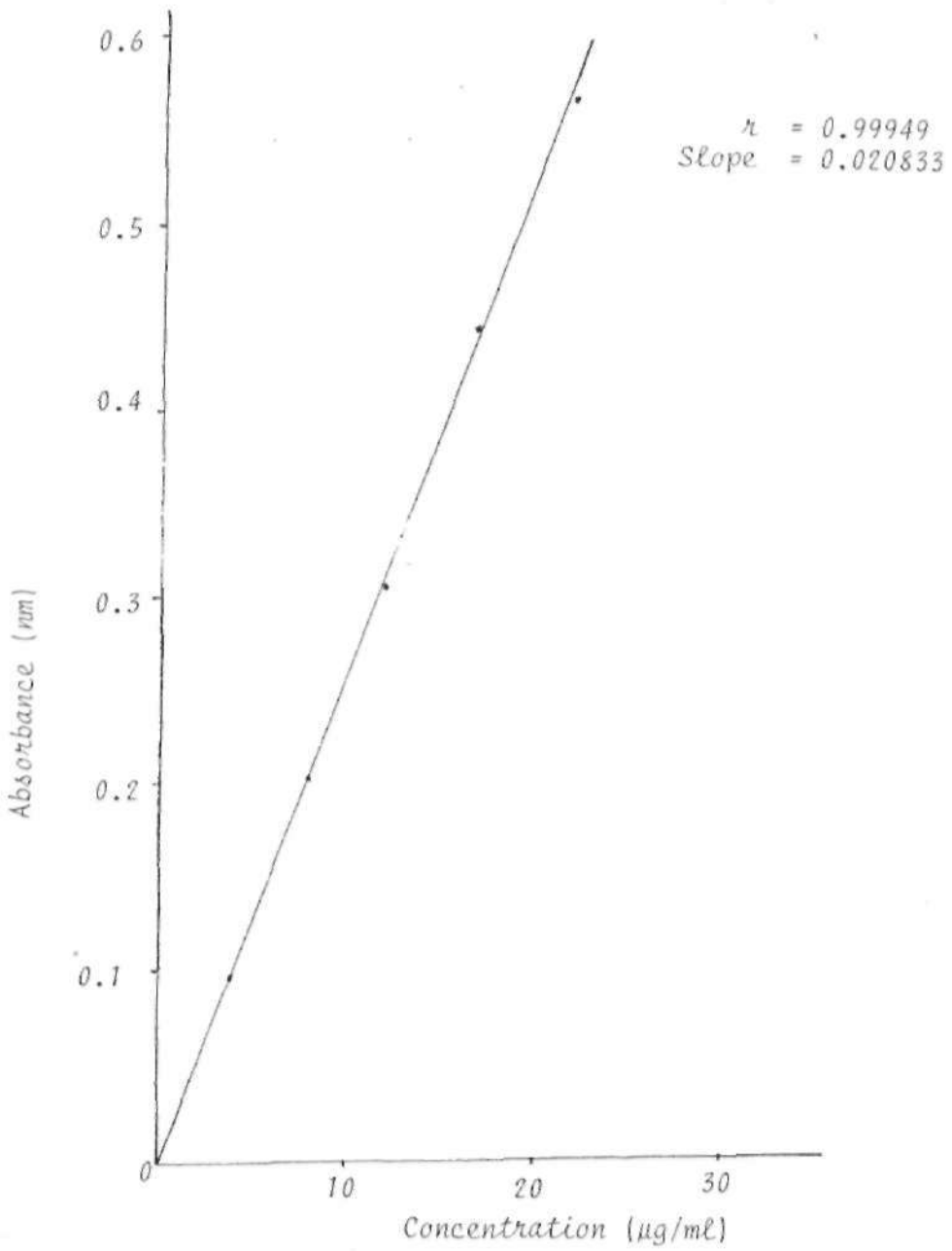


Fig. 13: Calibration graph for the salivary concentration of standard salicylic acid after extraction at 276nm and pH 3.9.

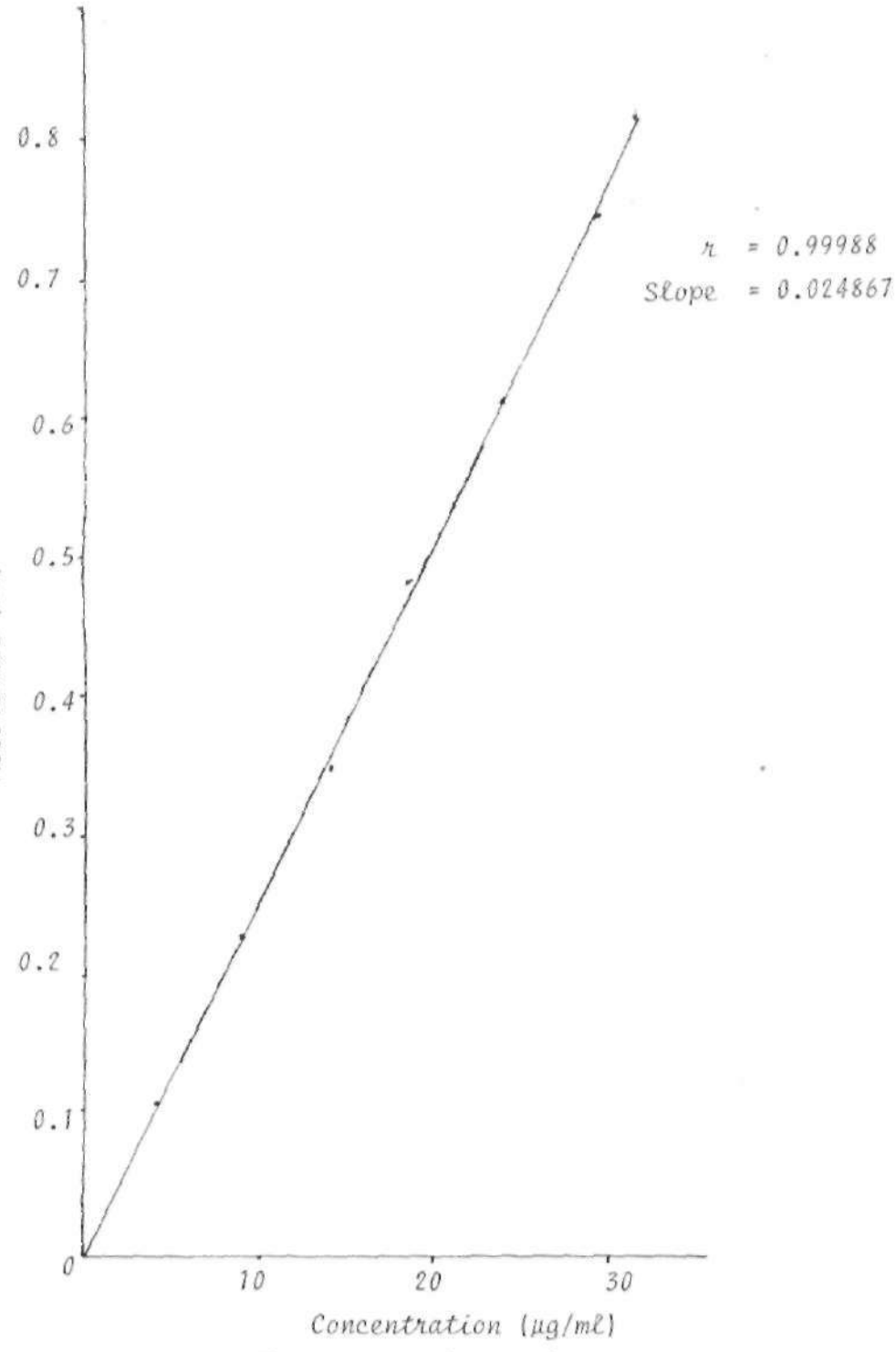


Fig. 14: Calibration graph for the salivary concentration of standard salicylic acid after extraction at 231nm and pH 3.9

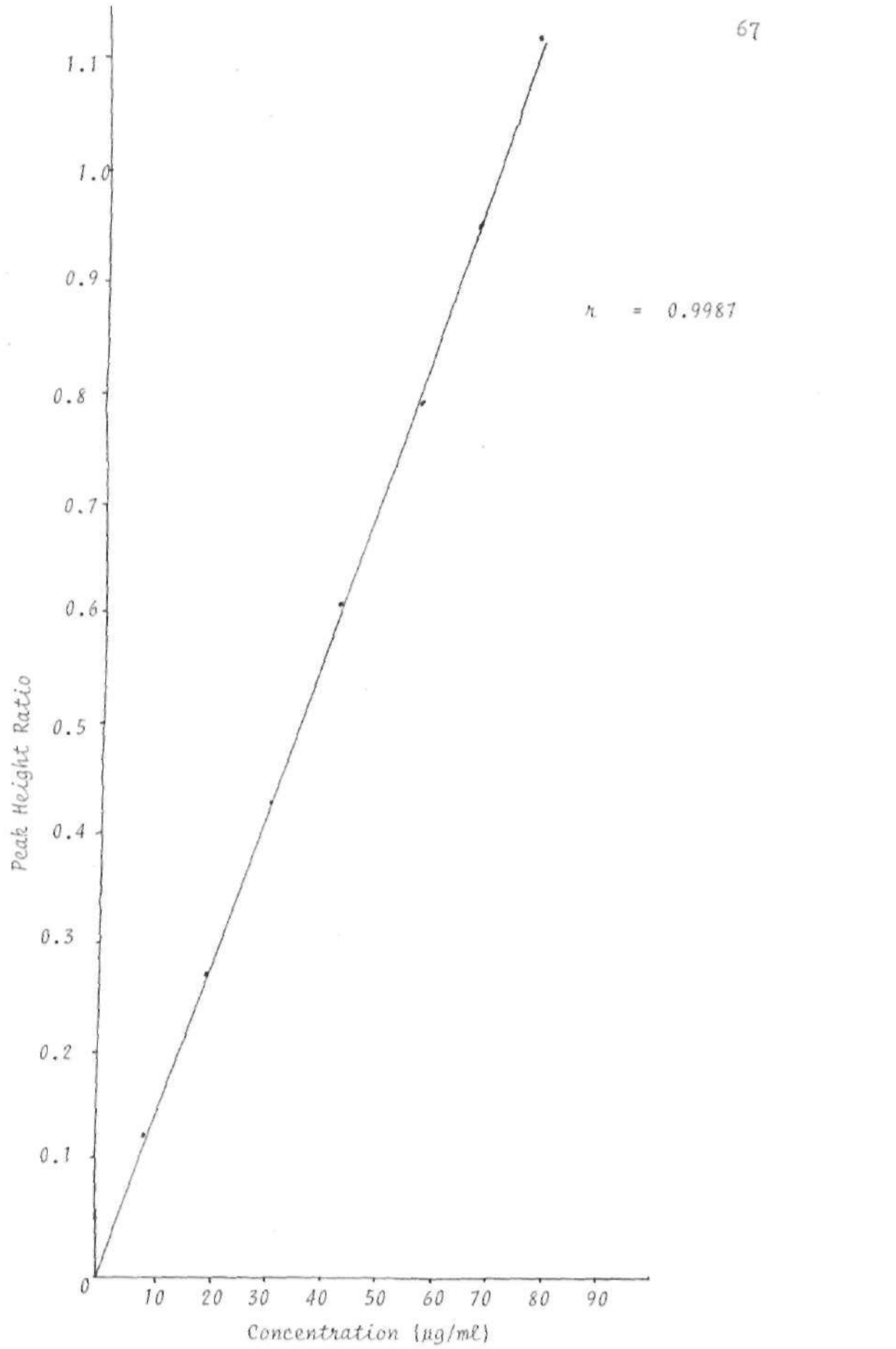


Fig. 15: Calibration graph for plasma aspirin assay using HPLC with the mobile phase methanol: 5% Acetic acid (25:75) pH 3.45.

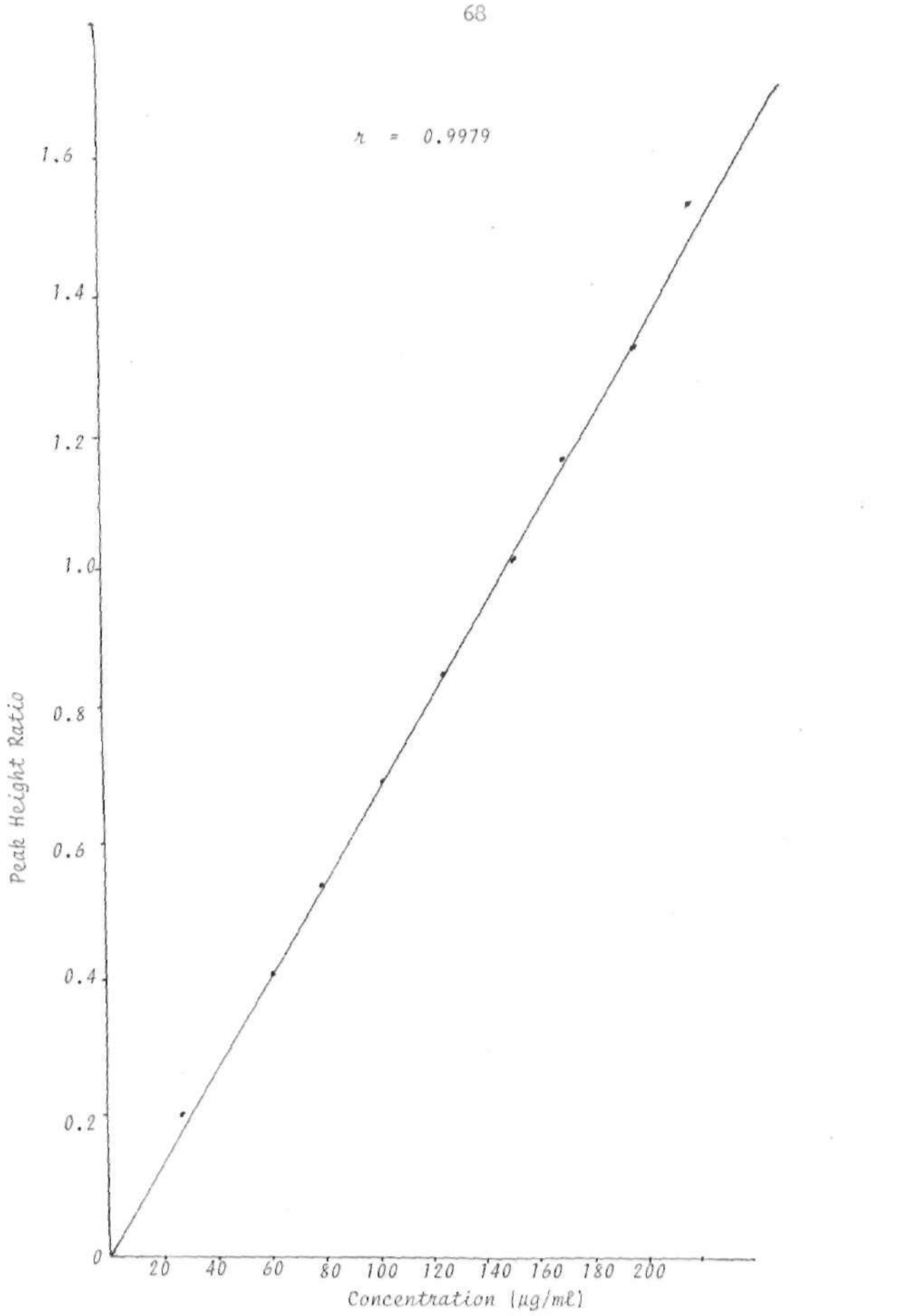


Fig. 16: Calibration graph for plasma salicylic acid assay using HPLC with the mobile phase methanol: 5% Acetic acid (25:75) pH 3.5,

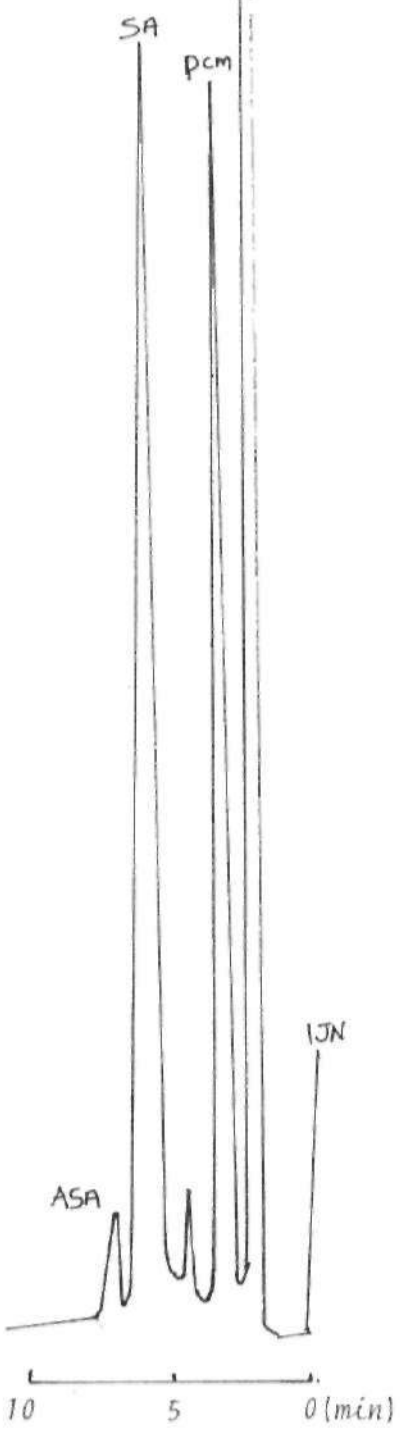


Fig. 17: HPLC Chromatogram of Plasma Aspirin and Salicylic acid at time of maximum concentration (Cmax) and Internal Standard (15µg/mℓ).

for salicylic acid and 0.2 to 0.4 ug/ml for aspirin respectively - Figure 19.

Drug with Starch

The mean plasma concentrations (C_{max}) for salicylic acid ranged from 40 to 60 ug/ml and 10 to 15 ug/ml for aspirin following oral ingestion of 600mg aspirin tablets with the starchy portion of the food drink - Figure 20. The mean salivary concentration for salicylic acid ranged from 10 to 14 ug/ml and 0.2 to 0.3 ug/ml for aspirin respectively - Figure 21.

Drug with Tamarindus indica

Following oral administration of 600mg aspirin tablets with T. indica extract (Tsamiya), the mean peak plasma concentration for salicylic acid ranged from 60 to 120 ug/ml and 30 to 40 ug/ml for aspirin respectively - Figure 22. While the mean salivary concentrations ranged from 18 to 21 ug/ml for salicylic acid and 1 to 2 ug/ml for aspirin respectively - Figure 23.

Drug with Sodium/Potassium Salt

The mean peak plasma concentrations following oral administration of 600mg aspirin tablets with sodium/potassium salt extract ranged from 20 to 24ug/ml for salicylic acid and 0.1 to 1.0 ug/ml for aspirin

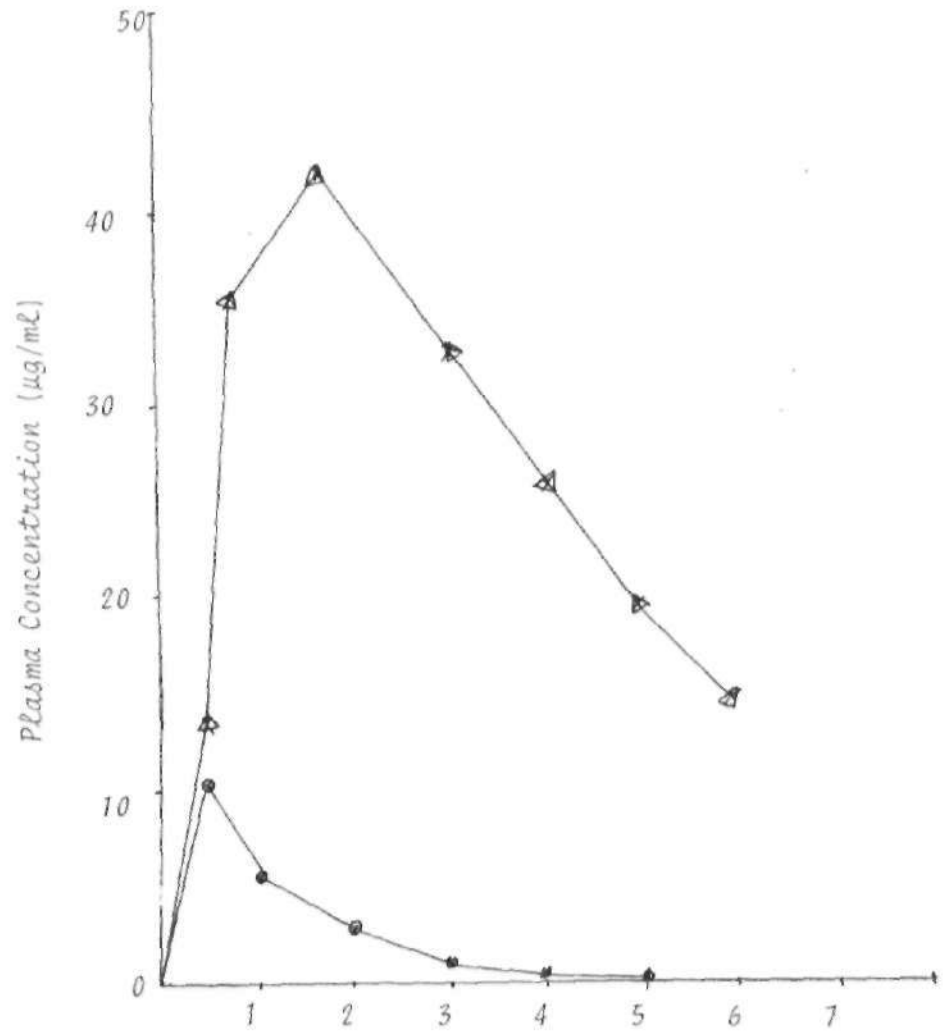


Fig. 18: Mean plasma concentrations of aspirin (-●-) and salicylic acid (-▲-) following oral ingestion of 600mg aspirin after overnight fast.

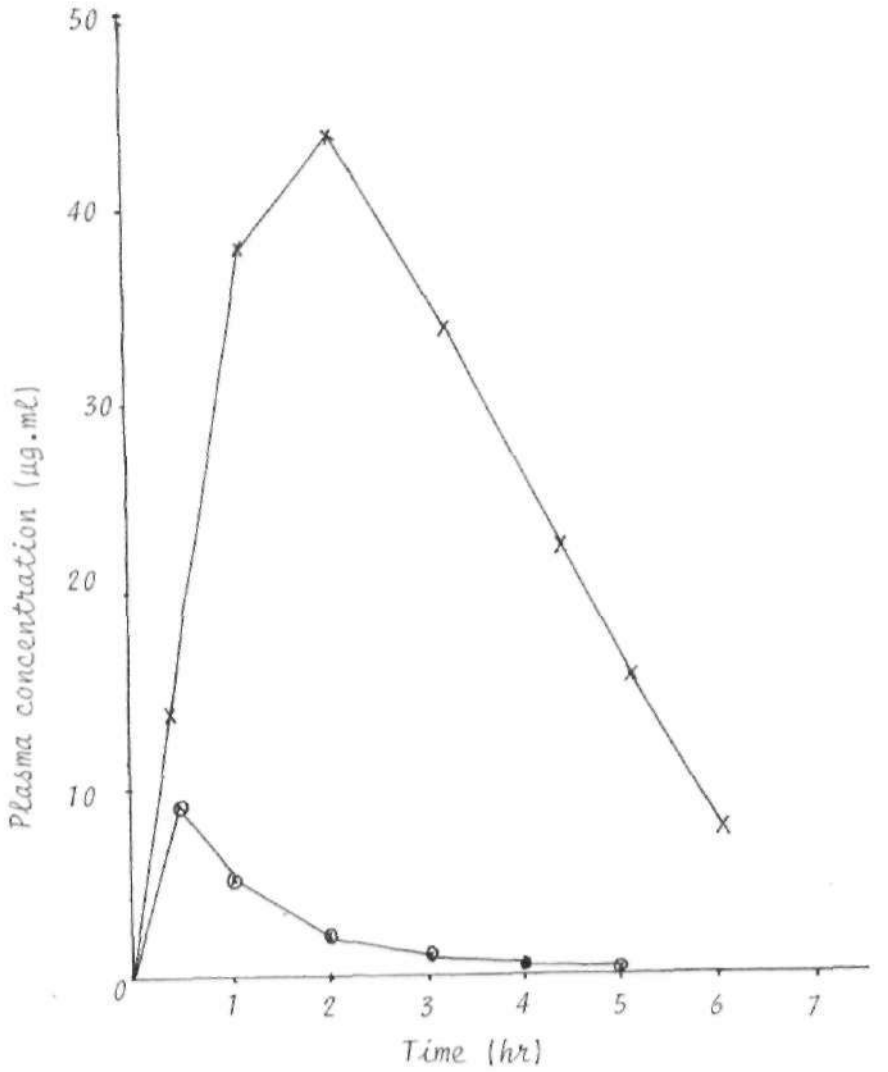


Fig. 20: Mean plasma concentration of aspirin (-o-) and salicylic acid (X) following oral ingestion of 600mg aspirin and starch after overnight fast.

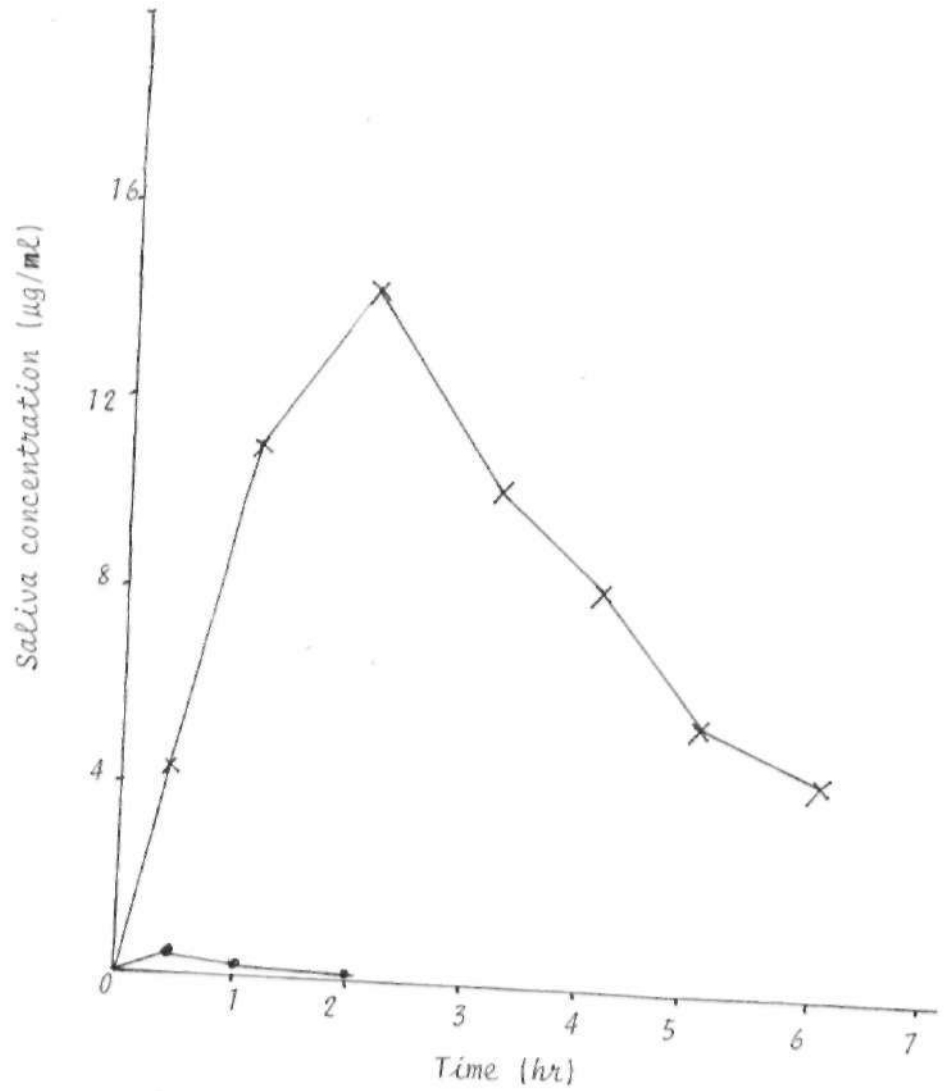


Fig. 21: Mean salivary concentration of aspirin (•) and salicylic acid (×) following oral ingestion of 600mg aspirin and starch after overnight fast.

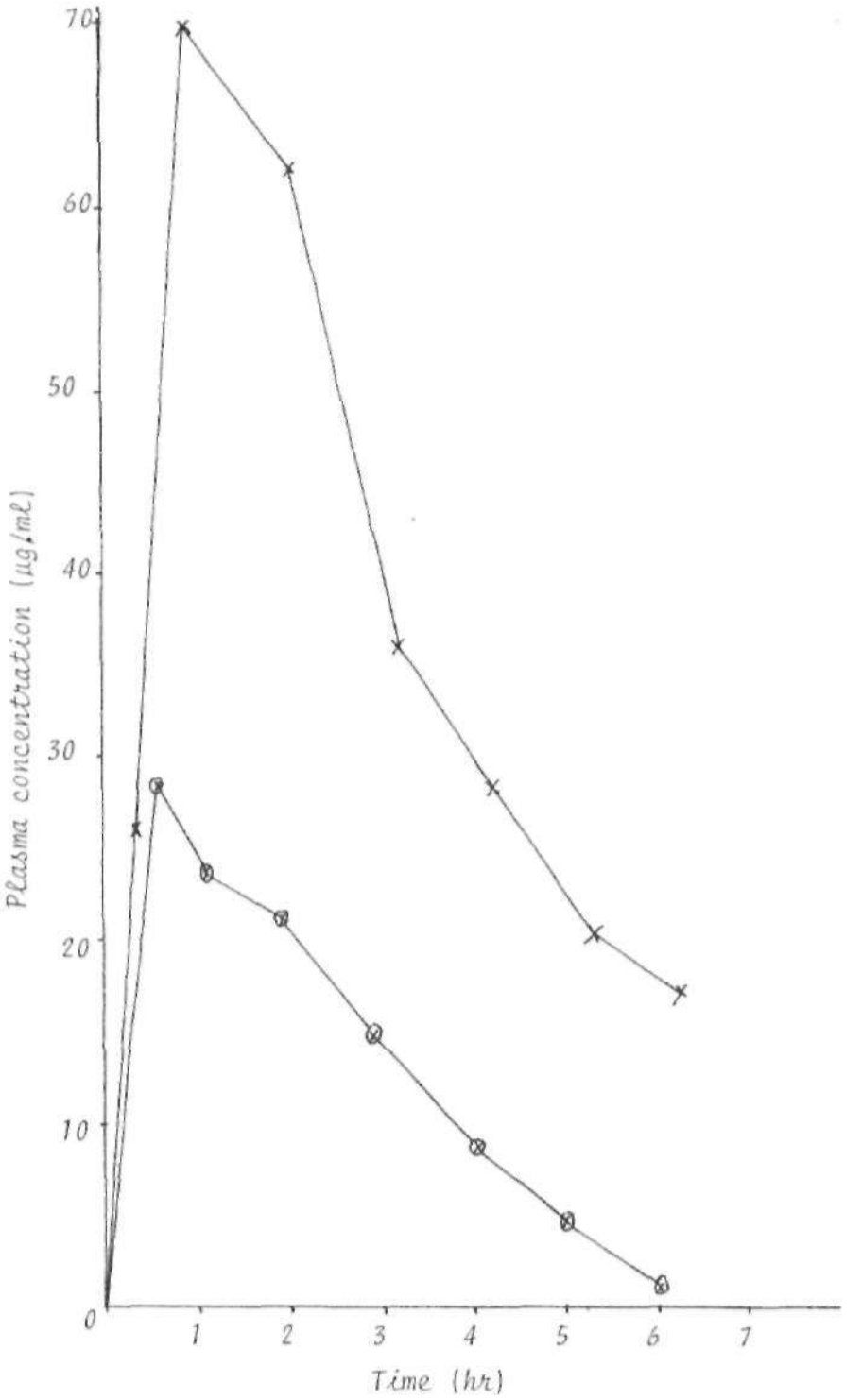


Fig. 22: Mean plasma concentration of aspirin (-⊗-) and salicylic acid (×) following oral ingestion of 600mg aspirin adn T.indica extract (tsamiya) after overnight fast.

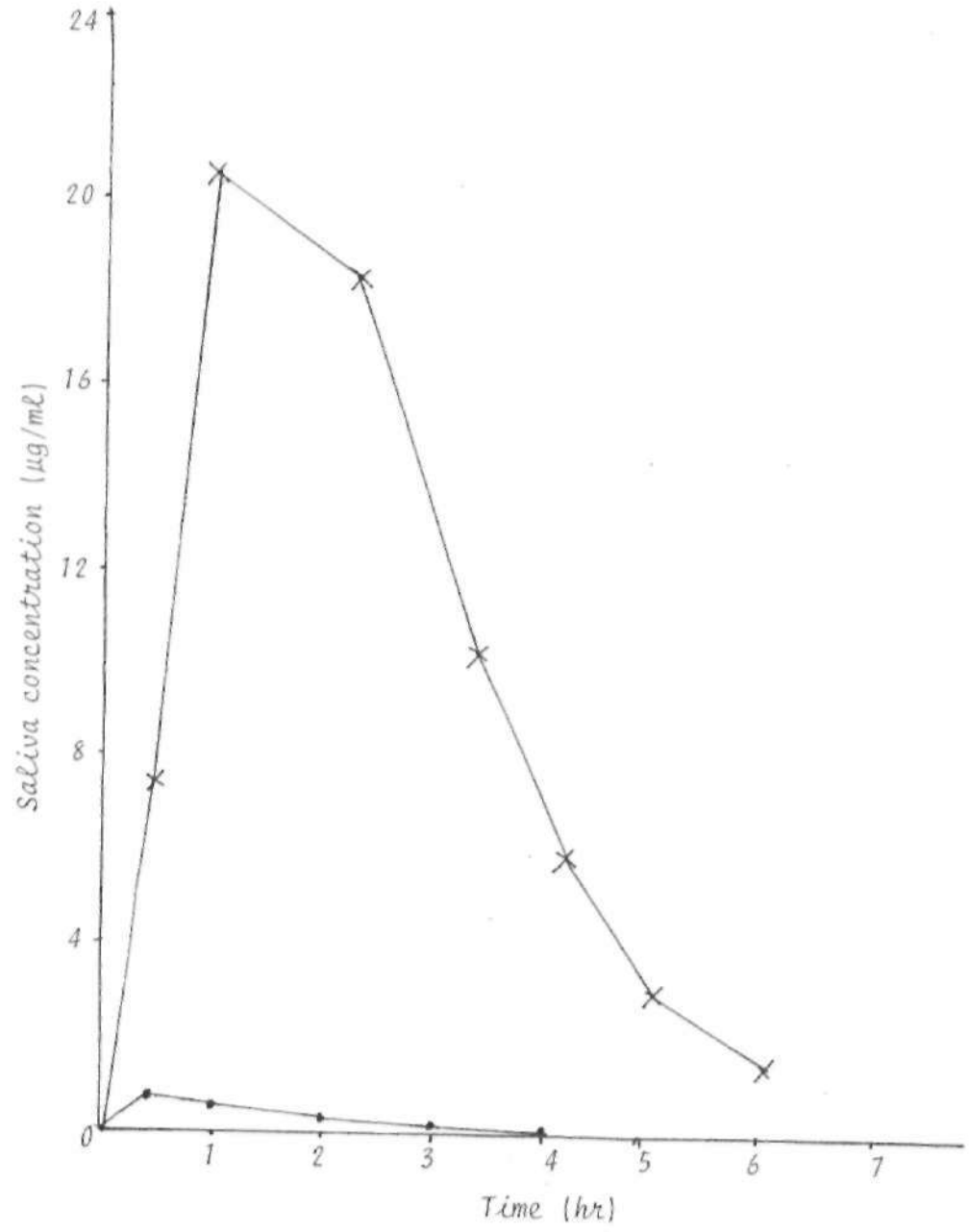


Fig. 23: Mean salivary concentration of aspirin (-•-) and salicylic acid (×) following oral ingestion of 600mg aspirin and T indica extract (tsamiya) after overnight fast.

respectively - Figure 24. While the mean salivary concentrations for salicylic acid ranged from 3.0 to 4.0 ug/ml and 0.2 to 0.3 ug/ml for aspirin respectively - Figure 25.

The mean recoveries for aspirin (ASA) and salicylic acid (SA) in plasma and saliva after oral ingestion of 600mg aspirin is summarised in Tables 6 and 7. The percent recoveries for both HPLC and UV spectrophotometric methods are summarised in Tables 8 and 9.

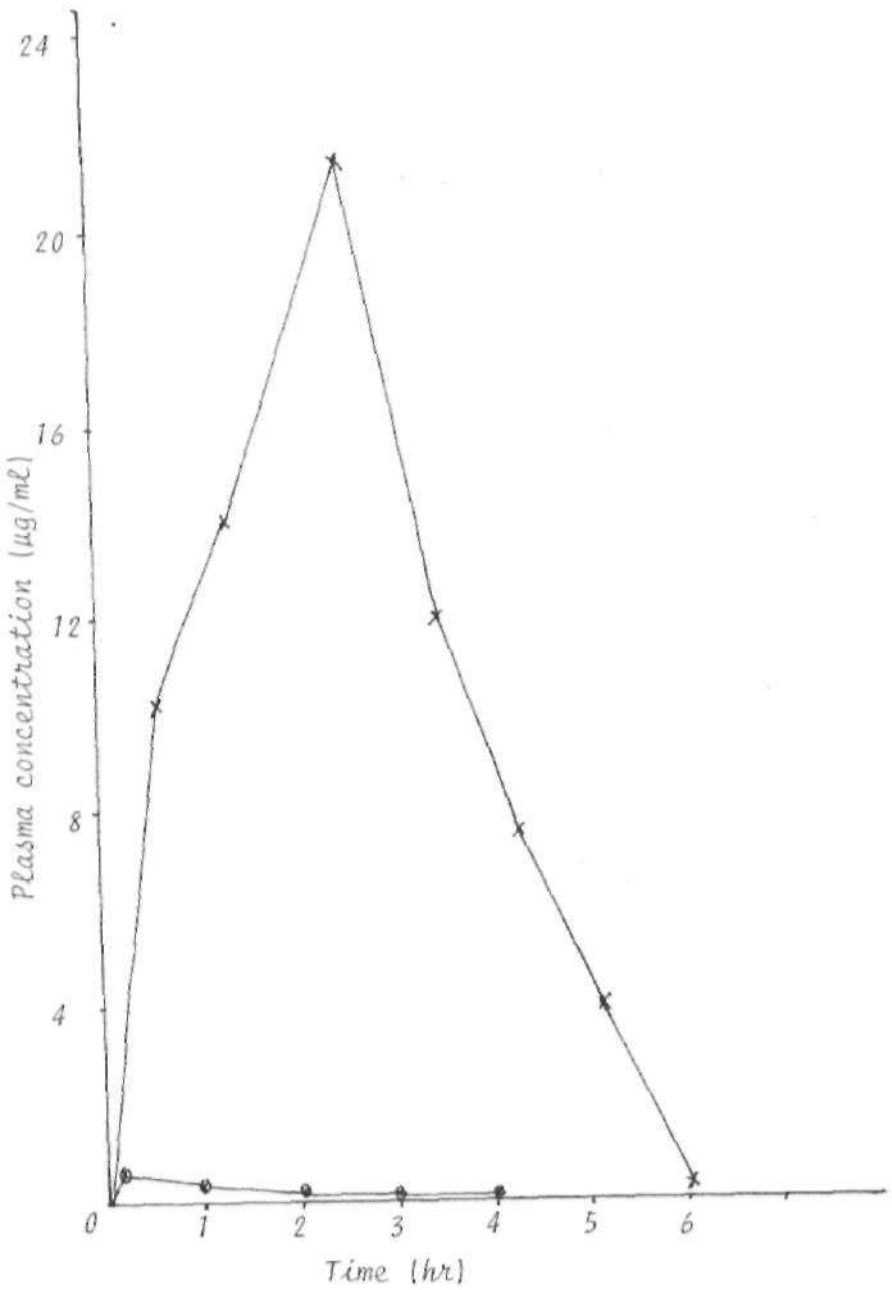


Fig. 24: Mean plasma concentration of aspirin (●) and salicylic acid (×) following oral ingestion of 600mg aspirin and sodium/potassium extract (Kanwa) after overnight fast.

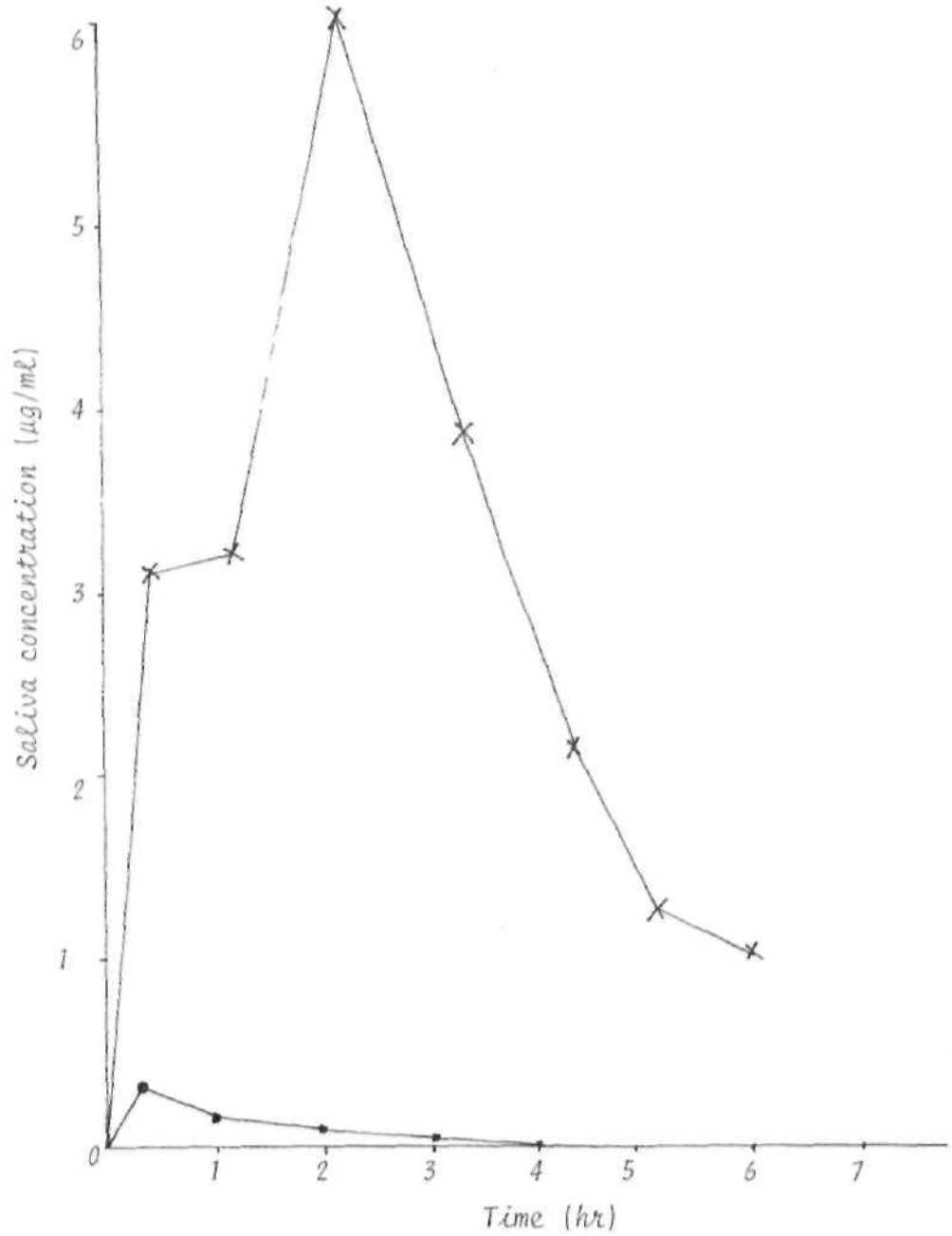


Fig. 25: Mean saliva concentration of aspirin (●) and salicylic acid (×) following oral ingestion of 600mg aspirin and sodium/potassium extract (Kanwa) after overnight fast.

Table 6: Mean plasma concentrations of aspirin (ASA) and salicylic acid (SA) in six healthy volunteers following oral ingestion of 600mg aspirin under different conditions.

Time (hr)	MEAN PLASMA CONCENTRATIONS (ug/ml) ± S.D.							
	DRUG ALONE		DRUG WITH STARCH		DRUG WITH T.INDICA		DRUG WITH Na/K SALT	
	ASA	SA	ASA	SA	ASA	SA	ASA	SA
0.5	10.31±0.11	13.25±0.71	9.48±0.70	13.49±0.27	28.62±0.62	24.73±0.24	0.78±0.38	10.50±1.11
1	5.42±0.39	36.32±1.01	5.21±1.11	37.74±0.85	24.98±0.24	68.19±0.12	0.66±0.76	14.01±1.21
2	3.20±0.04	43.84±0.27	2.22±0.12	44.46±0.38	22.21±0.47	61.07±0.30	0.49±0.36	22.21±0.98
3	1.10±0.70	32.50±0.85	1.01±1.11	32.21±0.24	15.40±0.27	34.69±0.17	0.34±0.11	11.41±0.71
4	0.39±0.85	26.03±0.12	0.05±0.12	20.82±0.12	8.99±0.76	27.65±0.24	0.09±0.21	6.77±0.85
5	0.11±0.24	19.32±0.38	0.03±0.81	14.08±0.76	5.82±0.11	20.18±0.24	0.04±0.10	4.42±0.17
6	0.04±0.30	15.91±0.12	0.03±0.24	7.89±0.85	0.74±0.11	18.34±0.47	0.02±0.11	0.22±0.16

Table 7: Mean saliva concentrations (ug/ml) of aspirin (ASA) and salicylic acid (SA) in six healthy volunteers following oral administration of 600mg aspirin under different conditions.

MEAN SALIVA CONCENTRATIONS (ug/ml) ± S.D.

Time (hr)	DRUG ALONE		DRUG WITH STARCH		DRUG WITH T. INDICA		DRUG WITH Na/K SALT	
	ASA	SA	ASA	SA	ASA	SA	ASA	SA
0.5	0.38±0.71	4.71±0.87	0.32±0.11	4.21±0.38	1.06±0.70	7.64±0.66	0.39±0.47	3.24±0.11
1	0.17±0.45	11.22±0.72	0.16±0.32	11.28±0.12	0.73±0.81	21.10±0.30	0.28±0.24	3.40±0.32
2	0.10±0.81	13.54±0.54	0.09±0.30	14.01±0.85	0.54±0.62	18.87±0.65	0.21±0.17	6.88±0.41
3	0.06±0.09	10.04±0.62	0.07±0.45	10.19±0.76	0.34±0.54	10.72±0.57	0.16±0.30	3.52.0.75
4	0.03±1.11	8.04±0.70	0.05±0.42	8.17±0.27	0.11±0.47	5.45±0.37	0.07±0.12	2.10±0.11
5	0.03±0.30	5.97±0.81	0.03±0.81	5.11±0.36	0.09±0.27	3.15±0.28	0.05±0.12	1.36±0.21
6	0.03±0.81	4.61±0.22	0.03±0.12	4.16±0.24	0.06±0.28	2.58±0.24	0.03±0.24	1.05±0.11

Table 8: Extraction recoveries for aspirin (ASA) and salicylic acid (SA) in plasma and saliva using U.V. spectrophotometer.

Sample	Medium	Wavelength nm	Concentration ug/ml	% Recoveries (Mean ± S.D) n = 5
ASA	Plasma	276	10	95.6 ± 0.11
			20	93.5 ± 0.14
ASA	Plasma	231	10	94.2 ± 0.94
			20	90.1 ± 0.23
ASA	Saliva	276	10	98.9 ± 0.41
			20	101.0 ± 1.02
ASA	Saliva	231	10	99.5 ± 0.11
			20	99.7 ± 0.72
SA	Plasma	276	10	81.8 ± 0.02
			20	83.7 ± 0.18
SA	Plasma	231	10	91.7 ± 0.11
			20	91.5 ± 0.23
SA	Saliva	276	10	89.3 ± 0.11
			20	81.4 ± 0.29
SA	Saliva	231	10	88.6 ± 0.21
			20	89.7 ± 0.44

Table 9: Extraction recoveries for aspirin (ASA), salicylic acid (SA) and paracetamol (internal standard) in plasma using HPLC.

Sample	Concentrations (ug/ml)	% Recoveries (Mean ± S.D) n=4
Aspirin	40	94.71 ± 0.04
	60	91.14 ± 0.32
	80	87.07 ± 1.11
Salicylic Acid	40	84.98 ± 0.91
	60	91.11 ± 0.04
	80	82.16 ± 0.16
Paracetamol	20	93.09 ± 0.12
	40	90.94 ± 0.38
	60	87.11 ± 0.23

Table 11: Pharmacokinetic parameters of aspirin derived from the mean plasma concentration data after a single 600mg oral dose under 4 conditions.

Parameters	Drug Alone	Drug + Starch	Drug + T.indica	Drug + Na/K Salt
Lag time (min)	0.100	0.100	0.100	0.200
Absorption rate constant Ka (hr^{-1})	1.098	1.034	4.880	1.009
Absorption half life $t_{\frac{1}{2}}\alpha$ (hr)	0.171	0.161	0.142	0.631
Elimination Rate Constant Ke (hr^{-1})	0.638	0.678	0.463	0.748
Elimination half life $t_{\frac{1}{2}}\beta$ (hr)	1.041	1.031	1.499	0.926
Maximum Concentration Cmax (µg/ml)	10.040	9.480	28.620	0.780
Time to peak t_{max} (min)	30.000	30.000	30.000	30.000
Area Under the Curve AUC (0-6hr) (ug/ml/hr)	13.930	13.050	85.000	1.855
AUC (0-∞)	14.030	13.180	86.510	1.908
Intercept on Y-Axis (Co)	40.000	17.000	56.000	2.600

Table 12: Pharmacokinetic parameters of aspirin derived from the mean saliva concentration data after a single 600mg oral dose under 4 conditions.

Parameters	Drug Alone	Drug + Starch	Drug + T. indica	Drug + Na/K Salt
Lag Time (min)	0.20±0.12	0.20±0.41	0.20±0.11	0.25±0.08
Absorption Rate Constant Ka (hr-1)	1.264±0.38	5.547±0.25	2.178±0.22	1.431±0.75
Absorption ½ life $t_{\frac{1}{2}}$ (hr)	0.548±0.11	0.125±0.08	0.318±0.31	0.484±0.01
Elimination Rate Constant Ke (hr-1)	0.413±0.39	0.174±0.11	0.614±0.001	0.370±0.21
Elimination ½ life $t_{\frac{1}{2}}$ B (hr)	1.678±0.21	3.983±1.02	1.129±0.41	1.873±0.17
Maximum Concentration Cmax (ug/ml	0.38±0.11	0.32±0.21	1.06±0.23	0.39±0.38
Time to peak tmax (min)	30.0±0.25	30.0±0.02	30.0±0.11	30.0±0.38
Area Under the Curve AUC (0 - 6hr)(ug/ml/hr)	9.48±0.02	9.24±0.43	13.71±0.21	1.97±0.21
AUC (0 - ∞)	9.55±0.44	9.42±0.77	14.04±0.23	1.04±0.4
Intercept on Y-Axis (Co)	37.0±0.11	13.0±0.01	130.0±0.31	15.0±0.21

Table 10: Pharmacokinetic parameters of Salicylic Acid derived from the mean plasma concentration data after a single oral dose of 600mg aspirin.

Parameters	Drug Alone	Drug + Starch	Drug + T. indica	Drug + Na/K Salt
Lag time (min)	0.25±0.77	0.25±0.03	0.25±0.41	0.25±0.21
Absorption Rate constant Ka (hr-1)	2.45±0.11	2.19±0.41	3.77±0.01	1.89±0.32
Absorption ½ life $t_{\frac{1}{2}}$ (hr)	0.2831±0.21	0.317±0.06	0.184±0.11	0.367±0.11
Elimination Rate constant Ke (hr-1)	7.37±0.39	12.71±0.44	19.30±0.21	3.16±0.38
Elimination ½ life $t_{\frac{1}{2}}$ B (hr)	0.094±0.21	0.054±0.72	0.036±0.24	0.22±0.22
Maximum concentration Cmax (ug/ml)	43.84±0.21	44.46±0.29	68.19±0.71	22.27±0.21
Time to peak tmax (min)	120±0.18	120±0.43	60±0.08	120±0.11
Area under the curve AUC 0-6hr (ug/ml/hr	171.59±0.07	158.29±0.12	266.22±0.21	58.98±0.04
Intercept on Y-Axis (Co)	60.0±0.44	52.51±0.12	94.0±0.21	72.0±0.12

Table 13: Comparison between HPLC and U.V. spectrophotometric plasma concentrations of aspirin and salicylic acid pharmacokinetic parameter after a single 600mg oral dose of aspirin.

PARAMETERS	DRUG ALONE				
	ASA		SA		
	HPLC	U.V.	HPLC	U.V	PROBABILITY STUDIES T.TEST
Lag time (min)	0.100	0.100	0.170	0.250	P.>0.1
Absorption Rate Constant Ka (hrs-1)	1.043	1.098	2.360	2.450	P.>0.1
Absorption half life $t_{\frac{1}{2}}$ (hr)	0.173	0.171	0.284	0.283	P.>0.1
Elimination Rate Constant Ke (hr-1)	0.637	0.638	6.090	7.370	P>.0.1
Elimination half life	1.068	1.041	0.142	0.094	P.>0.1
Maximum Concentration (Cmax)	15.280	10.040	65.050	43.840	P.>0.1
Time to peak tmax	30.000	30.000	120.000	120.000	P.>0.1
Area Under the Curve AUC (0.6hr) (ug/ml/hr)	17.980	13.930	180.310	169.570	P.>0.1

Table 14: Comparison between HPLC and U.V. spectrophotometric plasma concentrations of aspiring and salicylic acid pharmacokinetic parameters after concurrent administration of 600mg aspiring and 2000ml food drink containing T. indica extract.

	DRUG + T. INDICA				
	ASA		SA		PROBABILITY STUDIE T TEST
	HPLC	U.V.	HPLC	U.V.	
Lag time (min)	0.250	0.250	0.230	0.250	P>0.5
Absorption Rate Constant Ka (hr-1)	3.280	3.540	3.180	3.770	P>0.5
Absorption half life $t_{\frac{1}{2}}$ (hr)	0.317	0.239	0.211	0.184	P>0.5
Elimination Rate Constant Ke (hr-1)	12.740	13.800	23.040	19.300	P>0.5
Elimination half life $t_{\frac{1}{2}}$ B (hr)	0.096	0.044	0.046	0.036	P>0.5
Maximum Concentration Cmax (ug/ml)	38.180	28.620	120.010	68.190	P>0.5
Time to peak tmax (min)	30.000	30.000	60.000	60.000	P>0.5
Area Under the Curve AUC (0-6hr) (ug/ml/hr(	204.220	197.980	307.210	265.750	P>0.5

Table 15: Relationships between salivary and plasma concentrations of salicylic acid.

	Drug Alone	Drug + T.indica	Drug + Na/K Salt
Saliva-Plasma Ratio	0.3128	0.2472	0.3088
(± Coefficient of Variances)	(±5.76%)	(±3.97%)	(±0.02%)
Regression of salivary concentration versus plasma concentration: Slope	0.3094	0.2099	0.3041
Correlation coefficient between salivary and plasma concentration	0.9978	0.9919	0.9924

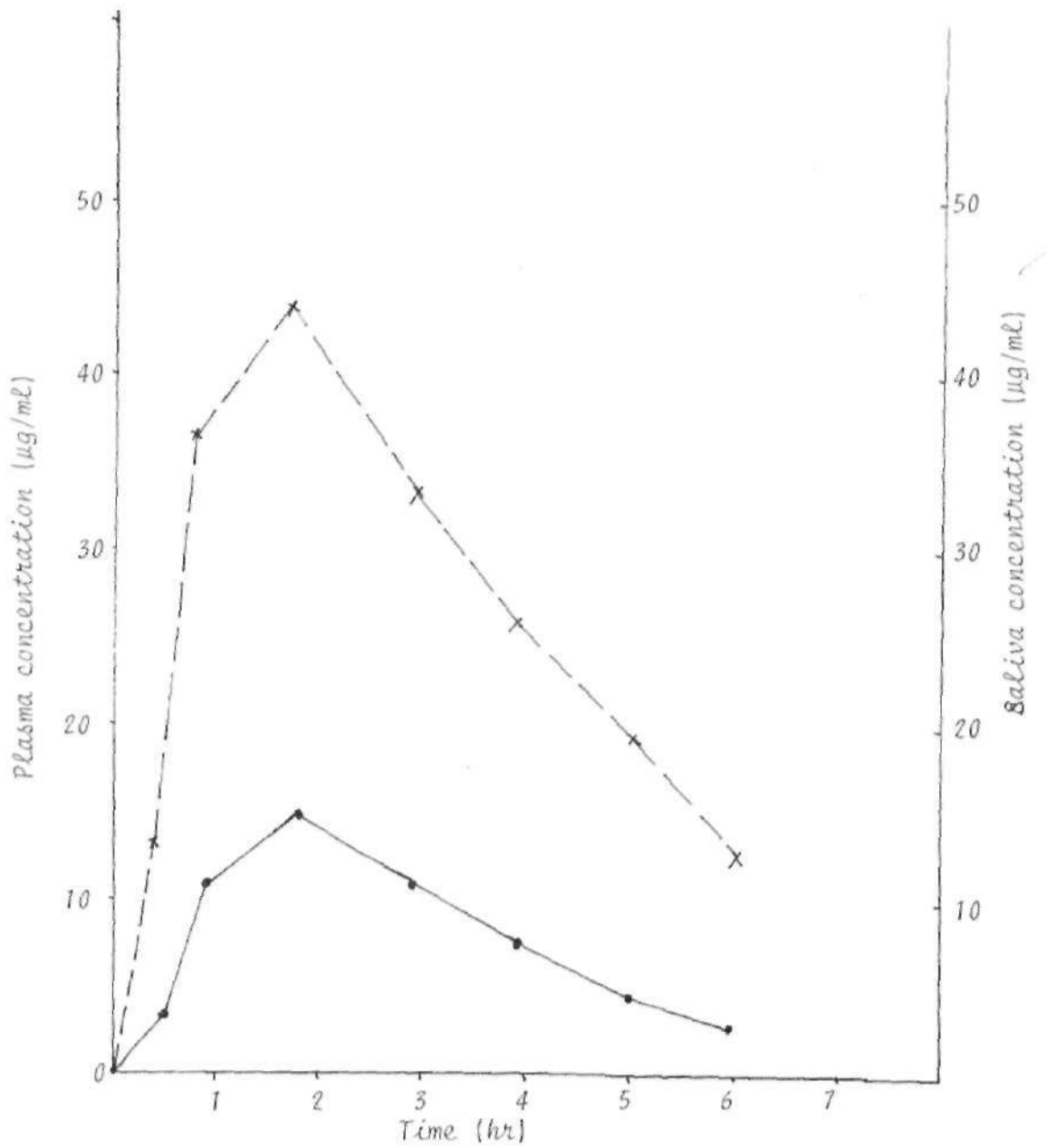


Fig. 26: Mean concentration of salicylic acid in saliva (•) and plasma (-x-) following oral ingestion of 600mg aspirin after an overnight fast.

CHAPTER FIVE

DISCUSSION

5.1 IDENTIFICATION TESTS (B.P. 1988)

All the standard samples of aspirin and salicylic acid powders as well as the aspirin tablets have passed the identification tests for aspirin and salicylic acid respectively. Hence all the samples used in this study contained either aspirin or salicylic acid.

5.2 CONTENT OF ACTIVE INGREDIENTS

The presence and the percentage of active ingredient in the aspirin tablets (Polfa and Vitalink brands) were quantitatively determined. The results obtained from this study showed that all the brands of the aspirin tablets tested complied with the official requirement (99.5 - 101.0%) of aspirin content.

5.3a Dissolution Rate Studies

The results from this study have illustrated the differences in the dissolution rates of the different brands of the aspirin tablets employed. For example, the Polfa aspirin tablets gave a dissolution rate value of 100%, while that of Vitalink tablets was 84.42% at 45 mins. Furthermore, these results have established the superiority of Polfa aspirin tablets than of Vitalink in attaining faster availability.

The variation found in the dissolution rate of the aspirin tablets could be explained by various formulation factors. Some of these factors could be the amount and type of diluent (37), binders and disintegrants and other adjuvants (38,39). Method of the incorporation of the ingredients, the compressional force and speed of compression have also been among other factors leading to large variations in the dissolution rates of tablets (40,41).

It has been observed that there was an unusual behaviour in the dissolution rate profile of aluminium acetylsalicylic acid in 0.1M HCl, due to formation of film coating on the drug (42). It has also been reported that the rate of release of an amine drug from a pellet of a weak acid salt of the amine, in acid solution was controlled by a coat which was formed by precipitation of a weak acid into the pellet surface (43).

However, it has been noted that the release of benzphentamine in 0.12M HCl was controlled by a layer of pamoic acid which was deposited on the surface (44). Also it has been observed that active lubricant sodium lauryl sulfate enhanced markedly the dissolution rate of salicylic acid contained in compressed tablets. The effect could be attributed to the interfacial tension lowering of the surfactant (45). These results showed that a surfactant can enhance the dissolution

rate of drugs contained in compressed tablets and cause them to be more rapidly and more completely absorbed. The observation can also be attributed to greater local solubilisation, increased drug wettability and particle size reduction. It has been established that a drug will dissolve more rapidly when its specific particle size is decreased (46). Since the dissolution rate is directly proportional to surface area, a reduction in particle size will result in increased surface area and consequently in rapid dissolution.

5.3b Disintegration Times

Dissolution of a drug from a tablet or other dosage form involves a disintegration or deaggregation step during which the solid drug particles are released to the dissolution medium. Once released, the individual drug particles undergo dissolution. Tablet dissolution is thus a composite of the sequential time-dependent processes of tablet disintegration and particle dissolution.

Disintegration tests conducted for both Polfa and Vitalink aspirin tablets is a reflection of what was found with their in-vitro dissolution rates. The mean disintegration time for Polfa aspirin was 2.92 ± 0.42 and that of Vitalink aspirin was 3.17 ± 0.98 respectively. The official B.P. (1988) time-limit for tablet

disintegration is 15 mins. Hence all the tablets passed the test, but Polfa tablet disintegrated faster than Vitalink aspirin tablets.

The type and amount of disintegrants employed may affect the disintegration time of a compressed tablet. It has been reported that in a comparative study of seven disintegrants compressed at three concentrations (5, 10 and 15% in a base of spray dried lactose using magnesium stearate as lubricant with direct compression), rapid disintegration was achieved with corn starch at 10 and 15% regardless of storage temperature.

5.4 IN-VIVO BIOAVAILABILITY STUDIES

Aspirin plasma levels following the ingestion of 600mg aspirin in all the four conditions were low compared with salicylic acid levels. Levels of aspirin rose sharply, reaching maximum between 20 to 30 minutes following administration. These levels then declined rapidly, with only small amounts remaining after two hours (Figures 20 to 25).

Associated with the hydrolysis of aspirin, salicylic acid levels rose rapidly, reaching maximum at 2 hours with maximum concentration (Cmax) of between 60 to 70 ug/ml exceeding those of aspirin with Cmax of between 10 to 15 ug/ml.

After simultaneous collection of saliva and blood, the time-course plots of salicylic acid levels in saliva and plasma showed good parallelism in each of the conditions (Figure 26). Ratios of the concentration of salicylic acid in saliva to the concentration in plasma are shown in Table 15.

It has been observed that the parotid plasma-saliva ratios of 0.31 - 0.76 was obtained when plasma levels ranged from 290 to 480 ug/ml after a dose of 6g sodium salicylate (47). These plasma levels and saliva-plasma ratios were considerably higher than those attained in the present study. However, lower saliva-plasma ratios of salicylic acid were anticipated at the lower dose employed in the present study, since the fraction of protein-free salicylic acid in plasma decreases as the plasma concentration decreases (46) and a diffusional equilibrium is established between the concentration of lipid-soluble drugs in saliva and the concentration of the protein-free, unionised forms of the drugs in plasma(49,50). Accordingly, the saliva-plasma ratio of salicylic acid might change slightly in serial samples of saliva following a single dose of aspirin or salicylic acid. However, in the present study, significant correlation was observed between the saliva-plasma ratios and the concentrations of salicylic acid in plasma ranging up to 90 ug/ml Proportionality between salivary and plasma levels of salicylic acid cannot be assumed outside this range of plasma concentrations (49,50).

The proportionality between salicylic acid levels in saliva and plasma indicated that measurement of salicylic acid levels in saliva may be a useful technique in the evaluation of different salicylate preparations.

Measurement of the excretion of salicylates in urine has been used as an alternative technique for the estimation of the plasma levels in the evaluation of different formulations of aspirin. The total recovery of salicylates in urine is a good index of the absorption of different preparations of aspirin, since over 95% of orally ingested doses can be recovered in urine (51). However, the rate of elimination of free salicylate in urine fluctuates considerably (51); and the rate of excretion of the major metabolite salicyluric acid does not parallel plasma levels of salicylic acid (52). While only a limited number of subjects were examined in the present study, measurement of salicylic acid level in saliva should give a more accurate estimate of changes in the plasma concentration of salicylic acid than can be obtained from urinary studies.

A wide variation in the absorption half-life of aspirin was observed following oral ingestion of the drug with T. indica and Na/k salt. Numerous reasons can be advanced for these findings. Since the absorption of salicylates is pH dependent (53), being slowest in

alkaline medium, the absorption half-life of aspirin following concurrent administration of the drug with sodium/potassium salt showed a longer half-life (0.63 hr). This may be attributed to the delayed absorption as most of the drug existed in an ionised form and hence less lipid soluble and poorly absorbed. Because of the delay in absorption and low concentration, the percentage hydrolysed during absorption is greatest.

Even though aspirin is a weak acid (pka = 3.5), salicylates are more rapidly absorbed in the duodenum due to enormous increase in surface area and hence when aspirin was concurrently administered with T. indica extract, the absorption half-life of the aspirin was short (0.142) even shorter than when the drug was given alone (Table 11). This may be as a result of an increase in acidity in the gastric and intestinal fluids which led to the drug to exist in an unionised form and hence lipid soluble and readily absorbed. As the concentration of non-ionised form of the drug in the intestinal fluid increased, enzyme saturation occurred for part of the absorption and this led to the decrease in the hydrolysis of the drug.

There were differences as well as similarities in the calculated aspirin plasma and saliva pharmacokinetic parameters for all the four conditions (Tables 10 to 12). For example, the mean time to reach peak concentration (T_{max}) and lag time were almost similar

for all the conditions (Table 10 to 12). However, there were slight differences in the absorption and elimination half-lives. When the drug was co-administered with T. indica extract, both the mean absorption ($t_{\frac{1}{2}}$) and elimination ($t_{\frac{1}{2}}$) half-lives were short (0.18 and 0.036). However, when the drug was co-administered with sodium/potassium salt extract, both the $t_{\frac{1}{2}}\alpha$ and $t_{\frac{1}{2}}\beta$ were long (0.367 and 0.22). The difference observed may be due to the difference in the chemical nature of the two food materials and their effects on the aspirin absorption as stated above. Other reasons for the difference may be due to individual variability or varying degree of the hepatid first-pass effect. An orally administered drug may be degraded or metabolised within the gut lumen by enzymes in the mucosa. Because of the anatomical arrangement of the hepatic portal circulation, drugs absorbed into the portal vein are exposed to the liver before reaching the site of action.

Comparing the bioavailability of the drug under the four conditions used in this study, it is obvious from the results obtained that the major cause of difference in absorption of aspirin is the difference in the chemical properties of the food materials used. This was reflected in the longer absorption half-life found in-vivo when the drug was given along with sodium/potassium salt extract (Table 11). However, measured

area under the concentration time-curve at 6 hours (AUC 0-6hr) using the trapezoid rule did not differ significantly for the drug when given along and when given with the starchy portion of the food drink; but was greater with T. indica extract (Tables 10 and 11).

Comparing the bioavailability of the drug under the two analytical methods employed in this study, it can be observed that higher concentrations were reflected with HPLC than U.V. spectrophotometric method. This may be as a result of the higher sensitivity of the HPLC method compared to the conventional U.V. spectrophotometric method which is less discriminatory and less sensitive. Also, the presence of other metabolites may interfere with the U.V. absorption of either aspirin or salicylic acid especially those metabolites which absorb at the same wavelengths (Tables 13 and 14).

However, the results obtained from these two analytical methods correlate with each other with the degree of probability ($0.05 < P < 0.1$) using student's t-test. Hence U.V. spectrophotometric method can also be used in the bioavailability studies of aspirin in the absence of high-performance liquid chromatography (HPLC).

5.5 CONCLUSION

Bioavailability of aspirin has been shown to be markedly affected by the presence of either Tamarindus indica extract or sodium/potassium salt extract when administered in the form of Hausa food drink (KUNU) concurrently with aspirin. The bioavailability is enhanced by T. indica extract and diminished by sodium/potassium salt extract. Hence these effects may be said to be potentiating in the case of T. indica extract; and inhibitory in the case of sodium/potassium salt extract on the bioavailability of aspirin. These findings may have clinical importance on the use of the drug for the treatment of common ailments associated with headaches, fever, rheumatic pains, etc.

However, in the analysis of aspirin and its metabolites, HPLC is the most ideal method compared with the U.V. method because of its higher sensitivity and reliability. But in the absence of HPLC, UV can be used as an alternative following the method employed in this study.

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APPENDIX 1

APPARATUS FOR THE DISINTEGRATION OF TABLET

The apparatus consists of

(a) a rig basket-rack assembly supporting 6 cylindrical glass tubes 77.5mm long, 21.5mm in internal diameter, and with a wall thickness of 2mm.

(b) a cylindrical disc for each tube, each 20.7mm in diameter and 9.5mm thick, made up of transparent plastic with a relative density of 1.18 to 1.20, and pierced with five holes, each 2mm in diameter, out in the centre and the other four spaced equally on a circle of radius 6mm from the centre of the disc. For equally-spaced grooves are cut in the lateral surface of the disc in such a way that at the upper surface of the disc they are 9.5mm wide and 2.55mm deep and at the lower surface 1.6mm square.

(c) The tubes are held vertically by two superimposed transparent plastic plates 90mm in diameter and 6mm thick, perforated by 6 holes having the same diameter as the tubes. The holes are equidistant from the centre of the plate and are equally spaced from one another. Attached to the underside of the lower plate of wooven guaze made from stainless steel wire 635 um in diameter and having nominal mesh aperture of 2.00mm. The upper plate is covered with a stainless steel disc perforated

by six holes each about 22mm in diameter, which fits over the tubes and holds them between the plastic plates. The holes coincide with those of the upper plastic plate and the upper open ends of the glass tube.

(d) The plates are held rigidly in position and 77.5mm apart by vertical metal rods at the periphery and a metal rod is also fixed to the centre of the upper plate to enable the assembly to be attached to a mechanical device capable of raising and lowering it smoothly at a constant frequency of between 28 and 32 cycles per minute, through a distance of 50 to 60mm.

(e) The assembly is suspended in the liquid medium in a suitable vessel, preferrably a 1000ml beaker. The volume of liquid is such that the wire mesh at its highest point is at least 25mm below the surface of the liquid, and at its lowest point is at least 25mm above the bottom of the beaker.

(f) A suitable device maintains the temperature of the liquid at 36° to 38°.

APPENDIX 2

ASPIRIN PLASMA ABSORPTION AND SALIVARY SECRETION STUDIES OF INDIVIDUAL VOLUNTEER FOLLOWING ORAL INGESTION OF 600mg ASPIRIN UNDER FOUR CONDITIONS.

(1) Name of Volunteer:- Ss
Age:- 19
Weight:- 52kg
Sex:- Male

Drug:- Aspirin
Dose:- 600mg

PLASMA ASPIRIN CONCENTRATIONS (ug/ml)

Time (hr)	Drug Alone	Drug + Starch	Drug + T. indica	Drug + Na/K Salt
0.5	10.22	9.48	28.72	0.78
1	5.18	5.11	24.91	0.64
2	3.14	2.28	22.62	0.44
3	1.10	1.09	15.44	0.32
4	0.34	0.36	8.18	0.11
5	0.12	0.13	5.67	0.09
6	0.08	0.03	0.92	0.04

PLASMA SALICYLIC ACID CONCENTRATIONS (ug/ml)

Time (hr)	Drug Alone	Drug + Starch	Drug + T. indica	Drug + Na/K Salt
0.5	13.28	13.18	24.71	10.50
1	37.14	36.74	68.11	14.01
2	43.41	43.09	61.28	22.22
3	32.05	32.11	34.66	11.39
4	26.11	20.72	27.67	6.71
5	19.32	14.17	20.11	4.42
6	15.91	12.78	18.34	0.21

(1) Name of Volunteer:- Ss
Age:- 19
Weight:- 52kg
Sex:- Male

Drug:- Aspirin
Dose:- 600mg

SALIVA ASPIRIN CONCENTRATIONS (ug/ml)

Time (hr)	Drug Alone	Drug + Starch	Drug + T. indica	Drug + Na/K Salt
0.5	0.34	0.32	1.06	0.16
1	0.17	0.16	0.73	0.11
2	0.10	0.09	0.54	0.09
3	0.07	0.07	0.34	0.07
4	0.06	0.04	0.11	0.05
5	0.04	0.03	0.09	0.04
6	0.02	0.02	0.06	0.02

SALIVA SALICYLIC ACID CONCENTRATIONS (ug/ml)

Time (hr)	Drug Alone	Drug + Starch	Drug T.indica	Drug + Na/K Salt
0.5	4.23	4.21	7.64	3.38
1	10.98	10.48	21.11	3.40
2	14.01	13.74	18.42	6.88
3	10.04	9.97	10.72	3.53
4	8.11	7.98	5.40	2.10
5	5.79	5.11	3.15	1.21
6	4.61	4.16	2.58	1.05

(2) Name of Volunteer:- Ys
Age:- 21
Weight:- 61kg
Sex:- Male

Drug:- Aspirin
Dose:- 600mg

PLASMA ASPIRIN CONCENTRATIONS (ug/ml)

Time (hr)	Drug Alone	Drug + Starch	Drug + T.indica	Drug + Na/K Salt
0.5	9.89	9.01	27.76	0.79
1	5.44	5.09	24.84	0.63
2	3.17	2.21	21.11	0.46
3	0.98	0.96	14.98	0.28
4	0.41	0.35	9.01	0.09
5	0.22	0.11	5.72	0.05
6	0.10	0.06	0.89	0.03

PLASMA SALICYLIC ACID CONCENTRATIONS (ug/ml)

Time (hr)	Drug Alone	Drug + Starch	Drug + T.indica	Drug + Na/K Salt
0.5	12.98	12.59	25.01	11.01
1	36.87	35.91	68.12	13.94
2	43.28	43.46	62.07	20.69
3	31.74	31.52	34.28	10.11
4	26.03	20.78	26.91	7.01
5	19.11	15.09	20.18	3.90
6	15.28	12.59	17.99	0.42

(2) Name of Volunteer:- Ys
Age:- 21
Weight:- 61kg
Sex:- Male

Drug:- Aspirin
Dose:- 600mg

SALIVA ASPIRIN CONCENTRATIONS (ug/ml)

Time (hr)	Drug Alone	Drug + Starch	Drug + T.indica	Drug + Na/K Salt
0.5	0.38	0.32	1.11	0.16
1	0.16	0.16	1.03	0.11
2	0.10	0.09	0.74	0,09
3	0.06	0.07	0.41	0.06
4	0.05	0.06	0.10	0.04
5	0.03	0.04	0.07	0.02
6	0.02	0.02	0.03	0.02

SALIVA SALICYLIC ACID CONCENTRATIONS (ug/ml)

Time (hr)	Drug Alone	Drug + Starch	Drug + T.indica	Drug + Na/K Salt
0.5	4.71	4.21	7.64	3.24
1	11.22	11.28	21.10	3.40
2	13.54	14.01	18.87	6.87
3	10.04	10.19	10.72	3.52
4	8.04	8.17	5.45	2.10
5	5.97	5.11	3.15	1.37
6	4.61	4.16	2.58	1.05

(3) Name of Volunteer:- Zs
Age:- 24
Weight:- 62kg
Sex:- Male

Drug:- Aspirin
Dose:- 600mg

PLASMA ASPIRIN CONCENTRATIONS (ug/ml)

Time (hr)	Drug Alone	Drug + Starch	Drug + T.indica	Drug + Na/K Salt
0.5	10.04	8.71	28.61	0.69
1	5.24	4.91	24.79	0.63
2	3.10	2.11	22.21	0.48
3	1.10	0.98	15.41	0.34
4	0.38	0.39	8.78	0.10
5	0.14	0.11	5.80	0.07
6	0.08	0.08	0.66	0.04

PLASMA SALICYLIC ACID CONCENTRATIONS (ug/ml)

Time (hr)	Drug Alone	Drug + Starch	Drug + T.indica	Drug + Na/K Salt
0.5	13.25	12.86	25.09	11.10
1	36.32	37.11	67.37	13.95
2	43.84	42.98	60.19	21.22
3	32.50	32.12	35.10	11.49
4	26.03	21.04	27.66	5.45
5	19.31	14.02	21.13	3.09
6	15.64	7.78	17.74	0.78

(3) Name of Volunteer:- Zs
Age:- 24
Weight:- 62kg
Sex:- Male

Drug:- Aspirin
Dose:- 600mg

SALIVARY ASPIRIN CONCENTRATIONS (ug/ml)

Time (hr)	Drug Alone	Drug + Starch	Drug + T.indica	Drug + Na/K Salt
0.5	0.37	0.38	1.06	0.17
1	0.17	0.16	0.72	0.12
2	0.09	0.10	0.54	0.06
3	0.06	0.06	0.34	0.04
4	0.03	0.04	0.11	0.03
5	0.01	0.02	0.08	0.02
6	0.01	0.01	0.05	0.02

SALIVA SALICYLIC ACID CONCENTRATIONS (ug/ml)

Time (hr)	Drug Alone	Drug + Starch	Drug + T.indica	Drug + Na/K Salt
0.5	4.71	4.22	7.66	3.24
1	11.22	10.74	21.10	3.40
2	13.55	14.01	18.91	6.88
3	10.01	10.19	10.27	3.52
4	8.04	8.17	5.45	2.10
5	5.97	5.11	3.15	1.36
6	4.61	4.16	2.58	1.05

(4) Name of Volunteer:- As Drug:- Aspirin
Age:- 24 Dose:- 600mg
Weight:- 64kg
Sex:- Male

PLASMA ASPIRIN CONCENTRATIONS (ug/ml)

Time (hr)	Drug Alone	Drug + Starch	Drug + T.indica	Drug + Na/K Salt
0.5	8.97	8.52	29.28	0.77
1	4.93	4.11	24.44	0.63
2	3.44	2.27	22.27	0.45
3	0.97	0.84	14.96	0.27
4	0.51	0.37	8.11	0.11
5	0.31	0.14	4.92	0.06
6	0.09	0.05	0.99	0.03

PLASMA SALICYLIC ACID CONCENTRATIONS (ug/ml)

Time (hr)	Drug Alone	Drug + Starch	Drug + T.indica	Drug + Na/K Salt
0.5	14.01	14.04	22.94	11.50
1	37.41	36.72	68.12	13.02
2	42.05	42.11	60.67	21.42
3	33.32	31.24	35.11	10.39
4	25.85	20.78	26.66	6.01
5	19.39	14.42	20.72	4.22
6	15.24	12.81	18.06	0.22

(4) Name of Volunteer:- As
Age:- 24
Weight:- 64kg
Sex:- Male

Drug:- Aspirin
Dose:- 600mg

SALIVA ASPIRIN CONCENTRATIONS (ug/ml)

Time (hr)	Drug Alone	Drug + Starch	Drug + T.indica	Drug + Na/K Salt
0.5	0.35	0.36	1.16	0.17
1	0.16	0.15	0.92	0.11
2	0.10	0.09	0.62	0.08
3	0.07	0.06	0.41	0.06
4	0.04	0.04	0.10	0.03
5	0.03	0.03	0.07	0.02
6	0.01	0.01	0.04	0.02

SALIVA SALICYLIC ACID CONCENTRATIONS (ug/ml)

Time (hr)	Drug Alone	Drug + Starch	Drug + T.indica	Drug + Na/K Salt
0.5	4.62	4.71	8.10	3.21
1	11.01	10.74	22.23	3.98
2	14.23	14.24	18.48	6.90
3	10.94	9.95	10.11	3.52
4	8.11	8.10	5.48	2.10
5	5.83	5.11	3.14	1.36
6	4.23	4.21	2.59	1.15

(5) Name of Volunteer:- Ws
Age:- 22
Weight:- 64kg
Sex:- Male

Drug:- Aspirin
Dose:- 600mg

PLASMA ASPIRIN CONCENTRATIONS (ug/ml)

Time (hr)	Drug Alone	Drug + Starch	Drug + T.indica	Drug + Na/K Salt
0.5	10.18	9.48	28.59	0.78
1	5.01	5.21	24.98	0.66
2	3.41	2.21	22.12	0.47
3	1.02	1.11	15.41	0.34
4	0.45	0.37	8.99	0.09
5	0.24	0.14	5.28	0.05
6	0.09	0.04	0.70	0.04

PLASMA SALICYLIC ACID CONCENTRATIONS (ug/ml)

Time (hr)	Drug Alone	Drug + Starch	Drug + T.indica	Drug + Na/K Salt
0.5	13.49	13.87	24.69	10.55
1	37.32	36.11	68.69	14.02
2	43.85	42.29	61.04	22.71
3	33.50	31.12	33.18	11.22
4	26.24	20.04	27.34	6.45
5	19.21	14.01	20.18	4.07
6	15.81	12.60	17.98	0.52

(5) Name of Volunteer:- Ws
Age:- 22
Weight:- 64kg
Sex:- Male

Drug:- Aspirin
Dose:- 600mg

SALIVA ASPIRIN CONCENTRATIONS (ug/ml)

Time (hr)	Drug Alone	Drug + Starch	Drug + T.indica	Drug + Na/K Salt
0.5	0.39	0.32	1.06	0.16
1	0.17	0.16	0.73	0.10
2	0.10	0.09	0.54	0.09
3	0.07	0.07	0.34	0.07
4	0.05	0.04	0.11	0.05
5	0.03	0.03	0.09	0.09
6	0.01	0.02	0.06	0.02

SALIVA SALICYLIC ACID CONCENTRATIONS (ug/ml)

Time (hr)	Drug Alone	Drug + Starch	Drug + T.indica	Drug + Na/K Salt
0.5	4.22	3.97	7.46	3.24
1	9.43	10.48	21.02	3.40
2	15.31	14.24	18.91	6.95
3	10.99	9.28	10.72	3.52
4	8.01	8.10	5.45	2.09
5	5.83	5.12	3.01	1.36
6	4.22	4.02	2.54	1.05

(6) Name of Volunteer:- Ns
Age:- 24
Weight:- 65kg
Sex:- Male

Drug:- Aspirin
Dose:- 600mg

PLASMA ASPIRIN CONCENTRATIONS (ug/ml)

Time (hr)	Drug Alone	Drug + Starch	Drug + T.indica	Drug + Na/K Salt
0.5	10.13	9.43	28.72	0.77
1	5.23	5.12	24.19	0.65
2	3.04	2.07	22.67	0.48
3	0.97	0.84	15.44	0.32
4	0.47	0.37	8.81	0.12
5	0.11	0.13	5.40	0.07
6	0.07	0.04	0.98	0.05

PLASMA SALICYLIC ACID CONCENTRATIONS (ug/ml)

Time (hr)	Drug Alone	Drug + Starch	Drug + T.indica	Drug + Na/K Salt
0.5	13.22	13.22	25.11	10.50
1	37.21	37.21	68.61	13.59
2	43.90	42.17	61.92	22.78
3	31.31	32.04	34.13	11.94
4	25.64	21.24	28.47	6.45
5	19.40	15.19	20.78	3.09
6	15.61	12.48	17.77	0.29

(6) Name of Volunteer:- Ns
Age:- 24
Weight:- 65kg
Sex:- Male

Drug:- Aspirin
Dose:- 600mg

SALIVA ASPIRIN CONCENTRATIONS (ug/ml)

Time (hr)	Drug Alone	Drug + Starch	Drug + T.indica	Drug + Na/K Salt
0.5	0.34	0.32	1.11	0.18
1	0.17	0.16	1.04	0.12
2	0.09	0.11	0.84	0.10
3	0.07	0.08	0.62	0.07
4	0.05	0.06	0.41	0.06
5	0.04	0.04	0.11	0.03
6	0.02	0.02	0.04	0.02

SALIVA SALICYLIC ACID CONCENTRATIONS (ug/ml)

Time (hr)	Drug Alone	Drug + Starch	Drug + T.indica	Drug + Na/K Salt
0.5	4.69	4.54	7.21	3.21
1	11.12	10.24	21.38	3.38
2	14.09	14.02	19.01	6.48
3	10.91	9.95	11.00	3.52
4	8.03	7.98	5.45	2.11
5	5.48	5.11	3.15	1.32
6	4.21	4.24	2.21	1.14

APPENDICE 3

COMPARISON BETWEEN THE VALUES OF CONCENTRATION OF ASPIRIN (ASA) AND SALICYLIC ACID (SA) IN SUBJECT Ss USING HPLC AND SPECTROPHOTOMETRIC METHODS OF ANALYSIS.

DRUG ALONE

Time (hr)	HPLC		U.V. SPECTRO.	
	SA	ASA	SA	ASA
0.5	22.17	15.28	13.28	10.22
1	42.28	11.11	37.14	5.18
2	65.05	7.02	43.41	3.14
3	43.81	5.11	32.05	1.10
4	31.02	4.16	26.11	0.34
5	16.95	1.09	19.32	0.12

DRUG + T. INDICA

Time (hr)	HPLC		U.V. SPECTRO.	
	SA	ASA	SA	ASA
0.5	43.86	38.18	24.71	28.72
1	120.01	26.23	68.11	24.91
2	83.92	21.21	61.28	22.62
3	50.81	14.11	34.66	15.44
4	39.00	12.14	27.67	8.18
5	24.68	7.38	20.11	5.67

PHARMACOKINETIC PARAMETERS OF ASPIRIN DERIVED FROM THE MEAN PLASMA CONCENTRATION DATA AFTER A SINGLE ORAL INGESTION OF ASPIRIN UNDER TWO CONDITIONS.

PARAMETERS	DRUG ALONE	DRUG +T.INDICA	PROBABILITY t. TEST
Lag time (min)	0.10+0.02	0.10+0.23	$P > 0.1$
Absorption Rate Constant Ka (hr)	1.098+0.12	4.880+0.02	$P < 0.05$
Time to Peak T max (min)	30.0+0.17	30.0+0.08	$P > 0.1$
Absorption ½life $t_{\frac{1}{2}}$&	0.171+0.38	0.142+0.72	$P < 0.05$
Elimination Rate Constant Ke(hr)	0.638+0.22	0.463+0.72	$P < 0.05$
Elimination ½life	1.041+0.12	1.499+0.44	$P < 0.05$
Max. Concentration Cmax (mg/ml)	10.04+0.11	28.62+0.21	$P < 0.05$
AUC (0-6hrs) Ug/ml/hr	17.98+0.11	38.14+0.21	$P < 0.05$

PHARMACOKINETIC PARAMETERS OF ASPIRIN DERIVED FROM THE MEAN PLASMA CONCENTRATION DATA AFTER A SINGLE ORAL INGESTION OF ASPIRIN UNDER TWO CONDITIONS.

PARAMETERS	DRUG ALONE	DRUG + NA/K-SAIL	PROBABILITY T-TEST
Lag time (min)	0.10+0.02	0.20+0.32	$P < 0.05$
Absorption Rate Constant ka(hr)	1.098+0.12	1.009+0.41	$P < 0.05$
Time to Peak tmax (min)	30.0+0.17	30.0+0.01	$P > 0.05$
Absorption $\frac{1}{2}$life $t_{\frac{1}{2}}$ & (hr)	0.171+0.38	0.631+0.75	$P < 0.05$
Elimination Rate Constant Ke(hr)	0.638+0.22	0.748+0.11	$P < 0.05$
Elimination $\frac{1}{2}$ life (hr)	1.041=0.12	0.926+0.21	$P < 0.05$
Max Concentration Cmax (mg/ml)	10.04+0.11	0.78+011	$P < 0.05$
AUC (0-6hr) Ug/ml/hr)	17.98+0.11	4.48+0.99	$P < 0.05$