HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF PARACETAMOL, CHLOROQUINE AND PROMETHAZINE IN SOME PHARMACEUTICAL PREPARATIONS

BY

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

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DECLARATION

I hereby solemnly declare that the work reported in this thesis was carried out by me under the supervision of Dr. Abdullahi Mustapha, Head of Pharmaceutical and Medicinal Chemistry Department, and Dr. Ibrahim Abdu-Aguye, Head of Pharmacology and Clinical Pharmacy Department.

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 "HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF PARACETAMOL, CHLOROQUINE AND PROMETHAZINE IN SOME PHARMACEUTICAL PREPARATIONS" by DANLAMI UZAMA meets the regulations governing the award of the degree of MASTER OF SCIENCE of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

Dedicated to my dear parents, brothers and sisters.

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ABSTRACT

A single solvent system was developed under the same conditions for the analysis of paracetamol, chloroquine and promethazine in some pharmaceutical preparations (Maxiquine and Daga-Quine syrups, and Daga-Quine tablets) using High Performance Liquid Chromatography (HPLC) Waters Model 204 fitted with U.V. detector Model 441 with a fixed wavelength (254nm) and Model U6K septumless injector. A pump Model 510 at a flow rate of 1.5ml/min, with a pressure of 2000 PSI. The column used was a uBonda-Pak (Radial-Pak) 0-18 column (l5cm x 3.9mm I.D.) with a mobile phase consisting of acetonitrile:sodium dihydrogen phosphate : triethylamine (60:39:l), pH 7.2 at an ambient temperature. The retention times (tR) of paracetamol, chloroquine and promethazine were 3.0, 5.8, and 12.0min. respectively, while that of the internal standards were 4.2min for acetoaceta-nilide and 8.0 min. for papaverine, respectively.

The extraction recoveries were 97% for paracetamol, and 90% for chloroquine and promethazine. Calibration curves were linear from 20 to 100 ug/ml (r = 0.990) for paracetamol, and 100 to 500 ug/ml (r = 0.999 and 0.990) for chloroquine and promethazine respectively.

The Maxiquine syrup contained 96%, 95% and 101% of paracetamol, chloroquine and promethazine respectively. The Daga-Quine syrup contained 96.7% and 96% of

paracetamol and chloroquine respectively, while the Daga-Quine tablets contained 101% and 103% of paracetamol and chloroquine respectively. The precision of the method was 1.7% (n = 5) within run and 1.9% (n = 5) for day-to-day run.

Spectrophotometric method employed for the determination of chloroquine, paracetamol and promethazine in Maxiquine syrup gave comparable results with the HPLC method (100%, 100% and 97% of chloroquine, paracetamol and promethazine respectively).

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ABBREVIATIONS

L.C.	=	Liquid Chromatography
G.C.	=	Gas Chromatography
H.P.L.C.	=	High-Performance Liquid Chromatography
T.L.C.	=	Thin-Layer Chromatography
L.L.C.	=	Liquid-Liquid Chromatography
L.S.C.	=	Liquid-Solid Chromatography
I.V.	=	Intravenous
ug/ml	=	Microgramme per millilitre
mg	=	Milligramme
uL	=	Microlitre
M	=	Molarity
kg	=	Kilogramme
ng/ml	=	Nanogramme per millilitre
Min.	=	Minute
g	=	gramme
I.D.	=	Internal Diameter
HCl	=	Hydrochloride
PCM	=	Paracetamol
PMZ	=	Promethazine
CQ	=	Chloroquine
B.P.	=	British Pharmacopoeia
U.S.P.	=	United States Pharmacopoeia
U.V.	=	Ultraviolet
hr	=	Hour
PSI	=	Pounds per square inch.
cm	=	Centimetre
mm	=	Millimetre
NaH_2PO_4	=	Sodium dihydrogen phosphate

CHAPTER 1

INTRODUCTION

1.1 METHODS OF PHARMACEUTICAL ANALYSIS

For an overall quality assessment of a drug, various analytical methods are applied like: statistical methods, physicochemical methods, biological methods and biopharmaceutical methods.

The physicochemical methods comprise

1. Titrimetry and gravimetry
2. Electroanalytical techniques
3. Chromatography
4. Spectrophotometry (U.V./Visible, I.R., N.M.R)
5. Mass spectroscopy
6. Atomic Absorption Spectroscopy and flame photometry
7. Refractometry and Polarimetry.

The analyst may use any of these methods provided the desired results are obtained. Though the method he may adopt to may depend on the instrument available to him.

In making a choice for an analytical method, the criteria to bear in mind are: Selectivity, Sensitivity, Reliability and Convenience.

1.2 CHROMATOGRAPHY

The physicochemical method chosen for this work is chromatography. Chromatography is a separation technique used in the analysis of the components of a mixture.

This is done by a continuous distribution of the components between the mobile and the stationary phases. An example of the earliest reported liquid chromatography is the separation of the pigments of green leaves in a column filled with powdered chalk. This is attributed to Tswett, a chemist in Russia, 1903. Reichstein in 1930 adopted this method for the isolation of natural products.

In 1941, Martin and Synge made a significant advancement in the technique by working on liquid partition chromatography. Moore and Stein in 1948 reported the use of ion-exchange chromatography for the separation of amino acids. Not until 1960, the technique practised involved using a fairly large column containing a packed bed of adsorbent, most commonly silica gel or alumina, coated with a stationary liquid for partition applications. The separation was carried out by percolating liquid through the bed under the force of gravity. The progress of the separation was most often monitored by collecting fractions of the column effluent and subsequently performing some independent method of quantitation. Because of the limitations of the existing technique, a number of closely related methods have been developed for separating mixtures of chemical substances in the liquid phase.

The chromatographic technique consists of:

Paper chromatography

Column chromatography

Thin-layer chromatography

Ion-exchange chromatography

Exclusion chromatography

Gas chromatography and

High performance liquid chromatography.

Paper chromatography and thin-layer chromatography may be considered as semi-micro techniques involving partition or adsorption mechanisms, respectively, capable of producing fairly good resolution of small quantities of sample but lacking, except in specialised instances, an easy method of obtaining quantitative results. Although separations performed by both of these methods may often take less than one hour, particularly in the case of TLC, measurement of the area or density of the spots must be performed after the completion of the separation. The step consumes time and seldom is a precision better than 5% achieved.

Paper chromatography and TLC are not suitable for large scale separations. This is because there will be a progressive deterioration of the separating power of the system as a result of trying to increase capacity by increasing the thickness of the paper or adsorbent layer.

For column chromatography, in certain preparative applications of this method, the column has to be dissected and the sample components extracted from the adsorbent bed in order to recover the sample. Clearly this approach does not lend itself to repetitive analysis as the column packing must be renewed for each sample.

For these and other reasons, GC was accepted for the analysis of suitable organic and biological samples. GC is not the ultimate in analytical methods, however, for the sample to be analyzable the sample must be both volatilizable and thermally stable in the vapour state. It has been estimated that only 20% of all organic compounds are suitable for GC analysis without derivatization.

As there was the need for a liquid phase separation system, just like the gas chromatography, the liquid chromatography evolved.

This method is a highly sophisticated analytical technique which is capable of rapidly separating complex chemical mixtures and providing simultaneously a continuous record of the separation from which the quality of the separation and when suitably calibrated, a quantitative assessment of the composition of the original sample may be deduced.

Separations which may have taken hours or days or may not have been possible with the previous techniques will be achieved with LC in few minutes. LC has much to offer in preparative chromatography, relative to GC. This is because in LC, the sample does not have to be vaporised when introduced into the column and conversely does not have to be condensed from the vapour phase in order to collect the sample after separation.

With the modern LC, a precision of bettern than 1% has been reported. This suggests that the technique is directly suitable for many assays of commercial importance.

The precision of the method does not normally vary a great deal from sample to sample unlike the GC. This could be because of the vaporisation of the sample, with attendant possibilities of decomposition or variations in the rate of evaporation which is seen with GC, but which is absent in the liquid phase.

1.3 HPLC AND MODE OF OPERATIONS

The type of chromatography where a liquid mobile phase is forced through the column (stationary phase) under pressure is known as High Performance Liquid Chromatography.

Separation occurs at the stationary phase. The stationary phase determines the mode of operation. When the stationary phase is a liquid, the mode is a liquid-liquid chromatography (LLC). When the stationary phase is a solid, the mode is a liquid-solid chromatography (LSC). When the stationary phase is an ion-exchange resin, the mode is an ion-exchange chromatography. When the stationary phase is a porous polymer, the mode is an exclusion (gel permeation or gel filtration) chromatography.

There are four basic types of operational modes

Liquid-solid (adsorption)

Liquid-liquid (partition)

Ion-exchange, and

Exclusion, or gel permeation.

The liquid-solid chromatography is adsorption chromatography. The solute is reversibly adsorbed by a solid stationary surface.

Polar solids such as alumina and silica gel are normally used as the stationary phase. The mobile phase is a relatively non-polar solvent such as benzene, hexane or chloroform.

The liquid-liquid chromatography is partition chromatography, with a liquid stationary phase coated on an inert solid support. The stationary phase must be immiscible in the mobile phase. The separation is based on the partition behaviour or relative solubility of the solute in the two phase.

In general, the stationary phases are relatively polar, whereas the mobile phases are relatively non-polar. Water, ethylene glycol, and β,β'-oxydipropionitrile have been used as stationary phases, whereas hexane, benzene and chloroform have been used as mobile phases.

Ion-exchange chromatography is a subgroup of the liquid-solid mode; where the polar solute dissolved in a polar mobile phase, such as water or alcohol, is

passed over a bed of solid ion exchange material. The ionic solute distributes itself between the mobile and stationary phases by exchanging ions with a counter ion of the solid electrolyte as it passes through the column. Each solute will have a different affinity for the ion-exchange material if separation is to occur and will pass through the column at a different rate.

Amino acids, nucleic acids, as well as many inorganic metallic compounds are separated by this mode.

The exclusion chromatography involves the use of a uniform, highly porous, non-ionic or resin gel as a stationary phase to separate materials according to their molecular size. When a mixture of solute is passed through the resin, the smaller particles can migrate into the pores whereas the larger particles cannot penetrate as deeply, if at all. The net result of this process is that the larger molecules will have a shorter retention time than the smaller ones, since they follow a more direct path through the column.

In the pharmaceutical industry, the HPLC is widely applied due to the versatility of the technique, which arises from the wide choice of separation modes coupled with the growing range and capability of the equipment modules.

The HPLC is applied in qualitative analysis to compare the retention times of samples with those of authentic samples, to help in the identification of a compound.

It is also applied in quantitative analysis. HPLC finds its use in clinical chemistry, forensic science and toxicology.

1.4 IMPORTANCE OF ASSAY

Drug assay is of great importance, more especially in quality assurance. In drug product quality assurance, the ultimate goals are:

1. To assure that the drug product contains the amount of active drug stated on the label, within limits.
2. To assure that the variation of drug levels between dosage units is minimized.
3. To assure that each ingredient used to formulate the finished dosage form is of suitable purity, generally the highest practical purity.
4. To assure that each finished dosage form is of the highest possible purity, that is, that no contaminants have entered during the manufacturing process.
5. To assure that the ingredients in the finished dosage form are stable, both individually and in the presence of each other, under normal conditions.
6. To assure that the finished product is therapeutically effective in the patient.

1.5 DEVELOPING A SOLVENT SYSTEM

Since the solvent system developed for these drugs was unknown, the work was a trial and error work. The combination of the solvents and their ratios was by trial and error.

The solvents were selected based on some rules given on the selection of components of the mobile phase. The seven rules kept in mind in the selection of components of the mobile phase are:

1. The sample compounds should be soluble in the binary mobile phase. The components of the mobile phase should be completely miscible and should not react with one another, with the sample, or with the column material over the whole concentration range used for elution.

2. The two components of the binary mobile phase should be chosen so as to cover the whole elution strength range necessary to elute all sample compounds.

3. The components of the mobile phase should not interfere with the detectors used.

4. The components of the mobile phase with a low viscosity should be preferred, because they yield better column efficiency and lower column back pressure.

5. The components of the mobile phase should be available in sufficiently pure grade.

6. If reciprocating pumps are used, the components of the mobile phase should not be very volatile (such as n-pentane or diethylether), otherwise they are evaporized partially by sunction pressure and by the heat evolved in the pump.

7. Last but not the least, the prevention of potential explosions and other health hazards should be considered as well as economic aspects when selecting appropriate solvents for gradient-elution chromatography.

They were also mixed based on solvent miscibility and how to use miscibility numbers (M-Numbers) rules:

Miscibility numbers are used to predict the miscibility of a liquid with one of the standard solvents.

To predict the miscibility of the two liquids, subtract the smaller value from the larger.

- If the difference between the two M-numbers equals 15 or less, the two liquids are miscible in all proportions at 15°C.
- A difference of 16 units shows that the two liquids possess a critical solution temperature between 25 and 75°C, with 50°C as the optimal temperature.
- If the difference equals 17 or more, the two liquids are immiscible or their critical solution temperature is above 75°C.

Interaction between the molecules of the two liquids can sometimes change the expected degree of miscibility. For example, ethers or tertiary amines show unpredicted miscibility with hydroxylic solvents due to hydrogen bonding. Usually strong hydrogen bonding is also responsible for the miscibility of long chain alcohols or carboxylic acids with standard solvents of low M-number. Conversely, they show anomalous immiscibility with aprotic solvents of low M-number.

Some solvents prove immiscible with solvents of both ends of the lipophilicity scale. These solvents receive a dual M-number. The first number, always lower than 16, indicates the degree of miscibility with highly lipophilic solvents. The second number applies to the apposite end of the scale. A large difference between these two numbers indicates a limited range of miscibility. For example, some fluorocarbons are immiscible with all the standard solvents and have M-numbers of 0,32. Two liquids with dual M-numbers are usually miscible with each other.

1.6 MOBILE PHASES

Mobile phase is that phase in any chromatographic system which carries the solutes along and over the column material or surface on which the separation is being carried out.

For gas chromatography the mobile phase is commonly an inert gas such as helium, argon or nitrogen, whilst in liquid chromatography it may be one of many solvents or mixtures of solvents, depending upon the form of the separation.

The term polarity has for many years been the yardstick of most chemists, particularly chromatographers, for the qualitative classification of organic solvents and samples.

Solvents such as low-molecular weight alcohols, water, acids and bases are considered to be highly polar, whereas normal paraffins, i.e n-pentane and n-hexane, are regarded as non-polar.

The polarity of the solvent system relative to that of the stationary phase determines the phase in which the liquid-liquid chromatography is being run. It is normal phase when the mobile phase is less polar than the stationary phase. The technique is referred to as reverse phase when the mobile phase is more polar than the stationary phase.

When the C_{18} or C_8 column is being used, a polar solvent system is indicated. Water (or buffer) mixed with methanol, acetonitrile, dioxan or tetrahydrofuran is the most popular for this purpose.

In liquid chromatography, the retention volumes and the resolution of sample compounds with very different

retention can be widely controlled by the appropriate selection of the mobile phase so as to achieve adequate chromatographic separation within a reasonable time.

The reliability and precision of quantitative results and identification of compounds in both isocratic and gradient-elution chromatography depend on the reproducibility and accuracy of three variables: the composition and the flow rate of the mobile phase and the temperature.

1.7 PHARMACOKINETICS

Pharmacokinetics, in mathematical terms, describe the relationship between the absorption, distribution, metabolism, and excretion of a drug, and its effects and duration of action.

Clinical pharmacokinetics is the application of pharmacokinetic studies to clinical practice and to the safe and effective therapeutic management of the individual patient.

Absorption is defined as the process by which a drug proceeds from the site of administration to the site of measurement within the body.

Distribution is the process of reversible transfer of a drug to and from the site of measurement, usually the blood or plasma. Any drug that leaves the site of measurement and does not return has undergone elimination, not distribution.

Elimination is the irreversible loss of drug from the site of measurement. Elimination occurs by two processes, metabolism and excretion.

Metabolism is the conversion of one chemical species to another.

Excretion is the irreversible loss of the chemically unchanged drug.

When a drug is administered, it is transferred in solution from the absorption site to the blood.

It is then distributed throughout the body and eliminated by metabolism and excretion. The amount of drug at an absorption site such as the small intestine is continuously reduced as drug is transferred from the dosage form into the blood, at the same time there is a continuous increase in the amount of drug converted to metabolites and in the amount of drug excreted.

The rate of elimination of a drug depends on the values of both total clearance, Cl, and plasma drug concentration, C, that is

$$\text{Rate of elimination} = Cl \cdot C$$

$$C = \frac{Ab}{V}$$

where Ab - the amount of drug in the body.

V - volume into which the drug appears to distribute.

C - plasma drug concentration.

Thus rate of elimination

$$= \frac{Cl}{V} \cdot Ab$$

The apparent volume of distribution, V, is obtained by dividing the amount of drug in the body by the plasma concentration.

$\log_{10} C = \log_{10} Co - Kst$

where ks is the slope of the line of the plot of $\log_{10} C$ against time, t.

Co is the concentration one would determine from this equation at zero time. The negative sign varies because the concentration is declining with time.

$$Dose = V.Co$$

In practice, Co is estimated by extrapolating the straight line back to zero time.

Dose is the amount of drug administered.

Volume of distribution, $V = \frac{Dose}{Co}$

Rate of elimination = Clearance C

= K.V.C.

V - volume of distribution.

C - plasma concentration.

Clearance = K.V.

Elimination half-life is the time taken for the plasma concentration, as well as the amount of the drug in the body, to fall by one-half.

$$t_{\frac{1}{2}} = \frac{0.693}{k}$$

$$t_{\frac{1}{2}} = 0.\frac{693 \times \text{Volume of distribution}}{\text{Clearance}}$$

The total amount of drug eventually eliminated, which for an intravenous dose equals the dose administered is assessed by adding up or integrating the amount eliminated in each time interval from time zero to time infinity, therefore,

Amount eliminated = Clearance. C. dt

where the term C.dt is the correspondence small area under the plasma drug concentration time curve.

Dose = Clearance x Area.

The best method for calculating the volume of distribution is by dividing the clearance by the elimination rate constant.

$$V = \frac{Cl}{k} = \frac{Dose}{Area.\ k}$$

Unlike the method of extrapolation, the present method of estimating V is not restricted to the intravenous bolus situation but can be obtained under a variety of conditions.

The oral absorption of drugs in man often approximates first-order kinetics. The same holds true for the absorption of drugs from many other extravascular sites including subcutaneous tissue and muscle.

Amount of drug absorbed equals the total amount estimated.

F. Dose = Clearance x Area

F - availability

$$F = \frac{Clearance \times Area}{Dose}$$

CHAPTER 2

LITERATURE REVIEW

2.1 HISTORY AND CHEMISTRY OF CHLOROQUINE, PARACETAMOL AND PROMETHAZINE

CHLOROQUINE

History and Chemistry

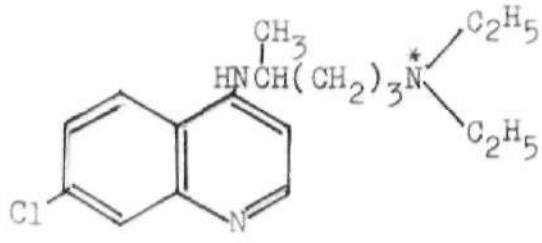
A great interest was shown by German researchers in malarial disease treatment. Ehrlich discovery in the use of methylene blue for malarial treatment gave limited success. This prompted the researchers to improve on it though they produced compounds which were of no practical value. They applied the experience they gained to other ring systems, and this led them to the discovery of pamaquine. This was reported in 1924 to be an active antimalarial drug. Pamaquine was first accepted with enthusiasm, but it was later found to be too toxic and inferior to quinine. By 1930, the drug was abandoned. In 1933, Mauss and Mietzsch prepared Quinacrine (Afabrine). Quinacrine was found to be a very active antimalarial with low toxicity.

Chemists at the Bayer Company in Germany prepared Chloroquine in 1939. Chloroquine was found to be superior to quinacrine for malarial treatment. Before the second world war, chloroquine has been used as an antimalarial. Chloroquine has been found to be highly active against all species of malarial parasites like: Plasmodium

vivax, plasmodium ovale, plasmodium malariae and plasmodium falciparium (Bruce-Chewatt, et al., 1986).

Chloroquine is a 4-aminoquinoline. For more than 30 years now, it has been used as the most common antimalarial drug in all developing countries. It is given both as a therapeutic and as a prophylactic agent, in hospitals as well as in the field, by doctors and other health personnel.

Structure



7-Chloro-4-(4'-diethylamino-1'-methylbutyl-amino)-quinoline.

Chloroquine phosphate has a molecular formula

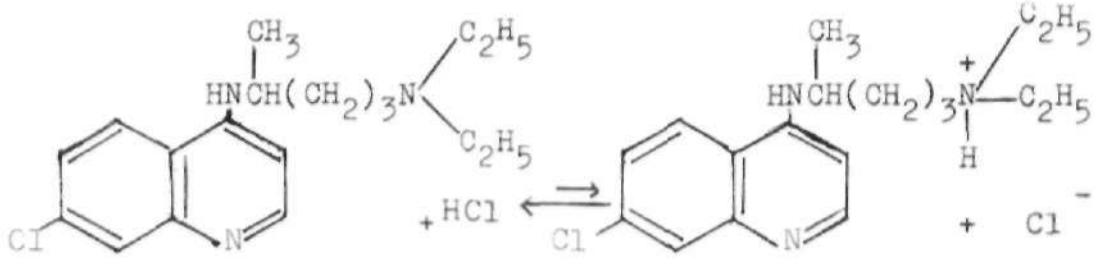
$$C_{18}H_{26}ClN_3,\ 2H_3PO_4.$$

It is a white, odourless powder which slowly discolours on exposure to light. It is soluble 1 in 4 of water; very slightly soluble or practically insoluble in alcohol; insoluble in chloroform and ether. A 10% solution in water has a pH of 3.5 to 4.5. Solutions are sterilised by autoclaving.

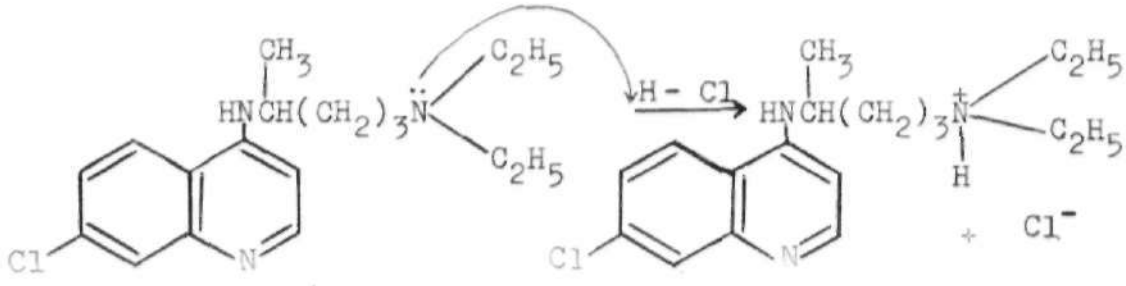
The presence of $\overset{*}{N}$ atom in the chloroquine drug, which contains a lone pair of electrons ($\ddot{N}$), makes the drug to act as a base and as a nucleophile in reactions. The $\overset{*}{N}$ atom is similar to that of an amine group, and their

reactions are similar. It acts as a nucleophile by donating its electron pair to an electrophilic reagent.

When the drug reacts with acid, an equilibrium is established as shown below.

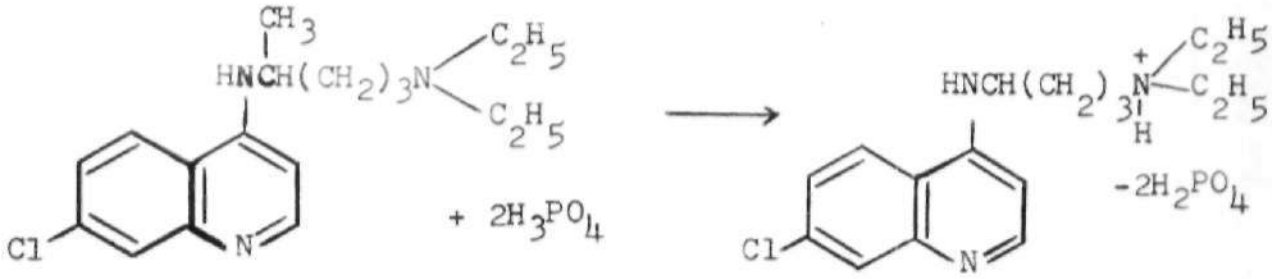


The mechanism of the reaction is



The drug has the tendency to accept a proton from acid. The alkyl groups release electrons, which stabilize the 4-diethylamino ion that results from the acid-base reaction by dispersing their positive charges.

The reaction of this drug with an acid produces a salt. The two salts usually produced are sulphate and phosphate salts. It also exist in hydrochloride salts. The salt form of the drug is more water soluble than the basic form.



Chloroquine Chloroquine phosphate

Clinical Uses

For more than 36 years now, chloroquine has been used as the most common antimalarial drug in all developing countries (Rollo, 1980).

This is largely because it is cheap, safe, widely available and until recently highly efficacious in the treatment of all the species of malarial parasites. It is also used for the treatment of rheumatoid arthritis and other collagen diseases for decades (McChesney and Rothfield, 1964; Stillman, 1981).

Mode of Action

Chloroquine forms molecular complexes with DNA, in which it is postulated that the quinoline ring is intercalated between base pairs of double stranded DNA involving electrostatic forces, while the 1,4-diamino-pentyl side chain falls outside the contour of the base pairs and bridges the minor groove of the double helix

by binding electrostatically to DNA phosphates. In this way the electronegative chlorine atom in the 7-position of the quinoline ring is brought into close proximity to the electropositive 2-amino group of guanine, whose presence is critical for chloroquine to interact with DNA. The binding of chloroquine to DNA appears to involve both electrostatic attraction between the positively charged chloroquine molecule and anionic phosphate groups of DNA (weakly reacting sites), and a more specific interaction involving the ring portions of the antimalarials and the nucleotide bases (strongly reacting sites).

DNA replication requires the separation of the two companion strands of the parental double-helical structure; substances that interfere with strand separation should inhibit the replication of DNA. The transcription of RNA from DNA requires the availability of certain base pairs, and constraint of these pairs will hinder the process. Thus the mode of action of chloroquine appears to be an inhibition primarily of DNA replication and secondarily of RNA transcription from DNA, mediated through the inhibition of the DNA and RNA polymerase reactions.

However, the current view of the mode of action of chloroquine is that the chloroquine blood schizontocide concentrates in and raise the pH of acidic vesicles within sensitive malarial parasites. There it interferes with lysosomal degradation of hemoglobin and histological

abnormalities characteristic of the particular drug class; these include rapid chloroquine-induced clumping of pigment or the more delayed and subtel pigment changes produced by arylamino alcohols like Quinine and Mefloquine (Warhurst, 1987).

Beyond the alteration of lysosomal pH, the molecular mechanisms by which this blood schizontocide acts is unknown; however, inhibition of the functions of Ca^{2+} - dependent proteins such as calmodulin has been implicated (Scheibel et al., 1987).

Side Effects and Toxicity

In recommended therapeutic dose, chloroquine is usually well tolerated. Occasionally, abdominal discomfort, nausea, vomiting, headache, dizziness, blurring of vision, mental and physical weakness, fatigue and prurities after a single therapeutic dose, do occur.

Severe adverse reactions like cardiovascular collapse, dyskinesia, neuromuscular disorders and loss of hearing rarely occur, which disappear on stopping the drug administration. Also, effects like skin eruption and itching do occur.

PARACETAMOL

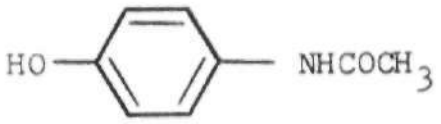
History and Chemistry

The parent member of this group of drugs is Acetanilide. In 1886, acetanilide was introduced as an antipyretic by Calm and Hepp. The drug proved to be

excessively toxic, and this prompted the search for less toxic compounds. With the belief that acetanilide is oxidised in the body to para-aminophenol, para-aminophenol was tried, but the toxicity was not lessened.

Derivatives of the para-aminophenol were also tested, out of which phenacetin (acetophenetidin) was discovered. This was introduced into therapy in 1887. In 1893, Von Mering introduced Acetaminophen (Paracetamol) into medicine. It gained popularity in 1949. It is the major active metabolite of acetanilide and phenacetin. Since 1955, paracetamol has been available in United States without prescription.

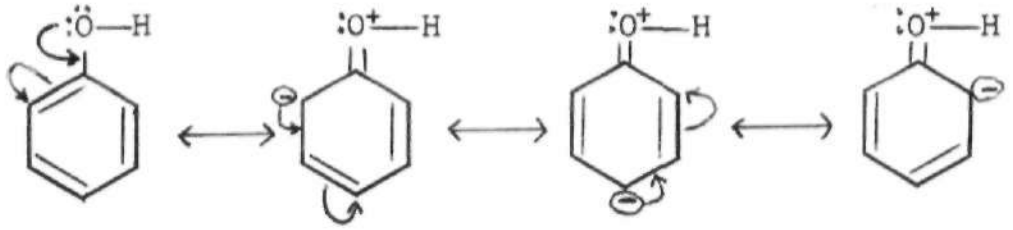
Structure



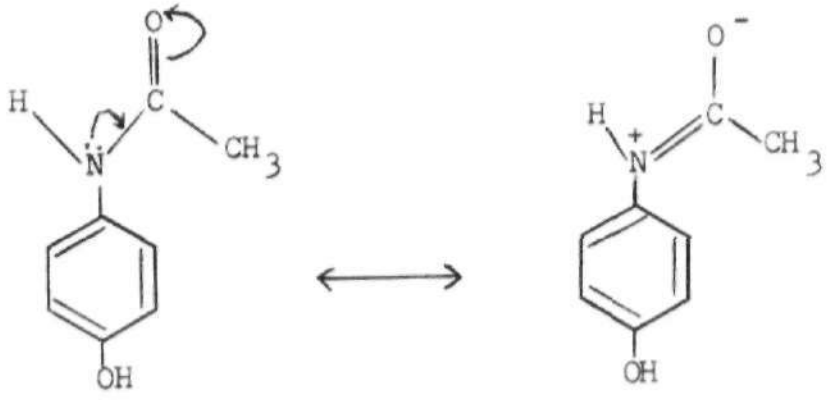
N-acetyl-p-aminophenol

Paracetamol is a white odourless crystalline powder, which has a slight bitter taste. It is soluble 1 in 70 of water, 1 in 20 of boiling water, and 1 in 7 to 10 of alcohols. It is very slightly soluble in chloroform and ether.

Paracetamol has a melting point of 169° to 172°. From the structure of paracetamol, the hydroxyl group that is directly attached to the benzene nucleus makes it to be weakly acidic. As can be seen from the mechanism below, the oxygen atom of the OH group acquires a positive charge because of resonance. This facilitates the release of proton.



The lone pair of electrons on the N-atom in the amine group in the $NHCOCH_3$ side chain, enters into resonance with the carbonyl group. As a result, the resonance with the ring is strongly diminished, and this makes the drug to be a very weak base. The mechanism is shown below.



The aminobenzene structure of the drug is responsible for its antipyretic activity.

Clinical Uses

Paracetamol has analgesic and antipyretic properties. It is an analgesic resembling phenacetin in effect, but less liable to cause side effects in those patients sensitive to phenacetin. Paracetamol as an analgesic, brings about insensibility to pain without loss of consciousness.

As an analgesic, it effectively relieves the pain of arthritis and other skeletal muscular conditions, headache (and it also relieves fever). It may reduce the post traumatic inflammatory response and may be preferrable to acetylsalicyclic acid in bilaterial oral surgery. The drug also has some antiinflammatory and antirheumatic as well.

Mode of Action

The antipyretic analgesic property of paracetamol is obtained by its inhibition of the prostaglandin E generation. It does this by inhibiting the action of the enzyme, prostaglandin synthetase, which is responsible for the biosynthesis of prostaglandin in the brain. By so doing, the fever is reduced. This is because fever is caused by the synthesis of endogenous pyrogens (prostaglandin E) which act upon the thermoregulatory neurones in the hypothalamus to increase the 'set-point' temperature (Parkhouse, 1979).

Side Effects and Toxicity

This is minimal at doses employed for therapy. Haematological reactions, skin rashes and other allergic reactions occur occasionally.

Symptoms of paracetamol over-dosage in the first 24 hours are pallor, nausea, vomiting, anorexia, and abdominal pain. Liver damage may become apparent 12 to 48 hours after ingestion.

For patients with impaired kidney or liver function, paracetamol should be given with care. Also, it should be given with care to patients taking other drugs that effect the liver.

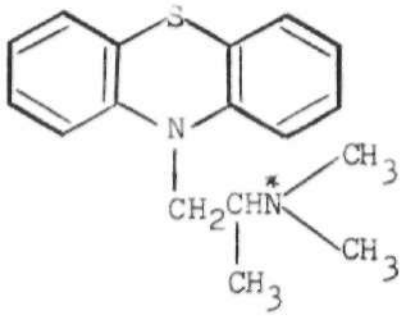
PROMETHAZINE

History and Chemistry

A number of phenothiazine derivatives were synthesized by French researchers during the World War II. Promethazine is a member of the phenothiazine derivatives. A search for effective antimalarials among phenothiazine derivatives and their subsequent testing as antihistaminic agents led to the discovery that the bridged structure, in which the aromatic rings on one of the nitrogen atoms were joined through a sulfur form, had good activity. Promethazine was the most active histamine antagonist in the series. Other drugs discovered initially were toxic, this gave room for further researches for the less toxic ones.

Promethazine has been in clinical practice for more than 30 years (Taylor, et al., 1983). It has been reported to be as much as 7 times more potent than certain other antihistaminics and to have approximately 3 times their duration of action.

Structure



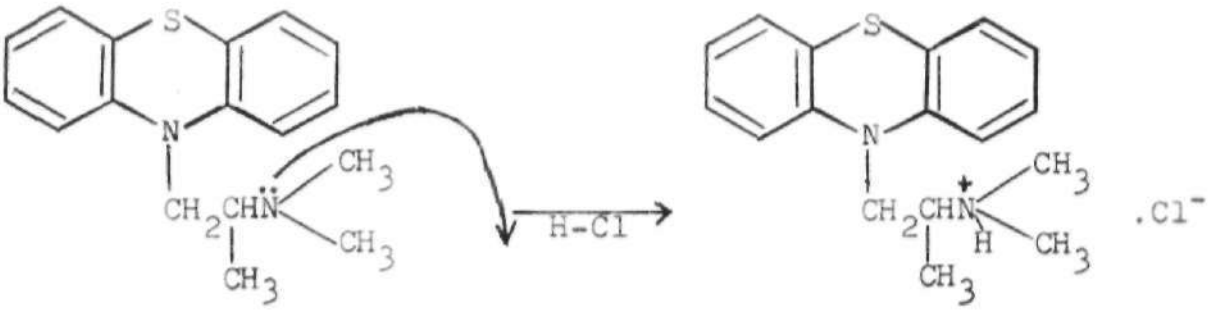
10-(2'-Dimethylaminopropyl)phenothiazine.

Promethazine hydrochloride is a white or faintly yellow almost odourless, crystalline powder. On prolonged exposure to air, it slowly oxidises becoming blue in colour.

It is soluble 1 in 0.6 of water, 1 in 9 of alcohol, and 1 in 2 of chloroform, very soluble in hot dehydrated alcohol, practically insoluble in ether, and ethylacetate. It has a melting point of about 220°C , and boiling point of 190° to 192° at 3mm Hg.

Promethazine is a basic drug. Its basicity is due to the presence of an $\overset{*}{N}$ atom which contains lone pair of electrons one or both of which can be donated to an electrophilic reagent in a reaction. The drug reacts with acids to produce salts. The drugs exists in salt

forms like: promethazine theoclate and promethazine hydrochloride, which are more water soluble than the basic form.



Promethazine Promethazine HCl

Clinical Uses

Promethazine is used as an antihistamine for the symptomatic relief of urticarial lesions, angioneuretic oedema, seasonal rhinitis and conjunctivitis such as hay fever, pollinosis in which it relieves sneezing, rhinorrhoea and itching of the eyes, nose and throat. It is also used for cough, cold and allergic dermatoses such as skin rashes as in acute urticaria, itching, pruritides, atopic dermatitis and contact dermititis. Promethazine is used as an antinausent and antiemetic reducing vomiting. It could also be used as a sedative.

Mode of Action

As an antihistamine, promethazine acts by diminishing, abolishing or preventing the pharmacological effects of histamine in the body. This it does by occupying the receptor site.

The mode of action may be considered as a competition in tissue between the promethazine and histamine for the receptor site.

The combining of the promethazine with the receptor site prevents histamine from exerting its characteristic effect on tissue. Promethazine does not combine with histamine to neutralize its action, nor does it prevent its liberation from cells. Also no indication has been found that this drug activates enzymes which catalyze the breakdown of histamine.

Side Effects and Toxicity

This is minimal at doses employed for therapy or suppression. Occasionally dizziness, hypotension, muscular weakness and incoordination occur. Sedation is the most common, manifesting as slight drowsiness or deep sleep. Gastrointestinal disturbance such as nausea, vomiting, diarrhoea or constipation and epigastric pain also occur. Cases of headache, blurred vision, tinnitus, depression, irritability, nightmares, anorexia, dryness of the mouth and throat have been reported. It rarely causes convulsion and hyperpyrexia in infants.

2.2 PHARMACOKINETICS OF CHLOROQUINE, PARACETAMOL AND PROMETHAZINE

CHLOROQUINE

Chloroquine is readily absorbed from the gastrointestinal tract. The protein binding of chloroquine is about 46 to 70% (mean 61 ± 9%). Binding is mainly to albumin and α_1-acid glycoprotein fractions, and is thought to be an important determinant of chloroquine pharmacokinetic or response (Walker, et al., 1983). Chloroquine accumulates in high concentrations in some tissues, such as the kidneys, liver, lungs, and spleen, and is strongly bound in melanin-containing cells such as those in the eyes and skin.

The elimination of chloroquine from the body is very slow and the drug may persist for a long period in tissues. The main route of excretion is urinary. About 75% is excreted as unchanged drug and about 25% as desethyl-metabolite. At low pH values excretion of chloroquine is increased.

Tulpule and Krishnaswamy (1982) after administering chloroquine (600mg base) orally to healthy adult volunteers with and without food, observed the drug to have enhanced bioavailability and reduced gastric side effects when administered with food. The peak plasma concentrations ranged from 240 to 800 µg/l during fasting, and 510 to 1,437 µg/l with food. Also in their kinetic study of

chloroquine (600mg oral dose) in normal and undernourished subjects, found that the bioavailability and blood concentration of chloroquine were not altered in moderately undernourished adults. In normal subjects considering plasma, the $t_{\frac{1}{2}}$ (h) was 47.3 ± 7.03, the AUC (µg-h/ml) was 23.66 ± 3.824, the clearance was 8.61 ± 1.031 ml/min/kg, while in the undernourished, the $t_{\frac{1}{2}}$ (h) was 45.4 ± 8.84, AUC (µg-h/ml) was 19.01 ± 1.814, and the clearance (ml/min/kg) was 13.09 ± 1.329.

A single dose of 300mg of chloroquine was given as an i.v. infusion, and oral solution as tablets to healthy volunteers and using HPLC the peak plasma chloroquine concentrations were determined to be 10 times higher after i.v. administration compared with oral administration.

The peak plasma concentration after the i.v. dose ranged between 478 to 1200 ng/ml (mean 837), while the peak concentrations after oral solution and tablets were 55 to 94 ng/ml (mean 73) and 56-102 ng/ml (mean 76), respectively.

The time to reach peak was 1.5 to 6 and 1 to 6h respectively (Gustafsson, et al., 1983).

PARACETAMOL

From the gastro-intestinal tract, paracetamol is readily absorbed. Its peak plasma concentrations after ingestion occurs at about 30 minutes to 2 hours. It is metabolished in the liver and excreted in the urine mainly as the glucuronide and sulphate conjugates.

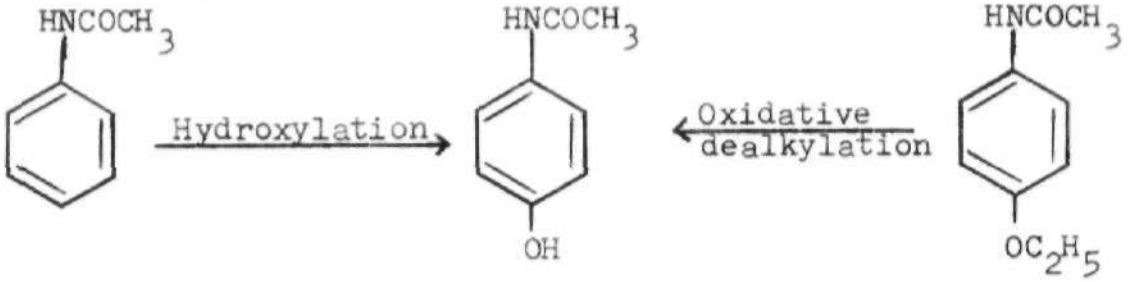


Acetaminophen — Conjugated Acetaminophen

R = Glucuronate (major in man)

R = Sulphate (minor in man)

Less than 5% is excreted as unchanged paracetamol. The major metabolites of phenacetin and acetanilide is paracetamol.



Acetanilide — Acetaminophen — Phenacetin

By mixed function oxidases, in the liver, a minor hydroxylated metabolite is produced, which following paracetamol overdosage accumulates to cause liver damage. This is usually detoxified by conjugation with liver glutathione.

At the usual therapeutic concentration (60 µg/ml) plasma-protein binding is negligible, but with toxic doses up to 43%, it could be bound to plasma proteins (Gazzard, et al., 1973). The elimination half-life varies from about 1 to 4 hours.

The peak plasma and saliva concentrations after the ingestion of 1g of paracetamol by fasting volunteers were found within 50 minutes.

Rawlings, et al. (1977) in their comparative study of the pharmacokinetics of paracetamol after i.v. and oral administrations suggested a considerable "first-pass" inactivation in the gut wall or the liver to have taken place.

PROMETHAZINE

The absorption of promethazine from the gastro-intestinal tract is quite good in most subjects (i.e. above 80%). It is metabolised in the liver and excreted mainly as metabolites in the urine.

Promethazine sulphoxide is the major metabolite of promethazine found in urine. A little of the unchanged

The disposition of promethazine is characterized by a large volume of distribution and a high blood clearance (1.141 min^{-1}). In the urine, less than 1% of the dose is excreted unchanged, therefore total body clearance is essentially metabolic clearance. In accord with this high clearance, the oral bioavailability of promethazine is only 25%, and it has an extensive first-pass metabolism in the liver (Taylor, et al., 1983).

In a kinetic study of promethazine and its sulphoxide metabolite after intravenous and oral administration to healthy volunteers, the oral administration resulted in maximum concentrations of promethazine within the range of 2.4 to 18.0 ng/ml which were achieved between 1.5 and 3h after dosing, while the promethazine blood concentrations were low following intravenous dosing. This was due to a large volume of distribution and decline in a bioexponential manner, $t_{\frac{1}{2}}$ 1.4h (Taylor et al., 1983).

The elimination half-life for promethazine following oral administration has been estimated to be within the range of 12-15h (Taylor, et al., 1979; Moolenaar, et al., 1981).

Gregorio and Ruch (1980) reported maximum blood concentration of 22 ng/ml after intramuscular dosing of 25mg of promethazine suggesting a large volume of distribution.

2.3 AIM AND OBJECTIVES OF THE STUDY

Chloroquine, a 4-aminoquinoline is used in the prophylaxis and treatment of malaria and is also indicated in rheumatoid arthritis and systemic lupus erythrometosis (Gunnar, et al., 1982; Gustafsson, et al., 1983; Ogunbona, et al., 1986; and Tulpule, et al., 1983).

Promethazine, a member of phenothiazine group of drugs, is commonly used in allergic conditions, as sedative and hypnotic, and as a premedicant in anaesthesia and obstetrics (Glyn, et al., 1982 and Taylor, et al., 1983).

Paracetamol has analgesic and antipyretic properties (George, et al., 1977, Martindale, 1989).

Chloroquine has been known to cause some side effects. Due to these side effects especially itching, some patients who react to chloroquine most have tended to abandon its use of malarial disease. The search for how to suppress these side effects and make it tolerable by those patients who react to it prompted the production of some samples which consists of these drugs.

Maxiquine sample consists of chloroquine phosphate, paracetamol and promethazine hydrochloride, while Daga-Quine sample consists of chloroquine phosphate and paracetamol (Vitabiotic, 1988).

HPLC method has been used for the simultaneous determinations of these drugs and their metabolites

individually under different conditions (Adriaenssens, et al., 1978; Glyn, et al., 1982; and Ogunbona, et al., 1986).

There has not been a report on the simultaneous determination of paracetamol, chloroquine and promethazine by HPLC using a single solvent system.

This study is designed to develop an analytical method for the simultaneous determination of paracetamol, chloroquine and promethazine, and to use the method to assay Maxiquine and Daga-Quine syrups, and Daga-Quine tablets.

In practice, these drugs have been co-formulated and are also co-administered. It therefore becomes necessary to develop a simple method by which the drugs can be assayed under the same conditions using the same method. This makes the work simple, less tedious and more accurate, unlike when the drugs are assayed under different solvent systems for each drug.

The separation of these drugs could have been achieved by a gradient elution method, where the composition of the mobile phase components has to be programmed, but the isocratic elution method was used, where the components of the mobile phase were premixed before using, for the separation of the drugs. This is of an advantage over the other, since it is simple and does not involve any sophistication of the instrument.

This study could be of importance in a situation where there is an overdosage of this combination, the drugs can be determined easily under the same conditions. This also can be used for pharmacokinetic study of the combination or for drug-drug interaction of any of the drugs in the combination.

For this study, the high performance liquid chromatographic technique would be adopted.

CHAPTER 3

MATERIALS AND METHODS

3.1 DRUGS

Compound	Manufacturer	Batch No.
1. Acetanilide	BDH Chemicals Ltd, Poole, England.	
2. Acetoacetanilide	Sigma Chemical Company, P.O. Box 14508, St. Louis, MO. 63178 U.S.A.	
3. Amodiaquine tablets (Camoquin^{R})	PHARMA-DEKO Ltd, Ogun	014
4. Chloroquine phosphate (authentic powder)	MAY & BAKER NIGERIA LTD, P.M.B. 21049, Industrial Estate, Ikeja.	2300939
5. Chlorpromazine HCl (authentic powder)	SIGMA Chemical Company, P.O. Box 14508, St. Louis, MO. 63178 U.S.A.	C-8138
6. Daga-Quine^{R} syrup	Farmex Ltd, Otta for Nigerian Hoechst Ltd, Otta.	S001
7. Daga-Quine^{R} tablets	Farmex Ltd, Otta for Nigerian Hoechst Ltd, Otta.	H004
8. Imipramine HCl Injection (Tofranil)	Swiss Nigerian Chemical Co. Ltd, 287 Agege Motor Road, P.O. Box 4310, Ikeja, Lagos.	B066100
9. Maxiquine^{R} syrup	VITABIOTICS (NIG.) LTD, 28, Oduyemi Street, P.O. Box 3020, Ikeja, Lagos, Nigeria.	19690

	Compound	Manufacturer	Batch No.
10.	Papaverine HCl (authentic powder)	BDH Chemicals Ltd, Poole, England.	29435
11.	Paracetamol (authentic powder)	Zazzau Pharmaceutical Ltd., Zaria Nigeria.	301
12.	Phenacetin	BDH Chemicals Ltd., Poole, England.	
13.	Promethazine HCl (authentic powder)	MAY & BAKER NIGERIA LTD., P.M.B. 21049, Industrial Estate, Ikeja, Lagos.	602
14.	Trifluoperazine tablets	LIFEPHARMA Milano-Italy.	24084

3.2 EQUIPMENT

1. High Performance Liquid Chromatography (HPLC) technic - Model 204 (Waters Associates Inc., Milford).
2. A 25 µL Syringe (Hamilton Reno, Nevada).
3. Whirl mixer (Gallenkamp, England).
4. Mechanical Shaker (Gallenkamp, England).
5. Centrifuge (Gallenkamp, England).
6. Centrifuge tubes (Capped, 12ml, 20ml).
7. Analytical balance, Type AE 240 Mettler.
8. µBonda Pak (Radial-Pak) C-18 Column, 15cm x 3.9mm I.D. (Waters Associates, Inc., Milford).
9. pH Meter (PW 2418, Philips, England).
10. Vacuum pump and compressor (RB5, Edwards, Britain).

3.3 REAGENTS

Acetic acid (Glacial)	- (BDH Chemicals, Poole, England)
Acetonitrile-Analar grade	- (BDH Chemicals, Poole, England)
Diethylamine	- (BDH Chemicals, Poole, England)
Disodium hydrogen phosphate	- (BDH Chemicals, Poole, England)
Ethanol - Anarlar grade	- (BDH Chemicals, Poole, England)
Ethylactate - Analar grade	- (SD's Lab-Chem Industry, Bombay, India).
Formic acid	- (Shermond, Sussex - England)
Hydrochloric acid	- (BDH Chemicals, Poole, England)
n-Hexane - HPLC grade	- (BDH Chemicals, Poole, England)
Methanol - Analar grade	- (BDH Chemicals, Poole, England)
Sodium dihydrogen phosphate	- (BDH Chemicals, Poole, England)
Tetrahydrofuran - HPLC grade	- (BDH Chemicals, Poole, England)
Triethylamine	- (BDH Chemicals, Poole, England)

3.4 EXPERIMENTAL PROCEDURE

3.4.1 SOLVENT SYSTEM

In developing the mobile phase used for this work, some solvent combinations were tried. The components of the mobile phase were premixed before use. Thin-layer chromatography was also applied in trying some solvent combinations. Columns, internal standards and salts

used were varied to aid the resolution of the drugs.

In the mobile phase: Acetonitrile-sodium dihydrogen phosphate-triethylamine, methanol, ethanol and acetic acid were tried in place of acetonitrile to ascertain the best solvent for the elution of the drugs. Also, the content (%, $^{v}/v$) of the components were varied to ascertain the optimum composition. The effect of pH, acetonitrile, NaH_2PO_4 and triethylamine on the capacity ratio of paracetamol, chloroquine and promethazine were studied.

3.4.2 CHEMICAL ASSAY

(A) SPECTROPHOTOMETRIC METHOD

For the assay of these drugs (paracetamol, chloroquine phosphate and promethazine hydrochloride), the procedure followed was that given in the U.S. Pharmacopoeia 1980 XX, and SP8-100 ultraviolet spectrophotometer was used.

Procedure

Chloroquine phosphate

6.25ml of Maxiquine^{R} syrup (equivalent to 100mg of chloroquine phosphate) was accurately pipetted into a

one litre volumetric flask. 5ml of freshly distilled water was added, shaken for 10 minutes and made up to mark with dilute hydrochloric acid.

10ml of this solution was pipetted into a 100ml volumetric flask and diluted to mark with dilute HCl to obtain a solution of 10 µg/ml. A standard solution of chloroquine phosphate of the same strength was prepared. The absorbance of both solutions in 1cm cells were concomitantly determined at the wavelength of maximum absorbance at about 343nm, using dilute HCl as the blank.

The quantity, in mg of $C_{18}H_{26}ClN_3.2H_3PO_4$ in the portion of chloroquine phosphate taken was calculated using the formula 10C(Au/As), where C is the concentration, in µg per ml of chloroquine phosphate in the standard solution, and Au and As are the absorbances of the solution of chloroquine phosphate and the standard solution, respectively.

Paracetamol

6ml of Maxiquine^R syrup (equivalent to 120mg of paracetamol) was accurately pipetted into a 500ml volumetric flask and 10ml of methanol was added and shaken for 5 minutes.

The volume was made up to mark with freshly distilled water. A standard solution of paracetamol of the same concentration (12 µg/ml) was prepared.

The absorbances of both solutions were concomitantly determined in the same medium, at a concentration of about 12 µg/ml in 1cm cells, at the wavelength of maximum absorbance at about 244nm, with a suitable spectrophotometer, using freshly distilled water as the blank.

The quantity in mg, of $C_8H_9NO_2$ in the paracetamol taken was calculated using the formula 10C(Au/As) where C is the concentration in µg per ml of paracetamol in the standard solution, and Au and As are the absorbances of the solution of paracetamol and the standard solution, respectively.

Promethazine hydrochloride

16.67ml of the Maxiquine^R syrup (equivalent to 10mg of promethazine HCl) was accurately pipetted into a 100ml separating funnel. 4ml of concentrated ammonium hydroxide was added and the promethazine base was extracted with 6 x 16ml portions of chloroform. The combined chloroform extract was washed with 10ml of dilute HCl. The acid solution was washed with 10ml of chloroform and added to the main chloroform extract.

With the aid of a current of air, the chloroform layer was evaporated on a water bath to a volume of about 2 to 4ml, then finally evaporated to dryness under air. The residue was dissolved with slight

warming in dilute sulphuric acid and then transferred to a 200ml volumetric flask with the aid of additional acid. This was cooled, and then diluted to mark with dilute sulphuric acid. The resulting solution was filtered, rejecting the first half of the filtrate. A standard solution of promethazine HCl was prepared using dilute sulphuric acid to produce a solution of known concentration of about 50 ug per ml.

The absorbances of both solutions were concomitantly determined in 1cm cells at the wavelength of maximum absorbance at about 298nm, using dilute sulphuric acid as the blank.

The quantity, in mg, of $C_{17}H_{20}N_2S.HCl$ in each ml of the drug taken was calculated using the formula 500 (C/V) (Au/As), where C is the concentration, in mg/ml, promethazine HCl in the standard solution, V is the volume, in ml, of group taken, and Au and As are the absorbances of the solution from the syrup and the standard solution, respectively.

(B) HPLC METHOD

Using the mobile phase: Acetonitrile-NaH_2PO_4-triethylamine (60:39:1) and the column: uBonda Pak column C-18 (15cm x 3.9mm I.D.) for paracetamol chloroquine and promethazine.

Procedure

Calibration curve

Paracetamol

A stock solution of 100 ug/ml was prepared of which a concentration range of 20 to 100 ug/ml were prepared for the calibration curve.

1ml of the desired paracetamol concentration was pipetted into a 20ml centrifuge tube and to it were added 1ml of internal standard, acetoacetanilide (100 ug/ml), 1ml of phosphate buffer (pH 7.2) and 7ml of ethylacetate. This was shaken for 10 minutes and centrifuged for 10 minutes at 2000g. The ethylacetate layer was transferred into a clean tube. The extraction was repeated with 3ml of ethylacetate. The ethylacetate layer was evaporated under nitrogen gas under a water bath controlled at 70°C. The residue was dissolved in 1ml of the mobile phase. The solution was shaken for six minutes and centrifuged for six minutes, and

then 10 µL was injected into the HPLC. The peak height ratios of paracetamol to internal standard were calculated, and this was used for the calibration curve.

Chloroquine and promethazine

Stock solutions of chloroquine phosphate (1mg/ml) and promethazine hydrochloride (1mg/ml) were prepared, of which concentration ranges of 100 to 500 µg/ml for chloroquine phosphate and promethazine hydrochloride each were prepared for the calibration curves.

Into a 20ml extraction tube were added 1ml of the desired chloroquine phosphate or promethazine hydrochloride concentration, 1ml of the internal standard, papaverine (600 µg/ml), 1ml of 1M sodium hydroxide, and 3ml of hexane. This was shaken for 10 minutes and then centrifuged for 10 minutes at 2000g. The hexane layer was transferred into another tube. The extraction was repeated with 2ml of hexane, and the hexane layer was added to the first hexane layer. 1ml of the mobile phase was added to the hexane layer, and this was shaken for 10 minutes and centrifuged for 10 minutes. The hexane layer was rejected, and a 10 µL aliquot of the aqueous phase was injected into the HPLC.

The peak height ratios of chloroquine to internal standard, and promethazine hydrochloride to internal standard were calculated and these were used for the

chloroquine phosphate and promethazine hydrochloride calibration curves, respectively.

(a) Maxiquine^{R} syrup

Paracetamol

5ml of the Maxiquine syrup (equivalent to 100mg of paracetamol) was pipetted into a 100ml volumetric flask and diluted to mark with filtered distilled water (1mg/ml of paracetamol in the Maxiquine^{R} syrup). Serial dilutions were made to obtain a solution of 50 µg/ml.

1ml of this solution was pipetted into a 20ml centrifuge tube and to it were added 1ml of internal standard, acetoacetanilide (100 µg/ml), 1ml of phosphate buffer (pH 7.2) and 7ml of ethylacetate, and was treated the same way as the paracetamol standard for the calibration curve. Using the calibration curve, the weight per 5ml of paracetamol in the Maxiquine^{R} syrup, and the percentage content were determined.

Chloroquine phosphate

1ml of the Maxiquine^{R} syrup (equivalent to 16mg of chloroquine phosphate) was pipetted into a 10ml volumetric flask and diluted to mark with freshly distilled water (1600 µg/ml of chloroquine phosphate). 2ml of this solution was pipetted into a 10ml volumetric flask and diluted to mark with distilled water (320 µg/ml of chloroquine phosphate). 1ml of this solution was

pipetted into a 12ml centrifuge tube and to it were added 1ml of the internal standard, papaverine (600 µg/ml), 1ml of 1M sodium hydroxide, and 3ml of hexane. This was treated the same way as was the case for the chloroquine phosphate standard for the calibration curve.

The weight per 5ml of chloroquine phosphate in the Maxiquine^{R} syrup, and the percentage content were obtained using the calibration curve.

Promethazine hydrochloride

5ml of the Maxiquine^{R} syrup (equivalent to 3mg of promethazine hydrochloride) was pipetted into a 10ml volumetric flask and diluted to mark with filtered distilled water (300 µg/ml of promethazine hydrochloride). 100 µg/ml and 200 µg/ml solutions were prepared from this solution.

1ml of this solution (100 µg/ml) was pipetted into a centrifuge tube and to it were added 1ml of the internal standard, papaverine (600 µg/ml), 1ml of 1M sodium hydroxide, and 3ml of hexane. The mixture was treated as was the case for the standard for the calibration curve.

The percentage content and the weight per 5ml of promethazine hydrochloride in the Maxiquine^{R} syrup were determined using the calibration curve.

(b) Daga-Quine^{R} syrup

Paracetamol

1ml of Daga-Quine^{R} syrup (equivalent to 10mg of paracetamol) was pipetted into a 10ml volumetric flask and diluted to mark with distilled water (1mg/ml of paracetamol in the Daga-Quine syrup). Serial dilutions were made to obtain a solution of 50 µg/ml. The solution was treated as was the case for the maxiquine syrup assay.

Chloroquine phosphate

1ml of Daga-Quine syrup (equivalent to 16mg of chloroquine phosphate) was pipetted into a 10ml volumetric flask and diluted to mark with distilled water (1600 µg/ml) of chloroquine phosphate in the Daga-Quine^{R} syrup). 2ml of this solution was pipetted into a 10ml volumetric flask and diluted to mark with distilled water (320 µg/ml). This solution was treated the same way as for Maxiquine^{R} syrup.

(c) Daga-Quine^{R} tablets

Paracetamol

Twenty tablets were weighed and powdered. An equivalent weight of 250mg of paracetamol was dissolved in 100ml of distilled water in a 250ml volumetric flask and mechanically shaken for 15 minutes. This was made

up to mark with filtered distilled water (1 mg/ml), shaken and filtered. 0.6ml of the filtered solution was pipetted into a 10ml volumetric flask and diluted to mark (60 µg/ml). This was treated the same way as for Maxiquine^{R} syrup.

Chloroquine phosphate

3ml of the above 1mg/ml solution was pipetted into a 10ml volumetric flask and diluted to mark (300 ug/ml). The solution was treated the same way as for Maxiquine^{R} syrup.

3.5 STATISTICAL ANALYSIS

The sets of data were compared with the manufacturers claim and their degree of reliability were expressed by applying standard deviation, the coefficient of variation, and the standard error of the mean.

Also the students "t" test for paired data and F-test were used for the analysis.

$P > 0.05$ was considered not significant. The larger the value of S, the less precise the sample. The value of S is an expression of the precision of the method. The coefficient of variation of above 10% is of less precision.

CHAPTER 4

RESULTS

4.1 SOLVENT SYSTEM

The solvent combinations tried in the development of the mobile phase used for this work are given in Table 1. The solvent combinations used for thin-layer chromatography are given in Table 2, with the Rf values of the drugs shown in Table 3. The internal standards, salts and columns tried in the course of optimizing the separation of the drugs are given in Table 4.

With 5µ spherical silica column (15cm x 3.9mm I.D.), the drugs (paracetamol, chloroquine and promethazine) were separated using the solvent system: Ethanol-Water-Formic acid-NaH_2PO_4 - Triethylamine (97:2.2:0.1:0.2:0.5). The retention times of paracetamol, promethazine, chloroquine and papaverine were 1.4, 2.6, 4.8 and 1.4 min. respectively (see Fig. 6). Separation was also achieved using µBonda-Pak C-18 column (15cm x 3.9mm I.D.) with solvent system: Acetonitrile-NaH_2PO_4-Triethylamine (60:39:1). The retention times (tR) of paracetamol, chloroquine and promethazine were 3.0, 5.8 and 12.0 min. respectively, while that of the internal standards were 4.2 min. for acetoacetanilide and 8.0 min. for papaverine (see Fig. 7).

TABLE 1

THE SOLVENT COMBINATIONS TRIED DURING THE DEVELOPMENT OF THE MOBILE PHASE THAT SEPARATED PARACETAMOL, CHLOROQUINE, PROMETHAZINE AND THEIR INTERNAL STANDARD.

No.	SOLVENT COMBINATION	RATIO
1.	Acetonitrile-Methanol-Diethylamine	80: 19.5 : 0.5
2.	Acetonitrile-Methanol-Diethylamine	1: 98 : 1
3.	Acetonitrile-Methanol-Diethylamine	1: 98.5 : 0.5
4.	Acetonitrile-Methanol-Diethylamine	9.7 : 90 : 0.3
5.	Acetonitrile-Methanol-Diethylamine	2 : 97 : 1
6.	Acetonitrile-Methanol-Diethylamine	8.5 : 90 : 1.5
7.	Methanol-Acetonitrile	90 : 10
8.	Methanol-Acetonitrile-Triethylamine	98.5 : 1 : 0.5
9.	Methanol-Acetonitrile-Triethylamine	97.5 : 2 : 0.5
10.	Methanol-Acetonitrile-Triethylamine	98 : 1 : 1
11.	Methanol-Acetonitrile-Diethylamine-Formic acid	98 : 1 : 1 : 0.1
12.	Methanol-Diethylamine-Water	19.5 : 0.5 : 80
13.	Methanol-Water	98 : 2
14.	Methanol-Water-Acetic acid	19.5 : 80 : 1
15.	Methanol-Water-Formic acid	98 : 1.7 : 0.3
16.	Methanol-Water-Triethylamine	98 : 1.5 : 0.5
17.	Methanol	100
18.	Methanol-Triethylamine-Acetic acid	98 : 0.5 : 1.5
19.	Methanol-Water-Formic acid-Triethylamine	97 : 1.5 : 1.0:0.5
20.	Methanol-Acetonitrile-Water	19.5 : 25 : 55.5

No.	SOLVENT SYSTEM	RATIO
21.	Methanol-Diethylamine-Water-Acetate buffer (pH 4.58)	19.5 : 0.5 : 79:1
22.	1-Propanol-Triethylamine-Water	79: 0.5:2.2: 0.1
23.	1-Propanol-Triethylamine-Water-Formic acid	79:0.5:2.2:0.1:0.2
24.	1-Propanol-Triethylamine-Water-Formic acid-NaH_2PO_4	97:0.5:2.2:0.1:0.2
25.	1-Propanol-Triethylamine-Water-NaH_2PO_4	97:0.5:2.2:0.2
26.	Tetrahydrofuran-Triethylamine-water	97 : 0.5 : 2.2
27.	Tetrahydrofuran-Triethylamine-Water Formic acid-NaH_2PO_4	97:0.5:2.2:0.1:0.2
28.	Tetrahydrofuran-Ethanol-Triethylamine-Formic acid	18:80: 0.5 : 1.5
29.	Ethanol-Triethylamine-Acetic acid	98.5 : 0.5 : 1.0
30.	Ethanol-Triethylamine-Acetonitrile	97.5:0.5 : 2.0
31.	Ethanol-Triethylamine-Acetonitrile	99 : 0.5 : 0.5
32.	Ethanol-Acetonitrile	98 : 2
33.	Ethanol-Diethylamine-Acetic acid	98.5 : 0.5 : 1.0
34.	Ethanol-Water-Triethylamine-Formic acid-NaH_2PO_4	97:2.2:0.5:0.1:0.2
35.	Ethanol-Diethylamine-Acetonitrile	98 : 1 : 1
36.	Ethanol-Water-Triethylamine-NaH_2PO_4	95:4.2:0.5:0.2
37.	Ethanol-Water-Triethylamine-Formic acid-NaH_2PO_4	95:4.2:0.5:0.1:0.2
38.	Ethanol-Water-Triethylamine-Formic acid-NaH_2PO_4	95:3:0.5:1:0.5
39.	Ethanol-Water-Triethylamine-Formic acid-NaH_2PO_4	60:38:0.5:1:0.5
40.	Ethanol-Water-Triethylamine-Acetic acid	50:19.5:0.5:30

No.	SOLVENT SYSTEM	RATIO
41.	Ethanol-Triethylamine-Water-Formic acid-NaH_2PO_4	90:0.5:9:0.2:0.3
42.	Ethanol-Water-Triethylamine-Formic acid-NaH_2PO_4	93:6.5:0.5:0.1:0.2
43.	Ethanol-Water-Triethylamine-Formic acid-NaH_2PO_4	97:2.2:0.5:0.1:0.2
44.	Ethanol-Water-Triethylamine-Formic acid-NaH_2PO_4	98:1.2:0.5:0.1:0.2
45.	Ethanol-Water-Triethylamine-Formic acid-NaH_2PO_4	97:2.3:0.5:0.1:0.1
46.	Acetonitrile-Water	60 : 40
47.	Acetonitrile-Water	40 : 60
48.	Acetonitrile-Water	70 : 30
49.	Acetonitrile-Water-Tetrahydrofuran	60 : 40: 1
50.	Acetonitrile-Water-Tetrahydrofuran	60 : 40 : 6
51.	Ethanol-NaH_2PO_4-Triethylamine	97 : 2 : 1
52.	Ethanol-NaH_2PO_4-Triethylamine-Tetrahydrofuran	90 : 2 : 1 : 1
53.	Ethanol-Diethylamine-NaH_2PO_4	97 : 1 : 2
54.	Ethanol-Water-NaH_2PO_4-Triethylamine	90 : 7 : 2 : 1
55.	Ethanol-Triethylamine-Acetate buffer	97 : 1 : 2
56.	Ethanol - Triethylamine-Carbonate buffer	90 : 1 : 2
57.	Acetonitrile-NaH_2PO_4-Triethylamine	60 : 39 : 1
58.	Methanol-NaH_2PO_4-Triethylamine	50 : 49 : 1
59.	Acetic acid - NaH_2PO_4-Triethylamine	60 : 39 : 1

TABLE 2

SOLVENTS USED IN THIN LAYER CHROMATOGRAPHY

No.	SOLVENT COMBINATION	RATIO
1.	Ammonium hydroxide solution-Benzene-Dioxan	5 : 60 : 35
2.	Acetic acid-Ethanol-Water	30 : 50 : 20
3.	n-Butanol-Methanol	40 : 60
4.	Strong ammonia solution-Methanol	1.5 : 100
5.	Methanol-NH_3 solution-Phosphate buffer	97 : 1.0 : 2.0
6.	Methanol-NH_3 solution-Phosphate-buffer Formic acid	97 : 1 : 2 : 0.5
7.	Methanol-NH_3 solution-Phosphate buffer-formic acid	98 : 1 : 1 : 1
8.	Methanol-NH_3 solution-Phosphate buffer (pH 7.4)	98.5 : 1.0 : 0.5
9.	Methanol-NH_3 solution-Phosphate buffer	98 : 1 : 1
10.	Acetonitrile-Water	60 : 40
11.	Acetonitrile-Water-Tetrahydrofuran	60 : 40 : 1
12.	Acetonitrile-Water-Tetrahydrofuran	60 : 40 : 6
13.	Acetonitrile-Methanol-Diethylamine	80 : 19.5 : 0.5
14.	Acetonitrile-Methanol-Diethylamine-Formic acid	80:19.5:0.5:0.5
15.	Methanol-0.2M NaH_2PO_4	50 : 50
16.	Acetonitrile-Methanol-Diethylamine-0.2M NaH_2PO_4	80:19.5:0.5:0.1
17.	Acetonitrile-Water	40 : 60

TABLE 3: THE RF VALUES OF PARACETAMOL, CHLOROQUINE, PROMETHAZINE AND THE VARIOUS INTERNAL STANDARDS TRIED USING TLC.

SAMPLE	RF VALUE																
	1	2	3	4	5	6	7	8	9.	10	11	12	13	14	15	16	17
CHLOROQUINE				0.31	0.20	0.30	0.30	0.20	0.21	0.47	0.63	0.61	0.20	0.55	0.51	0.10	0.62
PARACETAMOL				0.86	0.65	0.79	0.74	0.66	0.67	0.89	0.94	0.92	0.88	0.81	0.82	0.87	0.93
PROMETHAZINE	0.70	0.59	0.22	0.61	0.44	0.55	0.53	0.47	0.43	0.72	0.73	0.76	0.70	0.64	0.63	0.66	0.68
PAPAVERINE				0.84	0.59	0.55	0.60	0.60	0.60	0.71	0.71	0.75	0.85	0.62	0.62	0.70	0.64
AMODIAQUINE								0.76	0.76	0.38	0.40	0.43	0.50	0.13	0.59	0.23	0.42
CHLORPROMAZINE	0.94	0.70	0.30	0.56	0.39	0.55	0.53	0.41	0.38	0.79	0.72	0.68	0.61	0.62	0.68	0.61	0.69
PHENACETIN				0.00	0.00	0.00	0.00	0.00	0.00	0.00							
P-BROMOACE-TANILIDE										0.00			0.00	0.00	0.00	0.00	
ACETANILIDE				0.00	0.00	0.00	0.00	0.00	0.00								
ACETOACETANI-LIDE										0.92	0.91	0.90	0.90	0.86	0.81	0.90	0.86
IMIPRAMINE HCL	0.86	0.77	0.29	0.43	0.00	0.00	0.00	0.00									
TRIFLUO-PERAZINE	0.69	0.33	0.40	0.48				0.71	0.71	0.00	0.00					0.00	

TABLE 4: SOME OF THE PARAMETERS VARIED IN MOBILE PHASE DEVELOPMENT

(a) INTERNAL STANDARDS

Drug	Internal Standard
Paracetamol	Phenacetin
	Acetanilide
	Acetoacetanilide
	P-Bromoacetanilide
Chloroquine	Amodiaquine
	Papaverine
Promethazine	Chlorpromazine
	Imipramine HCl
	Trifluoperazine
	Papaverine

(b) SALTS

$MgSO_4 > KH_2PO_4 > Na_2SO_4 > NaH_2PO_4$

The order of increasing effectiveness

(c) COLUMNS

Column	Dimension
Steel column	15cm x 3.9mm I.D.
5µ spherical silica column	"
µBonda Pak C-18 column	"

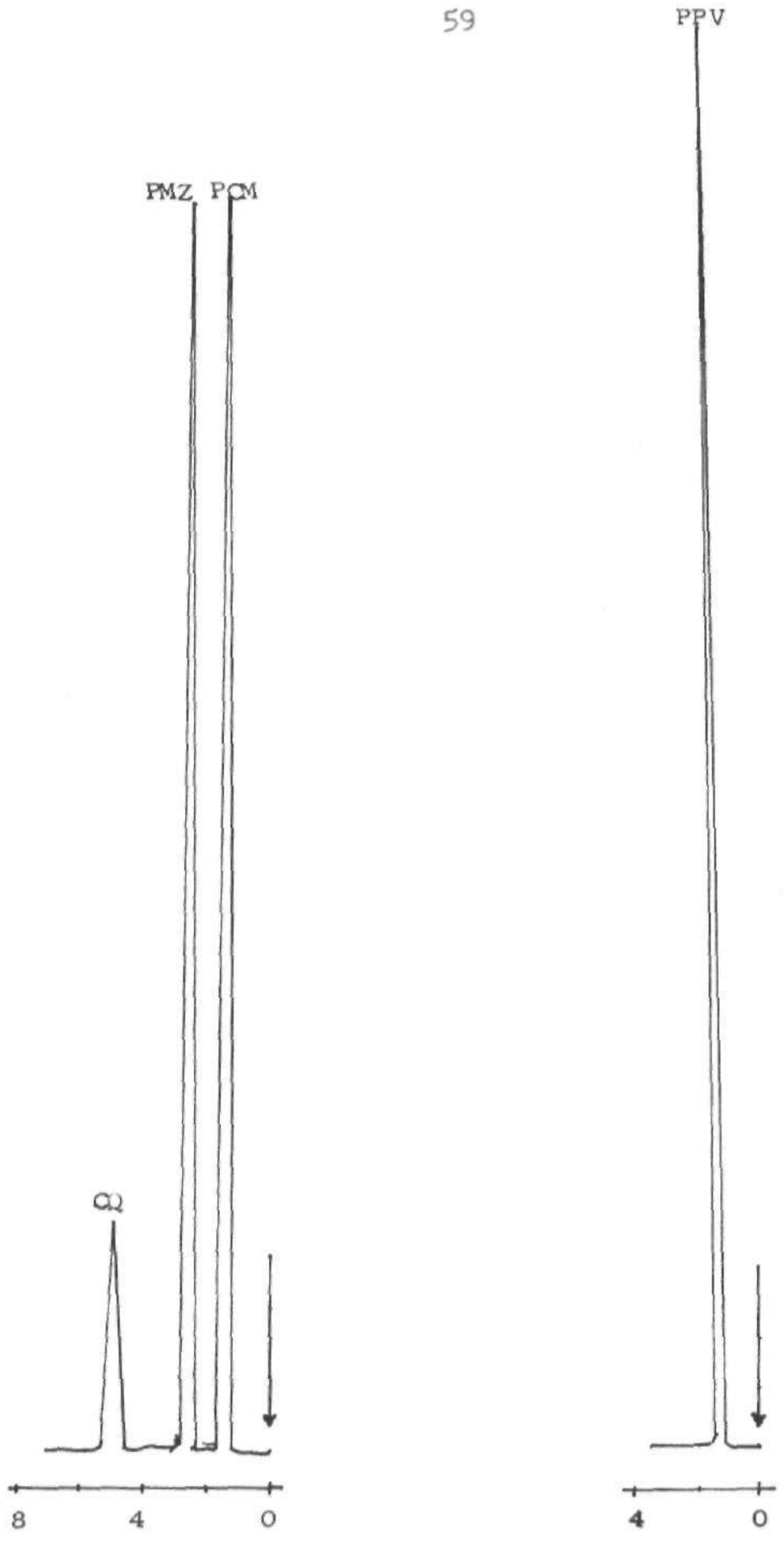


Fig. 6: Chromatograms of Paracetamol, Promethazine and Chloroquine.

Mobile Phase: Ethanol-Water-Triethylamine-Formic acid - NaH_2PO_4 (97:2.2:0.5:0.1:0.2)

Column: 5μ Spherical silica Column

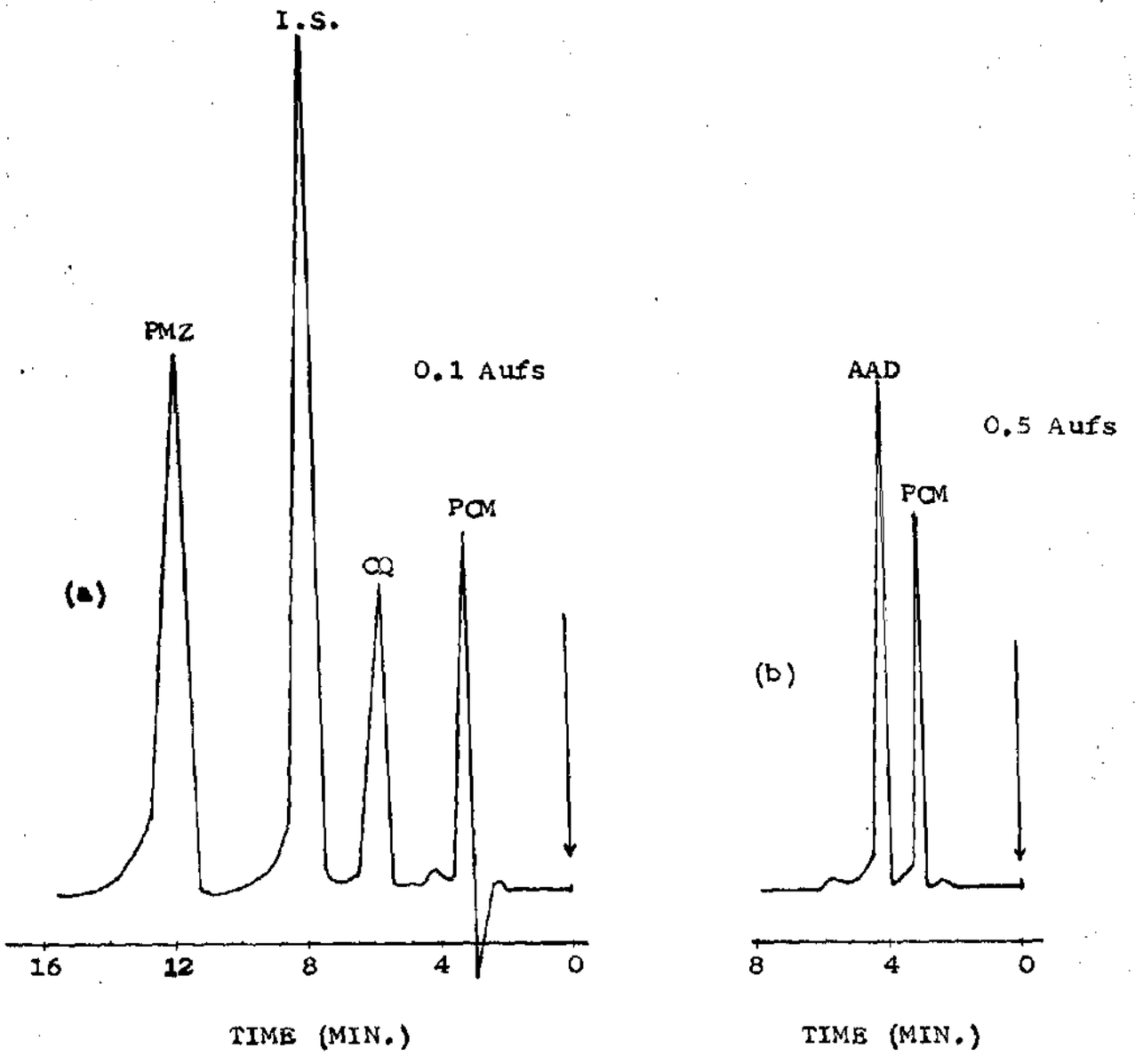


Fig. 7: (a) Chromatogram of the unextracted reference standards for the simultaneous determination of Paracetamol (PCM), Chloroquine (CQ) and Promethazine (PMZ). Paracetamol (40µg/ml), Chloroquine (200µg/ml), Promethazine (200µg/ml) and I.S. - Papaverine (600µg/ml).

(b) Chromatogram of the extracted reference standard of Paracetamol (PCM) with Acetoacetanilide (AAD). Paracetamol (40µg/ml), Acetoacetanilide (100µg/ml).

Mobile phase: Acetonitrile-NaH_2PO_4-Triethylamine (60:39:1)

Column: µBonda-Pak C-18 column

The effect of pH, NaH_2PO_4 and Acetonitrile in the mobile phase: Acetonitrile-NaH_2PO_4-Triethylamine are shown in Tables 6, 7 and 8 respectively.

With the exception of promethazine, it was observed that the capacity ratio of the drugs increases with an increase in the NaH_2PO_4 content (%, $^{v}/v$), and decreases with an increase in the acetonitrile content (see Fig. 1 and 2).

4.2 CHEMICAL ASSAY

4.2.1 U.V. SPECTROPHOTOMETRIC METHOD

The U.V. spectrophotometric method applied to assay Maxiquine syrup showed that the drug contained 100% of chloroquine phosphate, 100% of paracetamol and 97% of promethazine hydrochloride. The content of the active ingredients in this preparation complied with U.S.P. (1980, XX) specification of drug content (95 - 105%, 98 - 102%, and 90 - 110% for paracetamol, chloroquine phosphate and promethazine hydrochloride respectively). It also complied with the B.P. (1988) specification of drug content (95 - 105%, 93 - 107% and 90 - 110% for paracetamol, chloroquine phosphate and promethazine hydrochloride respectively). The values of the percentage of the active ingredients and the drug content are given in Table 9.

TABLE 5: SUBSTITUTION OF ACETONITRILE WITH METHANOL, ACETIC ACID AND ETHANOL IN THE MOBILE PHASE: ACETONITRILE NaH_2PO_4 - TRIETHYLAMINE.

No.	SOLVENT COMBINATION	pH	RETENTION TIME (MIN.)		
			CQ	PMZ	PPV
1.	Acetonitrile-NaH_2PO_4-Triethylamine (60:39:1)	7.2	3.8	7.2	5.2
2.	Methanol-NaH_2PO_4-Triethylamine (50:49:1)	7.1	8.6	35.2	44.4
3.	Acetic acid-NaH_2PO_4-Triethylamine (60:39:1)	1.8	3.2	5.7	3.8
4.	Ethanol-NaH_2PO_4-Triethylamine (97:2:1)	7.2	2.6	3.8	3.2

TABLE 6: THE EFFECT OF pH ON THE CAPACITY RATIO OF PARACETAMOL, CHLOROQUINE AND PROMETHAZINE IN THE MOBILE PHASE : ACETONITRILE-NaH_2PO_4 - TRIETHYLAMINE (60 : 39 : 1)

No.	pH	CAPACITY RATIO (K')		
		PCM	CQ	PMZ
1.	3.8	3.0	1.2	1.1
2.	4.5	4.5	0.2	5.6
3.	7.2	4.7	2.1	2.3
4.	7.5	3.8	1.1	2.4

TABLE 7: THE EFFECT OF NaH_2PO_4 ON THE CAPACITY RATIO OF PARACETAMOL, CHLOROQUINE AND PROMETHAZINE IN THE MOBILE PHASE : ACETONITRILE-NaH_2PO_4 - TRIETHYLAMINE (60 : 39 : 1).

No.	NaH_2PO_4 (%, v/v)	CAPACITY RATIO (K')		
		PCM	CQ	PMZ
1.	20	2.3	1.0	1.3
2.	39	4.7	2.1	2.3
3.	59	8.3	4.5	2.1

TABLE 8: THE EFFECT OF ACETONITRILE ON THE CAPACITY RATIO OF PARACETAMOL, CHLOROQUINE AND PROMETHAZINE IN THE MOBILE PHASE : ACETONITRILE NaH_2PO_4 - TRIETHYLAMINE (60:39:1)

No.	ACETONITRILE (%, v/v)	CAPACITY RATIO (K')		
		PCM	CQ	PMZ
1.	40	8.3	4.5	2.1
2.	60	4.7	2.1	2.3
3.	78	2.3	1.0	1.3

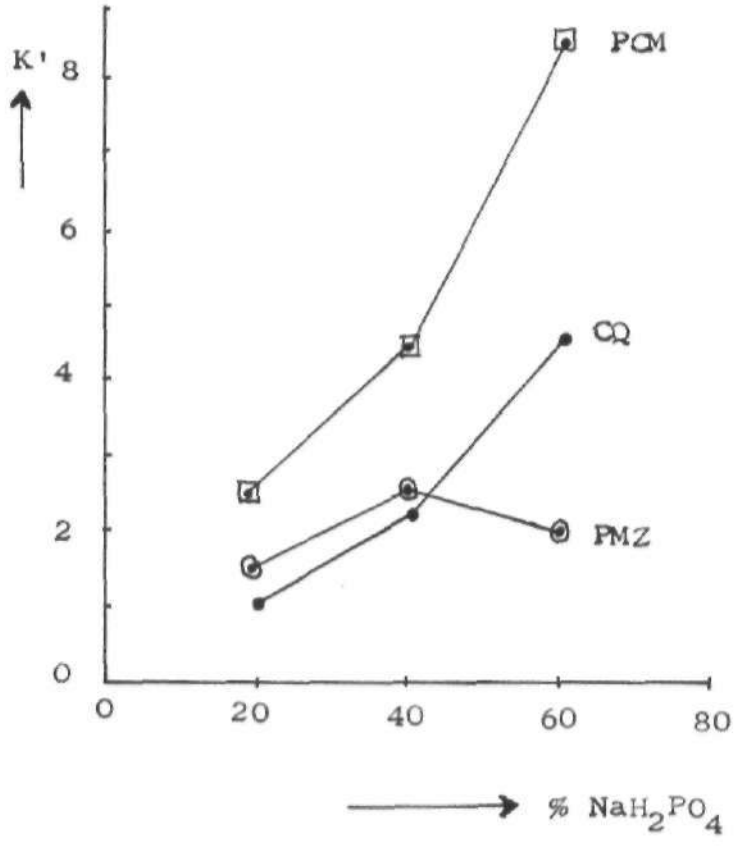


Fig. 1: Effect of NaH_2PO_4 content of the mobile phase on the capacity ratio (K').

Mobile phase: Acetonitrile -NaH_2PO_4-Triethylamine

PCM = Paracetamol
CQ = Chloroquine
PMZ = Promethazine

Column: µBonda-Pak C_{18} Column

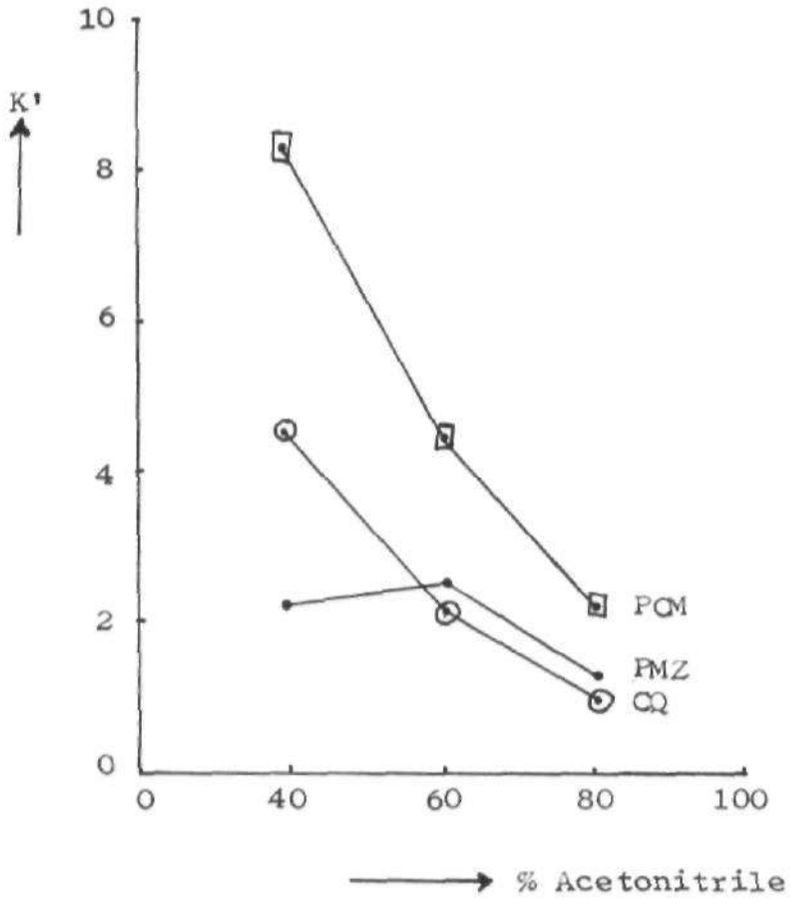


Fig. 2: Effect of Acetonitrile content of the mobile phase on the capacity ratio (K').

Stationary phase: Radial Pak μ Bonda Pak C-18 Column.

Mobile phase: Acetonitrile -NaH_2PO_4-Triethylamine.

PCM = Paracetamol
CQ = Chloroquine
PMZ = Promethazine

Column: μBonda-Pak C_{18} Column

4.2.2 HPLC METHOD

Using μBonda-Pak C-18 column with solvent system: Acetonitrile-NaH_2PO_4-Triethylamine (60:39:1), calibration curves were prepared for paracetamol, chloroquine phosphate and promethazine hydrochloride. Linear calibration curves with good correlation coefficients of 0.999, 0.990 and 0.990 for chloroquine phosphate, paracetamol and promethazine hydrochloride are shown in Figures 5, 4 and 3 respectively.

The Maxiquine syrup contained 95% of chloroquine phosphate, 96% of paracetamol and 101% of promethazine hydrochloride active ingredients. The content of the active ingredients in this preparation complied with the B.P. (1988) specification of drug content (95 - 105%, 93 - 107% and 90 - 110% for paracetamol, chloroquine phosphate and promethazine hydrochloride respectively). This result is in agreement with that obtained using the U.V. spectrophotometric method (see Table 9).

Under the same conditions as above, Daga-Quine syrup was found to contain 96.7% of paracetamol and 96% of chloroquine phosphate active ingredients. The values for the percentage content and weight of drug per 5ml of the syrup are given in Table 10 and 13 respectively.

Also Daga-Quine tablets contained 101% of paracetamol and 103% of chloroquine phosphate active ingredients. This complied with the official specifications for drug content in tablets (93-107% for chloroquine phosphate and 95 - 105% for paracetamol). The values for the percentage content of the active ingredients and the weight per tablet of each drug are shown in Tables 11 and 14 respectively.

The precision of the method was 1.7% (n = 5) within run and 1.9% (n = 5) for day-to-day run (Table 12). The extraction recoveries were 97% for paracetamol, and 90% for chloroquine and promethazine as shown in Table 15.

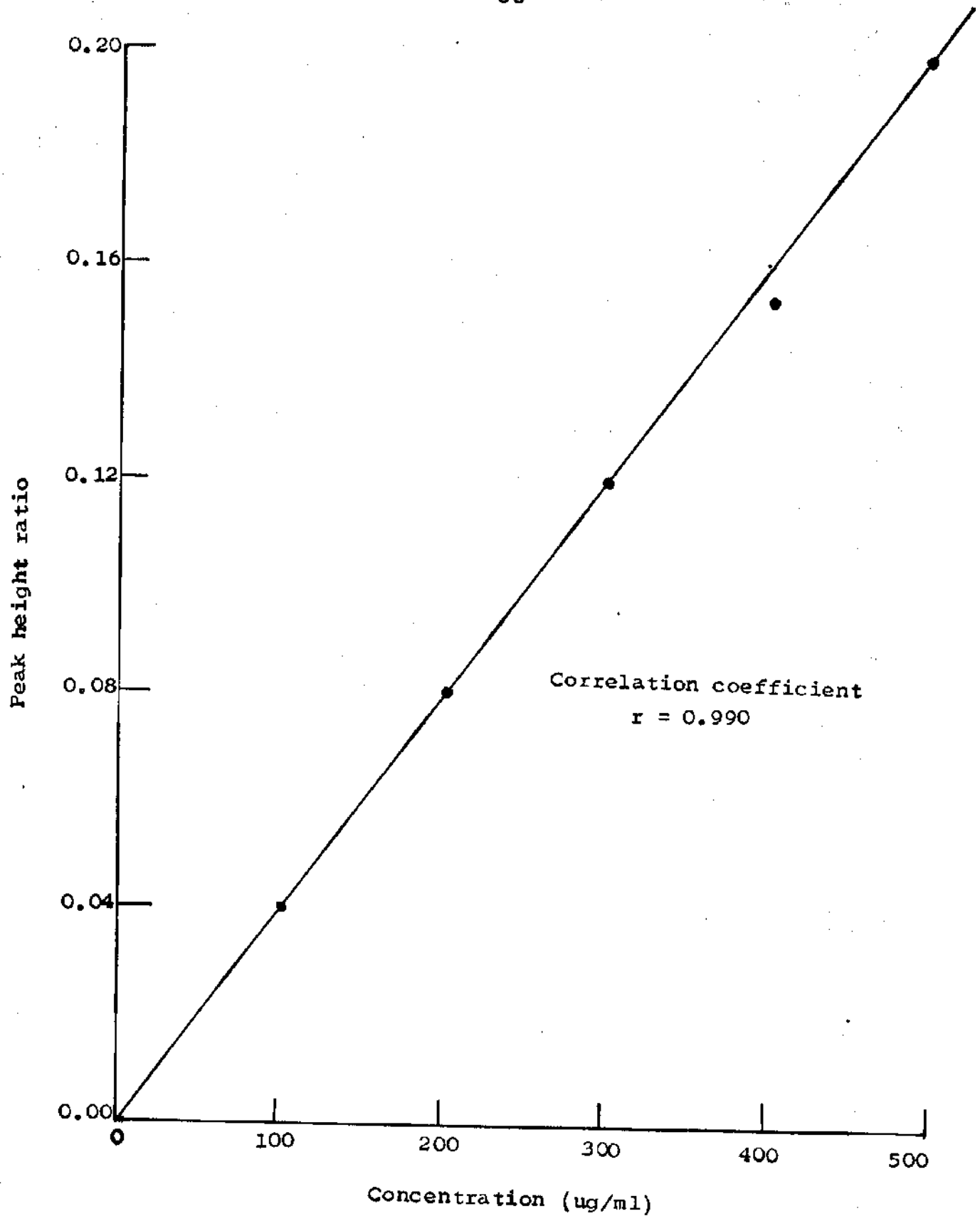


Fig. 3: Calibration curve for promethazine assay using HPLC with the mobile phase: Acetonitrile-NaH_2PO_4-Triethylamine (60:39:1).

Column: µBonda-Pak C_{18} Column

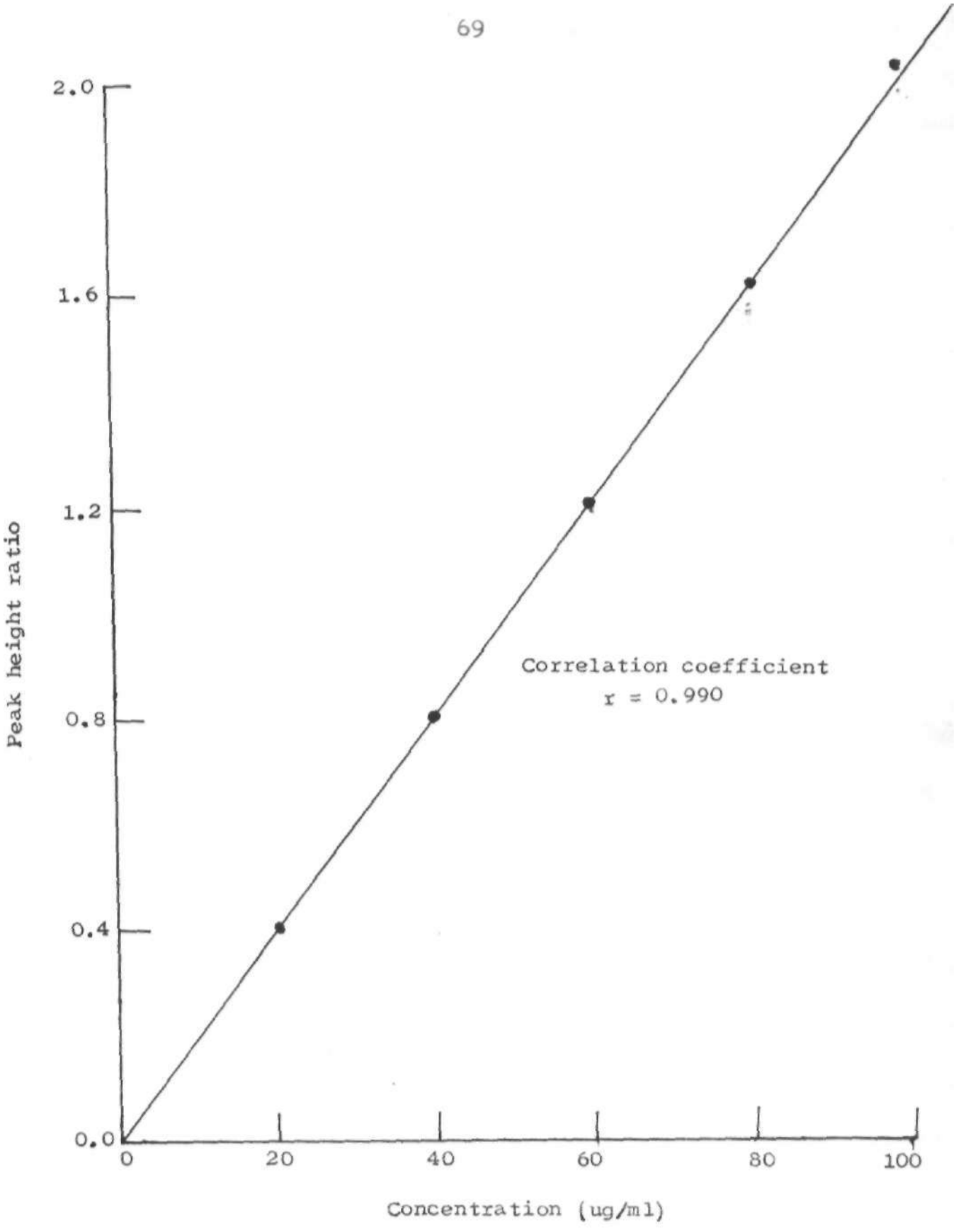


Fig. 4: Calibration curve for paracetamol assay using HPLC with the mobile phase: Acetonitrile-NaH_2PO_4-Triethylamine (60:39:1)

Column: µBonda-Pak C_{18} Column

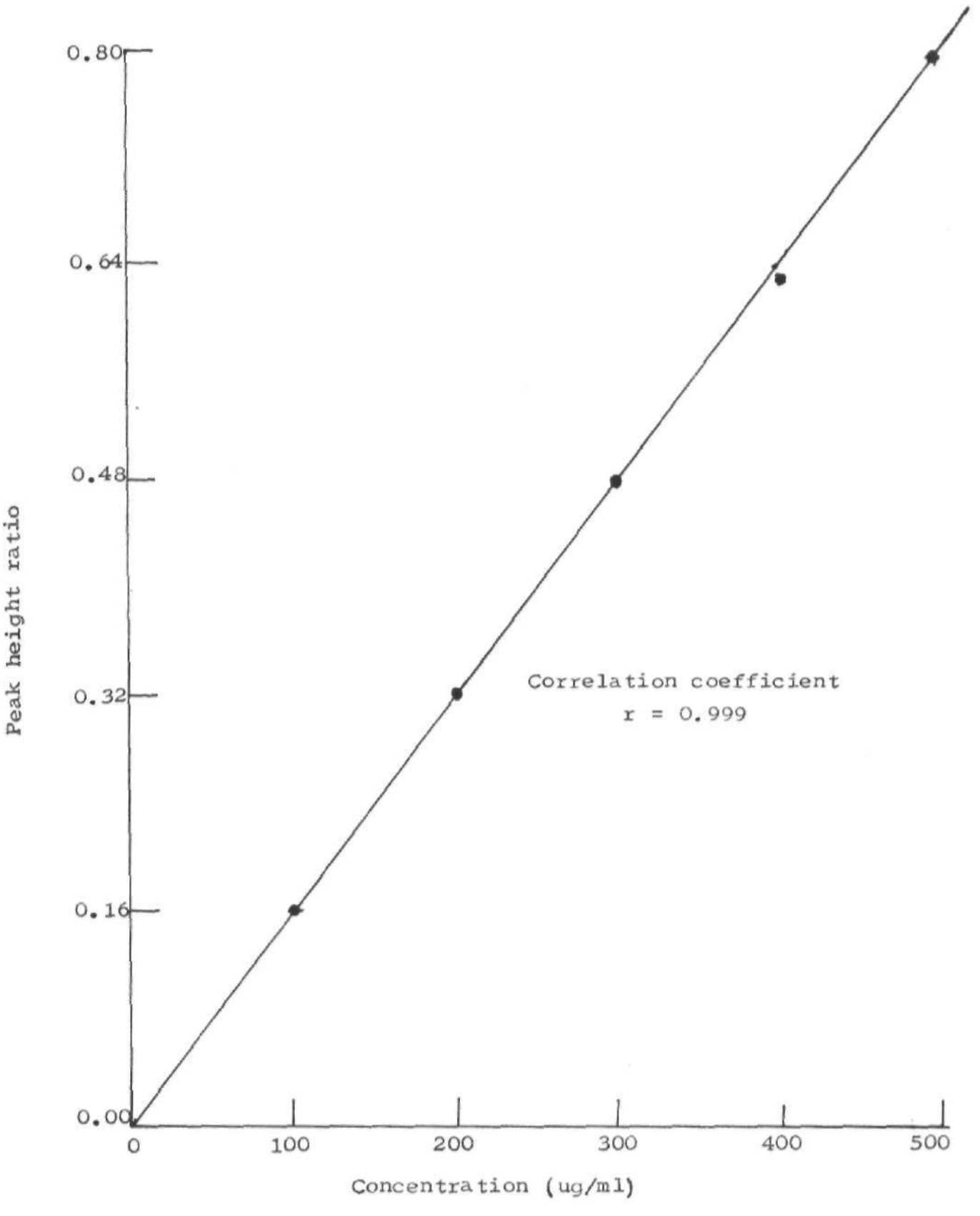


Fig. 5: Calibration curve for chloroquine assay using HPLC with the mobile phase: Acetonitrile-NaH_2PO_4-Triethylamine (60:39:1)

Column: μBonda-Pak C_{18} Column

TABLE 9: QUANTITATIVE DETERMINATION OF MAXIQUINE^{R} SYRUP (U.V. SPECTROPHOTOMETRIC AND HPLC METHODS OF ANALYSIS)

COMPOUND	DRUG CONTENT PER 5ml (mg)		PERCENTAGE ± S.D.	
	SPECTROPHOTO-METRIC METHOD	HPLC METHOD	SPECTROPHO-TOMETRIC METHOD	HPLC METHOD
Paracetamol	100	96	99.96±1.4	96
Chloroquine	80	76	100.0	95±1.4
Promethazine	3	3.03	97.00±0.58	101±1.4

TABLE 10: QUANTITATIVE DETERMINATION OF PARACETAMOL AND CHLOROQUINE PHOSPHATE IN DAGA-QUINE^{R} SYRUP USING HPLC METHOD

DRUG	PERCENTAGE CONTENT (%)			
	1	2	3	MEAN ± S.D.
Chloroquine phosphate	96	96	-	96
Paracetamol	96	98	96	96.7±1.2

TABLE 11: QUANTITATIVE DETERMINATION OF PARACETAMOL AND CHLOROQUINE PHOSPHATE IN DAGA-QUINE TABLETS USING HPLC METHOD.

DRUG	PERCENTAGE CONTENT (%)			
	1	2	3	MEAN ± S.D.
Chloroquine phosphate	104	102	-	103 ± 1.4
Paracetamol	100	102	-	101 ± 1.4

TABLE 12: PRECISION OF THE ANALYSIS USING HPLC METHOD WITH THE MOBILE PHASE: ACETONITRILE - NaH_2PO_4 - TRIETHYLAMINE (60:39:1)

COMPOUND	CONCENTRATION (ug/ml)	C.V. (%)
Within run		
Paracetamol	20 (n = 5)	1.7
	40 (n = 5)	3.9
Day-to-day run		
Chloroquine phosphate	100 (n = 2)	8.3
	200 (n = 2)	8.3
Promethazine hydrochloride	100 (n = 2)	1.9
	200 (n = 2)	9.4

TABLE 13: THE WEIGHT (mg) PER 5ml OF PARACETAMOL, CHLOROQUINE PHOSPHATE AND PROMETHAZINE HYDROCHLORIDE IN MAXIQUINE^{R} AND DAGA-QUINE^{R} SYRUPS.

DRUG	PARACETAMOL	CHLOROQUINE PHOSPHATE	PROMETHAZINE HYDROCHLORIDE
MAXIQUINE^{R} SYRUP	96	76	3.03
DAGA-QUINE^{R} SYRUP	49	77	-

TABLE 14: THE WEIGHT (mg) PER TABLET OF PARACETAMOL AND CHLOROQUINE PHOSPHATE IN DAGA-QUINE TABLETS

DRUG	PARACETAMOL	CHLOROQUINE PHOSPHATE
DAGA-QUINE^{R} TABLETS	252	257

TABLE 15: EXTRACTION RECOVERIES OF PARACETAMOL, CHLOROQUINE AND PROMETHAZINE

COMPOUND	CONCENTRATION (ug/ml)	RECOVERY (Mean ± S.D) % n = 5
Chloroquine	400	90 ± 1.0
	500	91 ± 1.0
Promethazine	100	90 ± 1.4
	200	90 ± 1.4
Paracetamol	10	93 ± 8.9
	20	109 ± 8.9
	25	97 ± 8.9

CHAPTER 5

DISCUSSIONS

5.1 SOLVENT SYSTEM

The solvent combinations used for TLC, given in Table 2, with Rf values of the drugs in Table 3, were tried on HPLC. They were found not to give good separation of the drugs despite the fact that the drugs were separated on TLC. This observation could be due to the differences in the nature and size of the support for the TLC and the packing material for the HPLC column. Also with the HPLC, the applied pressure and flow rate of the solvent system over the column could have altered the speed of separation observed with TLC. The solvent combinations tried in the development of the mobile phase was based on the rules given on the selection of the mobile phase (Giddings, et al, 1981) and the components were premixed based on their miscibility factors (Waters, 1989). The selection of the solvent combinations suitable for the separation of the drugs was based on their capacity ratios, retention times and symmetry of the peaks.

The internal standards tried for each drug, tended to be eluted at the same time with the drugs, only papaverine and acetoacetanilide were separated

with the drugs using the solvent system: Acetonitrile - NaH_2PO_4 - Triethylamine (60:39:1) with μBonda-Pak C_{18} column (15cm x 3.9mm I.D.).

Among the various salts tried, NaH_2PO_4 was found to be best for the resolution of the drugs. The base line was comparatively more stable and the solvent peaks were less prominent. Among the columns tried (see Table 4), Nova-Pak steel column was found to be leastefficient in separating the drugs. Paracetamol and promethazine both gave similar retention times, and all the drugs had the same retention times as their internal standards. The 5μ sperical silica column (15cm x 3.9mm I.D.) separated the three drugs, but could not discriminate between paracetamol and the internal standards. The separation of the drugs and their internal standards was achieved on μBonda-Pak (Radial-Pak) C_{18} column (15cm x 3.9mm I.D.) using an Acetonitrile-NaH_2PO_4 - Triethylamine (60:39:1) as mobile phase. The elution of the drugs was in relation to their polarity, with the highly polar compound being eluted first while the less polar one was eluted last. The separation also was based on the extent to which the drugs were adsorbed by the stationary phase. The less strongly adsorbed drugs travelled faster than the strongly adsorbed drugs.

To ascertain the best solvent for the elution of the drugs, methanol, ethanol and acetic acid were tried in place of acetonitrile in the solvent system: Acetonitrile-NaH_2PO_4-Triethylamine (see Table 5). With methanol, the retention times for promethazine and papaverine were too long for analytical purpose. With ethanol, the retention time for promethazine and papaverine were almost the same, while the retention times for chloroquine and papaverine were almost the same with acetic acid. The similarities in the retention times of the drugs could be due to similarity in the polarity of the drugs in the respective solvents.

The effect of pH on the capacity ratio of the drugs (see Table 6) showed that as the pH increases the capacity ratio increases (up to pH 7.2 for paracetamol and chloroquine and pH 4.5 for promethazine). The capacity ratio for all the three drugs was optimum at pH 7.2. The effect of NaH_2PO_4 on the capacity ratio of the drugs (see Table 7) showed that as the NaH_2PO_4 content (%, $^{v}/v$) increases, the value of the capacity ratio also increases for paracetamol, chloroquine and promethazine. Capacity ratio at 39% of NaH_2PO_4 was found optimal. The effect of Acetonitrile on the capacity ratio of the drugs (see Table 8) indicates that an increase in the content (%, $^{v}/v$) of acetonitrile, decreases the capacity

ratios of the drugs with an exception of promethazine. Capacity ratio at 60% of Acetonitrile was found optimal. This could be due to the polarity and solubility of the drugs in the solvent system which vary as the content (%, $^{v}/v$) of the components of the solvent system were varied.

5.2 CONTENT OF ACTIVE INGREDIENT

Linear calibration curves with good correlation coefficients of 0.999, 0.990 and 0.990 for chloroquine, paracetamol and promethazine are shown in Figures 5, 4 and 3 respectively. The precision of the method was 1.7% (n = 5) within run and 1.9% (n = 5) for day-to-day run (Table 12).

The HPLC method was compared with the U.V. spectrophotometric method for the determination of each drug in Maxiquine syrup. The percentage content of each drug in the syrup shown in Table 9, indicated that the percentage content determined by the HPLC method were similar to the U.V. spectrophotometric method. The percentage content determined for chloroquine, promethazine and paracetamol in Maxiquine syrup were in conformity with the specifications for the drugs in the B.P. (1988) 95 - 103% for paracetamol, 93 - 107% for chloroquine phosphate and 90 - 110% for promethazine hydrochloride, respectively.

The percentage content of the drugs in Daga-Quine^{R} syrup and tablets were also determined using the newly developed HPLC method (see Tables 10 and 11, respectively). The amount of the drugs (chloroquine phosphate and paracetamol) present in the syrup and tablets also met the official requirements specified for the drugs.

CONCLUSION

A high performance liquid chromatographic procedure for the determination of paracetamol, chloroquine and promethazine in some pharmaceutical preparation (Maxiquine and Daga-Quine syrups, and Daga-Quine tablets) was developed using the solvent system: Acetonitrile-Sodium dihydrogen phosphate-Triethylamine (60:39:1) with μBonda-Pak C_{18} column (15cm x 3.9mm I.D.).

The HPLC method developed was sensitive, selective, reproducible and simple. The pharmaceutical preparations assayed by this method gave results which complied with the B.P. (1988) official requirements. The results obtained were comparable with the U.V. spectrophotometric method.

There is the need for further studies on the determination of these drugs in the biological fluids using the developed solvent system in order to ascertain the suitability of the method in pharmacokinetic studies.

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Appendix 1

SPECTROPHOTOMETRIC ASSAY OF MAXIQUINE SYRUP

Compound:	Chloroquine	Promethazine	Paracetamol
Absorbance:	0.127	0.750	0.101

Wavelength of maximum absorbance (nm)

Chloroquine - 343

Paracetamol - 244

Promethazine - 298

(A) Chloroquine phosphate

Quantity, in mg, of $C_{18}H_{26}ClN_3.2H_3PO_4$ in the portion of chloroquine phosphate taken

= 10C(Au/As)

C = Concentration in ug/ml of chloroquine phosphate in the standard solution = 10ug/ml.

Au = Absorbance of solution of chloroquine phosphate in Maxiquine = 0.127.

As = Absorbance of solution of standard chloroquine phosphate = 1.309.

$$= 10 \times 0.01 \times \frac{0.127}{1.309} = 0.0097$$

= 0.01mg/ml

Percentage of chloroquine phosphate contained in the maxiquine syrup.

$$= \frac{\text{Amount of chloroquine phosphate in the Maxiquine}^R \text{ syrup} \times 100}{\text{Amount of chloroquine phosphate in the standard solution}}$$

$$= \frac{0.01 mg/ml}{0.01 mg/ml} \times 100 = 100\%$$

Percentage of the active ingredients contained in the drug.

Calculated = 100

Stated = 98 - 102

(B) Paracetamol

Quantity, in mg, of $C_6H_9NO_2$ in paracetamol taken

$$= 10C(Au/As)$$

C = Concentration, in ug/ml, of paracetamol in the standard solution = 12 ug/ml.

Au = Absorbance of solution of paracetamol in maxiquine syrup = 0.101

As = Absorbance of solution of standard paracetamol = 0.842

$$= 10 \times 0.01 \times \frac{0.101}{0.842} = 0.011995$$

$$= 0.012 \text{ mg/ml}$$

Percentage of paracetamol contained in the maxiquine syrup.

$$= \frac{\text{Amount of paracetamol in the maxiquine syrup}}{\text{Amount of paracetamol in the standard solution}} \times 100$$

$$= \frac{0.011995 \text{ mg/ml}}{0.012000 \text{ mg/ml}} \times 100$$

$$= \underline{\underline{99.96\%}}$$

Percentage of the active ingredient contained in the drug:

Calculated = 99.96

Stated = 98 - 101

(C) Promethazine hydrochloride

Quantity, in mg of $C_{17}H_{20}N_2S.HCl$ in each ml of the syrup taken = 500 ($^{C}/_{V}$) (Au/As)

C = Concentration, in mg/ml of promethazine hydrochloride in the standard solution = 0.05 mg/ml.

V = Volume, in ml, of syrup taken = 16.67ml.

Au = Absorbance of solution of promethazine hydrochloride in Maxiquine^{R} syrup = 0.750.

As = Absorbance of solution of standard promethazine hydrochloride = 1.938

$$= 1.938$$

$$= 500 \times \frac{0.05}{16.67} \times \frac{0.750}{1.938}$$

$$= \underline{0.58 mg/ml}$$

For the 16.67ml taken = 0.58mg/ml x 16.67ml

$$= \underline{9.7mg}$$

Percentage of promethazine hydrochloride contained in the Maxiquine^{R} syrup.

$$= \frac{\text{Amount of promethazine hydrochloride in Maxiquine}^{R} \text{ syrup} \times 100}{\text{Amount of promethazine hydrochloride in the standard solution}}$$

$$= \frac{9.7mg}{10.0mg} \times 100$$

$$= \underline{97\%}$$

Percentage of the active ingredients contained in the drug.

Calculated = 97.0

Stated = 98 - 101.

Appendix 2

SIMPLE CHEMICAL TESTS FOR THE IDENTIFICATION OF THE PHOSPHATE AND THE PHENOLIC GROUPS OF CHLOROQUINE PHOSPHATE, PARACETAMOL AND PROMETHAZINE HYDROCHLORIDE RESPECTIVELY

TEST	OBSERVATION	INFERENCE
CHLOROQUINE PHOSPHATE		
To 2ml of solution (chloroquine phosphate) was added few drops of 2M nitric acid to neutralise it. An equal volume of a 10% $^{w}/_{v}$ solution of ammonium molybdate was added. This was warmed for 5 minutes.	A yellow colloidal solution was observed when the ammonium molybdate solution was added, which gave rise to a yellow precipitate when warmed for 5 minutes.	This confirmed the presence of phosphate group in the chloroquine compound.
PARACETAMOL		
To 0.1g of substance in a test tube was added 1ml of concentrated HCl and allowed to stand for 3 minutes. 10ml of distilled water was added and cooled. 0.05ml of 0.0167M of potassium dichromate was added, and allowed to stand for 10 minutes.	A clear solution was observed. There was no precipitate, but a clear solution. A violet colour was observed, of which when allowed to stand for longer time, the colour remained unchanged.	Presence of phenolic group in the paracetamol compound confirmed.
PROMETHAZINE HYDROCHLORIDE		
5mg of the substance was weighed and its solution was made. To this solution was added 2ml of H_2SO_4 (Conc) and allowed to stand for five minutes.	A colourless solution was observed. A red colour was produced, which did not change.	Confirms the substance to be promethazine HCl.

Appendix 3

BUFFER PREPARATIONS

(a) Acetate Buffer (pH 6.0)

Dissolve 7.7g of sodium acetate in 300ml of water, add 0.32ml of glacial acetic acid, adjust the pH, if necessary, using 10M ammonia or 5M acetic acid and dilute to 500ml with water.

(b) Carbonate Buffer (pH 6.0)

Dissolve 2.12g of sodium carbonate and 1.68g of sodium hydrogen carbonate in sufficient water to produce 100ml.

(c) Phosphate Buffer (pH 7.2)

First prepare 0.2M of NaH_2PO_4 and 0.2M of $Na_2H\ PO_4$, then pipette 14ml of NaH_2PO_4 and 36ml of Na_2HPO_4 into a 100ml volumetric flask and dilute to mark with distilled water. This gives a phosphate buffer of pH 7.2