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DEVELOPMENT OF NEW ANALYTICAL METHODS FOR SELECTED ANALGESIC-ANTIINFLAMMATORY AND DIURETIC AGENTS IN THE COMBINATION FORMULATIONS

THESIS

Submitted in partial fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

By

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Under the supervision of

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CERTIFICATE

This is to certify that the thesis entitled **DEVELOPMENT OF NEW ANALYTICAL METHODS FOR SELECTED ANALGESIC-ANTIINFLAMMATORY AND DIURETIC AGENTS IN THE COMBINATION FORMULATIONS** and submitted by **A.BHARATHI**, ID. NO. **92PHXF004** for award of Ph.D. Degree of the Institute, embodies original work done by her under my supervision.

Signature in full of the Supervisor : P. Parimoo

Name in Capital block letters : **Dr. P. PARIMOO**

Designation : **PROFESSOR**

Date: 31st January 1996

LIST OF ABBREVIATIONS

C	= Degree Centigrade
ml	= Millilitre
mg	= Milligram
%	= Percentage
mt	= Minute
hr	= Hour
RT	= Room Temperature
>	= Greater than
<	= Less than
nm	= Nanometres
µg	= Micrograms
ng	= Nanograms
Rf	= Retardation Factor
µm	= Micrometres
cm	= Centimetres
AUFS	= Absorbance Units Full Scale range
gms	= Grams
N	= Normal
M	= Molar
w/v	= Weight / Volume
ppm	= Parts per Million
µ	= Micro
OTC	= Over the counter

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CHAPTER-1

INTRODUCTION

The chemical substances which interact at the molecular level altering the body functions therapeutically are generally referred to as drugs Drugs vary in molecular size and chemical nature Based on the therapeutic activity a compound has on the body, drugs have been variously classified as analgesics, antibiotics, vitamins, central nervous system (CNS) stimulants / depressants, tranquillisers, antipsycotics, diuretics etc

In a welfare state it is the responsibility of the national government to ensure that the drugs available to the consumers are of standard quality conforming to established specification regarding identity, strength, purity etc and also that it is safe and efficacious.

The manufacture of drugs in bulk and formulating them for pharmaceutical marketing requires analysis in the process control and quality control operations. Development of a new analytical method often occurs in response to specific need In many instances the new technique takes the form of an extension of some existing principle of measurement. Improvements in existing methods can be especially worthwhile if they improve the accuracy or lower the unit cost of an analysis. The procedures may be elaborate or simple. The various standards applied to the different pharmaceutical preparations depend upon the type of the formulation, whether in liquid form, tablet or capsule form or parenteral preparation.

For many years analytical chemistry was concerned with the chemical properties of the materials analysed It was common practice to dissolve the sample and then carry out some chemical reaction on the chemical component of interest, the extent of the reaction was then measured. Once the chemical reaction has been measured it was possible to calculate how much of the component was present in the sample Schemes of qualitative and quantitative analysis have been developed based on chemical reactions. The most important analytical fields involved are volumetric and gravimetric analysis. Unfortunately these demands a high degree of skill, patience and care on the part of the analytical chemist before he/she can get reliable results. But modern industry has grown so fast and control of manufactured products has become so important and vast a problem that the few skilled analytical wet chemists that are available can not provide the information needed.

Most pharmaceutical formulations are complex mixtures including, in addition to one or more medicinally active ingredients, a number of inert materials such as diluents, disintegrants,

colours and flavours. In order to ensure quality and stability of the final product the pharmaceutical analyst must be able to separate these mixtures into individual components prior to quantitative analysis and in the last two decades have seen the development of highly potent drugs of varied chemical structures. This fast and rapid development has created unanticipated problems for pharmaceutical analysts. Among the most powerful techniques available to the analyst for the resolution of these mixtures are a group of highly efficient methods collectively called chromatography. Because this technique is so intimately involved in all aspects of pharmaceutical research and development, the pharmacist should possess a working knowledge of chromatographic principles and techniques. But the recent advances in instrumental methods of analysis like UV-Visible, HPLC, Flourimeter etc, have helped to establish this rather new techniques, as the mainstream of the analytical laboratory. The conventional wet chemical methods gradually became obsolute or played a minor role in the analytical discipline which has led researchers to invent new methods and evolve new techniques.

The techniques widely used for the analysis of drugs, specially in the Asian Sub-continent are spectroscopy and chromatography. The absorption spectrum in the UV-Visible region is obtained when molecules absorb energies of electromagnetic radiation with a variation in electronic energy accompanied by changes translational energies. The molecular absorption therefore depends on the electronic structure of the molecule. Substances which show UV-Visible absorbance exhibit in general unsaturated character (chromophore). These chromophores when conjugated, exhibit an enhanced absorption with increase in absorption maxima. The spectrophotometric methods developed are therefore based on the introduction of conjugated systems (1,2).

The pharmaceutically important organic compounds are generally colourless or pale in colour. Colourimetric methods selectively transform the drug so as to shift the absorbance maximum to the visible region, away from the interference caused by another drug, formulation components or biological substances, thereby conferring a further degree of specificity to methods. A drug with little or no useful absorption can be more sensitively determined by modifying it to a more highly absorptive chromophore. The formation of such chromophore is mainly based on the active functional groups present in the drug structures. The most commonly encountered moieties are the phenolic, amino, aldehyde, ketonic and indole groups in addition to ether and heterocyclic residues (1,2). Many of the organic compounds absorb light in the UV and visible

regions of the electromagnetic spectrum when the electrons are excited from lower energy molecular orbitals to higher ones.

Fluorescence is defined as the radiation emitted in the transition of a molecule from a singlet excited state to a singlet ground state. With some compounds a process known as intersystem crossing can also occur. Here a molecule in the lowest vibrational level of the excited state converts to a triplet state, a state lying at an energy level intermediate between ground and excited states and characterised by an unpairing of two electrons. Once intersystem crossing has occured, a molecule quickly drops to the lowest vibrational relaxation. The triplet state is much longer lived than the corresponding singlet state with lifetimes of 10^{-4} to 10 sec. From triplet state a molecule can drop to the ground state by emission of radiation. This type of luminescence is termed phosphorescence. Fluorometric methods are limited to those compounds which possess a system of conjugated double bonds. A compound must absorb radiation in order to fluorescence, and it is the presence within a molecule of the mobile π electrons.

Molecules that have absorbed light are in a higher electronic state, they must lose their excess energy to return back to the ground state. If the excited molecule returns to the ground state by emitting light, it exhibits photoluminescence. The decay time of flourescence is of the same order of magnitude as the life time of an excited singlet state (10^{-9} to 10^{-7}). It is usually seen at moderate temperature in liquid solution. Flourimetry has assumed a major role in drug analysis in biologic samples because it has a greater sensitivity (by a factor of as great as 1000) and selectivity than absorption spectrophotometry.Fluorescence may be expected generally in molecules that are aromatic or contain multiple conjugated double bonds with a high degree of resonance stability. Both classes of substances have delocalised electrons that can be placed in low lying excited singlet states. In polycyclic aromatic systems where the number of electrons avialable is greater than in benzene, these compounds and their derivatives are usually much more flourescent than benzene and its derivatives. Those that are the most planar, rigid and sterically uncrowded are the most fluorescent. The fluorescent intensity and wavelength often vary with solvent (3, 4).

Chromatographic techniques by virtue of their scope, sensitivity, speed and reliability are useful in pharmaceutical and forensic chemistry. Chromatography in its various forms such as column,

paper, thin layer, gas and modern liquid chromatography (LC) has been extensively used in analytical chemistry. LC has the advantage of being convenient, rapid, accurate and versatile.

Thin layer chromatography in the conventional sense is rapidly becoming improved with high performance thin layer chromatography (HPTLC), separations and quantitative analysis can be carried out in as little as five minutes. The basic difference between conventional TLC and HPTLC plates is in the particle and pore size of the sorbents used. This ability has resulted in sorbents that are very reproducible and have high resolution. This means that results can be obtained in shorter times with less mobile phase (5) and are more accurate than TLC technique.

The analytical viability of the proposed methods was studied by comparing the results with those obtained using the official pharmacopoeial and standard procedures published in the text books. The results of the analytical studies using the methods were subjected to statistical analysis in order to critically evaluate the methods.Selection of method and procedures is based on their utility for the purpose of assuring the quality of the pharmaceuticals.

For various formulations several methods have been included so that the analyst can intelligently select and apply the method to suit his need and facilities available. Preliminary treatment of the samples for analysis utilising conventional isolation and extraction procedures have been applied wherever necessary to eliminate the presence of potentially interfering substances. In other cases, the methods are sufficiently specific to permit the analysis of individual ingredients without pretreatment. The procedures have been described in sufficient detail highlighting the important steps in methodology so that these can be reproduced without much difficulty.

One of the salient features of the thesis is that, simple methods based on differential solubilities, have been extensively used in separation and quantitation of individual components in several bicomponent drug formulations. Such methods have been found to give reasonably satisfactory results for routine quality assurance. However, the analyst may bear in mind that a procedure for an ingredient in a particular formulation may not always be applicable for the ingredient in other formulations of different composition, because of mutual interference of other ingredients.

Chemicals and Reagents

Reliability of the results partly depends upon the quality of the reagents used in assay procedures. All chemicals required during analysis are of reagent grade. Specific reagents are described under particular methods.

Reference substance / Standards

Reference substance are authentic specimens, which have been verified for use as standard for comparison. All solutions were freshly prepared in double distilled water.

Analytical techniques

The following analytical techniques have been employed for estimation of different components in formulations.

(1) Volumetric, based on stoichiometric relationship
(2) Ultraviolet and Visible absorption spectrometry
(3) Fluorimetry
(4) Thin Layer Chromatography
(5) High Performance Liquid Chromatography
(6) High Performance Thin Layer Chromatography

The drugs belonging to the categories of analgesics, antiinflammatory, and diuretics were chosen, as these are easily available in the market with out any restrictions and also widely used.

A good analytical procedure should be cheap, accurate and give reproducible results. The development of such new analytical procedures for complex drug products will be very useful in routine analytical procedures where simple and quick methods are usually preferred.

The work presented in this study is organised into various chapters as follows :

Chapter 1 : Gives the introduction and background of the investigations undertaken.
Chapter 2 . Discusses the statement of problem indicating the aims and objectives.
Chapter 3 : Gives the details on drug profiles.
Chapter 4 : Deals with the experimental, results, calculations and discussions of each investigation undertaken in this research work.
Chapter 5 : Gives the Conclusions & Summary.

CHAPTER-2

STATEMENT OF PROBLEM

Aims and Objectives

The non narcotic analgesics are most chosen group as they do not have any serious dependence and are free from unwanted effects of opiads on central nervous system. Besides analgesia they also possess antipyretic effects and some are antiinflammatory or antirheumatic.

Analgesics, antiinflammatory and antipyretics do constitute an important therapeutic group globally, both in terms of the number of patients it serves as well as the volume of production and hence the net market share it holds. Besides, this group also has the largest quantum and value of OTC and generic drugs on sale. Thus, it is obvious that most of the drugs in this category have been in use for decades though their patents have been expired long ago.

Similarly the diuretic agents were chosen for their extensive use by the patients in edematous effects and also in the treatment of patients with conditions such as congestive heart failure or hepatic or renal or pulmonary diseases where salt and water retention has resulted in edema or ascites.

Hence the drugs belonging to these two classess have been selected for the development of new analytical methods. For a chemical process to be successful, the participation and working of experts in different fields including analytical chemists is essential. Devising accurate assay procedures for each ingredient of complex dosage formulations containing several therapeutically and chemically compatible drugs with very similar chemical nature is a monumental undertaking. The presence of additives, excipients and decomposition products further complicates the development of analytical procedures. An analyst who is in the need of an analytical method may be obliged to survey tremendous amount of literature in order to select one procedure which may appear to suit his need and facilities available, but when he subjects the selected procedure to actual test, he/she may find that, he/she cannot reproduce the method. It is therefore essential that the practising analyst should have access to analytical procedures which are simple, reliable and accurate under routine laboratory conditions.

Modern analytical techniques rely increasingly on complex techniques of analysis that require expensive equipments and highly specialised personnel. Such methods are not of much help in countries lacking these resources. For the most part, modern analytical techniques merely permit analysis to be carried out more rapidly than the conventional method of analysis, which though not bad are tedious and time consuming. The selection of analytical method is a very

critical point. The method must be accurate, sensitive, selective, reproducible and convenient. Keeping in view the economic and technical limitations of drug manufacturers particularly in medium and small scale sector, it was felt that there exist a need to have compilation of simple, suitable and reliable methods of analysis of each ingredient of multi-component drug formulations and conform to the specification mentioned on the label.

The choice of analytical method depends on the range of concentration and percentage of the components to be determined. Sometimes it is determined by the composition of the substance and whether the determination is for a single canstituent or for several components. There are quite a number of individual methods of analysis for the drugs in literature but official methods of analysis for the simultaneous determination were not available for the combinations mentioned below. Therefore a need for a simple, sensitive, accurate and relatively inexpensive analytical procedure was felt and consequently being developed in this work.

The various new analytical procedures undertaken for investigation of drug combinations are :

1. Determination of oxyphenbutazone or ibuprofen in presence of paracetamol and dextropropoxyphene hydrochloride in dosage forms by quantitative thin layer chromatography.
2. Determination of paracetamol, dextropropoxyphene hydrochloride and dicycloamine hydrochloride in pharmaceutical formulations by quantitative thin layer chromatography.
3. Determination of propyphenazone and ketoprofen by quantitative thin layer chromatography, high performance thin layer chromatography and high performance liquid chromatography.
4. Simultaneous determination of mefenamic acid and paracetamol by UV-Absorption method.
5. Simultaneous and selective determination of paracetamol and diclofenac sodium in dosage forms by titrimetry.
6. Simultaneous determination of spironolactone in combination with frusemide and spironolactone in combination with hydroflumethiazide in formulations by UV-absorption spectroscopy.
7. Determination of spironolactone by spectrophotometry.
8. Fluorimetric determination of spironolactone in presence of frusemide and hydroflumethiazide in formulations.

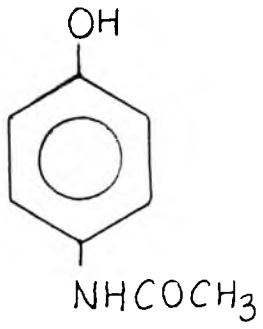
CHAPTER - 3

DRUG PROFILES

3.1 PARACETAMOL

4'-Hydroxyacetanilide; p-Hydroxyacetanilide; p-acetamidophenol; p-acetylaminophenol ; N-acetyl-p-amino-phenol ; Acetaminophen; N-P-Hydroxyphenylacetamide

Structure :



Empirical Formula : $C_8H_9NO_2$

Molecular Weight : 151.16

Melting Point : 169 - 170.5° C

Absorption Maxima : 250 nm in ethanol

The UV spectra of Paracetamol in water, acidified water, neutral methanol, acidified methanol and methanol containing sodium hydroxide has been obtained..In neutral water : 242 nm (major peak) 280 nm (minor peak).Addition of acid does not seem to effect either band. In neutral methanol : 243 nm. Its position is not affected by acid.Addition of sodium methoxide to methanolic solution caused bathochromic shift from 243 nm to 262 nm. The shift is due to the ionisation of Paracetamol to p-acetamidophenolate ion.

Appearance, Colour, Odour & Taste :

White, odorless, slightly bitter crystalline powder

Solubility :

Soluble in methanol, ethanol, dimethylformamide, ethylene dichloride, acetone, ethyl acetate, Slightly soluble in ether.Insoluble in petroleum ether, pentane, benzene. Soluble in solutions of alkali hydroxides.

1 in 70 of water

1 in 20 of boiling water

1 in 07 of alcohol

1 in 13 of acetone

1 in 50 of chloroform

1 in 40 of glycerol

1 in 10 of methyl alcohol

1 in 09 of propylene glycol

A saturated solution has a pH of about 6.0.

Therapeutic Category : Analgesic, Antipyretic

Use : Manufacture of azo dyes, photographic chemicals

Storage : Should be kept in a well closed container, protected from light.

Mechanism of Action :

Analgesic effect : Has an analgesic effect on the pain-producing actions of bradykinin. In addition it blocks prostaglandin synthesis and prostaglandins may sensatize nerve endings to the pain producing effect of bradykinin.

Antipyretic effect : Normal body temperature is maintained by the balance between heat production and heat dissipation. Central regulation of this process is accompanied in the hypothalamus. Under normal circumstances, increased heat production, such as is caused by muscular exercise, brings about increased heat dissipation through peripheral vasodilatation and sweating. Thus the constancy of body temperature is maintained within relatively narrow limits.In fever there appears to be primarily a defect in heat dissipation. Although the production of heat may be increased, it is not followed by a corresponding increase in heat dissipation. The temperature regulating centres behave under these conditions much as would a thermostat that has been set higher. The salicylates lower the temperature in fever by increasing heat loss through promotion of peripheral vasodilation and sweating. Sweating is not essential for the antipyretic action, since temperature can be lowered by the salicylates even when sweating is prevented by atropine.The possibility of the prostaglandins playing a role in temperature regulation is

of great interest. Prostaglandins E1 and E2 produced hyperthermia when injected. This is especially interesting in view of the inhibitory action of several antipyretics on prostaglandin synthesis.It has only weak antiinflammatory and antirheumatic actions presumably because it has little effect peripherally on prostaglandin synthetase.

Methods Of Analysis :

Numerous methods have been reported for the analysis of acetaminophen in pharmaceuticals and in biological fluids. Colorimetric techniques have been frequently employed in clinical laboratories (12-14) but they may be subject to interference by paracetamol metabolites, salicylate, phenacetin or uremic or icteric serum (15). Gas chromatography with (16-18) or without (19-20)derivatization, was found to be more specific technique. High pressure liquid chromatography was both sensitive and specific for clinical purposes (21-26) and has advantage of being applicable to the measurement of the conjugated metabolites of paracetamol in plasma and urine (27-29).

Titrimetric Methods:

A titrimetric method for the estimation of paracetamol alone or in powdered tablets was reported (30). The powder is boiled with dilute HCl for 30 minutes. The solution cooled to less than 20 °.A mixed indicator [(0.15% methylene blue-tropaeolin 00 (C.I. Acid orange 5) (1:1)] and KBr are added and the solution is titrated with 0.1M $NaNO_2$.

Analysis of paracetamol and barbiturate combination has been carried out by differentiating non-aqueous titration (31). Mixtures containing paracetamol, aspirin and salicylamide were assayed potentiometrically by non-aqueous titration (32). The difference in pKa values for these weak acids was sufficient to permit successful differentiation. The titrant was tetrabutylammonium hydroxide and the titration solvent was dimethylformamide. The procedure was applied to commercial dosage forms.

A titrimetric method was developed and used for the determination of paracetamol in tablet formulations. The method compared well with spectrophotometry and allowed a high percentage recovery of the drug (33).

Spectroscopic methods:

Ultraviolet methods

A differential spectrophotometric method involving the determination of the absorbance at 267 nm of an alkaline solution against a blank and Glenn's method of orthogonal function are described (34) for the assay of paracetamol in tablets, syrups and suppositories. Interferences from excipients in the formulations is thereby avoided.Paracetamol has been determined spectrophotometrically (35) in multicomponent tablets. A quantity of the powdered tablets is dissolved in methanol, diluted with water and extracted with ethyl ether. The aqueous layer is made alkaline with 1M NaOH, extracted with $CHCl_3$ and determined at 264 nm in the NaOH layer.

A spectrophotometric method was developed (36) for the determination of paracetamol in the preparation of its main degradation product, p-aminophenol. It involves measuring the absorbance in 0.1N HCl between 231 nm at 10 nm intervals. By substitution in a given formula, the calculated coefficient of the quadratic orthogonal polynomial will be proportional to the authentic samples was 100.31%.

Two spectrophotometric methods, an absorbance ratio technique and a difference spectrophotometric technique, are described for the simultaneous determination of chlorzoxazone and paracetamol in combined dosage forms. The difference between the results and the reproducibility of both methods were insignificant and the methods were simple to use for routine and control analysis of the drug combination (37).

An automated LC method with UV detection has been described for the quantitative determination of paracetamol and 6 of its metabolites in human urine (38).

A simple and rapid spectrophotometric method for the determination of paracetamol in drug formulations was developed by reaction with 2-iodyl benzoate in dil. acid produces an ornage yellow colour that reaches its maximum intensity with one minute, the λmax

absorbance being 444 nm. The method is suitable for routine analysis and unaffected by the presence of other drugs (39).

A sensitive HPLC method with UV detection was described for the determination of paracetamol and propoxyphene combination in human plasma (40).

A method for the determination of paracetamol by dansylation for direct fluorescence measurement of the derivative on TLC was described (41).

A simple, rapid, accurate and precise spectrophotometric method based on ion-pair extraction with rodamine B was described for the quantitation of paracetamol in pharmaceutical preparations (42).

A simple rapid procedure for simultaneous determination of chloroxazone and paracetamol in two component formulation has been developed. The absorbance maximas of chloroxazone in 0.02N NaOH were found to be at 244 nm and 288 nm and that of paracetamol in 0.02N NaOH is 257 nm. The Beer's law obeyed by both in concentration range of 0 µg/ml to 25 µ/ml. This method has been validated and found to be satisfactory (43).

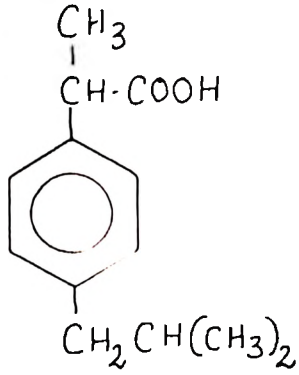
A first derivative spectroscopic method for the simultaneous determination of paracetamol and sodium salicylate in tablets was developed. The method, which can be used for tablet composite assay and content uniformity analysis, found to be linear for paracetamol concentrations ranging from 0.0-36.0 µ/ml. The potential of derivative spectroscopy as an analytical technique and its usefulness to rapidly & simultaneously quantitate active ingredients in multicomponent armaceuticals are demonstrated (44).

A coulometric method for the determination of paracetamol in pharmaceutical dosage forms, utilizing KBr and sulphuric acid as the electrolyte solution was presented (45).

3.2 IBUPROFEN

p-Isobutylhydratropic acid ; 2-(4-isobutyl-phenyl)propionic acid

Structure :



Empirical Formula : $C_{13}H_{18}O_2$

Molecular Weight : 206.27

Melting Range : 75-77.5 °C

UV-Spectra : 0.025% w/v solution in 0.1M NaOH exhibits maxima at 264 nm (0.93) & 273 nm (0.78)

Appearance, Colour, Odour & Taste :

A white or almost white powder or crystals with a characteristic odour.

Solubility : Practically insoluble in water, soluble 1 in 1.5 of alcohol (96%), in 1 part of $CHCl_3$ 2 parts of ether and 1.5 parts of acetone, also soluble in aqueous solutions of alkali hydroxides & carbonates.

Therapeutic Category : Antiinflammatory agent, Analgesic and Antipyretic.

Mechanism of Action :

Ibuprofen is a potent inhibitor of cyclo-oxygenase. In this action its potency is similar to that of aspirin but somewhat less than that of indomethacin. Ibuprofen also inhibits the synthesis of some products of the lipo-oxygenase pathway such as 11 and 15-monohydroxyeicotetranoic acid (HETE), but has no effect on the formation of leukotriene B4. Ibuprofen inhibits migration of polymorphonuclear leucocytes. Inhibition of superoxide radicals has been demonstrated in some studies.

Ibuprofen inhibits the formation of the vasodilator prostanoid, PGE2. This reduces the increased vascularity and permeability and consequent transudation of fluid which are typical of inflammation states. Inhibition of PGs is not restricted to inflamed areas.

Methods of Analysis :

(a) As per BP, 1980, Dissolve 0.5 gm in 100 ml of ethanol(96%) previously neutralised to phenolphthalein solution and titrate with 0.1M NaOH using phenolphthalein solution as indicator

Each ml of 0.1M NaOH = 0.02063g of $C_{13}H_{18}O_2$

(b) Gas Chromatography :

A capillary gas-liquid chromatographic method for the estimation of Ibuprofen in tablets using flame ionisation detection was presented and compared with the official method of the IP (47).

A high resolution gas chromatographic method using fused silica capillary column for the determination of methyl esters of ibuprofen in human plasma and urine samples has been described. The calibration curve was linear in the range of 2-40 μ/ml of plasma and 2-100 μ/ml of urine (48).

(c) Colorimetric Analysis :

A colorimetric method for the determination of Ibuprofen in tablet dosage forms on the conversion of carboxylic acid into an acid chloride and its coupling with hydroxylamine to get

hydroxamic acid which forms a violet colored complex with vanadium in an acidic medium was described (49).

A colorimetric assay for ibuprofen, naproxen, ketoprofen, indomethacin, pirprofen and flurbiprofen was described which is based on the development of the characteristic orange Griess pigment with sodium nitrite, p-nitroaniline and 1- naphthylamine. The method was applicable to the analysis of the pure compounds and to dosage forms (50).

A simple spectrophotometric method of determination of ibuprofen in presence of paracetamol based on formation of blue colored species having maximum absorbance at 640 nm with methylene blue was reported. The colour obeys Beer's law in concentration range of 40-240 µg/ml(51).

(c) High Performance Liquid Chromatography :

A selective reversed phase high pressure liquid chromatographic method for the determination of ibuprofen in 11 different ointment bases was described. The analysis of Ibuprofen decomposition products showed that the method also provides an indication of the stability of the drug (52).

A highly sensitive and sensitive column switching HPLC method with UV detection has been developed for the determination of two acidic drugs, ibuprofen and mefenamic acid, in spiked human serum. The detection limits were 0.5 ng/ml for ibuprofen and 0.1 ng/ml for mefenamic acid using 1 ml of serum (53).

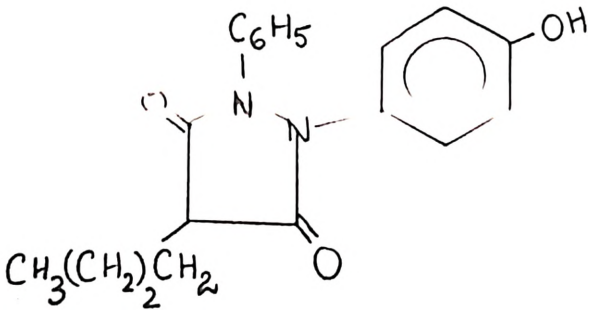
A sensitive, simple reversed phase ion-pair HPLC method with UV-detection was described for the determination of ibuprofen in human whole blood (54).

A reversed phase HPLC method has been developed for the estimation of ibuprofen and mefenamic acid in pharmaceutical dosage form. A phenomenex ODS column and a mobile phase consisted of CH_3 OH, water, acetic acid (80:20:01) were utilised. Evaluation was done at ambient temperature utilising UV detection at 235 nm. The results were calculated using standard curves of individual drugs (55).

3.3 OXYPHENBUTAZONE

p-Hydroxyphenylbutazone ; 4-Butyl-1-(p-hydroxyphenyl)-2-phenyl 3,5,pyrazolidinedione ; 4-Butyl-2(4 hydroxyphenyl)-1-phenyl pyrazolidine-3,5-dione.

Structure :



Empirical Formula : $C_{19}H_{20}N_2O_3 \cdot H_2O$

Molecular Weight : 324.37 (Anhy.)

342.39 (monohydrate)

Melting Point : 96 °C (monohydrate)

124 - 125 °C (anhy.)

UV-Spectrum :

In neutral methanol the drug exhibits two maxima at 243 and 265 nm and a maximum at 221 nm. A 0.001% w/v solution of Oxyphenbutazone in 0.01N NaOH solution exhibits a maximum only at 254 nm with an extinction of about 1.5. In ethanol it showed maxima at 242 nm (E1 %, 1 cm 564) and an inflexion at 275 nm.

Appearance, Colour, Odour & Taste :

White crystalline powder,odorless or almost odorless with bitter taste.

Solubility :

Practically insoluble in water, Soluble in 3 parts of alcohol (95%), 20 parts of chloroform, in 20 parts of solvent ether, 6 parts of acetone ; also soluble in solutions of alkali hydroxides, methanol, benzene and forms a water-soluble sodium salt.

Therapeutic Category : Antiinflammatory agent and Analgesic

Storage : Preserve in well closed containers.

Mechanism of Action :

Like salicylates it inhibits the biosynthesis of prostaglandins. It also diminishes the reabsorption of urate by proximal renal tubules and exerts the uricosuric effect. The compound, a metabolitic degradation product of phenylbutazone, is claimed to cause less gastric irritation than phenylbutazone.

Methods of Analysis :

(a) Titrime tric Methods :

The BP 1980 (46) describes a titrimetric method in which oxyphenbutazone solution in acetone is titrated with standard sodium hydroxide solution using bromothymol blue as indicator until a blue color persists for at least 30 seconds. The operation is reported without the drug; the difference between the titrations represents the amount of alkali required by oxyphenbutazone.

The USP 1980 (56) also recommends a standard sodium hydroxide titrimetric method but the drug solution is made in methanol and the end point is determined potentiometrically using a calomel-glass electrode system with a saturated salt bridge of potassium chloride in methanol. A blank determination is necessary.

Walash and Rizk (57) determined oxyphenbutazone in certain dosage forms by a nonaqueous titration procedure in tetramethylurea using standard sodium methoxide as a titrant. Recovery from compound preparations of the analgesic was quantitative and the end point could be located either potentiometrically using glass-calomel electrode system or visually using thymol blue as indicator.

(b) Electrochemical Methods :

Pelinard et al (58) studied the electrochemical behavior of antiinflammatory derivatives of pyrazolone and the application to determination in medicaments. Oxyphenbutazone and other 3,5-pyrazolinediones with mobile hydrogen atoms in position 4 were directly reduced

electrochemically in neutral ethanol. Monoketones, phenazone and aminophenazone were not reduced under similar conditions.

(c) Spectrophotometric Methods :

Herrmann (59) reported an ultraviolet method for the determination of phenylbutazolidine together with its metabolic products, chiefly oxyphenbutazone, in serum. The serum is shaken with 3N hydrochloric acid in 3 % isoamyl alcohol in heptane. After centrifugation an aliquot of the organic phase is shaken with 2.5N sodium hydroxide and the extinction of the alkaline extract is determined at 265 nm.

(d) Colorimetric Methods :

Svatek and Hradkova (60) determined oxyphenbutazone and related keto derivatives in serum using a colorimetric method involving the reaction of the drugs with diazotised sulfanilic acid. According to the procedure, the diluted serum is shaken with N hydrochloric acid, heptane and isoamyl alcohol. The heptane phase is shaken with 0.1N sodium hydroxide. The alkaline extract is shaken with a mixture of 1 % sulfanilic acid, N hydrochloric acid, 1 % sodium nitrite and methanol. The absorbance of this solution is measured at 525 nm against sodium hydroxide blank.

Wahbi et al (61) used sodium cobaltinitrite as a reagent for determining some phenolic drugs. When oxyphenbutazone is reacted with the reagent in aqueous acetic acid a yellow color measurable at 320 nm is produced. Substituting sodium hydroxide for acetic acid gives a color measurable at 380 nm. The method is applied to the determination in tablet form.

An accurate selective and simple method for the determination of oxyphenbutazone was proposed. The method is based on the formation of oxidised derivative of oxyphenbutazone in alkaline medium. In the proposed method, oxidised derivative of oxyphenbutazone is carried out by potassium ferricyanide in presence of 0.1N sodium hydroxide. The orange coloured complex shows maximum absorption at 460 nm (62).

Sanghavi et al (63) treated oxyphenbutazone with a mixture of anhydrous acetic acid and hydrochloric acid. The product of the heated mixture is reacted with vanillin or 4

dimethylaminobenzaldehyde in acetic acid. The resulting color is measured absorptiometrically or at 424 nm with vanillin or at 406 nm with 4-dimethylaminobenzaldehyde.

The analysis of oxyphenbutazone in drug formulations containing other analgesics and antiinflammatory drugs by derivatisation of the drug to indophenol, and its spectrophotometric determination are described (64).

(e) Spectrofluorimetric Method :

Miller et al (65) studied the luminescence properties of oxyphenbutazone and some other antiinflammatory and antipyretic drugs. Although oxyphenbutazone showed no significant fluorescence at room temperature it was strongly fluorescence at 77K. Submicrogram quantities of the drug could be readily detected. The wavelength of excitation maximum in ethanol was 280 nm and that of the fluorescence maximum was 430 nm.

(f) Chromatographic Methods :

Thin Layer Chromatography (TLC) :

Several TLC methods are available for the separation, identification and determination of oxyphenbutazone and its metabolites.

Schmidt (66) analysed Oxyphenbutazone together with other pharmaceuticals by staining on silica gel GF 254 using UV light visualisation. An Avicel layer chromatography was developed by Lee (67) for the separation and identification of several kinds of drugs. Acidic materials were separated by acidic solvents and basic materials were satisfactorily by basic solvents using mixed Avicel kieselgel plates. The procedure was applied to the different pharmaceutical formulations.

Paper Chromatography :

A paper chromatographic method was described by Clark (68).Clark described a paper chromatographic method for the identification of the drug using 3 different solvent systems.

System I : Consists of citric acid : water : n-butanol (4.8 gm : 130 ml : 870 ml). Detection carried out using UV light absorption. p-Dimethylaminobenzaldehyde or $KMnO_4$ can be used as spray to locate the drug. Rf = 0.96.

System II : Acetate buffer (pH 4.58). Detection was effected by UV light absorption. Rf = 0.04.

System III : Phosphate buffer (pH 7.4). Detection was effected by UV light absorption. Rf = 0.58.

An identification test for the drug by TLC was also supported by Clarke. The solvent system consists of strong ammonia solution and methanol (1.5 : 100). Detection was effected by $KMnO_4$ spray. Rf = 0.77.

Gas Chromatography :

Bruce et al (69) determined oxyphenbutazone and phenylbutazone in plasma and urine by gas-liquid chromatography.While phenylbutazone was determined directly after extraction from blood, plasma or urine, oxyphenbutazone was reacted with heptafluorobutyric anhydride prior to gas chromatography.

(f) High Performance Liquid Chromatography (HPLC) :

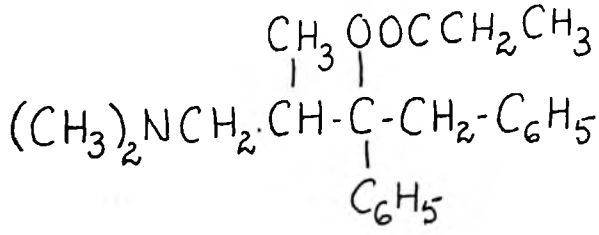
Pound and Sears (70) described a sensitive, specific, high-speed liquid chromatographic procedure for the simultaneous determination of phenylbutazone and its metabolite, oxyphenbutazone in blood plasma. Use of a UV detector permits quantitative analysis of samples containing less than 0.25 µg/ml of phenylbutazone or oxyphenbutazone.

Harzer and Barchet (71) separated various combinations of analgesics by HPLC, using both reverse-phase and adsorption columns and UV detection at 254 nm. Use of a variable wavelength detector enhanced the sensitivity by permitting adjustment to the absorption maximum of each drug.

3.4 PROPOXYPHENE

4- dimethylamino-3-methyl-1,2-diphenyl-2-butanol propionate

Structure :



Empirical Formula : $C_{22}H_{29}NO_2$

Molecular Weight : 375.94

Melting Point : 170 - 171° C

UV-Spectrum : The absorption maxima of 242, 247, 252, 258, 264 and 267 nm are typical of fine structure due to isolated benzene chromophore using 95 % ethanol.

λnm	Absorbance (corrected)	E
242	0.266	137
247	0.389	200
252	0.541	279
258	0.698	360
264	0.559	288
267	0.303	156

Appearance, Colour, Odour & Taste :

White crystalline powder with no noticeable odor and a bitter taste.

Solubility :

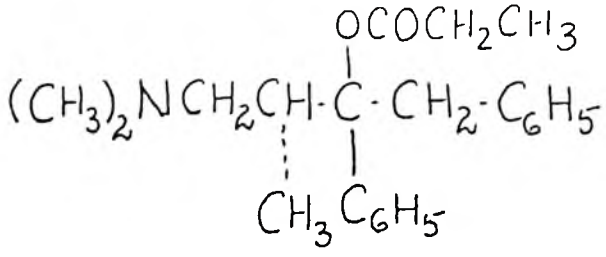
Soluble in water, alcohol, chloroform, acetone, practically insoluble in benzene, ether.In accordance with the usual practice the less soluble diastereoisomer is designated as the α isomer, and the more soluble as the β-form. The α-dl and d-diastereoisomers possess marked analgesic activity in contrast to the β-diastereoisomers which are substantially inactive.

DEXTROPROPOXYPHENE

α-d-4-Dimethylamino-3-methyl-1,2-diphenyl-2-butanol propionate ; d-propoxyphene

Used as a hydrochloride

Structure :



Empirical Formula : $C_{22}H_{29}NO_2.HCl$

Molecular weight : 339.48

Melting Point : 75 - 76° C

Melting Point : 163-168.5 °C (as hydrochloride obtained as bitter crystals from methanol and ethyl acetate)

Solubility : Soluble in water, alcohol, chloroform, acetone, practically insoluble in benzene, ether

Therapeutic Category : Analgesic

Mechanism of Action :

As an analgesic it is only 1/25-1/50th as potent as morphine and is 1/2 as potent as codeine. 65 mg of propoxyphene HCl is no more effective than 650 mg of aspirin. It has no antitussive action, antidiarrheal or antipyretic effect, thus differing from most analgesic systems. It is able to suppress the morphine abstinence syndrome in addicts but has shown a low level of abuse because of its toxicity. It is not very effective in deep pain and appears to be no more effective in minor pain than aspirin.

Methods of Analysis :

(a) Non-Aqueous Titration :

The non-aqueous titration (NAT) of propoxyphene hydrochloride was a fast & simple procedure(72). Accurately weigh about 600 mg of sample, dissolve in 40 ml of glacial acetic

acid and add 10 ml of mercuric acetate. Add crystal violet and titrate with 0.1N perchloric acid. Perform a blank titration and correct the sample titration when necessary.

Each ml of 0.1N perchloric acid is equivalent to 37.59 mg of propoxyphene hydrochloride.

(b) Infrared Absorption :

In most forms of quantitative spectroscopy, the sample and standard should be handled identically. In this IR assay (73) one accurately weighs about 130 mg of sample and of USP propoxyphene hydrochloride reference standard; transfer both (quantitatively) to 125 ml separatory funnels, containing 25 ml of water. Add 0.4 ml of sodium hydroxide solution (1 in 2) and 50 ml of chloroform. Extract for 3 minutes and then allow the layers to separate. Drain the organic phase through anhydrous sodium sulfate into a 250 ml beaker. Repeat the extraction with 3-50 ml portions of chloroform and pool them in the 250 ml beaker. Evaporate (on steam bath with air) to a small volume; then transfer (quantitatively) to a 50 ml volumetric flask, dilute to volume with chloroform and mix. Using a suitable IR spectrophotometer and 1 mm cells, read the sample and standard at the maximum (about 5.80 μ) using chloroform as the blank. The calculation used as follows :

$$\frac{\text{Sample Abs.}}{\text{Std Abs}} \times \frac{\text{Std. Wt}}{\text{Sample Wt}} \times 100 = \%\ \text{propoxyphene hydrochloride}$$

(c) Ultraviolet Absorption :

Accurately weigh about 25 mg of sample and USP propoxyphene HCl reference standard; transfer both (quantitatively) to 100 ml volumetric flasks, dilute to volume with purified water and mix. Determine the absorbance of both solutions at the maximum (about 257 mμ) using 1 cm silica cells with purified water as blank (74).

(d) Gas Chromatography :

For the assay of propoxyphene HCl, pyrroliphene HCl, is an excellent choice of internal standard (75). Individual analysts usually have preferences on operating conditions. For this assay a flame ionisation detector is desirable, because of its sensitivity. Detection of

microgram quantities is practicable under the conditions described by Wolen and Gruber (76). Other investigators have used much the same technique (77).

(e) Visible Absorption (With an autoanalyser) :

Propoxyphene HCl can be assayed by visible absorption spectroscopy. An automated assay of this type is found in literature (78). Since a variety of dyes will complex with tertiary amines, studies were undertaken to determine which dye is most specific and has the least retention on the analytical train. Bromocresol purple seems best suited for this type assay.

(f) Thin Layer Chromatography :

TLC of propoxyphene HCl (and other analgesics) is well documented (79). Emmerson and Anderson gave Rf values for thirteen solvent systems. The use of slightly alkaline absorption layers and ammonium saturated developing chambers is discussed in detail. The slight alkalinity facilitates spot movement on the plates. An iodoplatinate spray was used to locate the spots. For true quantitative results the spots should be removed and the propoxyphene HCl determined by UV or other instrumental methods (80).

Another source (81) gives Rf values for five solvent systems. These systems are unique because they are salted (with either ammonium chloride or sodium chloride), which results in greatly reduced tailing and zone diffusion.

(g) High Performance Liquid Chromatography :

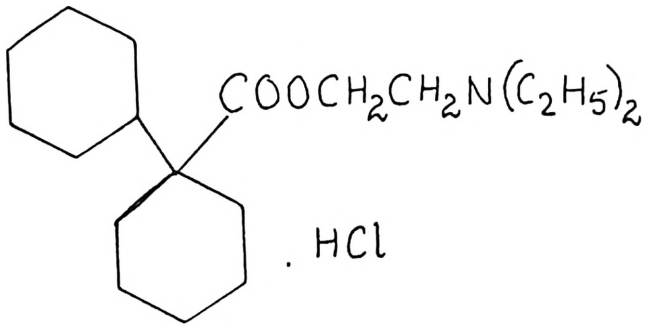
An HPLC method for tablets and capsules has been described (82).

A reversed phase high performance liquid chromatographic method for simultaneous quantitation of propoxyphene napsylate (dextropropoxyphene napsylate I) in 3 different capsule formulations containing 50 mg I plus 30 mg caffeine and 325 mg aspirin or 50 or 100 mg I plus 325 mg paracetamol was presented and its application to dissolution rate of studies of capsule formulation was described.The mobile phase consisted of acetonitrile (30 %) in acetate buffer. The assay was conducted at 280 nm wavelength. The method was found to be accurate for I in the presence of the other drugs and to be an acceptable alternative to the official gas chromatography method (83).

3.5 DICYCLOAMINE HYDROCHLORIDE

(Bicyclohexyl)-1-carboxylic acid 2-(diethylamino)ethyl ether hydrochloride, Dicycloverine HCl

Structure :



Empirical Formula : $C_{19}H_{35}NO_2.HCl$

Molecular Weight : 346.0

Melting Range : 169- 174° C

Appearance, Colour, Odour & Taste :

A white or almost white, odorless or almost odorless crystalline powder.

Solubility :

In Water about 25 % (1 in 13-20 of water), 1 in 5 of alcohol, and 1 in 2 of chloroform, practically insoluble in ether

pH : A 1 % solution is 5.0 - 5.5

Therapeutic Category : Anticholinergic, antispasmodic in GI disorders

Storage : Preserve in well closed containers

Mechanism of Action :

It is a tertiary amine, has a prominant direct relaxant effect on the smooth muscle. The acid portion is somewhat bulky in nature which is an indication for atleast one portion of the molecule to have the space occupying umbrella like shape which leads to firm binding at the receptor site area. Another important feature to be found in that to contain a quaternary nitrogen, presumably to enhance activity.

Methods of Analysis :

(a) As per USP,1995 (72), Dissolve 600 mg of a Dicycloamine HCl, accurately weighed in 70ml of glacial acetic acid previously neutralised using the indicator crystal violet, warming & cooling if necessary. As the substance is a salt of hydrochloric acid, add 10 ml of mercuric acetate solution and titrate with 0.1N perchloric acid using crystal violet solution (1 drop) as indicator to a blue end-point. Perform a blank determination, and make any necessary correction.

Each ml of 0.1N perchloric acid = 0.03460 gm of $C_{19}H_{35}NO_2.HCl$.

(b) An NMR method was described for the determination of Dicycloamine HCl in tablet, capsule and injection dosage forms that avoid many of the problems encountered with GC and titrimetric methods and which at the same time can be used for positive identification of this drug in sample tested (82).

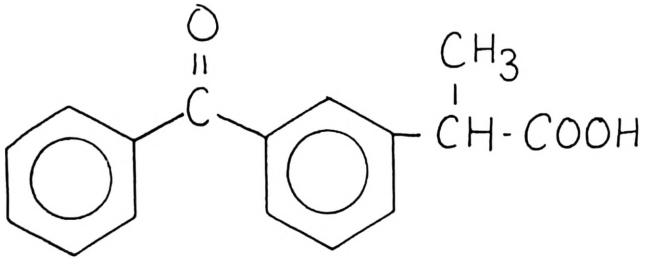
(c) A simple colorimetric estimation of dicycloamine from pharmaceutical formulations was based on ion-pair extraction technique using bromocresol purple solution at pH 5.3 ±0.1. The resulting yellow complex was extracted in chloroform and absorbance is measured at 408 nm along with colour formed by a standard solution of dicycloamine HCl under identical conditions (83).

(d) A GLC method was described for estimation of paracetamol and dicycloamine HCl in single and combined dosage forms using 5% OV-I column and flame ionisation detector, chloropheniramine maleate as internal standard (84).

3.6 KETOPROFEN

m-benzoyl hydratropic acid, α-(benzoyl phenyl) propionic acid

Structure :



Empirical Formula : $C_{16}H_{14}O_3$

Molecular Weight : 254.29

Melting Range : 93 - 95 °C

UV-Spectrum : Abdorption maxima appears at 261 nm in 0.1N HCl (1.2 pH), Distilled water (6.5 pH) and 0.1N NaOH (12.9 pH).

This maximum is independent of pH but the maximum absorbance is slightly decreased with increasing pH.The absorption maximum in methanol appears at 255 nm.The absorption maximum in ethanol reported at 255 nm and E 1%, 1cm is 640.

Appearance, Colour, Odour & Taste :

A slightly coloured,odorless, tasteless powder with an irritant dust.

Solubility :

Soluble in ether, ethanol, octanol, di-isopropyl ether, acetone, chloroform, DMF, methanol and ethyl acetate. Slightly soluble in water.

pK. : Dioxan : Water (2 : 1) is 7.2

Acetonitrile : Water (3 : 1) is 5.02

Methanol : Water (3 : 1) is 5.937

pH : The pH of a 3.95×10^{-4} M solution in water is 6.5.

Stability : Must be protected from light and moisture. It is stable at room temperature. Ketoprofen has been dissolved in ethyl acetate and stored for several weeks at 4° C with no detectable decomposition.

Therapeutic Category : Analgesic, Antiinflammatory and Antipyretic

Mechanism of Action :

Have properties similar to Aspirin but are better tolerated orally and the incidence of adverse reactions is low. They are particularly useful in patients with rheumatoid arthritis, osteo-arthritis and ankylosing spondylitis, who cannot tolerate aspirin.

Methods of Analysis :

(a) Thin Layer Chromatographic Analysis :

Thin layer chromatography on silica was found to be suitable as a fast preliminary purity test (85,86)

(b) Ultraviolet Spectroscopy :

Quantitative determinations of ketoprofen based on the peak maximum at 261 nm in distilled water (85) or 256 nm in methanol can be performed (86).

(c) Potentiometric Titration :

Blazevic, et al titrated ketoprofen with 0.1N NaOH by dissolving in acetonitrile : water (3:1) and titrated with 0.1N NaOH the potentiometric curves are recorded between pH 3.45-12.0. This method was convenient for measuring the purity of ketoprofen in the crystalline preparation and also the content in tablets (85).

(d) Gas Chromatography :

Gas chromatography was an inconvenient method for purity determination, as ketoprofen is partially decomposed by the procedure. This can be overcome by using the methylester or trimethylsilylester prepared quantitatively from ketoprofen (85,87).

(e) **Enantiomer Analysis :**

The ratio of (+)-ketoprofen to (-)-ketoprofen in a recemic mixture can be determined by reaction with a stereospecific molecule and the product was analysed by either gas chromatography (85) or high pressure liquid chromatography (88). The ratio of peak height or peak area respectively gives the ratio of the two enantiomers.

(f) **Colorimetric Analysis :**

Ketoprofen can be complexed with safranin and the absorption determined in $CHCl_3$ at 520 nm (89).

(g) **Potentiometric Titrations :**

300 mg of ground sample are dissolved in 5 ml of acetonitrile or ethanol and 15 ml of water added. The titration was performed with 0.1N NaOH and monitored using a glass electrode and a calomel reference electrode (85).

(h) **Pyrolysis-Gas Chromatography-Mass Spectrometry :**

Ground samples are dissolved in a solvent and a known amount applied to a rotating wire. After evaporation of the solvent the material was pyrolysed in a curie point pyrolyser at 770° C for 5 seconds, the temperature programmed from 100 to 240° C. On pyrolysis ketoprofen is rearranged to (3-benzoylphenyl)-ethane and (3-benzoylphenyl)-ethylene which have retention indices of 2.27 and 2.52 respectively.

(i) **High Performance Liquid Chromatography :**

A liquid chromatographic method for the determination of ketoprofen and 6 known related compounds have been developed. The lower limit of quantitation of the related compounds in the drug was 0.05 % or less for all compounds and the precision of the drug assay method was about 1.5 %. The highest levels of impurities found in 3 drug raw material samples and 4 solid oral dosage formulations were 1.37 and 0.43 % respectively (92).

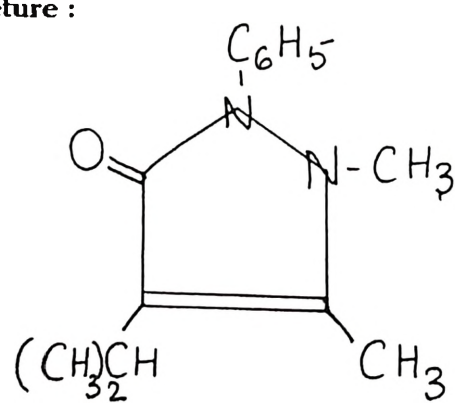
(j) As per BP (93), dissolve 0.5 gms in 25 ml of ethanol (96%), previously neutralised to phenolphthalein solution, add 25 ml water and titrate with 0.1M sodium hydroxide, using phenolphthalein solution as indicator.

Each ml of 0.1M sodium hydroxide ≡0.02543 gm of $C_{16}H_{14}O_3$

3.7 PROPYPHENAZONE

4-Isopropylantipyrine; 2,3-dimethyl-1-phenyl-4-isopropyl-pyrazolone ; Isopropylphenazone

Structure :



Empirical Formula : $C_{14}H_{18}N_2O$

Molecular Weight : 230.3

Melting Point : 103°C

UV-Spectrum : Absorption maxima in methanol shows at 245 and 276 nm.

Appearance, Colour, Odour & Taste :

White or slightly yellow crystalline powder.

Solubility : In water 0.24 gm/100 cc at 16.5 °C, readily soluble in alcohol, ether, methylene chloride.

Therapeutic Category : Analgesic

Storage : Protect from light.

Methods of Analysis :

(a) A reversed phase HPLC method has been developed to determine paracetamol, caffeine and propyphenazone in a typical tablet formulation with high accuracy and precision (94).

(b) Color reaction experiments for the differentiation of pyrazolone derivatives are described and identification of propyphenazone with UV, IR and NMR spectroscopy has been described (95).

(c) Two new spectrometric methods, a direct spectrophotometric method and a TLC spectrophotometric are described for the determination of propyphenazone and dipyrone combination and a propyphenazone and allobarbital combination (96).

(d) Propyphenazone dosage form analysis was carried out using a TLC separation procedure and UV-Spectrometry (97).

(e) Four different methods for determination of phenacetin, propyphenazone, caffeine and persedon in a combination preparation are described.

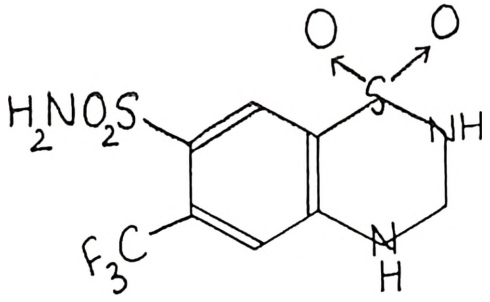
Two methods depend on UV-measurement of the active ingredient after separation by ion-exchange in one case and TLC in the other. A gas chromatographic procedure with a double flame ionisation detector was the most elegant method used. The last method was direct determination of the components using selective analytical reaction (98).

(f) A gas chromatographic method for the simultaneous determination of caffeine,paracetamol , propyphenazone in combination tablets has been developed (99).

3.8 HYDROFLUMETHIAZIDE

3, 4-Dihydro-6-(trifluoromethyl)-2-H-1, 2, 4-benzothiadiazine-7-sulfonamide 1, 1 dioxide; 6-trifluoro-methyl-7-sulfamyl-3,4-dihydro-1,2,4- benzothiadiazine 1,1-dioxide; 6-trifluoromethyl-3,4-dihydro-7-sulfamyl-2H-1,2,4-benzothiadiazine1,1-dioxide; 3,4-dihydro-7-sulfamyl-6-trifluoromethyl-1,2,4-benzothiadiazine1,1-dioxide;Triflouromethylhydrothiazide; Dihyydroflumethiazide; Methforylthiazidine

Structure



Empirical Formula : $C_8H_8F_3N_3O_4S_2$

Molecular Weight : 331.29

Melting Point : 272 - 273°C

pK$_1$: 8.9

pK$_2$: 10.7

Ultraviolet Spectrum :

(1) E(1%,1cm) is 450 at 276 nm at pH 10.0

(2) E(1%,1cm) is 580 at 273 nm at pH 02.0

(3) E(1%,1cm) is 560 at 274 nm in 70 % ethanol

(4) The absorptivity (log Σ) is reported as 4.182 at 275 nm in 0.1N sodium hydroxide

(5) The absorptivity (log Σ) is reported as 4.290 at 273.5 nm in 0.1N hydrochloric acid

(6) The absorptivity (log Σ) is reported as 4.229 at 272.5 nm in ethanol

(7) The absorptivity (log Σ) is reported as 4.286 at 272.5 nm in methanol

(8) The absorptivity of about 46 liter/g cm at 274 nm in 0.01N sodium hydroxide

Appearance, Colour, Odour :

White to cream colored, odorless,powder or crystals.

Solubility :

At room temperature solubilities are as follows :

Solvent	Approximate Solubility, mg/ml
Methanol	58
Ethanol	21
Ethyl acetate	11
Ethyl ether (anhy.)	0.2
Chloroform	0.1
Benzene	<0.1
Water	0.3 (15)
Acetonitrile	43
Acetone	>100

Hydroflumethiazide is easily soluble in the lower alcohols and ketones, tetrahydrofuran and pyridine. It is also easily soluble in bases. Hydroflumethiazide is soluble in a mixture of PEG 400, N-methyl-2-pyrrolidinone and water (40 : 5 : 55) or in a mixture of PEG 400, dimethylformamide and water (40 : 5 : 55 0) ; the solubilities are 11.2 and 15.8 mg/ml respectively. It forms water soluble salts with bases.

Therapeutic Category : Diuretic, Antihypertensive

Storage : Preserve in well closed containers.

Mechanism of Action :

Prevent the reabsorption of sodium and chloride. Believed to act mainly on the site proxinal to the sodium and potassium exchange region in the distal tubule. The glomerular filteration rate is not affected. Initially the drug causes a diminution in sodium reabsorption in the distal tubule and a gradual reduction in the extra-cellular fluid (ECF) volume. When the ECF volume falls to slightly below normal the reabsorption of sodium from the proximal tubule is stimulated, resulting in diminished amount of sodium delivered into the distal tubule. This causes a decrease in diuretic activity and resistance.Because of the marked inhibitory action on sodium reabsorption, a large amount of sodium is available to the distal segment where exchange of potassium with sodium takes place. This causes increased

potassium loss, particularly in the presence of excessive aldosterone which is known to stimulate this exchange mechanism.

Hypotensive Action :

Produce a mild hypotensive effect partly due to their action on sodium metabolism and partly due to their direct action on blood vessels.

Methods of Analysis :

(1) **Colorimetric Method :** Bermejo (100) determined by hydrolysing it to 2,4-disulfamyl-5-trifluoromethylaniline, diazotizing the hydrolysis product and coupling to chromotropic acid or to N-(1-naphthyl) ethylenediamine dihydrochloride. This technique has been used for quantitation of Hydroflumethiazide (101).

(2) **Titration Method** : DePaulis and Dipietromaria (102) titrated in anhydrous ethylene diamine solution with sodium methoxide in benzene-methanol (85 : 15), using a saturated solution of azo violet in benzene as indicator.Chiang (103) titrated hydroflumethiazide in dimethylformamide with sodium methoxide in benzene-methanol solution, using a saturated solution of p-nitrobenzene-azo-resorcinol in benzene as indicator; thymol blue indicator gave unsatisfactory endpoints.

(3) **Paper Chromatography**:Adam and Lapiere (104) used two systems for paper chromatography: (a) Whatman No.1 paper, with 1-pentanol, 12N ammonia and water (80:20:60) as mobile phase; (b) Whatman No.1 paper impregnated with formamide, with chloroform and 1-pentanol (80:20) as mobile phase. The spots were detected using short wavelength ultraviolet radiation.

Pilsbury and Jackson (105) also used two systems to differentiate hydroflumethiazide from other thiazide diuretics : (a) Whatman No.3 paper impregnated with tributyrin, developing for 20 minutes at 90°C with pH 7.4 phosphate buffer; (b) Whatman No.1 paper, with amyl alcohol and ammonia (9:1) as mobile phase. The spots were located using short wavelength radiation, and also by spraying with 0.1N sodium hydroxide solution followed by a saturated solution of sodium 1,2-naphthoquinone-4-sulfonate in ethanol and water (1:1); the latter treatment develops an orange-red color.

Column Chromatography:Fazzari (106.107) used a basic Celite column to purify tablet extracts prior to ultraviolet quantitation.

Ultraviolet Methods : Fazzari (106) developed a partition chromatography-ultraviolet method for hydroflumethiazide in drug formulations. The compound is held on an alkaline Celite column, which is washed with chloroform and ether, then eluted with acetic acid in ether. The eluate is then extracted with 0.2N sodium hydroxide and the strength of the sample is determined by the absorptivity at 273 nm. This method is now the NF assay procedure for hydroflumethiazide tablets (108).

A simple, rapid and sensitive spectrophotometric method was described for the determination of hydroflumethiazide. The proposed method was successfully applied to the determination of the drug in pharmaceutical preparations (109).

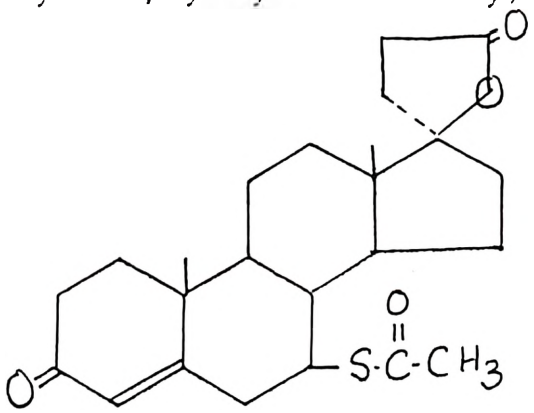
Thin Layer Chromatography : Garceau et al (110) reported a quantitative thin layer chromatography procedure with fluorometric detection, for the determination of hydroflumethiazide in urine and plasma. The limit of detection was reported to be 10 ng of drug per ml of plasma.

3.9 SPIRONOLACTONE

17-hydroxy-7α-mercapto-3-oxo-17α-pregn-4 ene-21-carboxylic acid-γ-lactone,7-acetate

3-(3-oxo-7α-acetylthio-17β-hydroxy-4-androsten-17α-yl) propionic acid γ-lactone

Structure :



Empirical Formula : $C_{24}H_{32}O_4S$

Molecular Weight : 416.59

Melting Point : 134-135° C (Resolidifies and dec. 201-202°)

[α] : - 33.5° C (1 % in chloroform)

Absorption Maximum : The molar absorptivity in methanol is 19.6 X 10^3 at the absorbance maximum 238 nm.

Appearance, Colour, Odour :

Yellowish-white crystalline powder, with a faint mercaptan odor.

Solubility : Practically insoluble in water, Soluble in organic solvents.

Solubilities of Spironolactone in various solvents at 25° C are given in the following table :

Solvent	**Solubility, mg/ml**
Methanol	6.9
Ethanol (USP)	27.9
Chloroform	50.0
Heptane	2.4 X 10^1

Therapeutic Category : Aldosterone antagonist, Diuretic

Storage : Preserve in well closed containers.

Mechanism of Action :

It is commonly employed aldosterone antagonist. Spironolactone is a steroid with structural similarity to aldosterone. It acts by competitive antagonism of aldosterone, in the distal part of the nephron, thereby preventing the potassium secretion and decreasing the sodium reabsorption. Obviously, the drug does not produce significant action in normal individuals or in those cases of edema which are not associated with rise in aldosterone concentration. Even in edematous status associated with an excess of circulatory aldosterone, its diuretic action is weak.

Methods of Analysis :

Spectrophotometric Analysis : The ultraviolet absorption spectrum of spironolactone was the basis for the USP XVIII assay for the compound. The absorbance maximum at about 238 nm in methanol is used for quantitation (58).

A simple and precise spectrophotometric method for the determination of spironolactone and hydrochlorothiazide in combination tablets was described (111).

Simple spectrophotometric methods for the simultaneous estimation of spironolactone and frusemide in combined dosage forms was developed. The total content of spironolactone and frusemide was determined at the isobestic points (255, 288, 258 nm for methods I, II and III respectively) and content for frusemide was determined at 330, 360 and 345 nm for methods I, II and III respectively (as at those wavelengths spironolactone has no absorbance). The solvents used were methanol, 0.1N NaOH and 0.1N HCl solutions I, II and III respectively. The proposed methods are linear, precise and are applicable to dissolution studies (112).

Colorimetric Analysis : Reaction of spironolactone with methanolic hydroxylamine hydrochloride and ferric perchlorate yields a red ferric hydroxamate complex having an absorbance maximum at about 515 nm (113).The absorbance at this wavelength is linear with concentration over a range of 5 mg/ml to 29 mg/ml. The colored complex was stable for up to 2 hrs. The method was used for analysis of the compound and a variety of its dosage forms. Canrenone, the principal degradation product of spironolactone, does not interfere.

A colorimetric assay for spironolactone based on its reaction with methanolic 4-amino antipyrine in an acidic medium was described. The method was accurate over the concentration range of 15-35 µg/ml (114).

Fluorometric Analysis : Spironolactone may be dethioacetylated under mild acid or alkaline conditions, yielding the corresponding 4,6-dienone (115), canrenone, which, in 62 % sulfuric acid, was converted to a fluorescent trienone (116). This compound has an excitation maximum at 483 nm and an emission maximum at 525 nm. The fluorecence is useful for quantitation of canrenone in plasma over the range 40-160 ng/ml (117). The procedure was used to determine spironolactone in the presence of its major dethioacetylated metabolites, by measuring fluorescence in 62% sulfuric acid both with and without prior dethioacetylation (115).

Chromatographic Analysis : High Pressure Liquid Chromatography : Spironolactone may be separated from canrenone on an octadecyl silane column using methanol-water mobile phase, and detected with the aid of a continuously variable wavelength UV detector. Spironolactone, which was eluted first, was detected at about 238 nm and canrenone observed at its absorption maximum at about 283 nm (118).

A HPLC method for the determination of spironolactone and frusemide in combination dosage forms is presented. A method was applied for the assay in tablets and capsules with mean recoveries of 99.39 % for spironolactone and 100.54 % for frusemide indicating high accuracy and precision of the method (119).

Thin Layer Chromatography : The solvent system Benzene : Ethyl acetate : Methanol (73 : 25 : 2) was used and absorbant as silica gel G sprayed with phosphomolybdic acid, heat at 80°C for 10 minutes. The Rf value was 0.67 and similarly by using solvent system 100 % ethyl acetate, the Rf value was 0.53 (120).

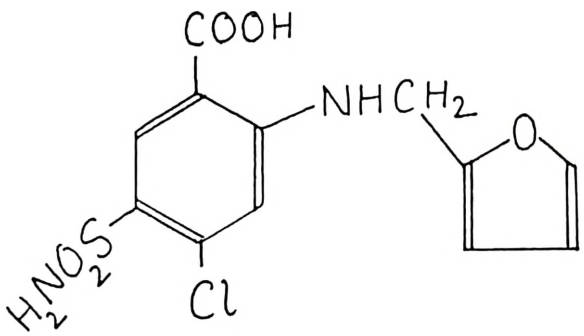
3.10 FRUSEMIDE (FURSEMIDE, FUROSEMIDE)

4-chloro-N-furfuryl-5-sulfamoylanthranilic acid

4-chloro-N-(2-furylmethyl)-5-sulfamoylanthranilic acid

5-(Aminosulfonyl)-4-chloro-2-[(2-furanylmethyl)amino]benzoic acid

Structure :



Molecular Weight : 330.77

Melting Point : 206°C

pH : pH of aqueous solutions is in between 8.9 to 9.3

Ultraviolet Spectrum : The UV spectrum in water exhibited a absorption maximum at 228 nm. Other reported UV spectral data are shown in table :

Solvent	λmax (nm)	E1%, 1cm
Ethanol (95%)	288	945
0.1N NaOH	276	588
	336	144
	226	1147
	273	557
	336	133

Solubility : Slightly soluble in water, chloroform. Soluble in acetone, methanol, methylformamide, aqueous solutions above pH 8.0 and solutions of alkali hydroxides. Less soluble in ethanol.

Therapeutic Category : Diuretic, especially in refractory edemas, Antihypertensive.

Packaging and Storage : Preserve in well closed, light resistant containers.

Mechanism of Action :

Potent, oral non-steroidal diuretic, the intense diuretic action starts within one hour and is complete by 4-6 hours. It produces more chloride loss than the sodium loss and it depresses both urinary dilution and concentration mechanism. Evidence suggests that frusemide acts along the entire nephron including the loop of henle with the exception only of the distal site where sodium is exchanged for potassium and hydrogen. In therapeutic doses, the drug has little effect on carbonic anhydrase and bicarbonates loss is not marked. It however causes increased phosphate excretion. It has greater efficacy and promote the excretion of a higher % of filtered salt than other diuretics. Frusemide is a potent high ceiling saluretic agent that produces a rapid diuretic response of comparatively short duration (6-8 hrs). Like the thiazides, frusemide promotes potassium excretion and is commonly used with potassium supplementation or a potassium sparing diuretic.

Methods of Analysis :

Titrimetric Method :

(a) Dissolve 0.25 gm in 20 ml of dimethylformamide and titrate with 0.1M sodium hydroxide, using bromothymol blue solution as indicator until a blue colour was obtained. Repeat the operation without the frusemide, the difference between the titrations represents the sodium hydroxide required.

Each ml of 0.1M sodium hydroxide is equivalent to 0.03307 gm of $C_{12}H_{11}ClN_2O_5S$(46).

(b) Dissolve about 600 mg of frusemide, accurately weighed in 50 ml of dimethylformamide to which has been added 3 drops of bromothymol blue and which previously has been neutralised with 0.1N sodium hydroxide. Titrate with 0.1N sodium hydroxide to a blue end-point. Each ml of 0.1N sodium hydroxide is equivalent to 33.07 mg of $C_{12}H_{11}ClN_2O_5S$(72).

(c) The titrimetric method for microdetermination of furosemide in pharmaceutical preparations involves titration of a solution of 1 to 5 mg of drug in 1.5M HCl with 0.02M bromosuccinamide, with methyl red as indicator (121).

Spectrophotometric Methods :

(a) Ultraviolet: A UV method for the assay (122) of frusemide in tablets involves the shaking of powdered sample containing more than 200 mg of frusemide with 0.1N NaOH (50 ml) for one hr, acidify a 10 ml portion with dil.HCl and extract with four 25 ml portions of $CHCl_3$ acetone (4:1). Evaporate the combined extracts to dryness, dissolve the residue in methanol, dilute to a concentration of about 5 µg/ml and measure the extinction at 274 nm. The mean recovery was found to be 99.2% with a maximum deviation of ±1.0%.

In the other method (123) Lasix (Frusemide) preparation(0.05 gm) was dissolved in water, ethanol or 0.1N NaOH and determined by spectrophotometry at 330, 334 and 335 nm respectively. Frusemide in tablets was determined in ethanol at 334 nm and in a 1% ampoule solution in water at 330 nm.

Frusemide in tablets is also determined (124) by spectrophotometry at 370 nm with 50% ethanol as solvent.

Colorimetric Method :

The colorimetric method involves the production of a blue color when frusemide reacts with butylamine, $CoCl_3$ and acetic acid in a medium of anhydrous methanol, the color is measured at 570 nm. Anhydrous solutions are essential, and the color was stable for 30 minutes (125).

Furosemide was detected by mixing with a solution of p-dimethylaminocinnamaldehyde in 62% H_2SO_4 containing $FeCl_2$ diluting with ethanol, and measuring the absorbance at 530 nm (126).

The extraction spectrophotometric determination of frusemide as its Fe(III) complex is described and used for analysing furosemide content in tablets and ampoules (127).

Nuclear Magnetic Resonance :

A quantitative PMR method (128) was described for the analysis of frusemide. The procedure reported gives accurate results 98.19 ±1.34% and 99.23 ±9.88 % for frusemide ampoules and tablets respectively. D_2O and DMSO-d6 were used as solvent system and (trimethylsilyl)propionic acid sodium as internal standard.

Chromatographic Methods :

(a) Column Chromatography (129) : 20 µl of urine was injected directly on to a stainless - steel column packed with LiChrosorb RP-8 or RP-18; various mobile phases; e.g. acetonitrile-phosphate buffer solution (pH 5 to 10) containing tetrabutylammonium hydrogen sulphate, were used. Under optimum conditions furosemide could be separated easily and detected by UV.

(b) Gas Liquid Chromatography (GLC) (130) : One ml plasma containing 0.3-5.4 µg of frusemide was diluted with water(1:2), acidified and furosemide was extracted with diethylether (2X5 ml). The residue containing the trimethyl derivative of furosemide was dissolved in hexane (0.2 ml) containing trimethyl furosemide as internal standard and 5 µl of the solution was subjected to GLC. The structure of the derivative was confirmed by combined GLC-MS.

(c) Thin-Layer Chromatography (TLC) (131) : 0.5 ml of plasma was treated with methanol (1 ml) and centrifuged. A 50 µl aliquot of the supernatant liquid was subjected to TLC on a kieselgel 60 plate together with standards (0.2 to 10 mg) of furosemide and its metabolites, with $CHCl_3$-ethyl acetate- formic acid (14:6:1) as solvent. The plate was dried and equilibrated with air for 3 hr, then sprayed with 10% citric acid in aq.ethanediol (1:1) and immediately scanned with a spectrophotometer with excitation at 365 nm.

(d) High Performance Liquid Chromatography (HPLC) :HPLC method has wide application for the estimation of furosemide (132-146).

(e) Potentiometric Methods : A potentiometric titration method for the determination of small quantities of frusemide (lasix) in pure form, tablet, injection solutions and combination tablets also containing reserpine was presented. The method is accurate and reproducible and can be applied for routine analysis of frusemide in quantities ranging from 1-15 mg (147).

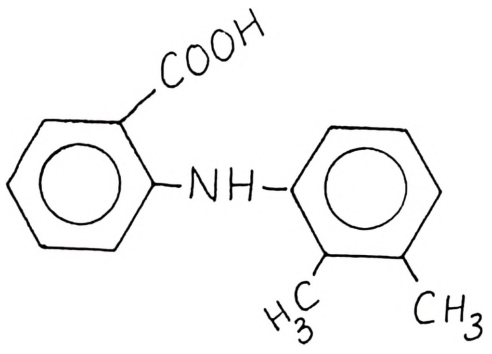
Determination of frusemide in anhydrous acetone by potentiometric titration with alcoholic KOH as titrant was developed. The titration were done using antimony sodium as indicator and glass electrode as a reference electrode. The method can give satisfactory results for assay of frusemide for the concentration range 0.6-1.5 mg/ml. The method was applied for assay of frusemide in single component tablets (148).

3.11 MEFENAMIC ACID

N-(2,3-Xylyl) anthranilic acid

2[(2,3-dimethylphenyl)amino]-benzoic acid

Structure



Empirical Formula : $C_{15}H_{15}NO_2$

Molecular Weight : 241.28

Melting Point : 230 - 231 °C (Effervescence)

pK.: 4.2

UV max (0.1N NaOH) : 285, 340 nm

Appearance, Colour & Odour :

White to greyish white, odourless or almost odourless microcrystalline powder

Solubility :

Soluble in solutions of alkali hydroxides, Sparingly soluble in ether (80 parts) & chloroform (150 parts), Slightly soluble in methanol and ethanol (185 parts),Practically insoluble in water.

Storage : Store in air tight containers and protect from light.

Therapeutic Category : Analgesic, Antiinflammatory agent and Antipyretic

Mechanism of Action :

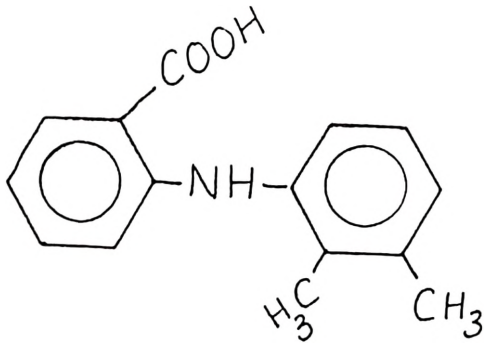
The anthranilic acid derivative useful in chronic and dull aching pains. However it is a weaker analgesic than aspirin. The action is mainly due to its inhibition of prostaglandin synthesis. It is a reversible inhibitor of cyclo-oxygenase. In addition, mefenamic acid may also inhibit production of lipo-oxygenase mediated products such as leucotrienes which are known to contribute to inflammation. Leucotrienes enhance chemotaxis and release of lysosomal enzymes which cause tissue damage. Cyclo-oxygenase and lipo-oxygenase have a common substrate in arachidonic acid. Supression of one enzyme could lead to increased

3.11 MEFENAMIC ACID

N-(2,3-Xylyl) anthranilic acid

2[(2,3-dimethylphenyl)amino]-benzoic acid

Structure



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substrate availability for the other. A third component in the anti-inflammatory action of mefenamic acid is its action at prostaglandin receptor sites. Fenamates act as antagonist at PG receptors. Mefenamic acid reversibly inhibited PGE1-induced contraction of smooth muscle in gerbil colon. PGH2 was inhibited by mefenamic acid. This may explain the effectiveness of this drug in dysmenorrhoea. Mefenamic acid 1-5 µg/ml had little effect on acetylcholine-induced contractions, but did antagonise those due to PGs. Mefenamic acid has been shown to antagonise PGF2α-induced contraction of isolated bronchial smooth muscle.

Methods of Analysis :

(a) As per BP, 1980, Dissolve 0.6 gm in 100 ml of warm absolute ethanol previously neutralised to phenol red solution and titrate with 0.1M NaOH VS, using phenol red solution as indicator. Each ml of 0.1M NaoH = 0.02413 gm of $C_{15}H_{15}NO_2$ (46).

(b) Two colorimetric methods utilising complexation with iron, are described for the determination of both acids in dosage forms (149).

(c) A colorimetric method for the determination of mefenamic acid in dosage forms, based on complexation with sodium nitroprusside and hydroxyl-ammonium chloride was described (150).

(d) A highly selective and sensitive column switching HPLC method with UV detection has been described for the determination of two acidic drugs ibuprofen and mefenamic acid in spiked human serum. The detection limits were 0.5 ng/ml for ibuprofen and 0.1 ng/ml for mefenamic acid using 1 ml of serum (53).

(e) A new spectrophotometric method for the determination of mefenamic acid in pharmaceutical preparations, based on the formation of orange yellow coloured species (λmax = 395 nm) with sodium cobaltinitrite in phosphoric acid media was described (151).

(f) The effect of pH on the UV spectra of mefenamic acid as measured by spectrophotometric methods was described and in addition the drug has been determined

in tablets for formation of a colored complex with Fe (III) in a methanol- water medium (152).

(g) A colorimetric method for the determination of famotidine and mefenamic acid in dosage forms has been described (153).

(h) A simple photometric method in the visible region was described for estimation of mefenamic acid in its pharmaceutical dosage form. Based on the reaction of the drug with 4-aminophenazone and potassium ferricyanide to yield a reddish green chromagen which exhibits absorbance max at 590 nm. The chromagen is stable for 40 minutes and Beer's law is obeyed in the concentration range of 0.5 µg/ml to 4.0 µg/ml (154).

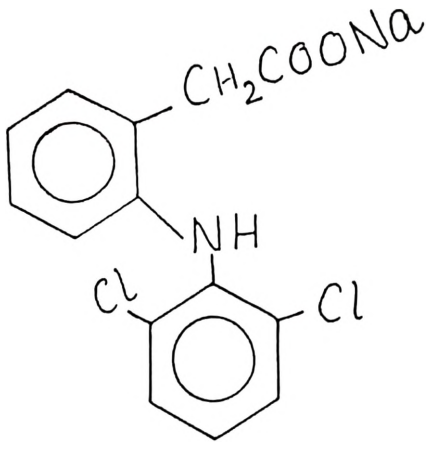
3.12 DICLOFENAC SODIUM

2-[2.6-Dichorophenyl amino]benzene acetic acid mono sodium salt

[o-(2,6-dichloroanilino)phenyl] acetic acid sodium salt

Sodium [o-[(2,6-dichlorophenyl)amino]phenyl] acetate

Structure :



Empirical Formula : $C_{14}H_{10}Cl_2NO_2Na$

Molecular Weight : 318.13

Melting Point : 283-285°C

UV-Spectrum : Absorption maximum in methanol appears at 283 nm and in aqueous phosphate buffer (pH 7.2) is 276 nm

Appearance, Colour, Odour :

Odorless, white to off-white crystalline, slightly hygroscopic powder

pK.: in water is 4.0

Solubility :

The equilibrium solubility performed in various solvents at the indicated temperature (RT) are shown below :

Solvent	Temperature	Solubility, mg/ml
Deionised water (pH 5.2)	RT	> 9
Methanol	RT	>24
Acetone	RT	6
Acetonitrile	RT	< 1
Cyclohexane	RT	< 1
pH 1.1 (HCl)	RT	< 1
pH 7.2, phosphate buffer	RT	6

Therapeutic Category : Non steroidal antiinflammatory and analgesic agent.

Mechanism of Action :

Diclofenac sodium inhibits the cyclo-oxygenase pathway in the metabolism of arachidonic acid and markedly reduces the synthesis of prostaglandins, prostacyclin and thromboxane products. Formation of the products of the lipo-oxygenase pathway such as leukotrienes and 5-hydroxyeicosatetraenoic acid is also reduced and this contributes to the antiinflammatory action.

The generally accepted view that the analgesic activity of NSAIDs depends on their ability to inhibit prostaglandin synthesis has been challenged recently.

Methods of Analysis :

(a) As per BP (155), dissolve 0.5 gms in 50 ml of anhydrous acetic acid and carry out method for non-aqueous titration, the end point determined potentiometrically.

Each ml of 0.1M perchloric acid ≡31.81 mg of $C_{14}H_{10}Cl_2NNaO_2$

(b) Gas-Liquid Chromatography :Gas-liquid chromatography has been used to analyse diclofenac sodium and its metabolites (156-161). Before injecting into the column, diclofenac or its metabolites are derivatized into the indolones or the methyl esters.

(c) Thin Layer Chromatography :Thin layer chromatography has been used to analyse diclofenac sodium in suppositories (162) and plasma (163).

(d) High Performance Liquid Chromatography :Analysis from solid dosage form (tablet) has been reported (164).

(e) Spectrophotometric Method :Diclofenac sodium may be assayed by simple spectrophotometric method at 600 nm in water (165). Diclofenac sodium reacted with 3-methyl-2-benzothiazolinone hydrazone hydrochloride and cerium ammonium sulfate to form a colored complex which exhibited absorption maximum at 600 nm

Harland et al (166) have also analysed diclofenac sodium spectrophotometrically at 275 nm from hydrophilic matrix (polyvinyl alcohol).

(f) Nuclear Magnetic Resonance :The proton magnetic resonance (PNMR) method to quantify diclofenac sodium in pure and tablet forms was described. The sharp singlet at 3.62 ppm which corresponded to the methylene protons in diclofenac was chosen for quantitative measurement. Anhydrous sodium acetate was used as internal standard. The methyl protons of sodium acetate gave a sharp singlet at 1.81 ppm. The amount of diclofenac could be calculated by comparing the peak ratio of diclofenac to that of the internal standard since the amount of internal was known. The PMR spectrum could also be used to examine the purity of the drug (167).

(g) Colorimetric Analysis :Sane et al described a method for determining diclofenac sodium from pharmaceutical preparations by reacting diclofenac sodium with potassium ferricyanide in the presence of sodium hydroxide to form a yellow complex which showed maximum absorbance at 450 nm (168).

Agrawal et al described a rapid method for the determination of diclofenac sodium and reported that the drug solution turned yellow color when reacted with sodium nitrite and hydrochloric acid and exhibited maximum absorbance at 390 nm (169).

Two simple spectrophotometric methods for the determination of diclofenac sodium in tablets, one of which the drug reduces ferric ions to ferrous ion and the other using methylene blue can be applied to the determination of diclofenac sodium in tablets containing paracetamol (170).

A simple spectrophotometric method has been developed for estimation of diclofenac sodium in pharmaceutical dosage forms. Diclofenac sodium when reacted with p-dimethyl amino benzaldehyde (p-DAB) and ammonium cerric sulphate in acidic medium gives an orange colour which exhibits maximum absorbance at 440 nm. It obeys Beer's law in the concentration range of 2-16 µg/ml. The colour is stable for 50 minutes (171).

A simple rapid, accurate and reproducible method for estimation of diclofenac sodium from tablets has been developed. This estimation method was based on the quantitative formation of a green coloured, chloroform extractable complex of the drug with copper (II) ions. The complex is formed using copper (II) chloride as the analytical reagent in the presence of pyridine. The complex formed showed absorbance maxima at 732 nm of concentration range of 0.0-10.0 µg/ml of diclofenac sodium (172).

(h) Gas Chromatography-Mass Spectrometry :It was most sensitive method reported in the analysis of diclofenac sodium (173). The lowest limit of detection of diclofenac is 0.2 ng/ml of plasma which is 10 times more sensitive than using gas chromatography alone.

(i) High Performance Liquid Chromatography :A liquid chromatographic method has been developed for determination of drug and related comps in diclofenac Na raw material, slow release and enteric coated tabs. The method specifies a 5 µm octadecyl silane bonded phase column, a mobile phase of tetrahydrofuran- acetonitrile- buffer, pH 5.0 (1:4:8.3) and detection at 229 nm. The titation of 0.2% or less. 17 drug raw material samples were evaluated. The method has also been used for determination of drug content in raw materials & formulations. Mean assay levels in drug raw materials ranged between 98.3% - 101.8% (174).

HPLC method was reported for estimation of paracetamol and diclofenac Na in combined dosage form using a µ Bondapak phenyl column with methanol, water, phosphoric acid (70 : 30 : 0.08) as mobile phase with xipamide as internal standard (175).

Diclofenac Na and paracetamol were chromatographed on a Novopac C18 column using mobile phase consisting of 0.01M Disodium hydrogen orthophosphate and methanol (25 :75). This method utilizes 80% of methanol and acetic acid with a total run time of 15 minutes. The recoveries reported are ranged from 92 - 102% (176).

CHAPTER - 4

EXPERIMENTAL

PART-A

ANALGESICS, ANTIINFLAMMATORY AND ANTIPYRETICS

(4.1)

DETERMINATION OF OXYPHENBUTAZONE OR IBUPROFEN IN PRESENCE OF PARACETAMOL & DEXTROPROPOXYPHENE IN DOSAGE FORMS BY QUANTITATIVE THIN LAYER CHROMATOGRAPHY

Instrument

A UV-Visible Cary 17D spectrophotometer was used for measuring the absorbance data equipped with two 1cm matched quartz cells.

Mobile Phase

Solvent system consisting of a mixture of ethyl acetate : chloroform : methanol : aq ammonia in the ratio of 55:40:5:0.5 was used for the combination of paracetamol, dextropropoxyphene hydrochloride and oxyphenbutazone and a mixture of ethyl acetate: chloroform : methanol in the ratio of 53 : 40 : 7 and addition of 4 drops of aq ammonia was used for the combination of paracetamol, dextropropoxyphene hydrochloride and ibuprofen.

Spraying Agents

1. **Phosphomolybdic Acid** : Five gms of phosphomolybdic acid was dissolved in 100 ml of water.

2. **Potassium Iodo-Bismuthate Solution :** Ten grams (10 gms) of (+) Tartaric acid was dissolved in 40 ml of water. To this solution was added 0.85 gms of Bismuth oxynitrate and continously stirred for one hour. Thereafter 20 ml of 40 % w/v solution of potassium iodide was added and shaken well. Finally, the mixture was allowed to stand for 24 hours and filtered.

3. **Acidified Potassium permanganate Solution :** One gram (1.0 gm) of Potassium permanganate solution was prepared in in 100 ml of N/10 sulphuric acid.

PROCEDURE

The TLC procedure developed for their separation and determination is described below :

Silica Gel G (7.5 gms) was triturated with 16 ml of potassium hydrogen phosphate buffer solution (KH_2PO_4) at pH 6.0 (178), to form a smooth paste and then uniformly spread on the glass TLC plates (20 X 20 cm) having thickness of 0.25 mm.

The plates were first air dried for about 15 minutes and then at 110°C for 30 minutes in an electric oven (179).

Various combination of solvents were attempted untill clear separation of the constituents with defined Rf values was observed. The solvent systems attempted and their combinations are stated below :

S.No	Mobile Phase Composition	Remarks
A	Ethyl Acetate : Chloroform : Methanol : Aq. Ammonia (55 : 45 : 5 : 0.1)	The Rf value for Ibuprofen was found to be very low
B	Ethyl Acetate : n-Hexane : Chloroform (50 : 40 : 10)	Tailing in case of dextropropoxyphene HCl was observed
C	Ethyl Acetate : Chlorform : Methanol (13.5 : 10 : 1)	Paracetamol spot was not bright
D	Ethyl Acetate : Chloroform : n-Hexane : Aq. Ammonia (12.5 : 10 : 10 : 1)	Ibuprofen spot was not clear.
E	Ethyl Acetate : Chloroform : n-Hexane : Methanol : Aq. Ammonia (40 : 55 : 10 : 5 : 0.2)	Tailing of dextropropoxyphene and ibuprofen spot was found.
F	Ethyl Acetate : Chloroform : n-Hexane : Methanol : Gla. Acetic Acid (55 : 40 : 10 : 5 : 5)	Rf of dextropropoxyphene was found to be very low.
G	Ethyl Acetate : Chloroform : n-Hexane : Gla. Acetic Acetic Acid (55 : 40 : 5 : 0.3)	Paracetamol spot was not bright.

NOTE : From the above solvent systems dextropropoxyphene was found not to move in the presence of glacial acetic acid but to be moving in the presence of aq.ammonia. However, ibuprofen was found to travel in acidic pH (i.e., in glacial acetic acid). At the same

time dextropropoxyphene was found to produce tailing in presence of n-hexane Therefore another set of solvent system was tried.

H	Ethyl Acetate : Chloroform : n-hexane (55 : 40 : 5)	The Rf values for paracetamol and ibuprofen were found to be very close.
I	Ethyl Acetate : Chloroform : n-hexane : Gla. Acetic Acid (53 : 40 : 5 : 0.3)	The Rf value of dextropropoxyphene was very low while in case of paracetamol and ibuprofen were almost identical.
J	Ethyl Acetate : Chloroform : Benzene (50 : 40 : 10)	Tailing in paracetamol and dextropropoxyphene was observed.
K	Ethyl Acetate : Chloroform : Toluene (50 : 40 : 10)	The above difficulties were encountered.
L	Ethyl Acetate : Chloroform : Benzene : Methanol (40 : 40 : 10 : 10)	Tailing in case of dextropro poxyphene was observed.
M	Ethyl Acetate : Chlorform : Benzene : Aq. Ammonia (50 : 40 : 10 : 0.2)	Tailing was produced in ibuprofen.
N	Ethyl Acetate : Chloroform : Methanol : Aq. Ammonia (53 : 40 : 7 : 0.2)	The spots were very clearly seperated for all the pure drugs

The final combination of solvents in the ratios which gave the best separation for the determination of Paracetamol, Dextropropoxyphene HCl and Oxyphenbutazone in formulation was :

SOLVENT SYSTEM O:

Ethyl acetate	: 55 Vol.
Chloroform	: 40 Vol.
Methanol	: 0.5 Vol.
Aq. Ammonia	: 0.5 Vol

Similarly the solvent system which gave best separation for the determination of Paracetamol, Dextropropoxyphene HCl and Ibuprofen in formulation was :

SOLVENT SYSTEM P:

Ethyl acetate	: 53 Vol.
Chloroform	: 40 Vol.
Methanol	: 07 Vol.
Aq. Ammonia	: 0.2 Vol.

STANDARD GRAPH FOR PARACETAMOL :

Pure paracetamol solutions in concentrations of 30, 40, 50, 60 and 70 mg/ml in ethanol were prepared by dissolving 300, 400, 500, 600 and 700 mg of paracetamol in 10 ml of ethanol separately. Exactly 0.05 ml (50 µl) of these solutions were spotted on the TLC plates in duplicate. The plates were slowly placed in the chamber containing the solvent system as mentioned in solvent system "O" maintained at room temperature by ascending technique. When the solvent front had moved to a distance of 15 cms (3/4 of the TLC plates) the plates were carefully removed, dried and spots developed are identified by spraying with a solution of 5% w/v phosphomolybdic acid solution and subsequently heating the plates at 80° C to obtain purple coloured spots. The coloured and uncoloured duplicate spots were then scraped and collected in dry centrifuge tubes. To these 7.0 ml of methanol was transferred and were centrifuged for five minutes for complete dissolution of Paracetamol. After centrifugation the supernatant solutions were removed and the adsorbant was again extracted with 3.0 ml of methanol. Both extracts were combined, filtered and 2.0 ml of filterate was further diluted to 10.0 ml with methanol. The absorbance (Table 1) of the clear solutions with respect to the blank (prepared by scraping of silica gel G from an equivalent area of the TLC plates to that of the sample spot from a point at the same

migration distance from the start as the sample spot and parallel to it and by processing it exactly in the same manner as the sample) was measured at 242 nm, the spectrum measurement of which was carried out for one of the clear solution (f ig. 1) on a spectrophotometer. A standard graph was plotted with concentration against absorbance. A straight line graph was obtained as shown in Fig.2.

STANDARD GRAPH FOR DEXTROPROPOXYPHENE HYDROCHLORIDE

Stock solutions of 50, 40, 30 and 20 mg of pure dextropropoxyphene HCl in 5.0 ml each of ethanol were prepared separately giving concentrations of 10, 8, 6 and 4 mg/ml respectively. Exactly 0.05 ml (50 µl) of these solutes were spotted on TLC plates (20 X 20 cm) separately in duplicate. The development of the chromatograms were done in the same solvent system as in case of paracetamol under similar conditions. The plates were removed after their development to a distance of 15 cms and dried. The spots are identified by spraying with Potassium-iodo bismuthate solution. The spots became reddish orange in colour. The coloured and uncoloured duplicate spots were scraped and dissolved in 10 ml of methanol. The solutions were centrifuged and the supernatant solution was used measuring absorbance with respect to the blank at 258 nm (Table 2 and Fig. 3). A standard graph was plotted with concentration against absorbance. A straight line graph was obtained as shown in Fig. 4.

STANDARD GRAPH FOR OXYPHENBUTAZONE

A stock solution of concentrations ranging from 150, 125, 100, 75 and 50 mg in 5.0 ml of ethanol were prepared separately giving concentrations of 30, 25, 20, 15 and 10 mg/ml respectively. The loading of the TLC plates was done in duplicate using 0.05 ml of each of the above concentrations. The development of the chromatogram was done in the same solvent system as in Paracetamol under similar conditions of temperature by ascending technique. The spots were developed by spraying with 1 % acidified $KMnO_4$ solution. The colour of the spots was colorless with pink in background. The coloured and uncoloured duplicate spots of various concentrations were scraped and dissolved in 10 ml of methanol. The solutions were centrifuged for five minutes and 3.0 ml of the supernatant solutions were diluted to 10.0 ml with methanol. The absorbance of the clear solutions was then measured at 265 nm (Table 3 and Fig. 5) with respect to the blank. A standard graph

was plotted with concentration against absorbance. A straight line graph was obtained as shown in Fig. 6.

STANDARD GRAPH FOR IBUPROFEN

The stock solutions of pure ibuprofen ranging in concentrations from 600, 540, 480, 420, 360, 300, 240 and 180 mg in 10 ml of ethanol were prepared. The loading of the TLC plates were done in duplicate using exactly 0.05 ml (50 µl) per spot of the above dilutions. The plates were placed in the chromatographic chamber containing the solvent system P at room temperature by ascending technique. The spots developed were detected by spraying with 1 % acidified $KMnO_4$. The spots became colourless with pink in the background. The coloured and uncoloured duplicate spots were scraped and dissolved in 10 ml of methanol. The solutions were centrifuged for 10 minutes, the supernatant solutions were removed and the adsorbant was again extracted with 5.0 ml of methanol. The extracts were combined and the absorbance of the clear solutions with respect to the blank was measured at 265 nm (Table 4 and Fig. 7). A standard graph was plotted with concentration against absorbance. A straight line graph was obtained as shown in fig. 8.

ASSAY PREPARATION FOR COMBINATION OF OXYPHENBUTAZONE, PARACETAMOL AND DEXTROPROPOXYPHENE HYDROCHLORIDE

Twenty capsules were weighed and the powder equivalent to the average weight of the capsule was dissolved in 5 ml of ethanol giving a concentration of 50 mg/ml of paracetamol, 20 mg/ml of oxyphenbutazone and 6.5 mg/ml of dextropropoxyphene HCl. The solution was centrifuged for 10 minutes and 0.05 ml (50 µl) of the supernatant solution was used for spotting on the TLC plates. The loading was done in duplicate. The plates were placed in the chromatographic chamber containing the solvent system O. When the solvent front had moved a distance of 15 cm on the plate, the plate was carefully removed and dried. The spots were developed by spraying with 5 % phosphomolybdic acid for paracetamol (Rf = 0.45), with potassium iodo bismuthate for dextropropoxyphene HCl (Rf = 0.80) and 1 % acidified $KMnO_4$ for oxyphenbutazone (Rf = 0.15). The uncoloured duplicate spots which were not sprayed were also collected into 3 separate centrifuge tubes. The extraction of the separated constituents were followed in a similar manner as that for the individual pure drugs. The absorbance of the solutions was measured at 242, 258 and 265 nm for paracetamol, dextropropoxyphene HCl and

oxyphenbutazone respectively. The results of estimation and statistical analysis were given in Table 5 & 6..

ASSAY PREPARATION FOR COMBINATION OF IBUPROFEN, DEXTROPROPOXYPHENE HYDROCHLORIDE AND PARACETAMOL

Twenty tablets were finely ground and the powder equivalent to the average weight of the tablet was dissolved in 5 ml of ethanol giving a concentration of 50 mg/ml of paracetamol, 6.5 mg/ml of dextropropoxyphene hydrochloride and 40 mg/ml of ibuprofen respectively. The solution was centrifuged and the supernatant solution was used for spotting on the TLC plates. Exactly 0.05 ml (50 µl) of the above centrifuged solution was spotted on the TLC plate. The loading was done in duplicate. The plates were placed in the chromatographic chamber containing the solvent system P. When the solvent front had moved to a distance of 15 cm on the plate, the plate was carefully removed and dried. The spots were developed by spraying with 5 % phosphomolybdic acid for paracetamol (Rf = 0.50), with potassium iodobismuthate for dextropropoxyphene HCl (Rf = 0.80) and 1 % acidified $KMnO_4$ for ibuprofen (Rf = 0.20). The uncoloured duplicate spots which were not sprayed were collected into 3 separate centrifuge tubes. The extraction of the separated constituents was followed in a similar manner as that for the individual pure drugs. The absorbance of the solution was measured at 242, 258 and 265 nm for paracetamol, dextropropoxyphene HCl and ibuprofen respectively. The results of estimation and statistical analysis were given in Table 7 & 8.

RESULTS

Table 1

Calibration curve for Paracetamol

Concentration (µg/ml)	Absorbance						SD*	CV**
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Mean		
30	0.092	0.099	0.097	0.090	0.098	0.095	0.0039	4.162
40	0.119	0.131	0.121	0.130	0.124	0.125	0.0053	4.270
50	0.154	0.164	0.155	0.162	0.155	0.158	0.0076	4.841
60	0.192	0.196	0.186	0.189	0.187	0.190	0.0133	7.012
70	0.218	0.226	0.219	0.220	0.217	0.220	0.0193	8.796

* Standard Deviation

** Coefficient of variation (%)

Covariance = 1.04999

Correlation Coefficient = 0.99986

Regression = 0.9997

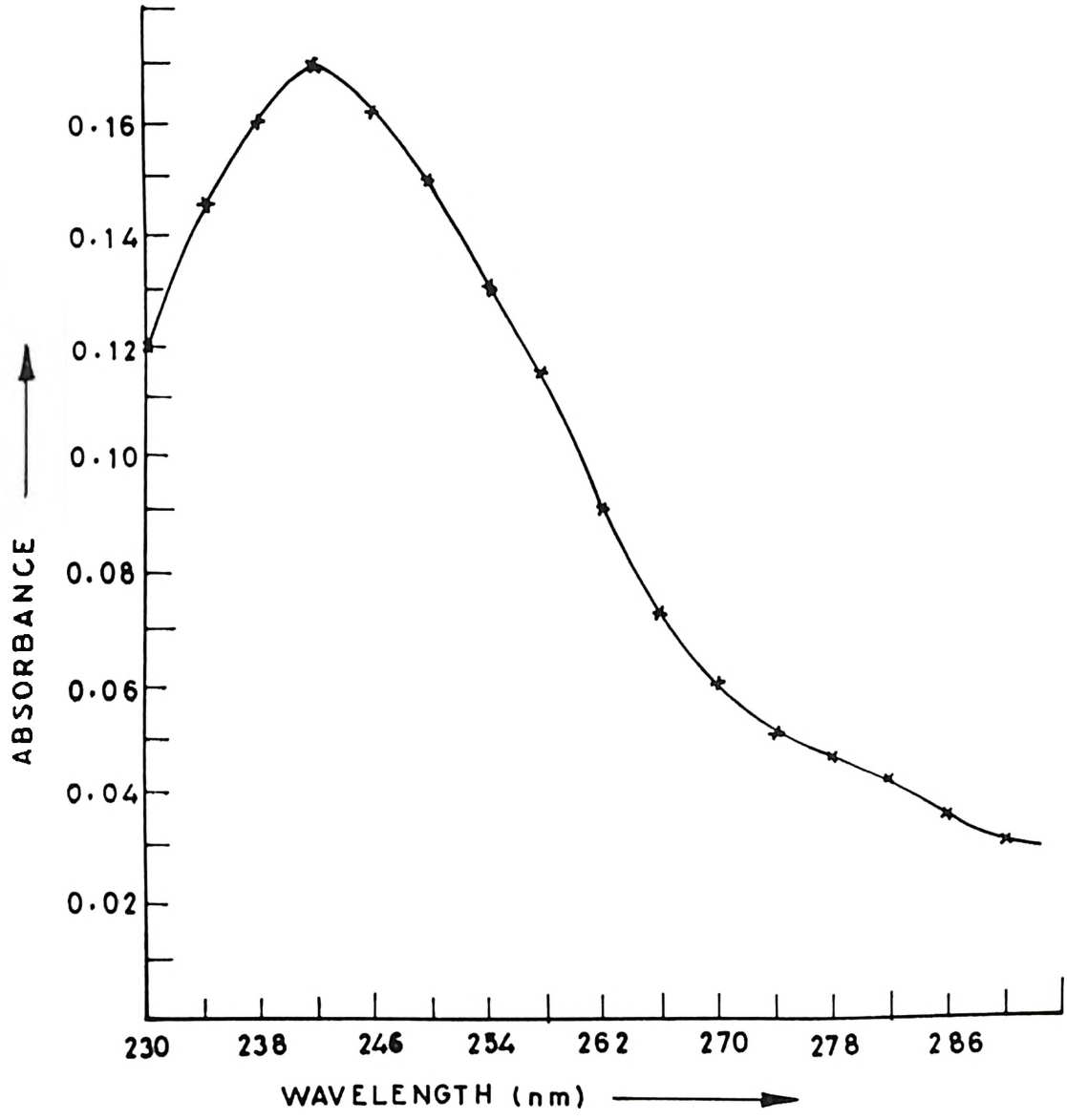


Fig. 1 ABSORPTION SPECTRUM FOR PARACETAMOL

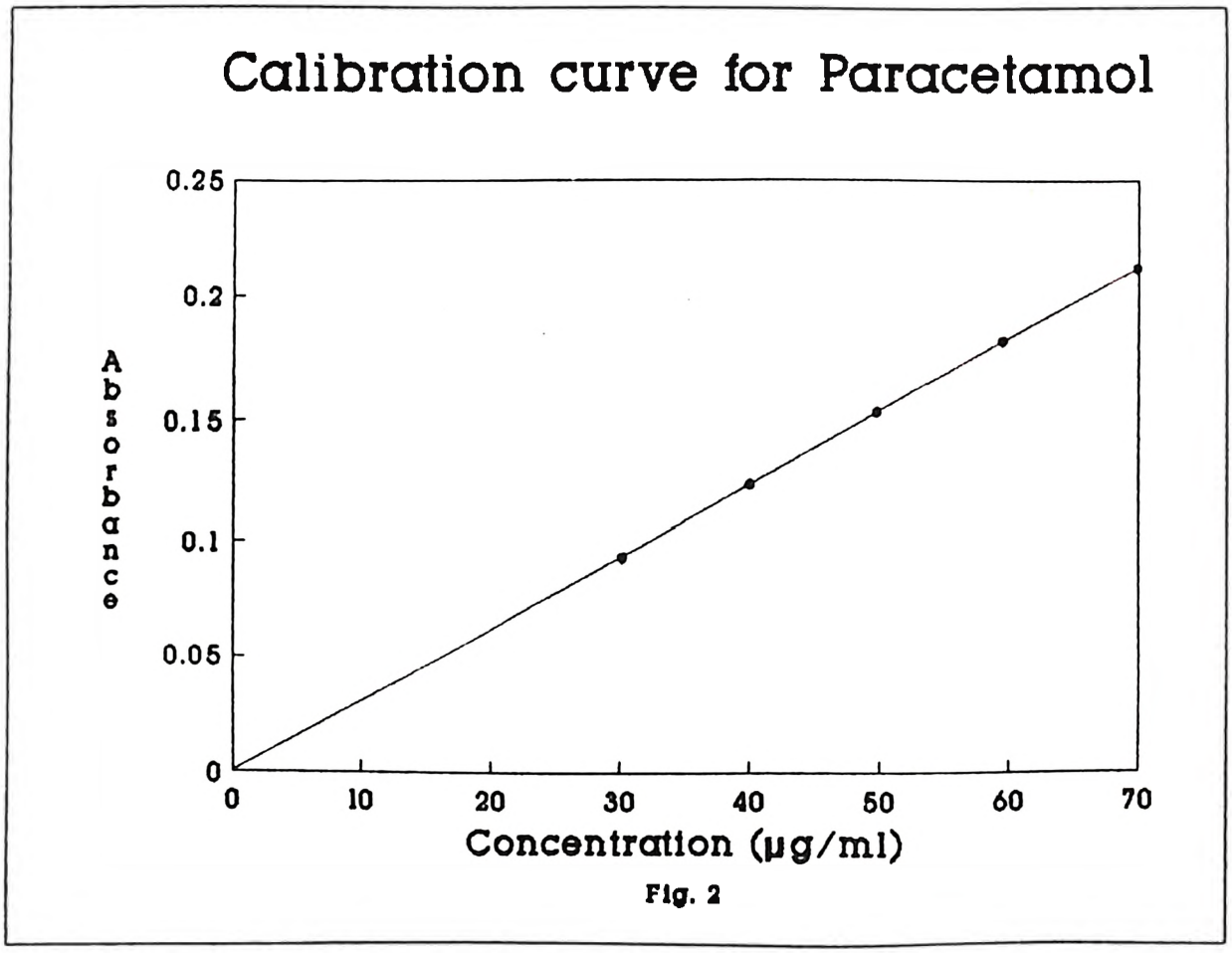

Calibration curve for Paracetamol
0.25
0.2
0.15
0.1
0.05
0
Absorbance
0
10
20
30
40
50
60
70
Concentration (µg/ml)

Fig. 2

Table 2

Calibration curve for Dextropropoxyphene hydrochloride

Concentration (µg/ml)	Absorbance				SD*	CV**
	Trial 1	Trial 2	Trial 3	Mean		
20	0.110	0.105	0.107	0.108	0.037	34.57
30	0.263	0.263	0.260	0.262	0.039	15.08
40	0.419	0.420	0.418	0.419	0.031	7.58
50	0.566	0.568	0.561	0.565	0.040	7.13

*Standard Deviation

**Coefficient of Variation (%)

Covariance = 3.8175

Correlation Coefficient = 0.99988

Regression = 0.9997

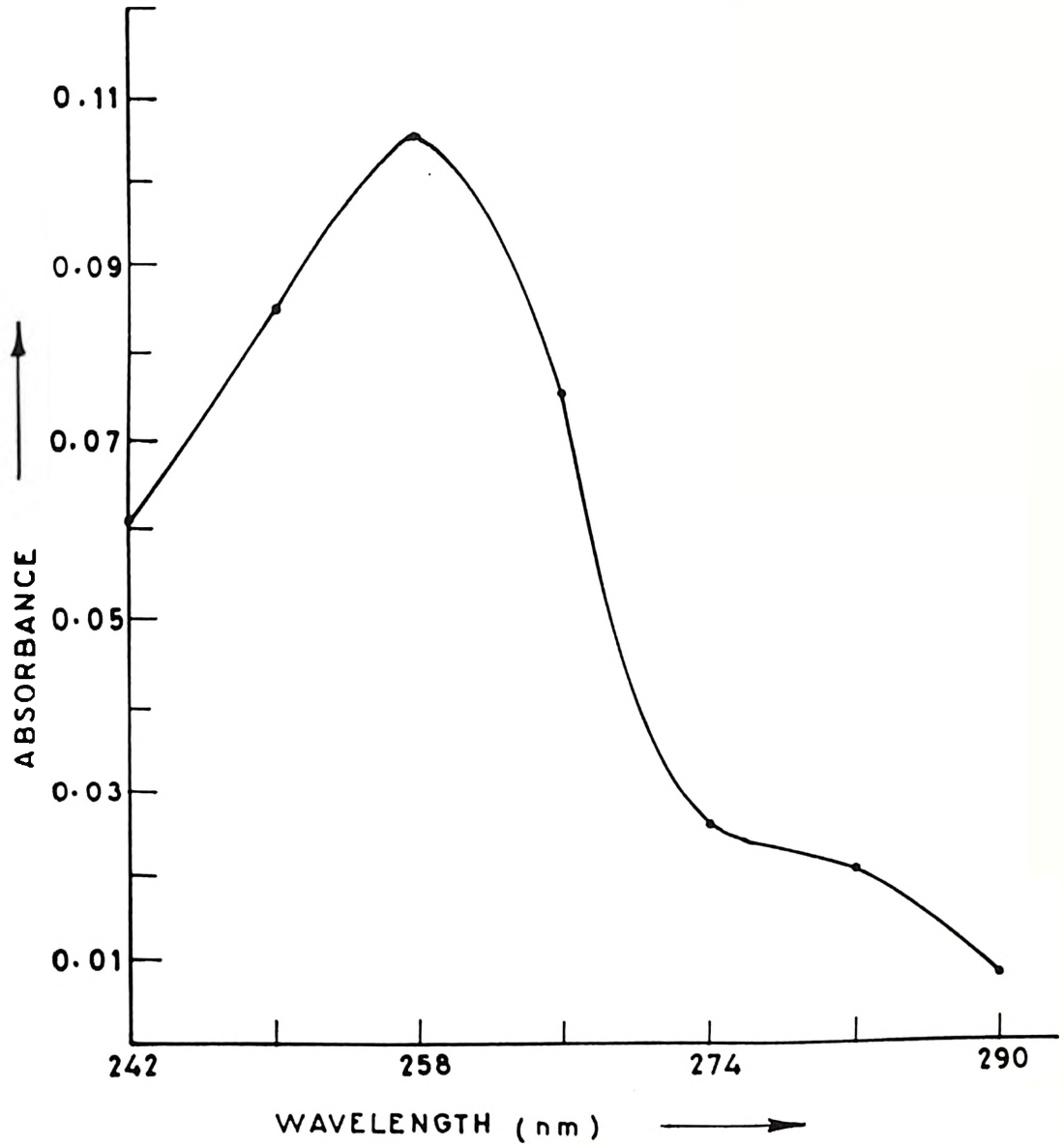


Fig.3 ABSORPTION SPECTRUM FOR DEXTROPROPOXYPHENE HYDROCHLORIDE

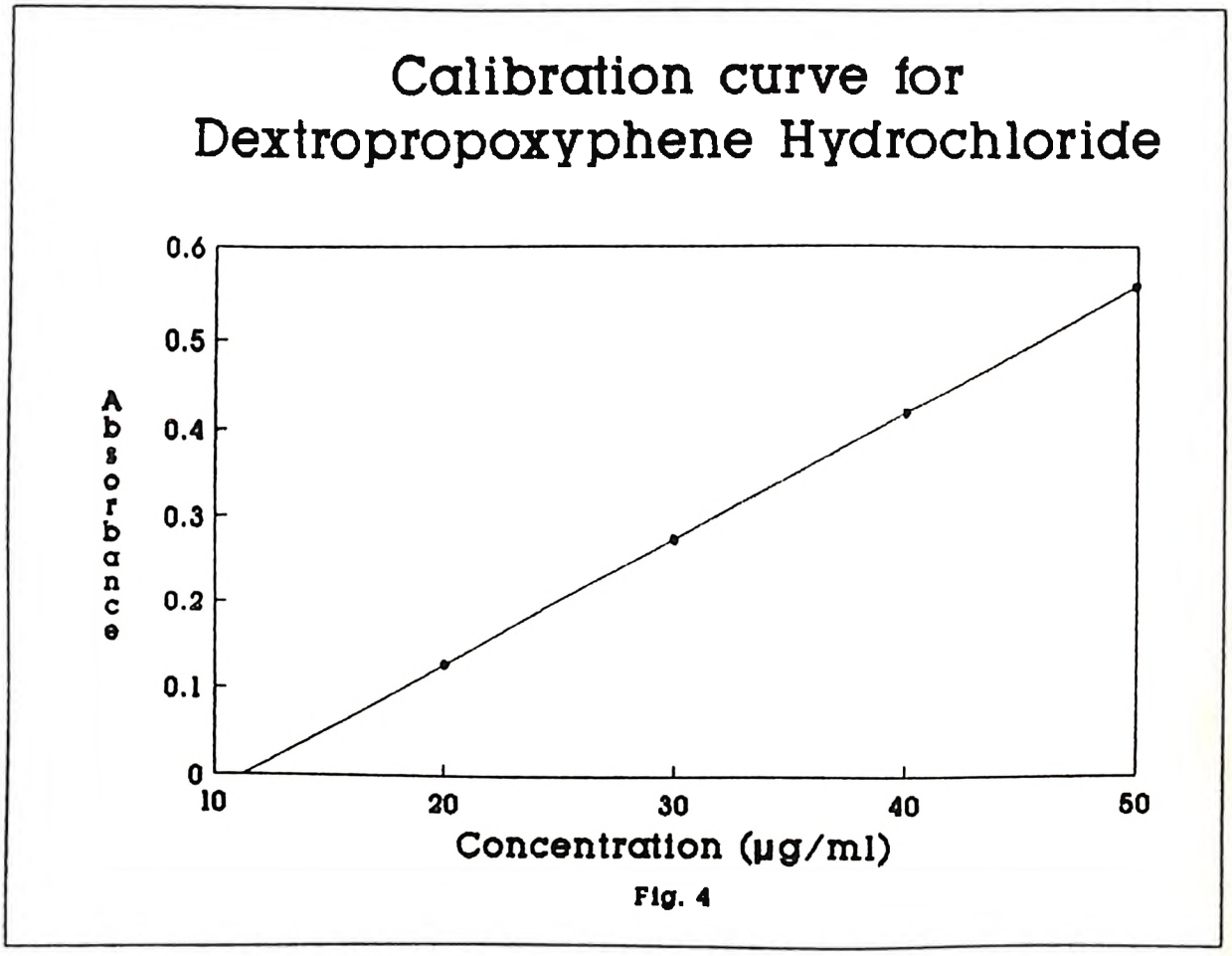

Calibration curve for
Dextropropoxyphene Hydrochloride
Absorbance
0.6
0.5
0.4
0.3
0.2
0.1
0
10
20
30
40
50
Concentration (µg/ml)

Fig. 4

Table 3

Calibration curve for Oxyphenbutazone

Concentration (μg/ml)	Absorbance						SD*	CV**
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Mean		
15	0.101	0.109	0.105	0.104	0.106	0.105	0.0029	2.777
22.5	0.165	0.160	0.156	0.155	0.164	0.160	0.0045	2.830
30	0.210	0.222	0.217	0.220	0.206	0.215	0.0067	3.154
37.5	0.262	0.275	0.274	0.275	0.264	0.270	0.0075	2.784
45	0.323	0.326	0.330	0.333	0.328	0.330	0.0142	4.306

* Standard Deviation

** Coefficient of Variation (%)

Covariance = 1.3999

Correlation Coefficient = 0.99983

Regression = 0.9996

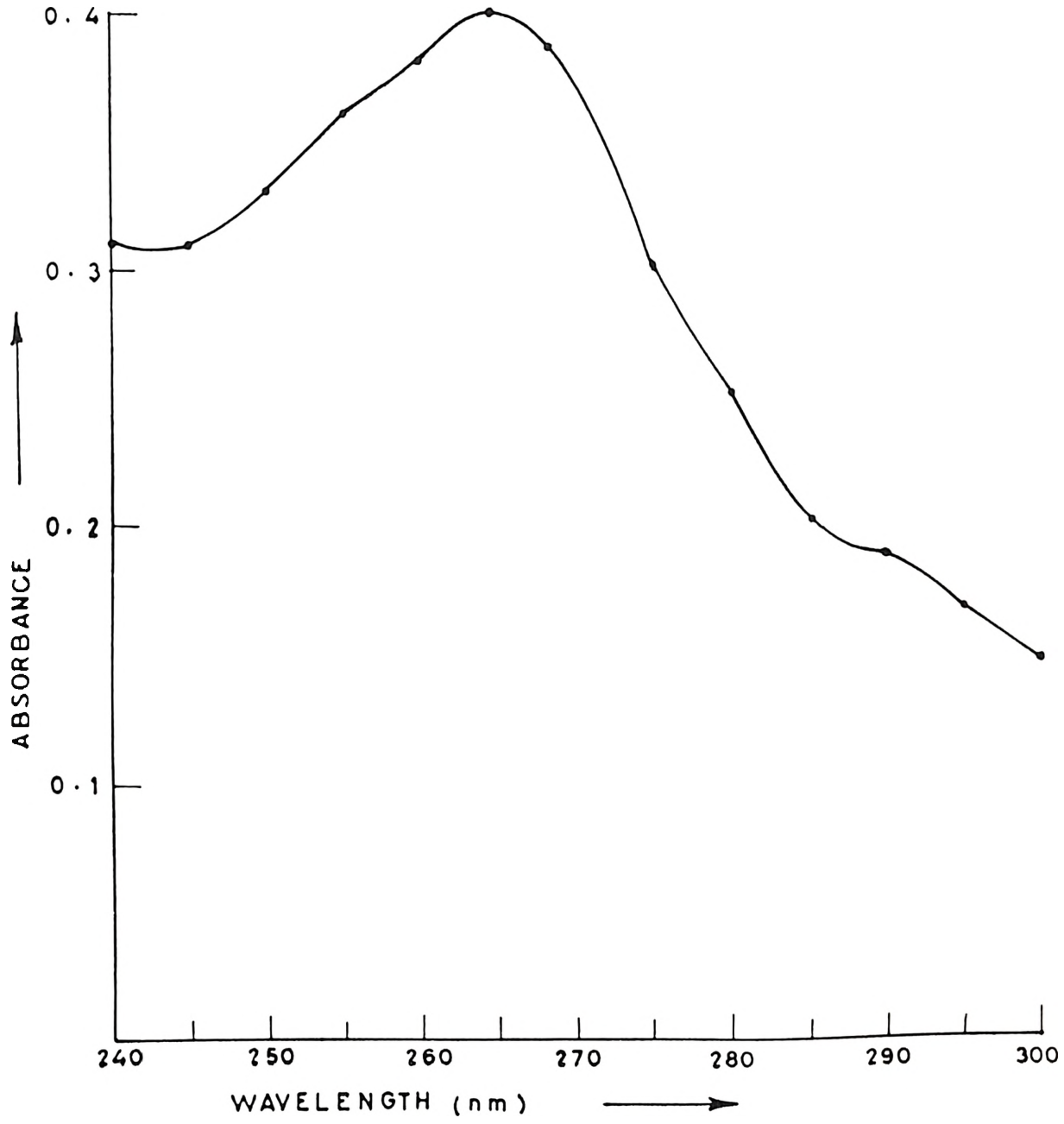


Fig.5 ABSORPTION SPECTRUM FOR OXYPHENBUTAZONE

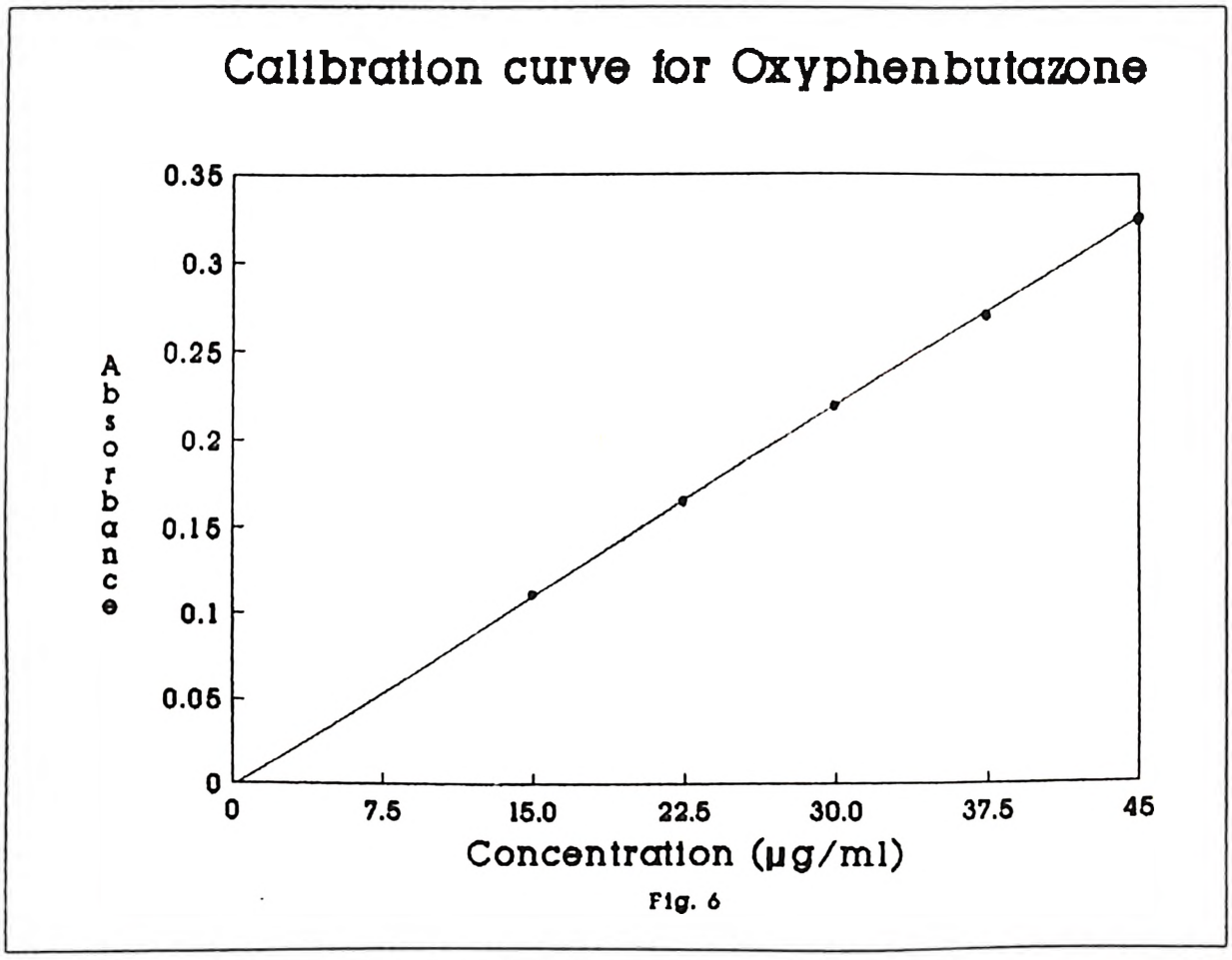

Calibration curve for Oxyphenbutazone
Absorbance
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
7.5
15.0
22.5
30.0
37.5
45
Concentration (µg/ml)

Fig. 6

Table 4

Calibration curve for Ibuprofen

Concentration (µg/ml)	Absorbance						SD*	CV**
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Mean		
60	0.061	0.053	0.059	0.050	0.066	0.057	0.0063	11.037
80	0.077	0.069	0.076	0.079	0.072	0.074	0.0040	5.411
100	0.089	0.083	0.095	0.091	0.082	0.088	0.0055	9.634
120	0.110	0.105	0.115	0.123	0.092	0.109	0.0115	10.639
140	0.126	0.121	0.127	0.134	0.117	0.125	0.0064	5.153
160	0.142	0.138	0.140	0.144	0.141	0.141	0.0033	2.404
180	0.158	0.156	0.161	0.158	0.152	0.157	0.0033	2.112

*Standard deviation

**Coefficient of Variation (%)

Covariance = 1.884

Correlation Coefficient = 0.99933

Regression = 0.99867

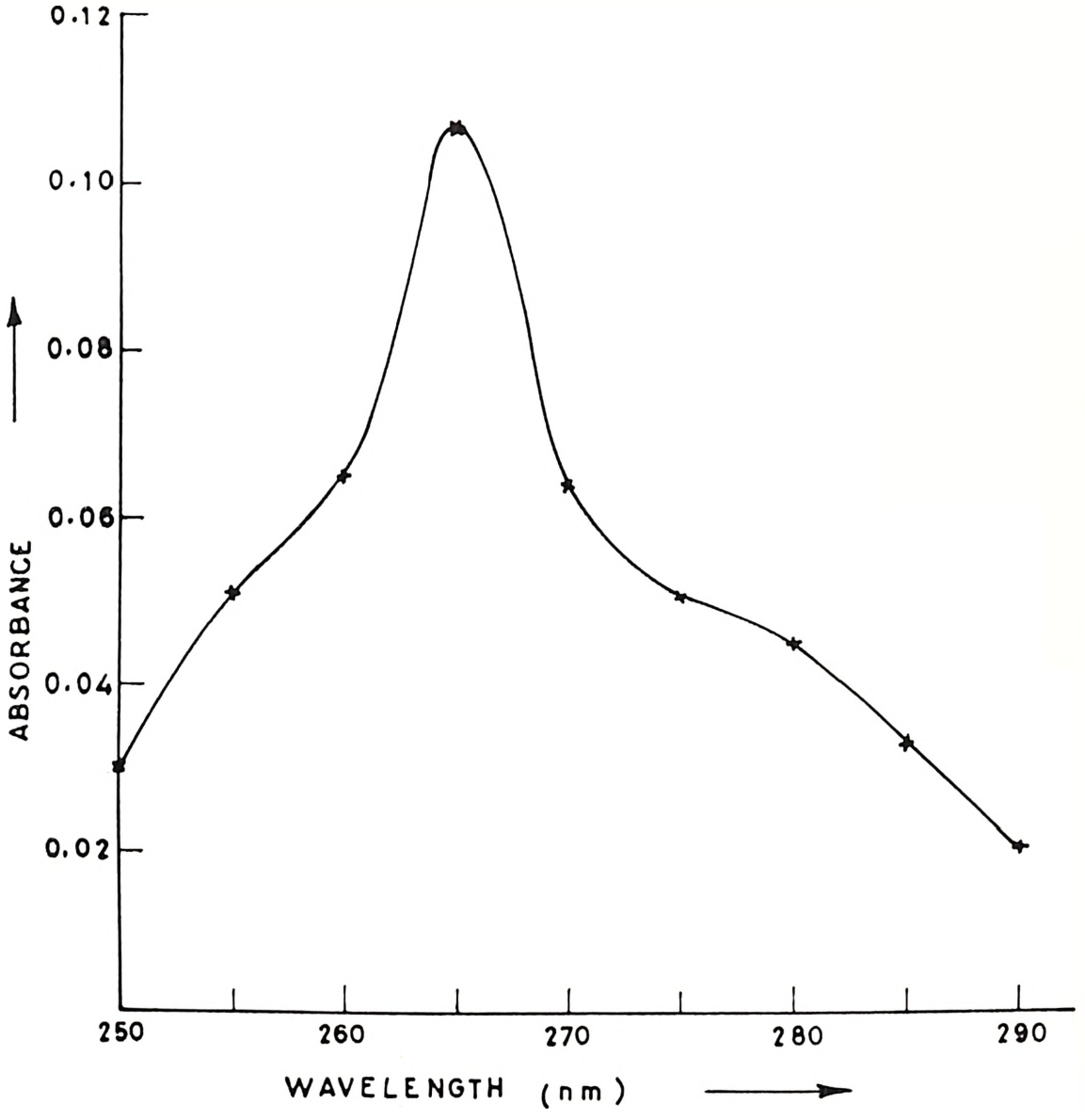


Fig.7 ABSORPTION SPECTRUM FOR IBUPROFEN

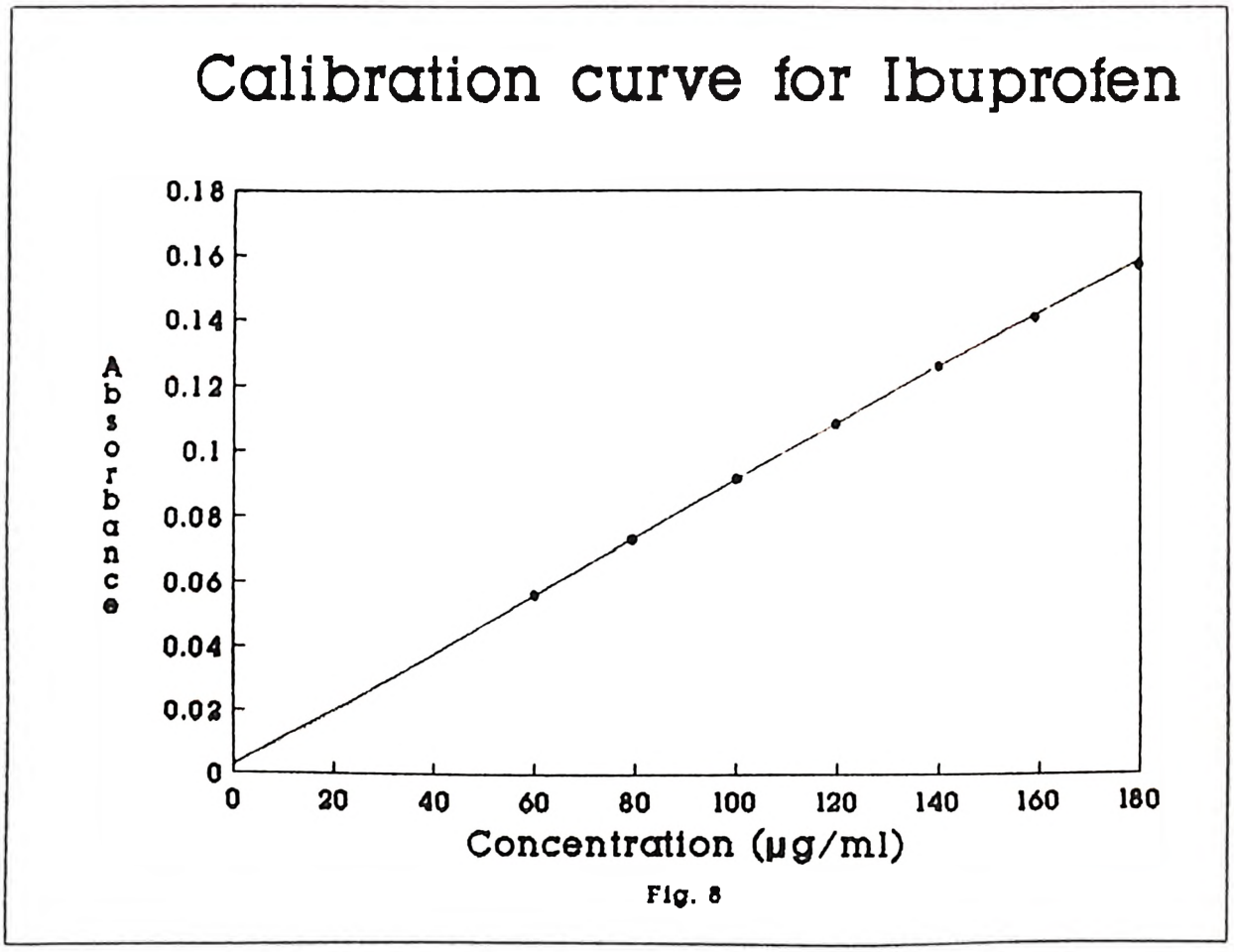

Calibration curve for Ibuprofen
Absorbance
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
0
20
40
60
80
100
120
140
160
180
Concentration (µg/ml)

Fig. 8

Table 5

For combination of Paracetamol, Dextropropoxyphene hydrochloride and Oxyphenbutazone

Commercial Formulation

Each capsule contains

Brand A

Oxyphenbutazone IP : 100 mg

Dextropropoxyphene hydrochloride IP : 32.5 mg

Paracetamol IP : 250 mg

Formulation	Trial No.	Absorbance	Concentration from std graph (µg/ml)	Amount in each sample (mg)	% Recovery
Paracetamol	1	0.151	50.9	254.50	101.80
Dextropropoxyphene hydrochloride		0.290	31.7	32.30	99.00
Oxyphenbutazone		0.217	29.9	98.32	98.32
	2	0.153	51.5	257.50	103.00
		0.293	31.8	32.90	102.81
		0.219	30.1	100.50	100.50
	3	0.151	50.9	249.50	99.80
		0.290	31.7	31.10	97.18
		0.217	29.9	99.00	99.00

For Paracetamol (label claim 250 mg)

Average weight of the powder present in each capsule = 499.01 mg
499.01 mg of the sample was dissolved in ethanol = 5 ml
Loaded volume on TLC plate = 0.05 ml (50 µl)
Scraped sample extracted with methanol = 10 ml
Secondary dilution = 2 ml to 10 ml

Calculations :

$$1.\ 50.9 \times \frac{10}{0.05} \times \frac{10}{2} \times \frac{5}{1000} = 254.5 \text{ mg}$$

$$2.\ 51.5 \times \frac{10}{0.05} \times \frac{10}{2} \times \frac{5}{1000} = 257.5 \text{ mg}$$

$$3.\ 50.9 \times \frac{10}{0.05} \times \frac{10}{2} \times \frac{5}{1000} = 249.5 \text{ mg}$$

Average result :

$$\frac{254.5 + 257.5 + 254.5}{3} = 255.50 \text{ (237.5 - 262.5 mg)}$$

$$\frac{255.50}{250.00} \times 100 = 102.20\ \% \text{ (95 - 105 \%)}$$

For Dextropropoxyphene hydrochloride : (label claim 32.5 mg)

Average weight of the powder present in each capsule = 499.01 mg

499.01 mg of the sample (from capsule) dissolved in ethanol = 5 ml

Loaded volume on TLC plate = 0.05 ml (50 µl)

scraped sample extracted with methanol = 10 ml

Calculations

$$1.\ 31.7 \times \frac{10}{0.05} \times \frac{5}{1000} = 31.7 \text{ mg}$$

$$2.\ 31.8 \times \frac{10}{0.05} \times \frac{5}{1000} = 31.8 \text{ mg}$$

$$3.\ 31.7 \times \frac{10}{0.05} \times \frac{5}{1000} = 31.7 \text{ mg}$$

Average result :

$$\frac{31.7 + 31.8 + 31.7}{3} = 31.73 \text{ mg } (30.875 - 34.125 \text{ mg})$$

$$\frac{31.73}{32.5} \times 100 = 97.69\ \% \ (95 - 105\ \%)$$

For Oxyphenbutazone : (Label Claim 100 mg)

Average weight of the powder present in each capsule = 499.01 mg

499.01 mg of sample dissolved in ethanol = 5 ml

Scraped sample was extracted with methanol = 10 ml

Secondary dilution = 3 ml in 10 ml of methanol

Calculations :

$$1.\ 29.9 \times \frac{10}{0.05} \times \frac{10}{3} \times \frac{5}{1000} = 99.66 \text{ mg}$$

$$2.\ 30.15 \times \frac{10}{0.05} \times \frac{10}{3} \times \frac{5}{1000} = 100.5 \text{ mg}$$

$$3.\ 29.9 \times \frac{10}{0.05} \times \frac{10}{3} \times \frac{5}{1000} = 99.66 \text{ mg}$$

Average result :

$$\frac{99.66 + 100.5 + 99.66}{3} = 99.94 \text{ mg (95 - 105 mg)}$$

$$\frac{99.94}{100} \times 100 = 99.94\ \% \text{ (95 - 105 \%)}$$

Each capsule contains

Brand B

Oxyphenbutazone IP : 100 mg

Dextropropoxyphene hydrochloride IP : 32.5 mg

Paracetamol IP : 650 mg

Formulation	Trial No.	Absorbance	Concentration from std graph (µg/ml)	Amount in each sample (mg)	% Recovery
Paracetamol	1	0.195	65.7	657.00	101.08
Dextropropoxyphene hydrochloride		0.063	16.0	32.00	98.46
Oxyphenbutazone		0.115	14.9	99.33	99.33
	2	0.197	65.8	658.00	101.23
		0.065	16.2	32.40	99.69
		0.113	14.8	98.66	98.66
	3	0.195	65.7	657.00	101.08
		0.063	16.0	32.00	98.46
		0.115	14.9	99.33	99.33

For Paracetamol (label claim 650 mg)

Average weight of the powder present in each capsule = 1031.6 mg

1031.6 mg of the sample was dissolved in ethanol = 5 ml

Loaded volume on TLC plate = 0.05 ml (50 µl)

Secondary dilution = 2 ml to 10 ml

$$1.\ 65.7 \times \frac{10}{0.05} \times \frac{10}{2} \times \frac{5}{1000} = 657.0 \text{ mg}$$

$$2.\ 65.8 \times \frac{10}{0.05} \times \frac{10}{2} \times \frac{5}{1000} = 658.0 \text{ mg}$$

$$3.\ 65.7 \times \frac{10}{0.05} \times \frac{10}{2} \times \frac{5}{1000} = 657.0 \text{ mg}$$

Average result :

$$\frac{657.0 + 658.0 + 657.0}{3} = 657.33 \text{ (237.5 - 262.5 mg)}$$

$$\frac{657.33}{650.00} \times 100 = 101.128\ \% \text{ (95 - 105 \%)}$$

For Dextropropoxyphene hydrochloride : (label claim 32.5 mg)

Average weight of the powder present in each capsule = 1031.6 mg

1031.6 mg of the sample (from capsule) dissolved in ethanol = 5 ml

Loaded volume on TLC plate = 0.05 ml (50 µl)

Scraped sample extracted with methanol = 10 ml

Calculations

$$1.\ 16.0 \times \frac{10}{0.05} \times \frac{5}{1000} = 32.0 \text{ mg}$$

$$2.\ 16.2 \times \frac{10}{0.05} \times \frac{5}{1000} = 32.4 \text{ mg}$$

$$3.\ 16.0 \times \frac{10}{0.05} \times \frac{5}{1000} = 32.0 \text{ mg}$$

Average result :

$$\frac{32.0 + 32.4 + 32.0}{3} = 32.13 \text{ mg } (30.875 - 34.125 \text{ mg})$$

$$\frac{32.13}{32.50} \times 100 = 98.86\ \% \ (95 - 105\ \%)$$

For Oxyphenbutazone : (Label Claim 100 mg)

Average weight of the powder present in each capsule	= 1031.6 mg
1031.6 mg of sample dissolved in ethanol	= 5 ml
Scraped sample was extracted with methanol	= 10 ml
Secondary dilution	= 3 ml in 10 ml of methanol

Calculations :

$$1.\ 14.9 \times \frac{10}{0.05} \times \frac{10}{3} \times \frac{5}{1000} = 99.33 \text{ mg}$$

$$2.\ 14.8 \times \frac{10}{0.05} \times \frac{10}{3} \times \frac{5}{1000} = 98.66 \text{ mg}$$

$$3.\ 14.9 \times \frac{10}{0.05} \times \frac{10}{3} \times \frac{5}{1000} = 99.33 \text{ mg}$$

Average result :

$$\frac{99.33 + 98.66 + 99.33}{3} = 99.106 \text{ mg (95 - 105 mg)}$$

$$\frac{99.106}{100} \times 100 = 99.106\ \% \text{ (95 - 105 \%)}$$

Table 6

Statistical Analysis

Formulation	Label Claim (mg)	Amount Found* (mg)	% Recovery	SD**	CV***	t-test
Brand A						
Paracetamol	250	255.50	102.20	1.7321	0.6779	255.500
Dextropropoxyphene Hydrochloride	32.5	31.73	97.69	0.0595	0.1875	923.788
Oxyphenbutazone	100	99.94	99.94	0.4841	0.4844	357.556
Brand B						
Paracetamol	650	657.33	101.13	0.5590	0.0850	2036.673
Dextropropoxyphene Hydrochloride	32.5	32.13	98.86	0.2312	0.7196	240.699
Oxyphenbutazone	100	99.11	99.11	0.3865	0.3900	444.087

* Average of three determinations

** Standard Deviation

*** Coefficient of Variation (%)

Table 7

For combination of Ibuprofen, Dextropropoxyphene hydrochloride and Paracetamol

Commercial Formulation

Each coated tablet contains :

Paracetamol IP : 250 mg

Dextropropoxyphene hydrochloride IP : 32.5 mg

Ibuprofen IP : 200 mg

Formulation	Trial No.	Absorbance	Concentration from std graph (µg/ml)	Amount in each sample (mg)	% Recovery
Paracetamol	1	0.151	51.3	256.5	102.60
Dextropropoxyphene Hydrochloride		0.240	32.7	32.7	100.61
Ibuprofen		0.119	133.0	199.5	099.75
	2	0.153	51.5	257.5	103.00
		0.243	32.8	32.8	100.92
		0.118	132.5	198.75	99.38
	3	0.151	51.3	256.50	102.60
		0.240	32.7	32.70	100.62
		0.118	132.5	198.75	099.38

Similar calculations were followed as shown in the previous brand

Table 8

Statistical Analysis

Formulation	Label Claim (mg)	Amount Found* (mg)	%Recovery	SD**	CV***	t-test
Brand A						
Paracetamol	250	256.83	102.73	0.5796	0.2257	767.508
Dextropropoxyphene Hydrochloride	32.5	32.73	100.70	0.0585	0.1786	969.766
Ibuprofen	200	199.00	99.50	0.4330	0.2176	796.000

*Average of three determinations

**Standard Deviation

***Coefficient of Variation %

DISCUSSION

Various difficulties were encountered while devising a simultaneous assay procedures for the drug formulations described in this research work. Since official methods are not available for the analysis of drug preparations containing three constituents simultaneously, the proposed methods which have been developed are found to be precise, sensitive and reproducible. A quantitative thin layer chromatography (TLC) method has been developed for the determination of oxyphenbutazone and ibuprofen in the presence of paracetamol and dextropropoxyphene hydrochloride in drug preparations. The method involves the separation of the three constituents by TLC technique, extraction of the components with methanol and the measurement of their absorption in the ultra-violet region. The absorbance curve is linear in the concentration range of 30-70 µg/ml for paracetamol, Rf value being 0.45 and absorbance maximum was observed at 242 nm. Similarly the concentration range for dextropropoxyphene hydrochloride, oxyphenbutazone and ibuprofen were 10 - 50 µg/ml, 15 - 45 µg/ml and 60-200 µg/ml, the Rf values being 0.80, 0.15 and 0.20 respectively and corresponding absorption maxima are 258 nm, 265 nm and 265 nm. The results of estimation were evaluated by the standard deviation and coefficient of variation. The Rf is the ratio of distance moved by sample to that of the distance moved by solvent front.

The areas of the spots of drugs are compact and separation is easier. Hence the drug is made to react with a spraying reagent to identify the location and movement of drug by the mobile phase. Phosphomolybdic acid, potassium-iodo-bismuthate and acidified potassium permanganate (oxidising agent) are used as spraying agents for the paracetamol, dextropropoxyphene hydrochloride, oxyphenbutazone and ibuprofen.

Before analysis is performed with spectrophotometer the readout system of the instrument is calibrated quantitatively, the instrument is zeroed by using blank. When monochromatic radiation passed through a cell which contains a solution of an absorbing substance the effects occuring are reflection at cell surfaces, absorption by the solute and transmission of unabsorbed radiation. It should obey the Lambert-Beer's law.

To justify the suitability of method, known amounts of the pure drug were added to the previously analysed formulation samples and the mixture was again analysed by the assay method. The difference between the two absorbance readings was noted which showed the quantity of the drug added. It was calculated from the standard curve. The % recoveries are found to be more than 99 % for paracetamol, ibuprofen, dextropropoxyphene hydrochloride and oxyphenbutazone.

Paracetamol has been evaluated and standard graph was drawn by adopting the technique (177) followed by the group of workers in the laboratory ie, the silica gel G was triturated with distilled water to prepare the plates and the solvent system used in the developing chamber consisted of chloroform, ethyl acetate, isopropanol (50 : 30 : 20). As far as paracetamol and oxyphenbutazone are concerned, there was no difficulty in analysis and a standard graph was prepared. But attempts made to obtain a linear curve for dextropropoxyphene hydrochloride which is not successful. After several trials, it was found that dextropropoxyphene hydrochloride being a salt was found to have high affinity for silica gel G and produced tailing effect which in turn affected the linearity of the standard curve. Thus silica gel G plates buffered with potassium hydrogen phosphate buffered (pH 6.0) were prepared.

The results of our study performed on combination preparations show that the amounts of paracetamol, dextropropoxyphene hydrochloride, ibuprofen and oxyphenbutazone contained in the aforesaid formulations conform to the label claim specifications.

4.2 (a)

DETERMINATION OF PARACETAMOL, DEXTROPROPOXYPHENE HYDROCHLORIDE AND DICYCLOAMINE HYDROCHLORIDE IN PHARMACEUTICAL FORMULATIONS BY QUANTITATIVE THIN LAYER CHROMATOGRAPHY

Instrument

A UV-Visible Cary 17D spectrophotometer was used for measuring the absorbance data equipped with two 1 cm quartz cells .

Mobile Phase

Solvent system consisting of a mixture of ethyl acetate : chloroform : methanol : Water : Ammonia (75:15:5:3:2) was used..

Spraying Agents

1. Phosphomolybic Acid : Five grames (5.0 gms) of phasphomolybic acid was dissolved in 100 ml of water.

2. Potassium Iodo-Bismuthate solution : Ten grams (10 gms) of (+) Tartaric acid was dissolved in 40 ml of water. To this solution was added 0.85 gm of bismuth oxynitrate and continuously stirred for one hour. Thereafter 20 ml of 40% w/v solution of potassium iodide was added and shaken well. Finally, the mixture was allowed to stand for 24 hrs and filtered.

3. Colouring Agent : A new colouring reagent was developed for the identification of dicycloamine hydrochloride prepared by mixing 24.25 gms of potassium thiocyanate, 20 gms of cobaltous chloride, 13.6 gms of sodium acetate and 10 ml of 1N HCl. The solution was transferred into 100 ml volumetric flask and volume made upto 100 ml with distilled water. The colour of the reagent thus prepared was intense blue.

PROCEDURE

The TLC method developed for their separation and determination is described below :

Silica gel G (7.5 gms) was triturated with 16 ml of distilled water to form a smooth paste which was uniformly spread on the glass plate (20 X 20 cm) to form a 0.25 mm thickness of the adsorbant layer. The plates were air dried for 15 minutes and then at 110° C for 30 minutes in an electric oven (179). Various combination of solvents were attempted until a clear separation of the 3 constituents with defined Rf values was achieved. The final solvent system was selected by doing many trials on the previous solvent system development as described in the previous determination of this work. The final combination of the solvents which gave best separation with Rf values for the determination of paracetamol, dextropropoxyphene hydrochloride and dicycloamine hydrochloride in formulation was :

Ethyl acetate	: 75
Chloroform	: 15
Methanol	: 05
Water	: 03
Ammonia	: 02

STANDARD GRAPH FOR PARACETAMOL

A stock solution of 2.5 gm of pure paracetamol in 25 ml of ethanol giving a concentration of 10 % w/v was prepared. A series of dilutions ranging in concentration of 5, 10, 15, 20, 25, 30, 40, 50, 60, 70 and 80 mg/ml in ethanol were thereafter prepared. Exactly 0.2 ml (200 µl) of this solution was spotted in duplicate on the TLC plates. The plates were carefully placed in the chromatographic chamber containing the solvent system as mentioned above which is maintained at room temperature by ascending technique. When the solvent front had moved a distance of 15 cm the plates were carefully removed, dried and the spots developed were identified with 5 % phosphomolybdic acid solution and subsequently heating the plates at 80°C. Purple coloured spots were obtained. The coloured and uncoloured duplicate spots were scraped and collected in a clear dry centrifuge tubes. 5.0 ml of the extraction solvent (99 % methanol + 1.0 % 1N HCl) was added and vigorously shaken for 5 minutes to achieve proper dissolution. The supernatant solution was further diluted to 100 ml with extraction solvent. The absorbance of the clear solution with respect to the blank (extracted by same method) was measured at 242 nm (Fig. 9). This procedure was followed for all the concentrations and standard graph was plotted (Table 9). Straight line graph as shown in Fig.10 was obtained. The absorbance data, calculations and quantitation are reported under Results.

STANDARD GRAPH FOR DEXTROPROPOXYPHENE HYDROCHLORIDE

A stock solution having a concentration 2.0 % w/v was prepared by dissolving 500 mg of pure drug in 25 ml of absolute alcohol. From this a series of dilutions ranging in concentrations of 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 % w/v in ethanol were prepared. The loading of the TLC plates was done in duplicate using 0.2 ml (200 µl) of solution per spot. The rest of the procedure was similar to the previous experiment. The plates were carefully removed and the spots were developed by spraying with potassium iodo-bismuthate solution. Red coloured spots were

produced. The coloured and uncoloured duplicate spots were scraped and collected in dry clean centrifuge tubes. The drug was extracted for 4 times with 25 ml portions of methanol. All the four extracts were combined, filtered and the absorbance of the clear solutions with respect to the blank was measured at 258 nm (Fig. 11). The procedure was followed for all the concentrations and a standard graph was prepared which is shown in Table 10 & Fig. 12.

STANDARD GRAPH FOR DICYCLOAMINE HYDROCHLORIDE

A stock solution of 250 mg of the pure drug was prepared in 25 ml of chloroform and a series of dilutions ranging in concentrations of 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0 and 4.5 mg/ml were prepared in the same solvent. The rest of the procedure was similar to the previous experiment. The spots were developed by spraying with potassium iodo-bismuthate solution. The spots obtained were orange in colour. The coloured & uncoloured duplicate spots were scraped and collected in dry clean centrifuge tubes. These were dissolved in 4ml of chloroform and centrifuged for 10 minutes. The supernatant was removed and transferred to 250 ml seperating flask. A 10 ml solution of colouring reagent was measured at 620 nm (Fig. 13) on a spectrophotometry using pure chloroform as the blank. The procedure was repeated for all concentrations and graph was plotted, which gave a straight line as shown in Table 11 & Fig.14.

ASSAY PREPARATION FOR COMBINATION OF PARACETAMOL, DEXTROPROPOXYPHENE HCl & DICYCLOAMINE HCl

Ten capsules were accurately weighed and the contents were finely powdered. The powder equivalent to an average weight of the capsule was extracted with 10 ml of ethanol giving a concentration of 40 mg/ml of paracetamol , 6.5 mg/ml of dextropropoxyphene HCl and 1.0 mg/ml of dicycloamine HCl. The solution was centrifuged and the supernatant solution was used for spotting the TLC plates.The spotting, development, detection and extraction of the separated constituents were done in the same manner as described for the pure drug samples.The absorbance of the solutions were measured at 242 nm, 258 nm and 620 nm for paracetamol, dextropropoxyphene hydrochloride and dicycloamine hydrochloride respectively. The results of the estimation are shown in Table 12 & 13.

RECOVERY PROCEDURE : In order to establish the suitability of this method, known amounts of the pure drug was added to previously analysed formulation and the mixture was again analysed by the assay method. The difference between the two absorbance readings was noted which showed the quantity of the drug added. It was calculated from the standard curve and is reported under results.

Result

Table 9

Calibration curve for Paracetamol

Concentration (µg/ml)	Absorbance				SD*	CV*
	Trial 1	Trial 2	Trial 3	Mean		
10	0.050	0.045	0.047	0.045	0.0035	7.909
20	0.095	0.096	0.094	0.095	0.0041	4.297
30	0.143	0.145	0.141	0.143	0.0008	0.570
40	0.190	0.195	0.194	0.193	0.0029	1.526
50	0.242	0.245	0.239	0.242	0.0029	1.217
60	0.292	0.295	0.288	0.291	0.0029	0.989
80	0.391	0.387	0.386	0.388	0.0069	1.798
100	0.490	0.488	0.486	0.486	0.0106	2.184
120	0.583	0.586	0.583	0.584	0.0086	1.473
140	0.683	0.685	0.678	0.682	0.0054	0.803
150	0.733	0.735	0.725	0.731	0.0063	0.867

* Standard deviation

** Coefficient of variation (%)

Covariance = 6.477122

Correlation coefficient = 0.9999981

Regression = 0.9999962

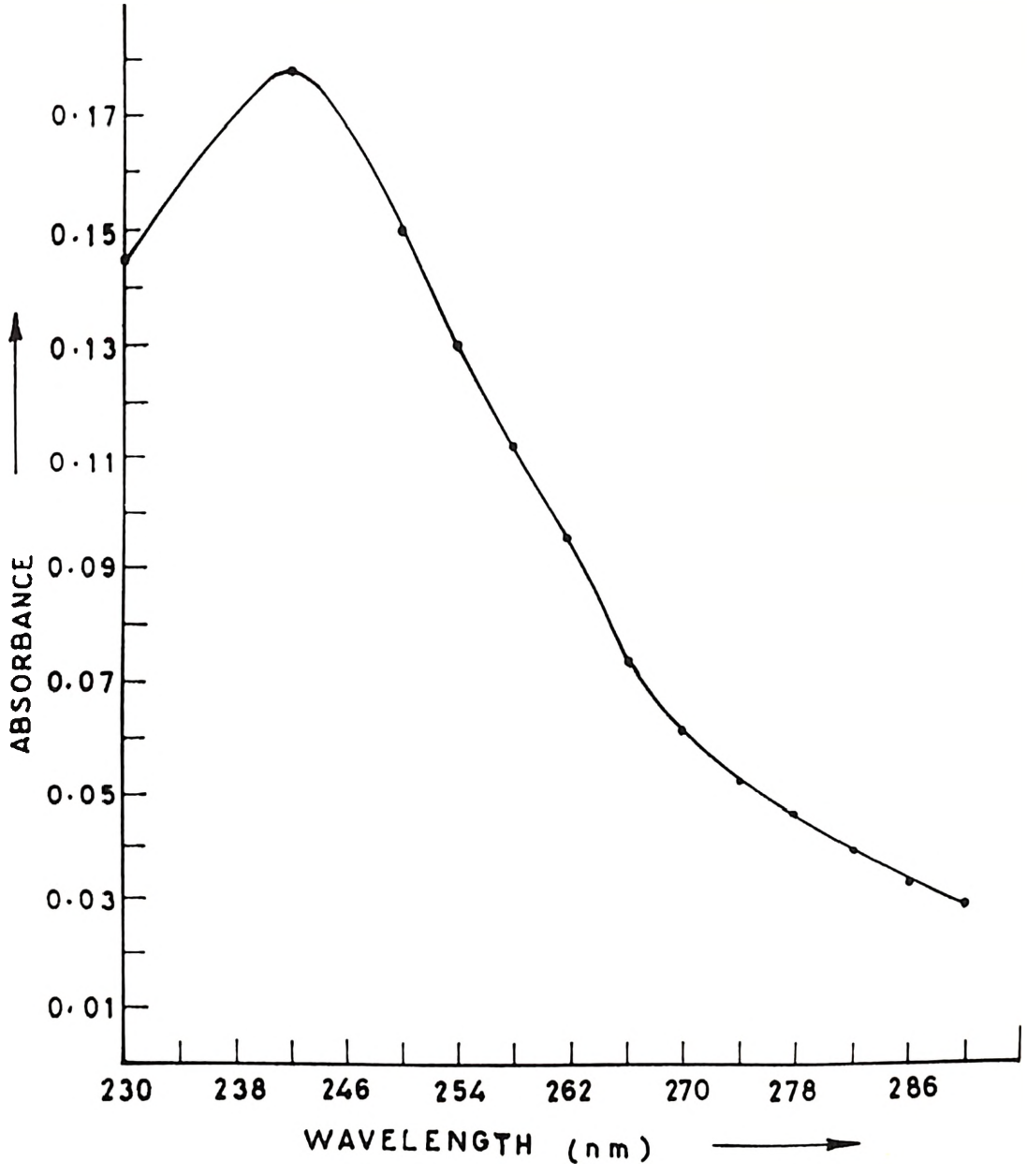


Fig. 9· ABSORPTION SPECTRUM FOR PARACETAMOL

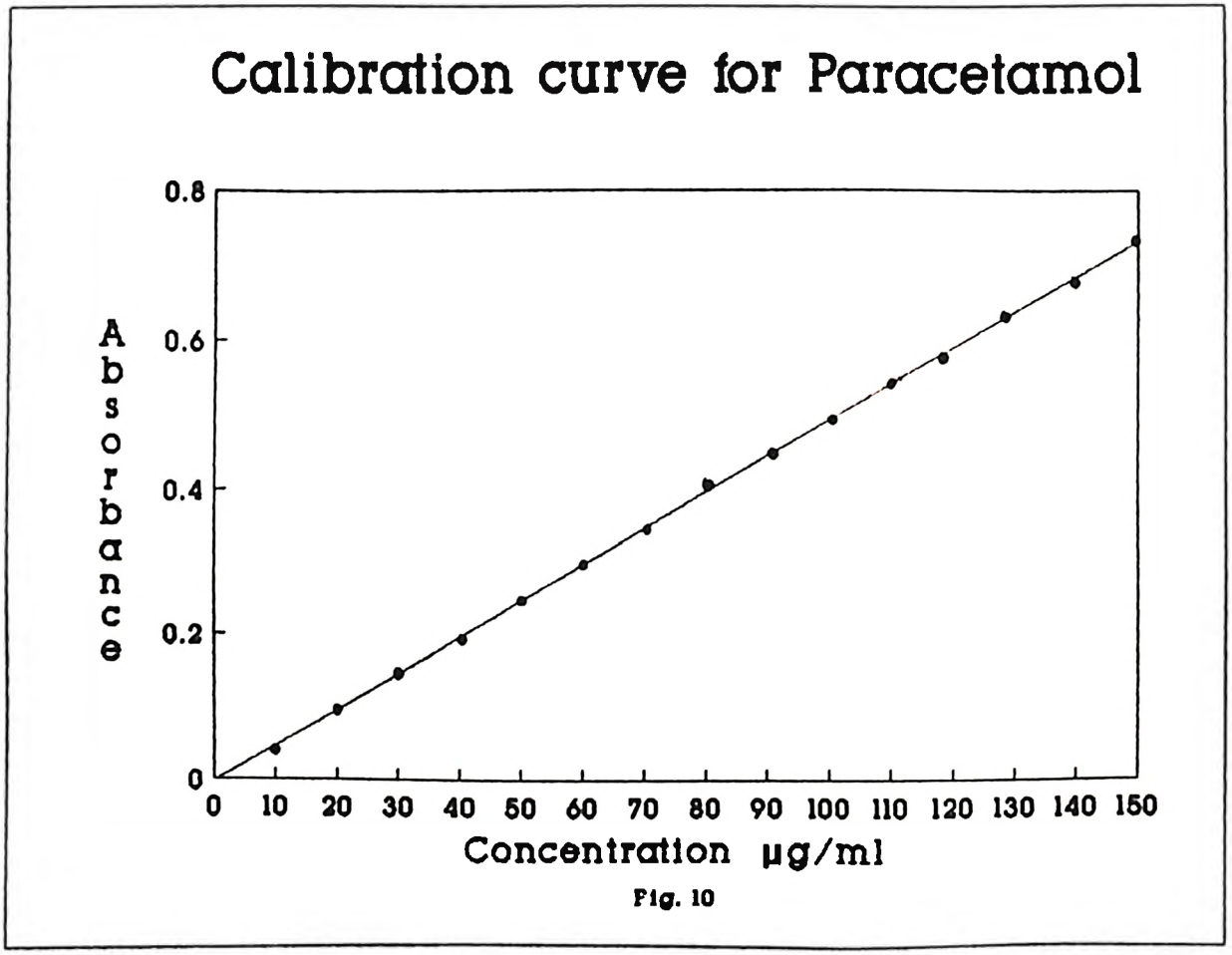

Calibration curve for Paracetamol
Absorbance
0.8
0.6
0.4
0.2
0
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150
Concentration µg/ml

Fig. 10

Table 10

Calibration curve for Dextropropoxyphene Hydrochloride

Concentration (µg/ml)	Absorbance				SD*	CV**
	Trial 1	Trial 2	Trial 3	Mean		
6	0.036	0.038	0.034	0.036	0.0018	5.071
8	0.048	0.051	0.045	0.048	0.0021	4.272
10	0.059	0.063	0.058	0.060	0.0022	3.600
12	0.073	0.074	0.072	0.073	0.0014	1.937
14	0.086	0.087	0.083	0.085	0.0018	2.148
16	0.096	0.100	0.095	0.097	0.0018	1.882
18	0.108	0.111	0.108	0.109	0.0015	1.379
20	0.121	0.124	0.118	0.121	0.0022	1.786

* Standard deviation

** Coefficient of variation (%)

Covariance = 0.08524998

Correlation coefficient = 0.9999569

Regression = 0.9999138

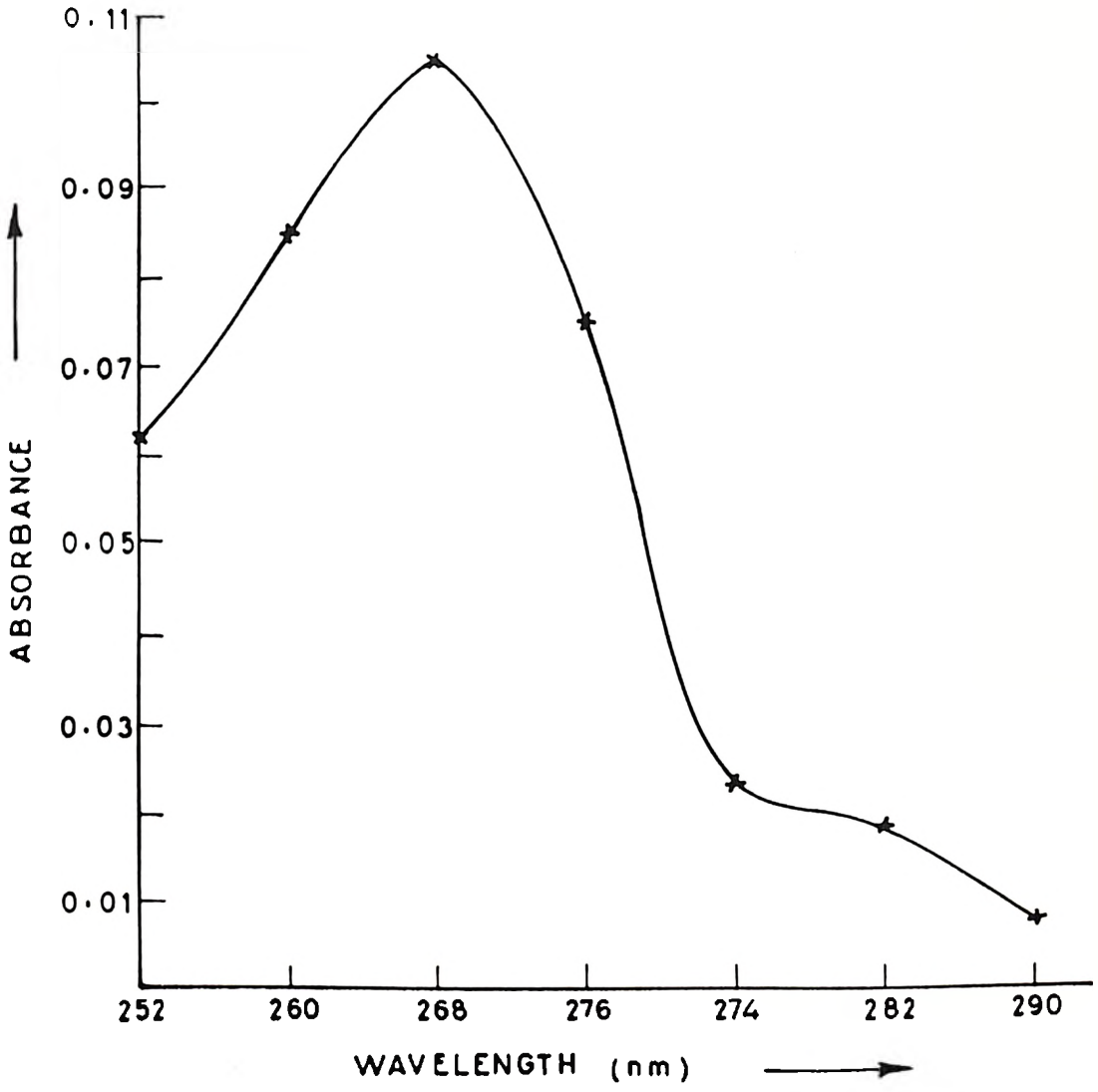


Fig. 11. ABSORPTION SPECTRUM FOR DEXTROPROPOXY-PHENE HYDROCHLORIDE

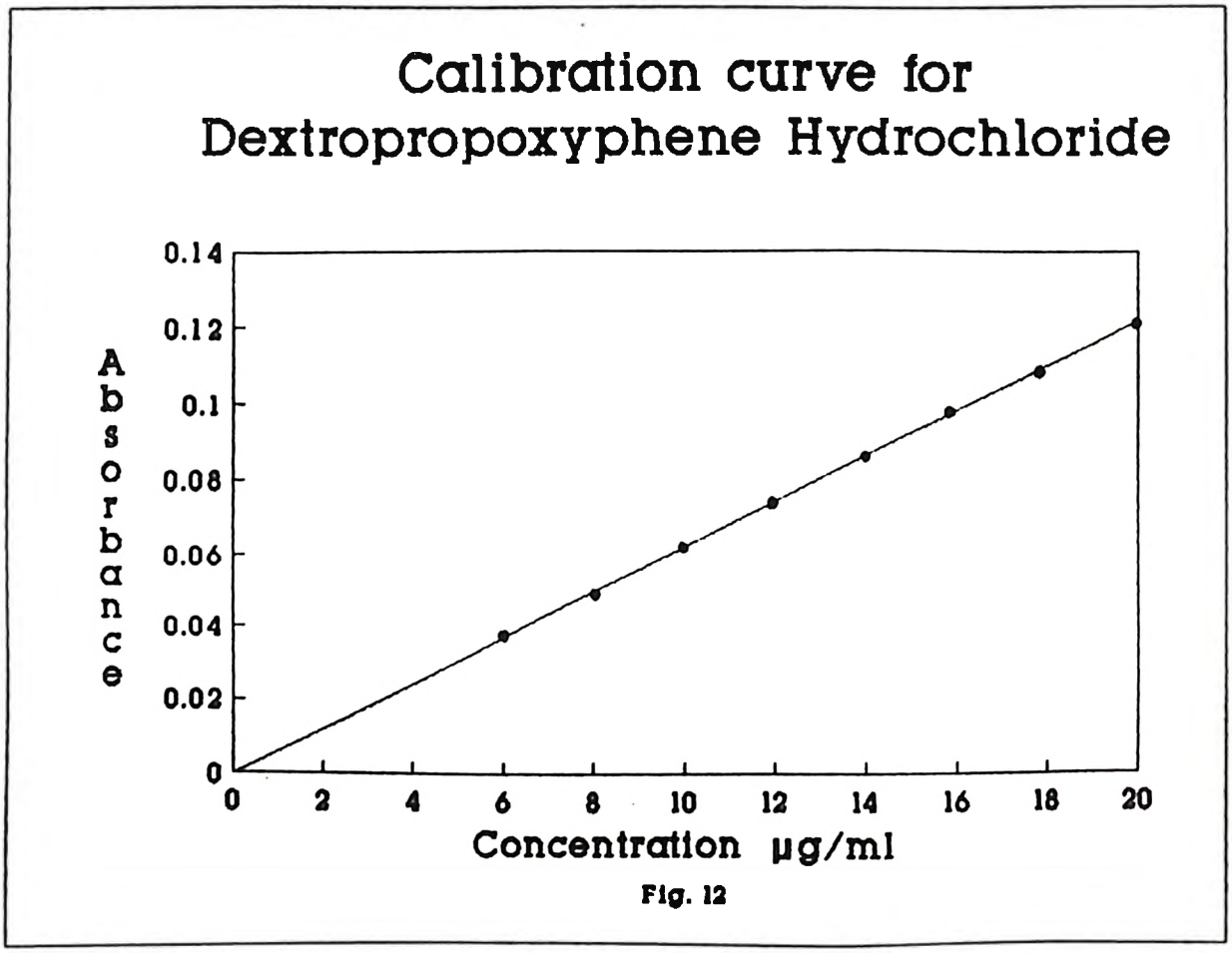

Calibration curve for
Dextropropoxyphene Hydrochloride
Absorbance
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
0
2
4
6
8
10
12
14
16
18
20
Concentration µg/ml

Fig. 12

Table 11

Calibration curve for Dicycloamine Hydrochloride

Concentration (µg/ml)	Absorbance				SD*	CV**
	Trial 1	Trial 2	Trial 3	Mean		
2.5	0.008	0.010	0.012	0.010	0.0021	19.820
5.0	0.019	0.020	0.024	0.021	0.0022	10.680
7.5	0.033	0.031	0.032	0.032	0.0029	9.199
10.0	0.041	0.043	0.045	0.043	0.0026	6.152
12.5	0.053	0.054	0.055	0.054	0.0029	5.452
15.0	0.066	0.065	0.067	0.066	0.0046	6.903
17.5	0.075	0.077	0.079	0.077	0.0035	4.622
20.0	0.084	0.089	0.091	0.088	0.0026	2.934
22.5	0.096	0.101	0.103	0.100	0.0026	2.582

* Standard deviation

** Coefficient of variation (%)

Covariance = 48.14286

Correlation coefficient = 0.9999369

Regression = 0.9998738

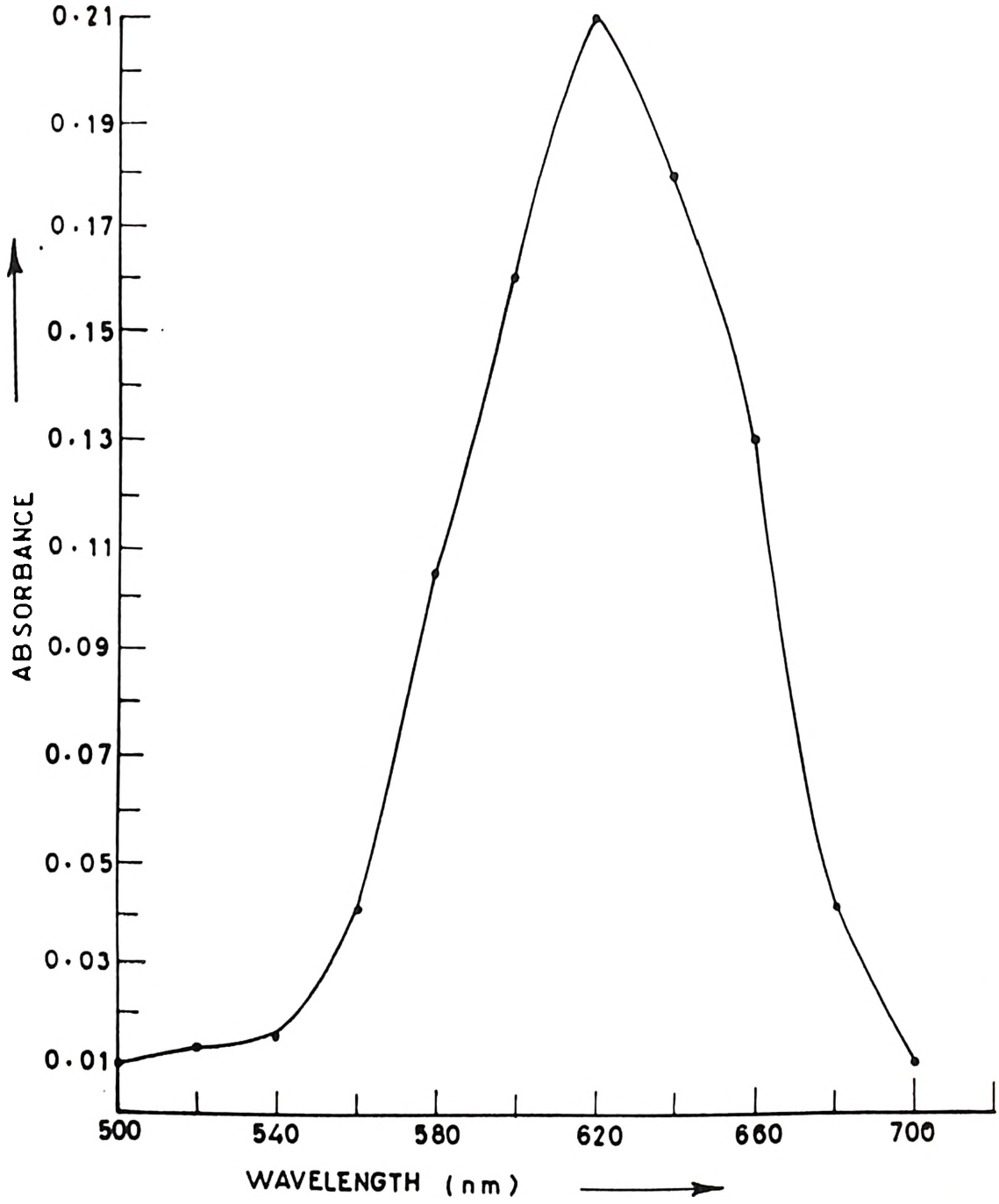


Fig.13 ABSORPTION SPECTRUM FOR DICYCLOAMINE HYDROCHLORIDE

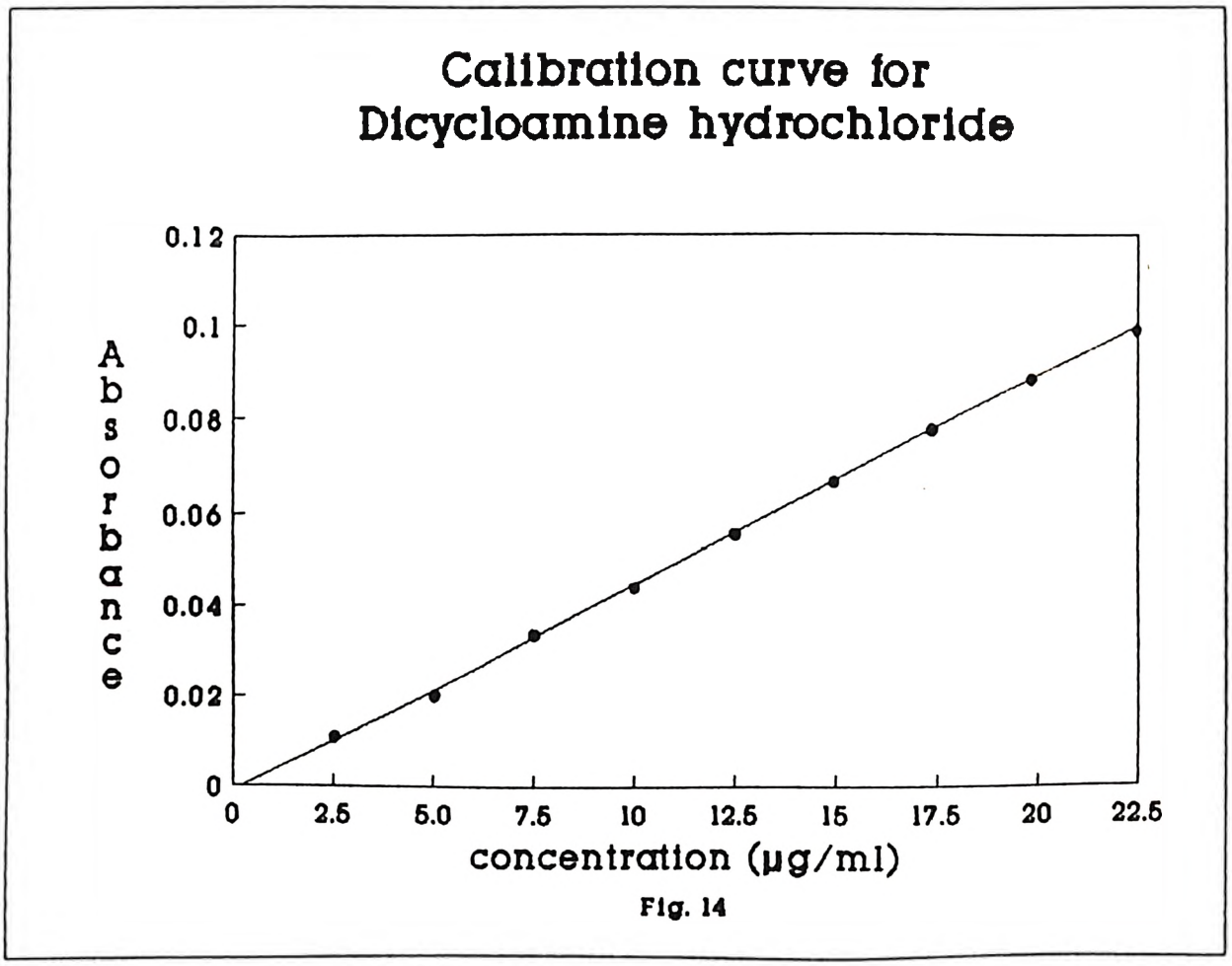

Calibration curve for
Dicycloamine hydrochloride
Absorbance
0.12
0.1
0.08
0.06
0.04
0.02
0
0
2.5
5.0
7.5
10
12.5
15
17.5
20
22.5
concentration (µg/ml)

Fig. 14

Table 12

For combination of Paracetamol, Dextropropoxyphene hydrochloride and Dicycloamine hydrochloride

Commercial formulation

Each capsule contains

Paracetamol IP : 400 mg

Dextropropoxyphene Hydrochloride IP : 65 mg

Dicycloamine Hydrochloride IP : 10 mg

Formulation	Trial No.	Absorbance	Concentration from std graph (µg/ml)	Amount in each sample (mg)	% Recovery
Paracetamol	1	0.376	79.00	395.00	98.75
Dextropropoxyphene hydrochloride		0.082	12.80	64.00	98.46
Dicycloamine hydrochloride		0.023	5.11	10.23	102.30
	2	0.379	79.60	398.00	99.50
		0.083	12.94	64.70	99.53
		0.021	4.86	9.72	97.26
	3	0.378	79.40	397.00	99.25
		0.082	12.80	64.00	98.46
		0.023	5.11	10.23	102.30

For Paracetamol (label claim : 400 mg)

Average weight of the powder present in each capsule = 535.03 mg

535.03 mg of the sample was dissolved in ethanol = 10 ml

Loaded volume on TLC plate = 0.2 ml

Scraped sample extracted with solvent mixture (99% methanol and 1% 1N HCl) = 100 ml

Calculations :

$$1.\ 79 \times \frac{10}{1} \times \frac{100}{0.2} \times \frac{1.0}{1000} = 395 \text{ mg}$$

$$2.\ 79.6 \times \frac{10}{1} \times \frac{100}{0.2} \times \frac{1.0}{1000} = 398 \text{ mg}$$

$$3.\ 79.4 \times \frac{10}{1} \times \frac{100}{0.2} \times \frac{1.0}{1000} = 397 \text{ mg}$$

Average result

$$\frac{395 + 398 + 397}{3} = 396.66 \text{ mg (380 - 420 mg)}$$

$$\frac{396.66}{400} \times 100 = 99.1665\ \% \text{ (95 - 105 \%)}$$

For Dextropropoxyphene hydrochloride (label claim : 65 mg)

Average weight of the powder present in each capsule = 535.03 mg

535.03 mg of the sample dissolved in ethanol = 10 ml

Loaded volume on TLC plate = 0.2 ml

Scraped sample extracted with methanol = 100 ml

$$1.\ 12.8 \times \frac{10}{1.0} \times \frac{100}{0.2} \times \frac{1}{1000} = 64 \text{ mg}$$

$$2.\ 12.94 \times \frac{10}{1.0} \times \frac{100}{0.2} \times \frac{1}{1000} = 64.7 \text{ mg}$$

$$3.\ 12.8 \times \frac{10}{1.0} \times \frac{100}{0.2} \times \frac{1}{1000} = 64 \text{ mg}$$

Average result

$$\frac{64 + 64.7 + 64}{3} = 64.23 \text{ mg } (61.75 - 68.25 \text{ mg})$$

$$\frac{64.23}{65} \times 100 = 98.82\ \% \ (95 - 105\ \%)$$

For Dicycloamine hydrochloride (label claim : 10 mg)

Average weight of the powder present in each capsule = 535.03 mg

535.03 mg of sample was dissolved in ethanol = 10 ml

Loaded volume on TLC plate = 0.2 ml

Scraped sample extracted with 4 ml chloroform and 10 ml colouring reagent was added.

$$1.\ 5.11 \times \frac{10}{1} \times \frac{4.0}{0.2} \times \frac{10}{1} \times \frac{1}{1000} = 10.23 \text{ mg}$$

$$2.\ 4.86 \times \frac{10}{1} \times \frac{4.0}{0.2} \times \frac{10}{1} \times \frac{1}{1000} = 9.72 \text{ mg}$$

$$3.\ 5.11 \times \frac{10}{1} \times \frac{4.0}{0.2} \times \frac{10}{1} \times \frac{1}{1000} = 10.23 \text{ mg}$$

Average result

$$\frac{10.23 + 9.72 + 10.23}{3} = 10.06 \text{ mg } (9.53 - 10.54 \text{ mg})$$

$$\frac{10.06}{10} \times 100 = 100.6\ \% \ (95 - 105\ \%)$$

Table 13

Statistical Analysis

Formulation	Label Claim (mg)	Amount Found* (mg)	% Recovery	SD**	CV***	t-test
Brand A						
Paracetamol	400	396.67	99.17	0.002	0.406	426.32
Dextropropoxyphene hydrochloride	65	64.23	98.82	0.001	0.700	247.32
Dicycloamine hydrochloride	10	10.06	100.60	0.001	2.805	61.75

* Average of three determinations

** Standard deviation

*** Coefficient of Variation %

4.2(b)

DETERMINATION OF DICYCLOAMINE HYDROCHLORIDE BY SPECTROPHOTOMETRY

PROCEDURE

The following spectrophotometric procedure was developed for the determination of dicycloamine HCl using colouring agent described in the previous experiment.

A stock solution of 25 mg of pure dicycloamine HCl in 25 ml of chloroform was prepared to give a concentration of 1.0 mg/ml.Further dilutions ranging from 50, 100, 200, 300, 400, 500, 600, 700 and 800 (g/ml in chloroform were prepared from the stock solution by transferring 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0 ml respectively into different 10 ml volumetric flasks. The volume was made up with chloroform. These solutions were transferred into separately flasks separately and 10 ml of colouring reagent was added to each one of them. The mixture was well shaken and allowed to stand for 5 minutes. The chloroform layers were separated by filteration using Whatmann filter paper No.41. The absorbance of the above solutions was measured at 620 nm (Fig. 13) with respect to the blank prepared by same method expect for the addition of pure drug. A standard graph was plotted (Table 14) and a straight line was obtained (Fig. 15) which was found to obey Beer's law in the concentration range from 50 - 700 (µg/ml).

ASSAY PREPARATION

Ten tablets were accurately weighed and their contents were finely powdered. A quantity equivalent to 20 mg of drug was extracted with 25, 15 and 10 ml portions of chloroform. The extracts were filtered on a Whatman filter paper No.1 and combined. The volume was made upto 50 ml with chloroform. The solutions were transferred to 250 ml separating flask and 10 ml of colouring reagent was added. The mixture was vigorously shaken to achieve proper dissolution & allowed to stand for 5 minutes. The chloroform layer was removed, filtered and the absorbance of the clear solution with respect to the blank measured at 620 nm on a spectrophotometer. The amount of Dicycloamine Hcl present in the tablet preparation was calculated from the absorbance using the standard curve.

RECOVERY PROCEDURE

In order to establish the suitability of this method, known amounts of the pure drug was added to previously analysed formulation and the mixture was again analysed by the assay method. The difference between the two absorbance readings was noted which showed the quantity of the drug added. It was calculated from the standard curve and is reported under results (Table 15 & 16).

RESULTS

Table 14

Calibration curve for Dicycloamine Hydrochloride

Concentration	Absorbance				SD°	CV°°
(µg/ml)	Trial 1	Trial 2	Trial 3	Mean		
05	0.008	0.010	0.013	0.011	0.0100	0.002
10	0.045	0.050	0.038	0.044	0.0060	13.596
20	0.084	0.091	0.080	0.085	0.0056	6.550
25	0.105	0.110	0.102	0.105	0.0040	3.824
30	0.126	0.130	0.125	0.127	0.0026	2.083
40	0.170	0.172	0.165	0.169	0.0036	2.133
50	0.210	0.212	0.205	0.209	0.0020	1.726
60	0.254	0.252	0.248	0.251	0.0031	1.215
70	0.295	0.294	0.290	0.293	0.0026	0.904
80	0.340	0.344	0.345	0.343	0.0026	0.771

° Standard Deviation

°° Coefficient of Variation (%)

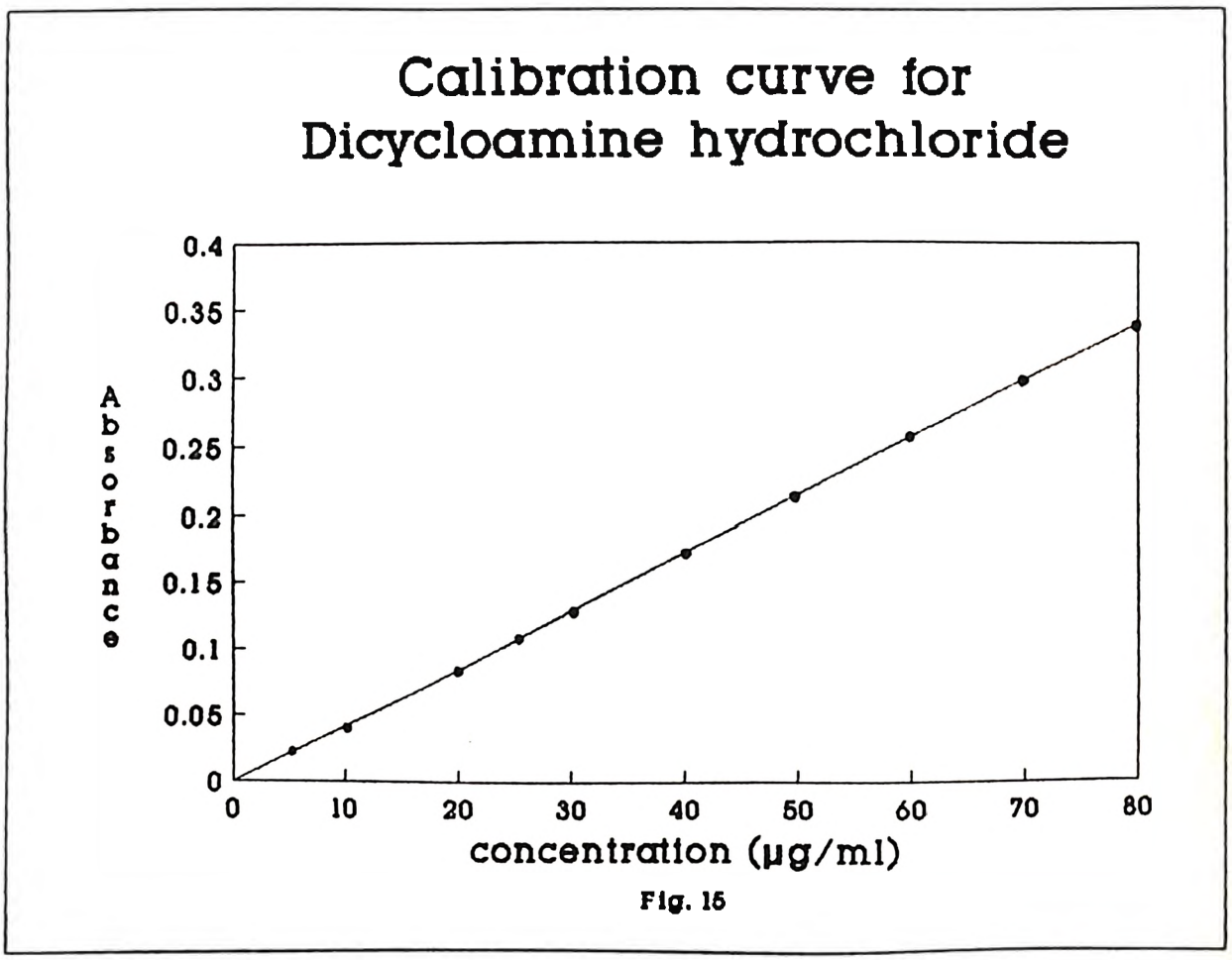

Calibration curve for
Dicycloamine hydrochloride
Absorbance
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
10
20
30
40
50
60
70
80
concentration (µg/ml)

Fig. 15

Table 15

Recovery Assay Data for Dicycloamine hydrochloride

Commercial formulation

Each tablet contains

Paracetamol IP : 500 mg

Dicycloamine hydrochloride IP : 20 mg

Formulation (µg/ml)	Trial No.	Absorbance	Concentration from std graph (µg/ml)	Amount in each sample (mg)	% Recovery
Brand A					
Paracetamol	1	-	-	-	-
Dicycloamine hydrochloride		0.168	39.6	19.81	99.25
	2	-	-	-	-
		0.170	40.2	20.12	100.50
	3	-	-	-	-
		0.167	39.5	19.75	98.75

For Dicycloamine hydrochloride (label claim : 20 mg)

Average weight of the tablet = 612.5 mg

612.5 mg of sample was extracted with 50 ml of chloroform.

The solution was transferred to 250 ml separating flask and 10 ml of colouring reagent was added.

Calculations

$$1.\quad 39.7 \times \frac{50}{1} \times \frac{10}{1} \times \frac{1}{1000} = 19.85 \text{ mg}$$

$$2.\quad 40.2 \times \frac{50}{1} \times \frac{10}{1} \times \frac{1}{1000} = 20.10 \text{ mg}$$

$$3.\quad 39.5 \times \frac{50}{1} \times \frac{10}{1} \times \frac{1}{1000} = 19.75 \text{ mg}$$

Average result

$$\frac{19.85 + 20.10 + 19.75}{3} = 19.90 \text{ mg (19.0 - 21.0 mg)}$$

$$\frac{19.90}{20.0} \times 100 = 99.50\ \%\ (95 - 105\ \%)$$

Table 16

Statistical Analysis

Formulation	Label Claim (mg)	Amount Found° (mg)	% Recovery	SD°°	CV°°°	t-test
Brand A						
Paracetamol	500	-	-	-	-	-
Dicycloamine hydrochloride	20	19.90	99.50	0.00153	0.90791	190.77

° Average of three determinations

°° Standard deviation

°°° Coefficient of Variation (%)

DISCUSSION

Various difficulties were encountered while devising a simultaneous assay procedures for the drug formulations described in this research work. Although individual assay methods are available for these Various difficulties were encountered while devising a simultaneous assay procedures for the drug formulations described drugs, since an official methods are not available for the analysis of drug preparations containing three constituents simultaneously. Further the individual reported methods are time consuming therefore the proposed methods which have been developed found to be cheap, precise, sensitive and reproducible.

A quantitative thin layer chromatography (TLC) procedure was established for the determination of paracetamol, dextropropoxyphene hydrochloride and dicycloamine hydrochloride in commercial formulations. The method comprised of separating the 3 constituents by TLC technique, extraction of the components and the measurement in the UV-Visible region. The concentration range for paracetamol was 10-160 µg/ml whereas the concentration range for dextropropoxyphene hydrochloride and for dicycloamine hydrochloride are 6-20 µg/ml and 0.5-22.5 µg/ml. The Rf values being 0.40, 0.60 and 0.80 and observed at 242 nm, 258 nm and 620 nm. The standard graphs were found to be linear and obeyed Lambert-Beer's law. The results of estimation were evaluated by the standard deviation and coefficient of variation.

The drugs are made to react with spraying reagent whereby form a coloured complexes in order to identify the location and movement of drugs by the mobile phase. The areas of the spots of drugs are compact and the seperation is easier and the development time is much shorter.

Although some of the individual methods like non-aqueous titrations, spectrophotometry are available for the estimations of dicycloamine hydrochloride but these could not be worked out in a simultaneous determination because dicycloamine hydrochloride is a very saturated organic compound and does not contain any chromophore. It is also contained in very small amounts in the formulations.

Since dicycloamine HCl contains a -NH(C_2H_5)$_2$ group, it was thought that could be coupled to a complex made from cobaltous chloride, potassium thiocyanate and sodium acetate in presence of HCl which was devised to be a new colouring reagent and be stable enough to be determined in visible region at 620 nm. This study was also found to be reproducible on individual drug preparations of dicycloamine hydrochloride performed by us on several determinations with recovery studies.

Reactions

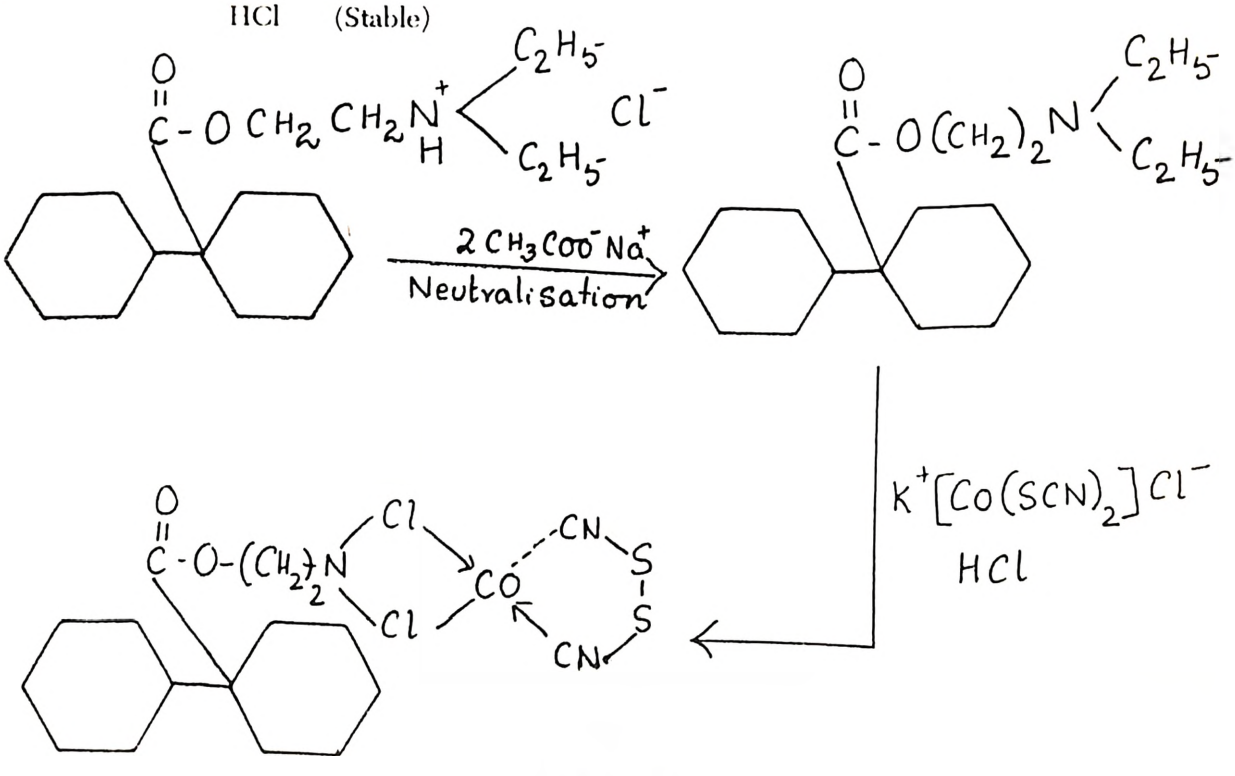
$$CoCl_2 + 2KSCN = Co(SCN)_2 + KCl$$
(unstable)

To prevent the reversible rections (due to deficiency of Chloride ions) HCl was added which is having the common ion chloride.

$$HCl = H^+ + Cl^-$$

$$KCl = K^+ + Cl^-$$

$$Co(SCN)_2 + KCl \rightarrow K^+[Co(SCN)_2]Cl^-$$
HCl (Stable)



BLUE COLOUR METAL COMPLEX

Reasons :

1. To prevent the reversible reaction (due to deficiency of Cl). hydrochloric acid was added which is having the common ion Cl

2. Colour of cobaltous chloride in aqueous solution is pink to red. To prevent the change of colour hydrochloric acid was added it forms stable blue colour.

3. Potassium thiocyanate reacted with cobaltous chloride to form complex and it increases the intensity of the colour.

The results of our study showed that the amounts of paracetamol, dextropropoxyphene hydrochloride and dicycloamine hydrochloride contained in the aforesaid formulations conform to the label claim specifications.

(4.3)

SIMULTANEOUS DETERMINATION OF PROPYPHENAZONE AND KETOPROFEN IN DOSAGE FORMS BY QUANTITATIVE THIN LAYER CHROMATOGRAPHY, HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

The TLC, HPTLC and HPLC methods developed for the simultaneous determination of propyphenazone and ketoprofen in formulations is described below :

QUANTITATIVE THIN LAYER CHROMATOGRAPHY

Instrument

Jasco model 7800 (MHT-344) autoscan UV-Visible spectrophotometer equipped with two 1 cm matched quartz cells..

Chromatographic Plates

Thin layer chromatographic glass plates 20X20 cm coated with silica-gel G having a thickness of 0.05 mm were prepared.

Chromatographic Chamber

Glass tank 20X20 cms with glass lid and lined with whatman filter paper No.1 was found suitable.

Mobile Phase

Solvent system consisted of a mixture of butyl acetate chloroform : formic acid : benzene : toluene (60:20:20:10:10).

Spraying Agent

0.1N Iodine Solution : Iodine (1.4 gms) and potassium iodide (3.6 gms) were dissolved in 10 ml of distilled water. To this solution, 2-3 drops of hydrochloric acid was added and the volume was made upto 100 ml with distilled water.

PROCEDURE

Silica gel G (7.5 gms) was triturated with 16 ml of distlled water to form a smooth paste and was uniformly spread on the TLC plates. The plates were first air dried for 15 minutes and then activated at 110°C for 30 minutes (179). Various combination of solvent systems was attempted untill clear separation of the true constituents with defined Rf values was achieved. The solvent systems attempted and their combination are stated below :

S.No	Mobile Phase Composition	Remarks
A	Benzene : Ether : Gla. Acetic Acid : Methanol (60 : 30 : 9 : 1)	The spot was not clear in case of propyphenazone .
B	Butyl Acetate : Chlorform : Formic Acid (60 : 20 : 20)	The distance between the propyphenazone & Ketoprofen was very near.
C	Methyl Alcohol : St. Ammonia Solution (93 : 7.5)	The spot of propyphenazone was not clear.
D	Toulene : Di - isopropyl Ether : Formic Acid (70 : 30 : 1)	The spot of propyphenazzone was not clear.
E	Butyl Acetate : Chloroform : Formic Acid : Benzene (60 : 20 : 20 : 20)	The distance between propyphenazone & Ketoprofen were very close.
F	Butyl Acetate : Chloroform : Formic Acid : Toulene (60 : 20 : 20 : 20)	The spot of propyphenazone was not clear.
G	Butyl Acetate : Chloroform : Formic Acid : Toulene : Benzene (60 : 20 : 20 : 10 : 10)	The spots for the two pure drugs were very clearly separated.

The individual solvents were mixed together as per the given quantities and placed in a chromatographic chamber lined with a filter paper and allowed to saturate for a period of 20 minutes.

STANDARD GRAPH FOR PROPYPHENAZONE

The stock solutions of 2.0, 4.0, 6.0, 8.0, 10.0, 12.0, 14.0, 16.0 and 18.0 mg of pure propyphenazone in 10 ml of methanol were prepared separately giving concentrations of 0.2, 0.4, 0.6, 0.8, 1.0, 1.4, 1.6 and 1.8 mg/ml. Exactly 0.1 ml (100 µl) of these solutes were spotted on TLC plates (20 X 20 cm) separately in duplicate. The plates were placed in a chromatographic chamber containing a saturated solution of the mobile phase and developed similarly as described in the previous experiment. The plates were removed after the spots had separated and moved to a distance of 15 cm. They were air-dried and spots identified by spraying with 0.1N iodine solution. The spots became brownish yellow in color. The coloured and uncoloured duplicate spots were scraped and collected in dry centrifuge tubes containing 10 ml of methanol. These were centrifuged for 10 minutes and filtered. The absorbance of the clear solution was measured with respect to the blank at 245 nm (Table 17 & Fig. 16). A standard graph with concentration against absorbance was plotted which gave a straight line (Fig. 17).

The absorbance data, calculations and quantitation for the determination is reported under results.

STANDARD GRAPH FOR KETOPROFEN

The stock solutions of 6,8,10,12,14 & 16 mg of pure ketoprofen in 10 ml of methanol were prepared separately giving a concentrations of 0.6, 0.8, 1.0, 1.2, 1.4 & 1.6 mg/ml. Exactly 0.1 ml (100 µl) of these solutes were spotted on the TLC plates (20 X 20 cm) in duplicate. The rest of the procedure was same as given under propyphenazone except for the absorbance of the clear solution measured with respect to the blank at 255 nm (Table 18 & Fig. 18). A standard graph with concentration against absorbance was plotted which gave a straight line (Fig. 19).The absorbance data, calculations and quantitation for the determination is reported under results.

ASSAY PREPARATION BY QUANTITATIVE THIN LAYER CHROMATOGRAPHY

Ten capsules were accurately weighed and their contents were finely powdered. The powder equivalent to the average weight of the capsule was extracted with 10 ml of methanol and the volume was made upto 100 ml with methanol giving a concentration of 1.5 mg/ml of propyphenazone and 0.5 mg/ml of ketoprofen. The solution was centrifuged and the supernatant

solution was used for spotting the TLC plates. Exactly 0.1 ml (100 µl) of the solution was applied on TLC plates in duplicate. The rest of the chromatographic procedure was similar to as described for pure drugs. The absorbance of the solutions was measured at 245 and 255 nm for propyphenazone (Rf = 0.56) and ketoprofen (Rf = 0.84) respectively. The results of estimation are shown in Table 19 & 20.

RECOVERY PROCEDURE

In order to establish the suitability of this method, known amounts of the pure drug was added to previously analysed formulation (25 ml) and the mixture was again analysed by the assay method. The difference between the two absorbance readings was noted which showed the quantity of the drug added. It was calculated from the standard curve and is reported under results.

HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY

Instrument

Camag TLC scanner II

Chromatographic Plates

Commercial available high performance thin layer chromatographic plates 10X10 cm coated with silica gel 60 F 254 having a thickness of 200 μm were used (CAMAG)

Chromatographic Chamber

A 5X6 inch with glass lid twin trough chamber was employed. A Linomat IV, automatic sampler was used for spotting the chromatographic plates.

Mobile Phase

In HPTLC the solvent system as used in TLC was modified to a less polar system and comprised of butyl acetate : chloroform : formic acid : benzene : toluene in the ratio of 16 : 8 : 4 : 4 : 6.

PROCEDURE

STANDARD CURVE BY HPTLC DENSITOMETRY

Ten milligrams (10 mg) of propyphenazone and ketoprofen were weighed separately and dissolved in 10 ml of methanol giving a concentration of 1 mg/ml each. A 0.5 ml of each of these solutions were further diluted to 10 ml to give final concentrations of 50 ng/μl for each of them. One ml of each of these solutions were mixed together and the amounts of 2, 3, 4, 5, 6 and 7 μl were spotted bandwise using Linomat IV automatic sampler on commercially prepared HPTLC plates. The chromatograms were developed in a closed twin trough chromatographic chamber (10X12X4 inch) containing the mobile phase by ascending technique. The chamber was lined with Whatman filter paper No.1 and closed with a glass-lid.The developed

plates were dried and scanned at 265 nm for the absorbance by reflectance. The individual pure drugs showed absorbance maximum at 273 nm for propyphenazone and at 261 nm for ketoprofen as shown in Fig. 20 & 23.The spot areas and heights were evaluated and a plot of area or height vs concentration was obtained by the camag cats software program (Table 21-24 & fig. 22).

ASSAY PROCEDURE

Ten capsules were accurately weighed and powder equivalent to the average weight was extracted with 25 ml of methanol giving a concentration of 6 mg/ml of propyphenazone and 2 mg/ml of ketoprofen. The solution was centrifuged and concentrations of 2.0 µl were spotted on TLC plates as per above procedure. The development, detection and extraction were done in a same manner as described for the pure drugs.The absorbance of the solutions containing propyphenazone (Rf = 0.52) and ketoprofen (Rf = 0.73) was measured at 265 nm on a Camag scanner. The results of the estimation are shown in table 25 & 26 under results.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Apparatus

Liquid Chromatograph

Waters associate HPLC system equipped with dual piston reciprocating pumps (model 501), rheodyne injector and LC spectrophotometer (model 481) was used. The chromatograms were recorded on an omniscribe recorder B 5000. Injections were done using a 20 µl Hamilton's microsyringe.

Operating Conditions

Column: A stainless steel, 300X3.9 inch i.d. column packed with µ Bondapak C 18, 10 µm partical size, equipped with an end capped guard column-PAK packed with µ Bondapak C18 bulk material (10 µm) was used (Waters Associate)

Temperature: Ambient

Solvents : HPLC grade methanol, water and acetonitrile

Mobile Phase : Methanol : Acetonitrile : Water (10:20:30)

Mode : Isocratic

Flow rate : 2 ml/mt

Chart Speed : 10 cm/mt

Detector Wavelength : 254 nm (UV)

Range : 0.2 AUFS

Internal Standard Solution : Pure pharmacopoeial sample of phenobarbitone (50 mg) was accurately weighed in a 250 ml volumetric flask, dissolved in methanol and the final volume was made upto the mark.

PROCEDURE

The procedure developed for the separation and determination of propyphenazone and ketoprofen by HPLC is described below :

Various combinations of mobile phase was attempted in this study untill clear separation of the true constituents with defined Rf values was achieved. Firstly the solutions were prepared in pure HPLC grade methanol and further dilutions in combination with acetonitrile and triple distilled water.

S. No	Mobile Phase Composition	Remarks
A	Methanol : Acetonitrile (90 : 10)	The peaks of propyphenazone & phenobarbitone are not clear.
B	Methanol : Acetonitrile (80 : 20)	The peaks of propyphenazone & phenobarbitone are not clear.
C	Methanol : Water (80 : 20)	The peaks of propyphenazone & Ketoprofen are not clear.
D	Acetonitrile : Water (80 : 20)	The peak of Ketoprofen & phen -obarbitone are not clear.
E	Methanol : Acetonitrile : Water (50 : 30 : 20)	Phenobarbitone peak is not clear.
F	Methanol : Acetonitrile : Water (20 : 30 : 50)	Phenobarbitone peak is not clear.
G	Methanol : Acetonitrile : Water (20 : 40 : 60) or Methanol : Acetonitrile : Water (10 : 20 : 30)	The peaks were clearly resolved

The mobile phase No.G gave the best separation of the pure drugs along with phenobarbitone which was used as internal standard. In the other cases of mobile phase, the separation is not clear. The mobile phase was prepared by mixing methanol, acetonitrile, water and 10 mg of sodium acetate (to obtain sharp peaks) in the ratio of 10 : 20 : 30. The solution was used after filtering it through a membrane of 0.22 μ porosity (millipore, USA).

STANDARD SOLUTION FOR PROPYPHENAZONE

Pure reference standard propyphenazone (75.0 mg) was accurately weighed into a 250 ml volumetric flask & dissolved in 100 ml methanol. The final volume was made upto the mark with methanol giving a concentration of 0.3 mg/ml.

From this stock solution volumes of 0.5, 1.0, 1.5 & 2.0 ml each were transferred separately into 10 ml volumetric flasks and 1.0 ml of internal standard was added to each one of them. The final volumes were made upto the mark with the mobile phase.

In a similar manner, 25.0 mg of pure ketoprofen was accurately weighed in a 250 ml volumetric flask and dissolved in 10 ml methanol giving a concentration of 0.1 mg/ml. Similar dilutions as described for propyphenazone were prepared.

SAMPLE PREPARATION

Twenty capsules were weighed, their contents finely powdered. The powder equivalent to the average weight of a capsule was calculated. One hundred and twenty milligrams (120 mg) of the powder was accurately weighed into a 250 ml flask and dissolved in 100 ml of methanol. The powder was dispersed and the volume was made upto the mark with methanol giving a concentration of 0.3 mg/ml of propyphenazone and 0.1 mg/ml of ketoprofen. The solution was centrifuged and filtered to obtain a clear solution.

One millilitre (1.0 ml) of the above solution and 1 ml of the internal standard were transferred into 10.0 ml volumetric flask and diluted with mobile phase upto the mark. The solutions of standard preparation and sample preparation were injected separately through a rheodyne injector of 20 µl loop and the chromatograms were recorded on an omniscribe recorder. The eluent was monitored at 254 nm. The results of estimation are shown in Table 27-34 under results.

RECOVERY PROCEDURE

In order to establish the suitability of this method, known amounts of the pure drug was added to previously analysed formulation and the mixture was again analysed by the assay method. The difference between the two absorbance readings was noted which showed the quantity of the drug added. It was calculated from the standard curve and is reported under results (Table 34).

RESULT

Quantitative Thin Layer Chromatography

Table 17

Calibration curve for propyphenazone

Concentration (μg/ml)	Absorbance				SD*	CV**
	Trial 1	Trial 2	Trial 3	Mean		
6.0	0.250	0.248	0.252	0.250	0.0049	0.8000
8.0	0.340	0.346	0.344	0.343	0.0031	0.8902
10.0	0.431	0.430	0.438	0.433	0.0044	1.0063
12.0	0.523	0.520	0.532	0.525	0.0062	1.1892
14.0	0.615	0.616	0.620	0.617	0.0027	0.4298
16.0	0.707	0.706	0.702	0.705	0.0026	0.3746
18.0	0.799	0.786	0.795	0.793	0.0067	0.8383

* Standard deviation

** Coefficient of variation (%)

Covariance = 1.0148

Regression = 0.99991

Correlation coefficient = 0.999957

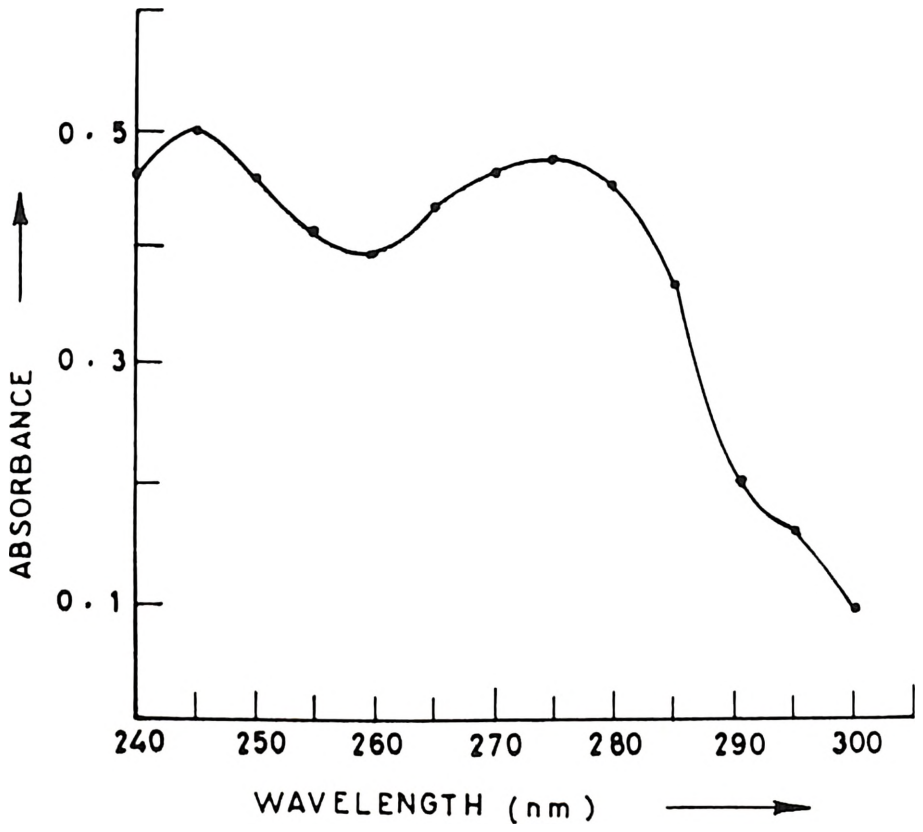


Fig.16 ABSORPTION SPECTRUM FOR PROPYPHENAZONE

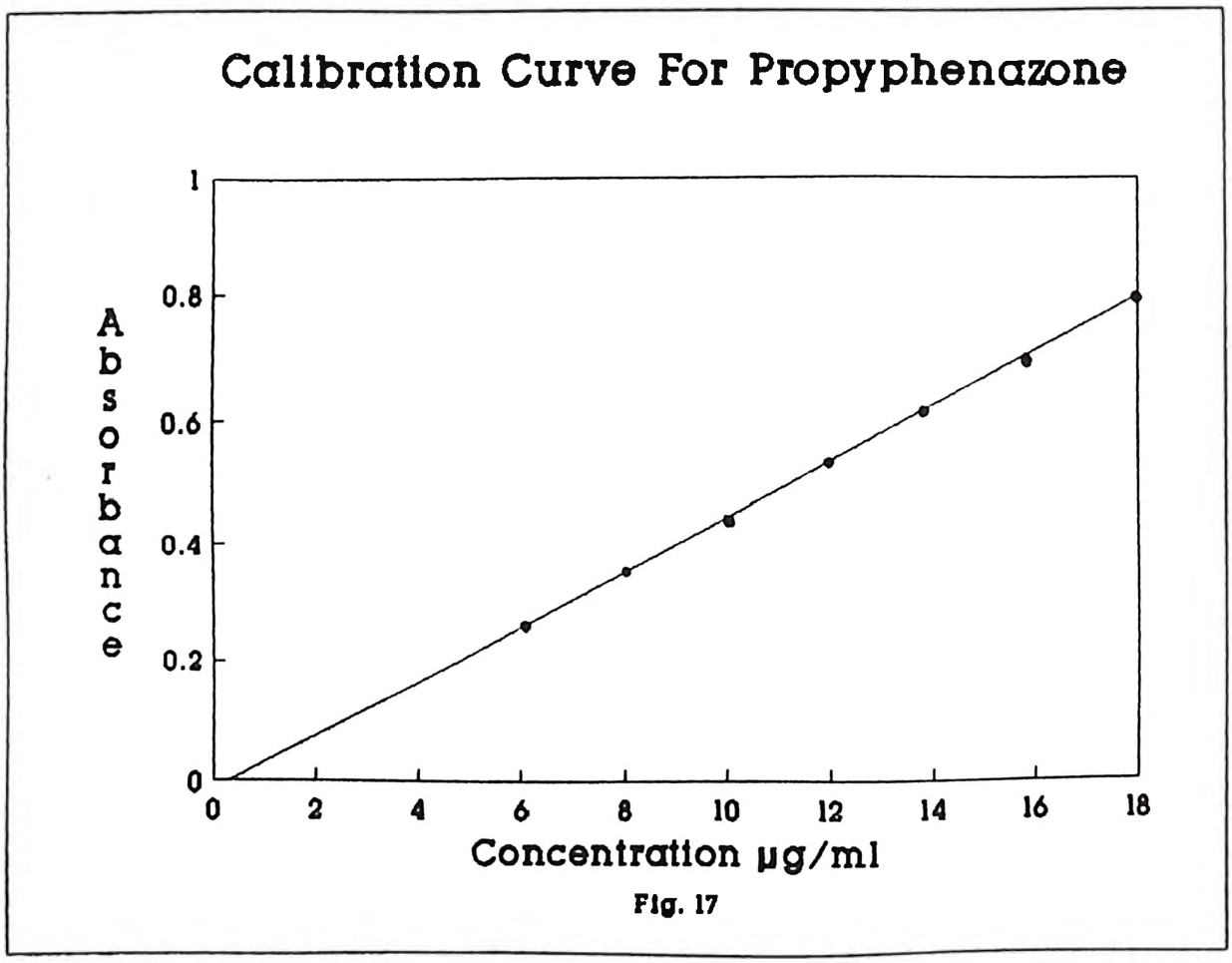

Calibration Curve For Propyphenazone
Absorbance
1
0.8
0.6
0.4
0.2
0
0
2
4
6
8
10
12
14
16
18
Concentration µg/ml

Fig. 17

Table 18

Calibration curve for ketoprofen

Concentration (µg/ml)	Absorbance				SD*	CV**
	Trial 1	Trial 2	Trial 3	Mean		
6.0	0.361	0.360	0.365	0.362	0.0060	0.8123
8.0	0.515	0.519	0.526	0.520	0.0056	1.0717
10.0	0.645	0.649	0.656	0.650	0.0056	0.8573
12.0	0.775	0.778	0.787	0.780	0.0062	0.8012
14.0	0.905	0.910	0.915	0.910	0.0050	0.5498
16.0	1.256	1.261	1.251	1.256	0.0051	0.5513

* Standard deviation

** Coefficient of variation (%)

Covariance = 0.8666

Regression = 1.0000

Correlation coefficient = 1.0000

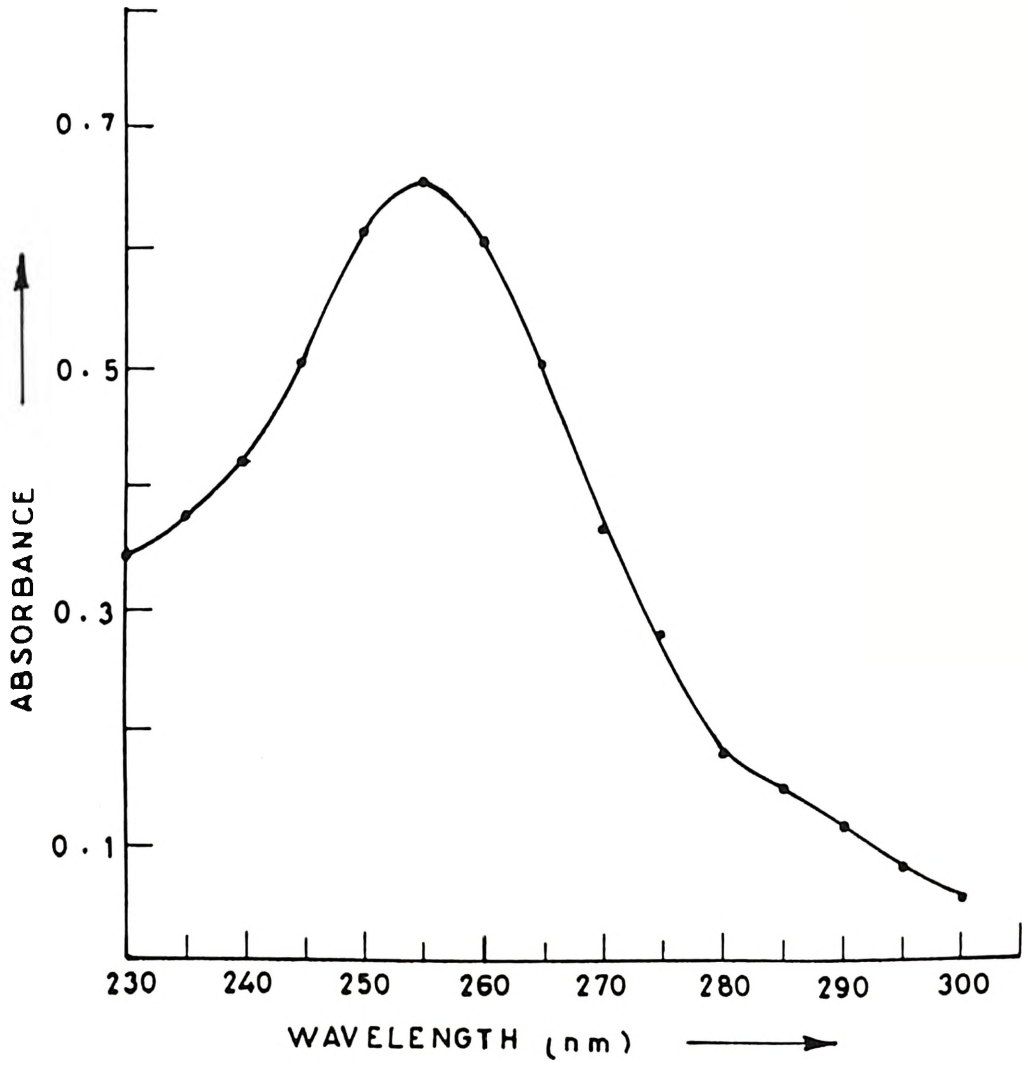


Fig. 18. ABSORPTION SPECTRUM FOR KETOPROFEN

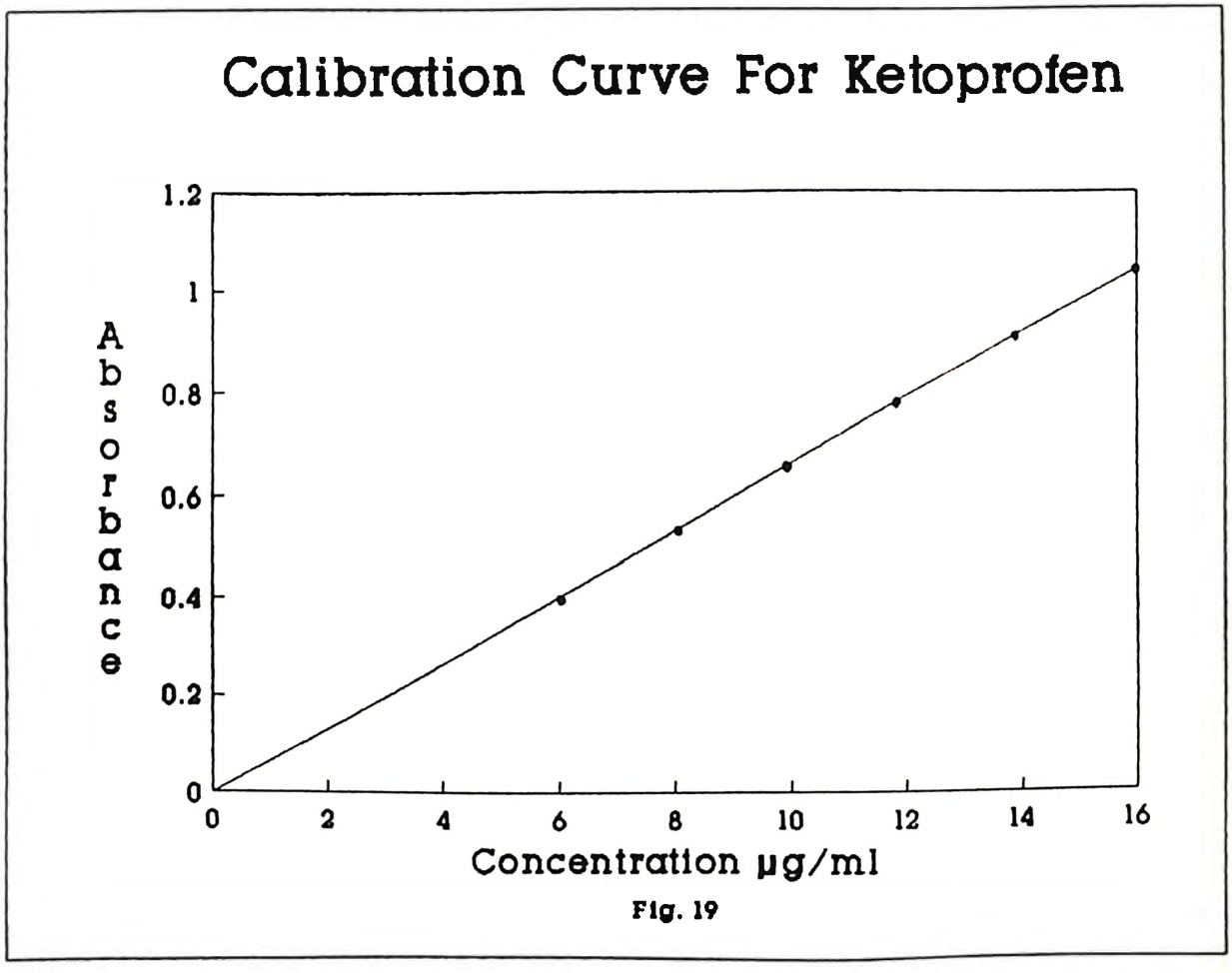

Calibration Curve For Ketoprofen
Absorbance
1.2
1
0.8
0.6
0.4
0.2
0
0
2
4
6
8
10
12
14
16
Concentration µg/ml

Fig. 19

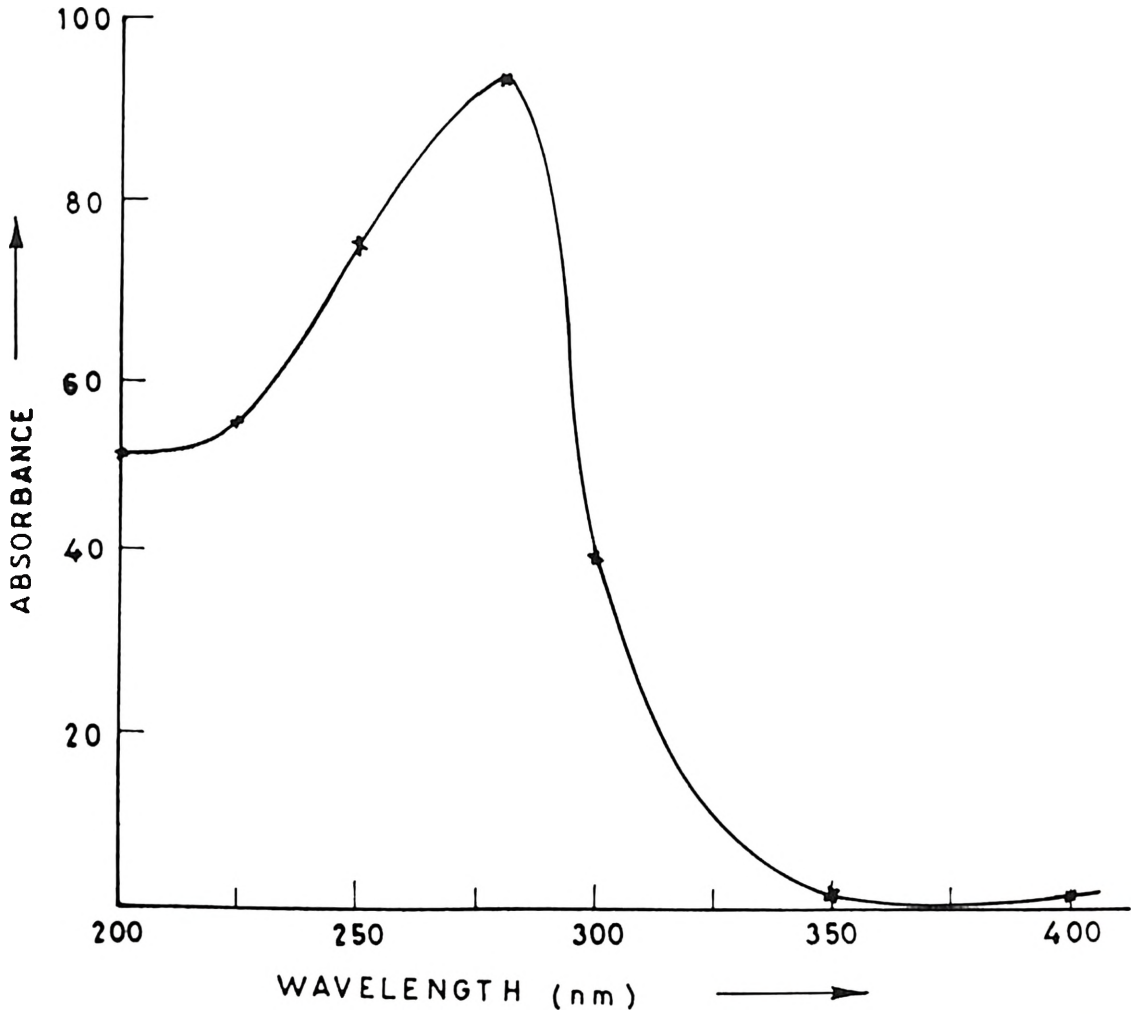


Fig. 20 ABSORPTION SPECTRUM FOR PROPYPHENAZONE

Table 19

For combination of ketoprofen and propyphenazone by QTLC

Commercial formulation

Propyphenazone = 150 mg

Ketoprofen BP = 50 mg

Formulation	Trial No.	Absorbance	Concentration from std graph (µg/ml)	Amount in sample (mg)	%Recovery
Propyphenazone	1	0.647	14.85	148.50	99.00
Ketoprofen		0.340	4.90	49.00	98.00
	2	0.645	14.80	148.00	98.66
		0.337	4.85	48.50	97.00
	3	0.649	14.90	149.00	99.33
		0.347	5.00	50.00	100.00

Calculations

For Propyphenazone (label claim : 150 mg)

Average weight of the capsule = 270 mg

270 mg of the sample was dissolved in 100 ml of methanol

Loaded volume in TLC plate = 0.1 ml

Scraped sample extracted with 10 ml methanol

$$1.\ 14.85 \times \frac{100}{0.1} \times \frac{10}{1000} = 148.50 \text{ mg}$$

$$2.\ 14.80 \times \frac{100}{0.1} \times \frac{10}{1000} = 148.00 \text{ mg}$$

$$3.\ 14.90 \times \frac{100}{0.1} \times \frac{10}{1000} = 149.00 \text{ mg}$$

Average Result

$$\frac{148.50 + 148.00 + 149.00}{3} = 148.50 \text{ mg (142.5 - 157.5 mg)}$$

For Ketoprofen (label claim : 50 mg)

Average weight of the powder in each capsule = 270 mg

270 mg of the sample dissolved in 100 ml of methanol

Loaded volume on TLC plate = 0.1 ml

Scraped sample extracted with 10 ml methanol.

$$1.\ 4.9 \times \frac{100}{0.1} \times \frac{10}{1000} = 49.00 \text{ mg}$$

$$2.\ 4.85 \times \frac{100}{0.1} \times \frac{10}{1000} = 48.50 \text{ mg}$$

$$3.\ 5.0 \times \frac{100}{0.1} \times \frac{10}{1000} = 50.00 \text{ mg}$$

Average Result

$$\frac{49.0 + 48.5 + 50.0}{3} = 49.17 \text{ mg (47.5 - 52.5 mg)}$$

% Purity for ketoprofen

49.17
-------- X 100 = 98.34 % (95 - 105 %)
50.00

% Purity for propyphenazone

148.50
-------- X 100 = 99.00 % (95 % - 105 %)
150.00

Table 20

Statistical Analysis

Formulation	Label Claim (mg)	Amount Found* (mg)	SD**	CV***	t-test
Propyphenazone	150.0	148.50	0.5000	0.3367	514.4191
Ketoprofen	50.0	49.17	0.7637	1.5533	111.5073

* Average of three determinations

** Standard deviation

*** Coefficient of variation (%)

For High Performance Thin Layer Chromatography

Table 21

Calibration curve for Propyphenazone

Concentration (ng/ml)	Height				SD*	CV**
	Trial 1	Trial 2	Trial 3	Mean		
100	16.0	16.9	16.9	16.6	0.5195	3.1298
150	23.5	23.9	23.6	23.66	0.2080	0.8789
200	30.9	30.4	30.2	30.5	0.3606	1.1822
250	36.9	37.5	36.9	37.1	0.3466	0.9342
300	42.3	42.2	41.5	42.0	0.4358	1.0377
350	48.5	48.8	48.8	48.7	0.1726	0.3544

* Standard deviation

** Coefficient of variation (%)

Covariance = 1388.25

Correlation of coefficient = 0.99867

Regression = 0.997345

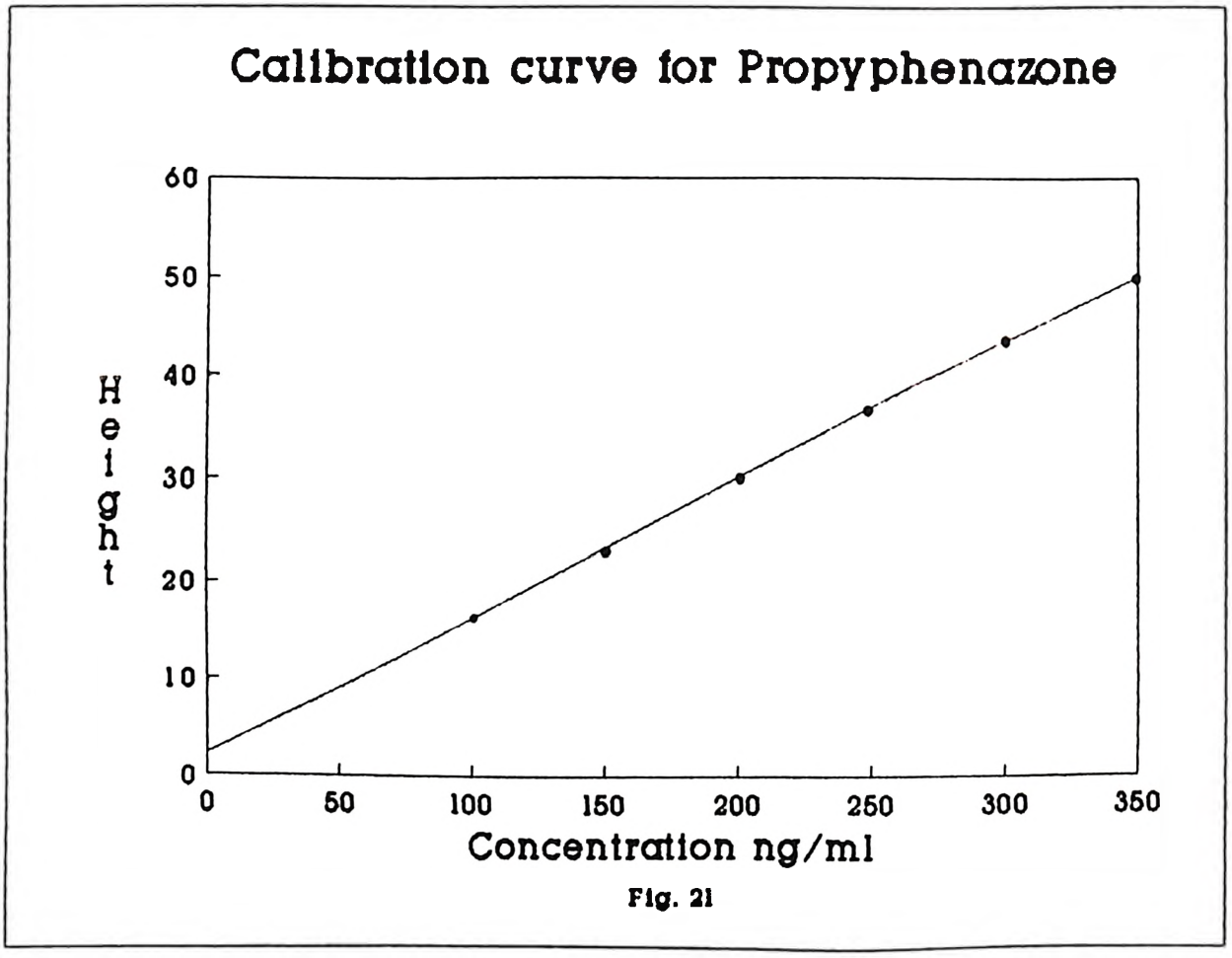

Calibration curve for Propyphenazone
Height
60
50
40
30
20
10
0
0
50
100
150
200
250
300
350
Concentration ng/ml

Fig. 21

Table 22

Calibration curve for Propyphenazone

Concentration (ng/ml)	Area				SD*	CV**
	Trial 1	Trial 2	Trial 3	Mean		
100	358.0	356.0	357.0	357.0	1.0000	0.2801
150	484.1	483.2	482.0	483.1	1.0607	0.2196
200	623.3	625.1	630.5	626.3	3.7583	0.6001
250	771.8	772.6	774.3	772.9	1.2990	0.1681
300	867.9	869.7	868.8	868.8	0.9354	0.1077
350	1020.0	1020.2	1020.4	1020.2	0.0000	0.0000

* Standard deviation

** Coefficient of variation (%)

Covariance = 28873.13

Correlation of coefficient = 0.99881

Regression = 0.997629

Table 23

Calibration curve for Ketoprofen

Concentration (ng/ml)	Height				SD*	CV**
	Trial 1	Trial 2	Trial 3	Mean		
100	19.0	19.2	19.4	19.2	0.2000	1.0422
150	28.1	28.0	28.2	28.1	0.1000	0.3560
200	34.4	36.2	35.9	35.5	0.9645	2.7168
250	43.6	43.1	45.0	43.9	0.9849	2.2437
300	50.4	50.7	50.8	50.6	0.2067	0.4082
350	57.0	57.2	57.4	57.2	0.1989	0.3477

* Standard deviation

** Coefficient of variation (%)

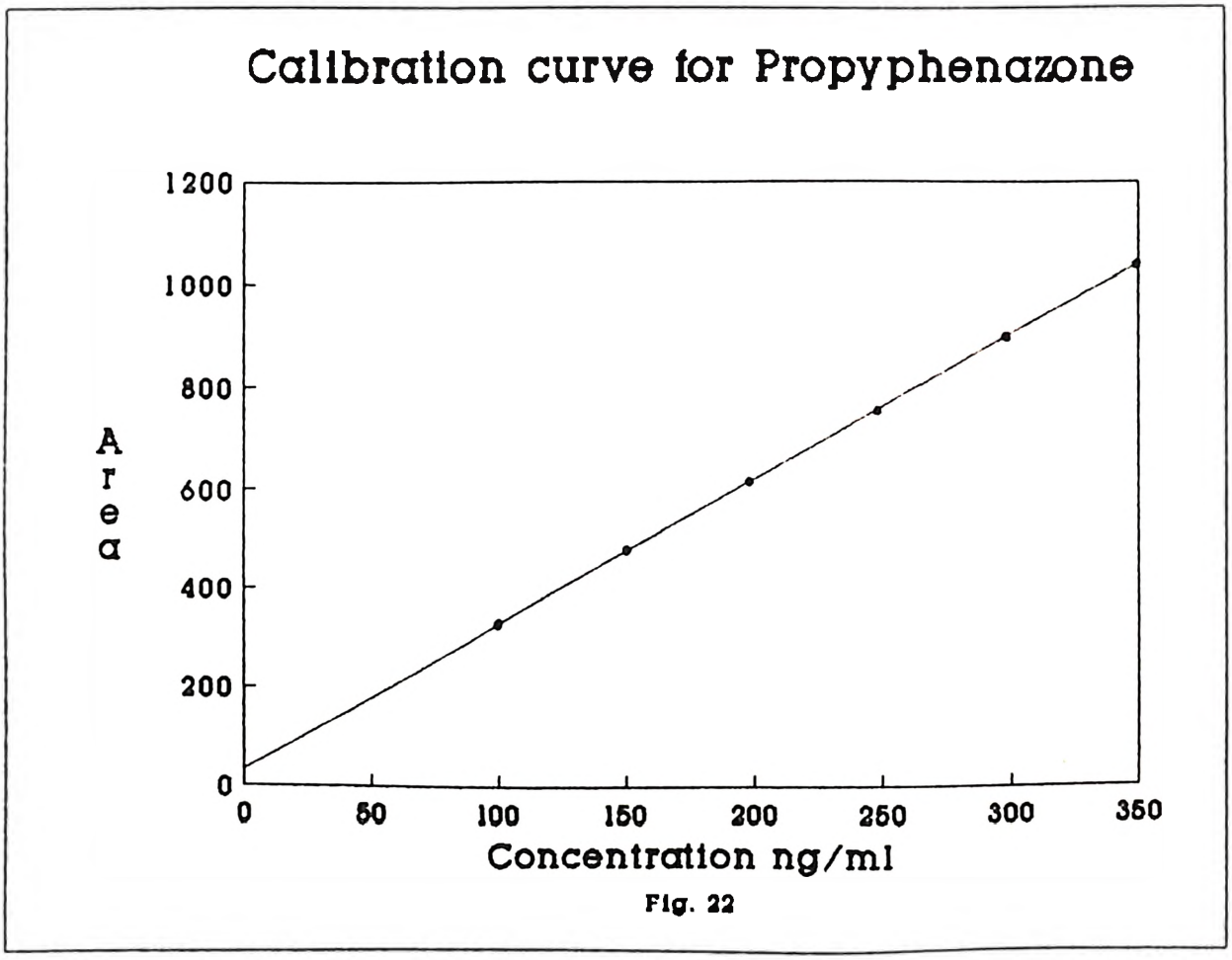
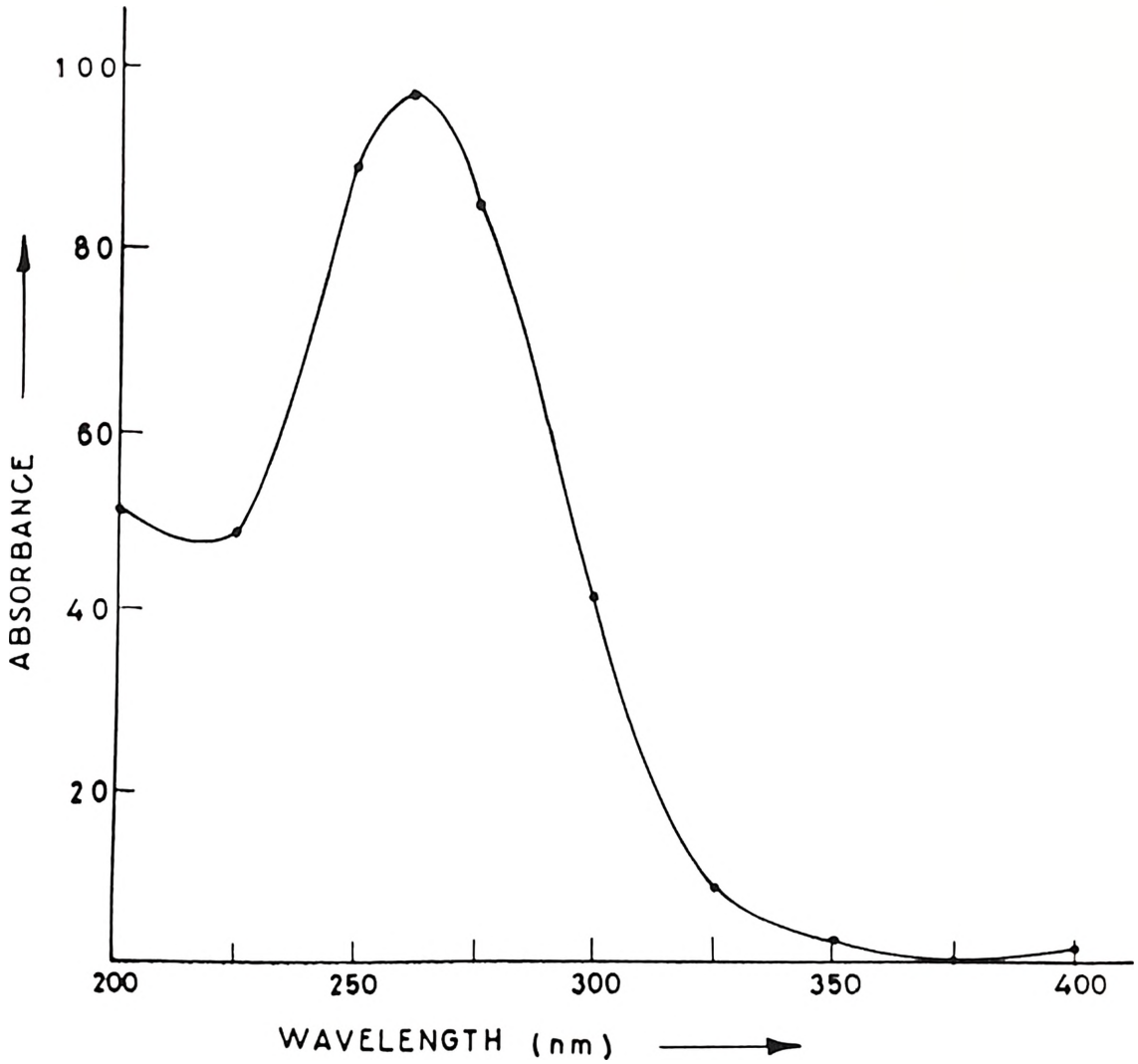

Calibration curve for Propyphenazone
1200
1000
800
600
400
200
0
Area
0
50
100
150
200
250
300
350
Concentration ng/ml

Fig. 22



Fig·23 ABSORPTION SPECTRUM FOR KETOPROFEN

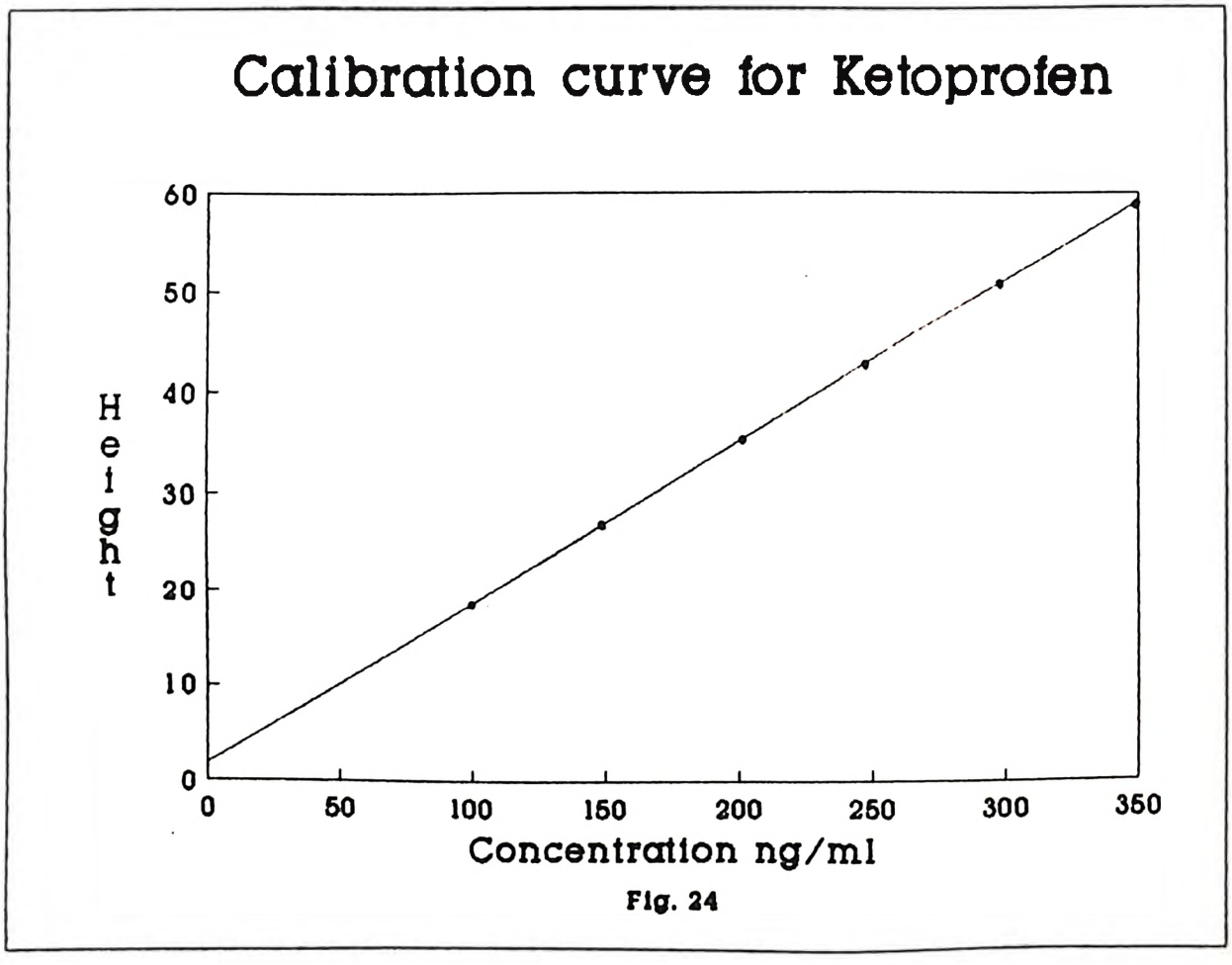

Calibration curve for Ketoprofen
Height
60
50
40
30
20
10
0
0
50
100
150
200
250
300
350
Concentration ng/ml

Fig. 24

Covariance = 1661.875

Correlation of coefficient = 0.99860

Regression = 0.997208

Table 24

Calibration curve for Ketoprofen

Concentration (ng/ml)	Area				SD*	CV**
	Trial 1	Trial 2	Trial 3	Mean		
100	574.0	574.1	574.8	574.3	0.4677	0.0814
150	837.3	837.1	837.5	837.3	0.3536	0.0422
200	1049.6	1049.8	1050.3	1049.9	0.5000	0.0476
250	1298.0	1298.6	1298.6	1298.4	0.0000	0.0000
300	1504.7	1504.2	1503.7	1504.2	0.7071	0.0470
350	1761.5	1760.9	1760.6	1761.0	0.0000	0.0000

* Standard deviation

** Coefficient of variation (%)

Covariance = 51141.88

Correlation of coefficient = 0.99953

Regression = 0.999058

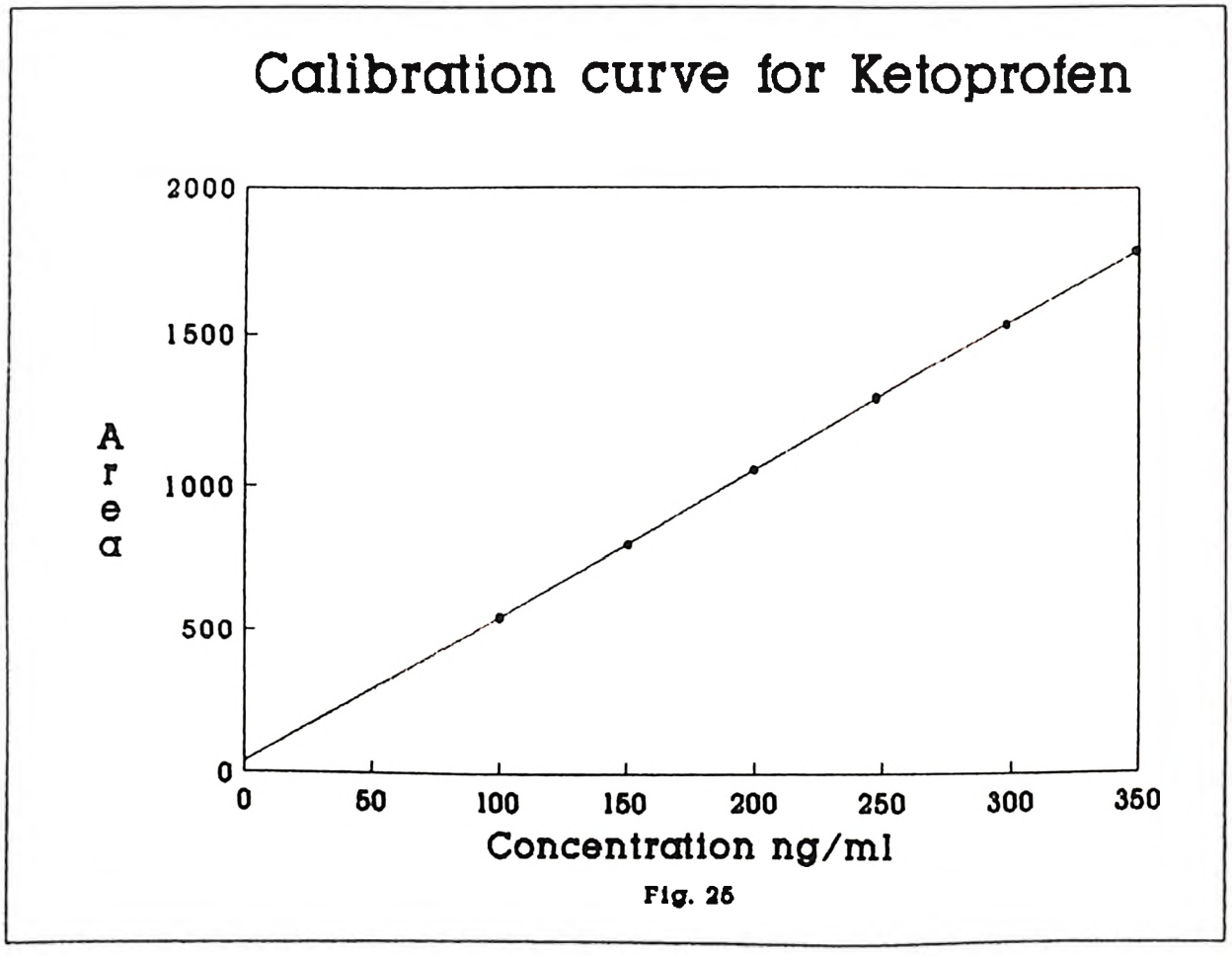

Calibration curve for Ketoprofen
2000
1500
1000
500
0
Area
0
50
100
150
200
250
300
350
Concentration ng/ml

Fig. 25

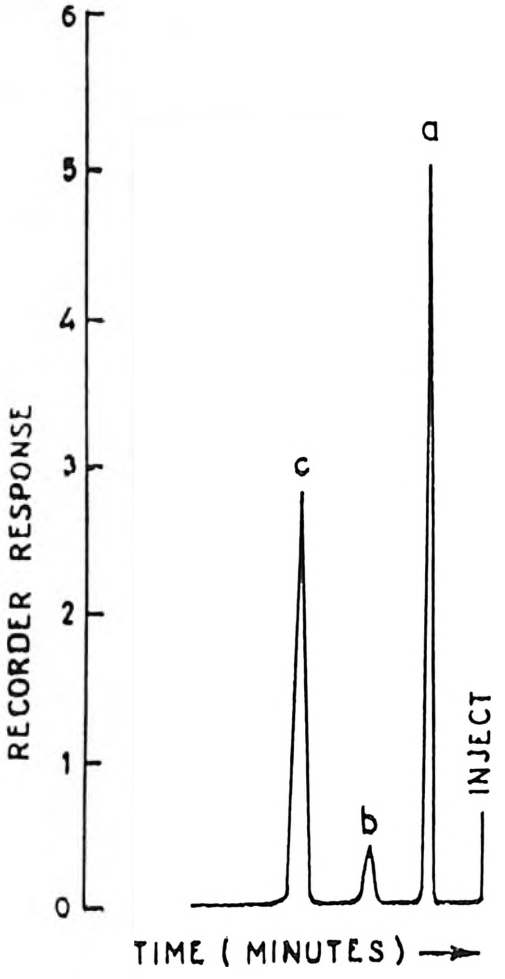


Fig. 26 HPLC RECORDINGS OF (a) KETOPROFEN (b) PHENOBARBITONE AND (c) PROPYPHENAZONE

Table 25

For combination of Propyphenazone and Ketoprofen by HPTLC

Commercial Formulation

Each capsule contains

Propyphenazone = 150 mg

Ketoprofen BP = 50 mg

Formulation	Trial No.	Absorbance (Area)	Amount in each Sample (mg)	% Recovery
Propyphenazone	1	2464.1	150.05	100.03
Ketoprofen		2944.1	50.62	101.24
	2	2461.0	149.86	99.91
		2936.8	50.50	101.00
	3	2469.0	150.29	100.19
		3019.2	51.91	103.82
	4	2476.9	150.83	100.55
		2793.5	48.03	96.06
	5	2571.2	153.29	102.19
		2834.8	48.74	97.48

Average Result

For Propyphenazone

$$\frac{150.05 + 149.86 + 150.29 + 150.83 + 153.29}{5} = 150.86 \text{ mg}$$

150.86
--------- X 100 = 100.57 %
150.00

For Ketoprofen

50.62 + 50.50 + 51.91 + 48.03 + 48.74
---------- = 49.96 mg
5

49.96
------- X 100 = 99.92 %
50.00

Table 26

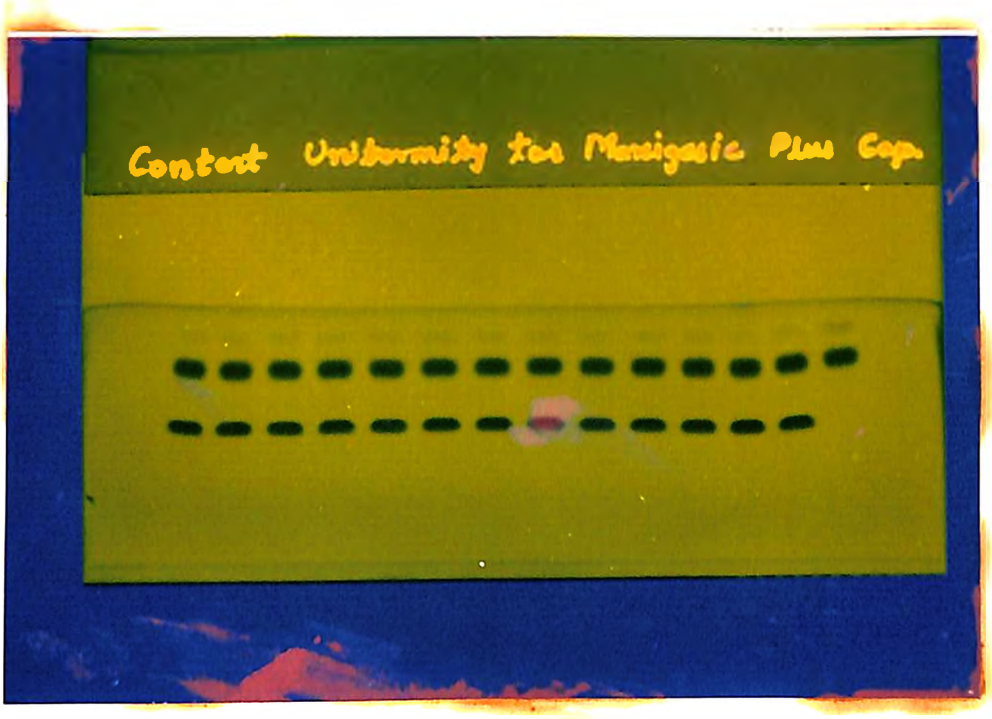
Statistical Analysis

Formulation	Label Claim (mg)	Amount Found* (mg)	SD**	CV***	t-test
Propyphenazone	150.0	150.86	1.4038	0.9305	240.3035
Ketoprofen	50.0	49.96	1.5607	3.1239	71.5793

* Average of three determinations

** Standard deviation

*** Coefficient of variation (%)



A photograph showing the separation of propyphenazone and ketoprofen by HPTLC in drug formulations of Mexigesic plus capsules.

High Performance Liquid Chromatography

Table 27

Area under curve for Propyphenazone

Concentration (µg/20 µl)	Area under curve (A1)			
	Trial 1	Trial 2	Trial 3	Mean
0.3	1.83	1.86	1.99	1.88
0.6	4.43	4.50	4.54	4.49
0.9	8.09	8.01	8.05	8.05
1.2	8.86	8.99	9.00	8.95

Table 28

Area under curve for phenobarbitone

Concentration with respect to Propyphenazone (µg/20 µl)	Area under curve (A2)			
	Trial 1	Trial 2	Trial 3	Mean
0.3	0.46	0.49	0.47	0.48
0.6	0.50	0.53	0.53	0.52
0.9	0.62	0.63	0.63	0.64
1.2	0.48	0.51	0.51	0.50

Table 29

Calibration curve for propyphenazone

Concentration (µg/ 20 µl)	A1/A2 Mean	Covariance	Correlation Coefficient	Regression
0.3	3.91			
0.6	8.63	6.3770	0.9944	0.9887
0.9	12.57			
1.2	17.21			

Table 30

Area under curve for Ketoprofen

Concentration (µg/20 µl)	Area under curve (A1)			
	Trial 1	Trial 2	Trial 3	Mean
0.1	0.830	0.823	0.822	0.825
0.2	1.820	1.770	1.810	1.800
0.3	2.170	2.140	2.170	2.160
0.4	3.140	3.121	3.120	3.127

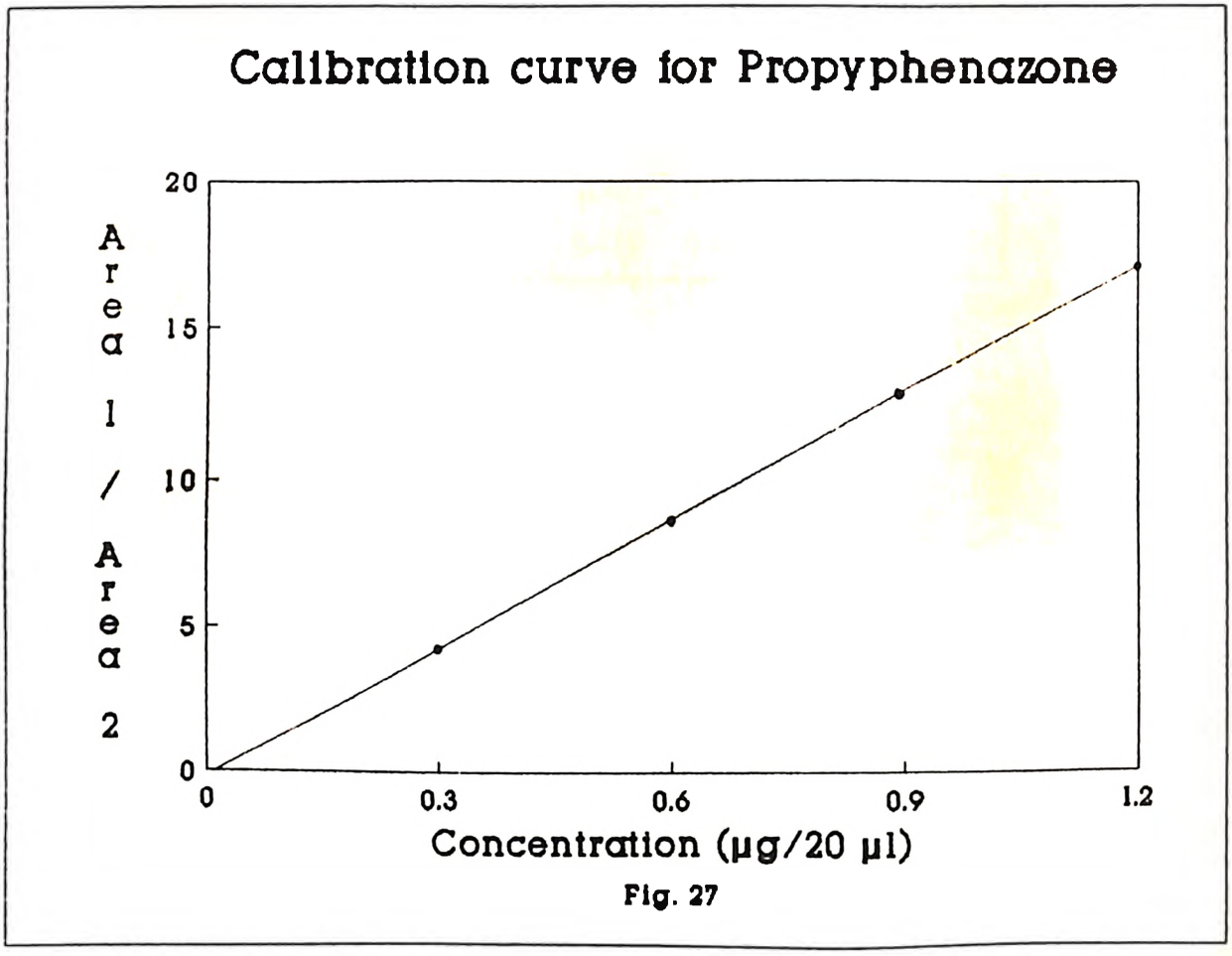

Calibration curve for Propyphenazone
Area 1 / Area 2
20
15
10
5
0
0
0.3
0.6
0.9
1.2
Concentration (µg/20 µl)

Fig. 27

Table 31

Area under curve for Phenobarbitone

Concentration with respect to Ketoprofen (μg/20 μl)	Area under curve (A2)			
	Trial 1	Trial 2	Trial 3	Mean
0.1	0.54	0.51	0.51	0.52
0.2	0.54	0.51	0.51	0.52
0.3	0.402	0.407	0.406	0.405
0.4	0.421	0.427	0.427	0.425

Table 32

Calibration curve for Ketoprofen

Concentration (μg/ 20 μl)	A1/A2 Mean	Covariance	Correlation Coefficient	Regression
0.1	1.269			
0.2	3.461			
		1.0067	0.9996	0.9991
0.3	5.330			
0.4	7.357			

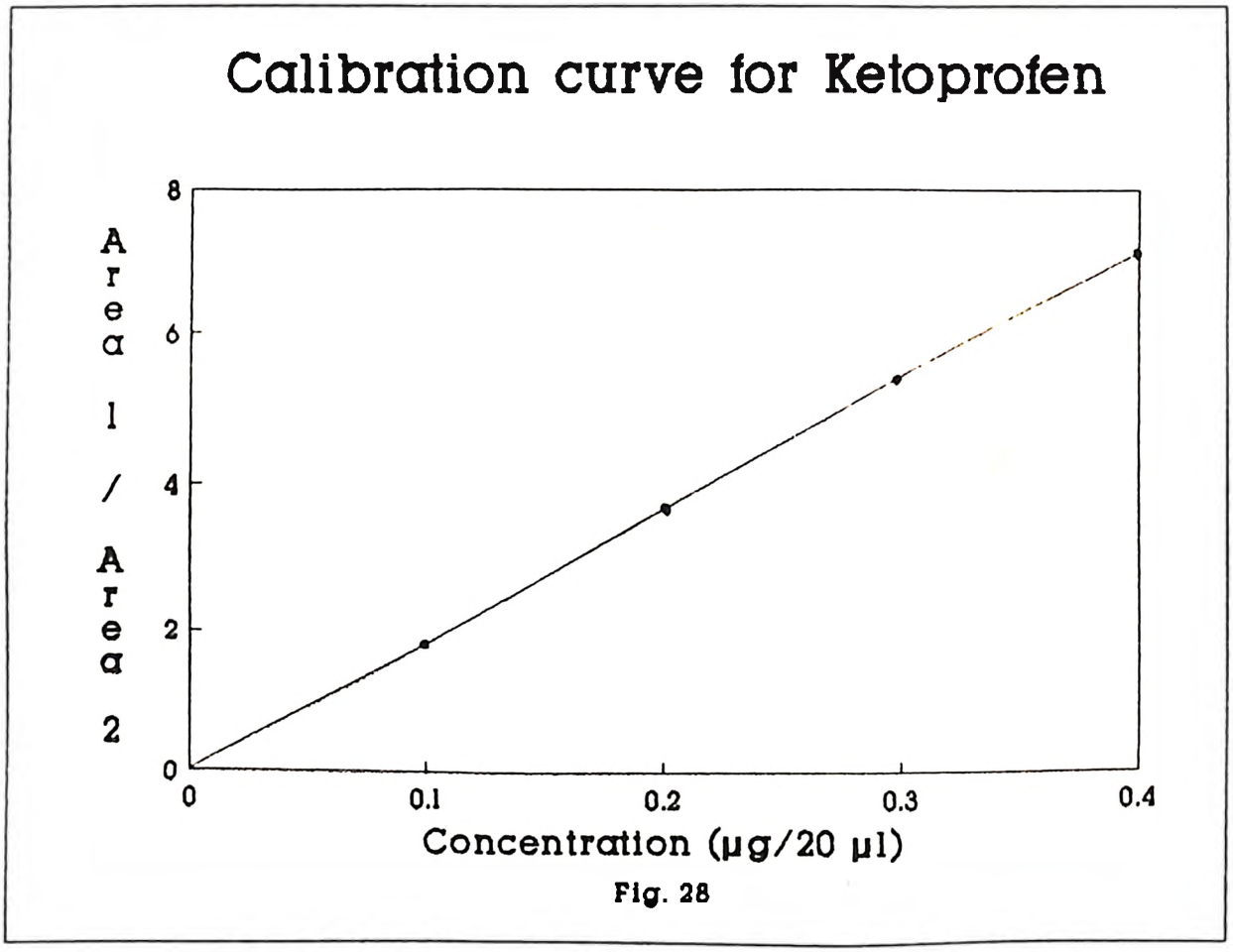

Calibration curve for Ketoprofen
Area 1 / Area 2
8
6
4
2
0
0
0.1
0.2
0.3
0.4
Concentration (µg/20 µl)

Fig. 28

Table 33

For combination of Propyphenazone and Ketoprofen

Commercial formulation

Each capsule contains

Propyphenazone = 150.00 mg

Ketoprofen BP = 50.00 mg

Formulation	Trial No.	A1/A2	Concentration from std graph mg or µg/ 20 µl	Amount found (mg)	% Recovery
Propyphenazone	1	6.943	0.051 or 1.02	149.91	99.94
Ketoprofen		2.716	0.017 or 0.34	49.95	99.90
	2	6.965	0.0511 or 1.022	150.95	100.63
		2.737	0.0171 or 0.342	50.60	101.20
	3	6.920	0.0508 or 1.016	148.86	99.24
		2.697	0.0168 or 0.336	49.20	98.40

Average Result for propyphenazone

$$\frac{149.91 + 150.95 + 148.86}{3} = 149.908 \text{ mg}$$

Average Result for ketoprofen

$$\frac{49.95 + 50.60 + 49.20}{3} = 49.92 \text{ mg}$$

Table 34

Statistical Analysis

Formulation	Label Claim (mg)	Amount Found* (mg)	CV**	SD***	t-test
Brand A					
Propyphenazone	150.00	149.91	0.6952	1.0421	249.1605
Ketoprofen	50.00	49.92	1.4034	0.7001	123.4208

* Average of three determinations

** Coefficient of variation (%)

*** Standard deviation

DISCUSSION

The results of our study show that the amounts of propyphenazone and ketoprofen contained in the aforesaid formulation conform to the label claim specifications .

Various difficulties were encountered while devising a simultaneous assay procedures for the drug formulations described in this research work. Since an official methods are not available for the analysis of drug preparations containing two/three constituents simultaneously, the proposed methods of QTLC, HPTLC and HPLC densitometry which have been developed found to be precise, sensitive and reproducible.The method comprised of separating the two constituents by TLC technique, extraction of the components with methanol and the measurement of their absorption in UV region whereby the absorption maximum for ketoprofen and propyphenazone were found to be 255 nm and 245 nm and Rf values being 0.84 and 0.56 respectively. 0.1N iodine solution being a oxidising agent is used as an spraying agent. The standard graphs were found to be linear and obeyed Lambert-Beer's law. The results of estimation were evaluated by the standard deviation and coefficient of variation.

The high performance thin layer chromatographic densitometry has the added advantage of high efficiency, sensitivity and requires small sample amounts. The developed spots were scanned on a Camag TLC scanner II at 265 nm for absorbance by reflectance. The HPTLC determination was performed by modifying the solvent system developed for TLC and scanning the spots by densitometry.

A comparison of the analysis data obtained from the quantitative TLC and HPTLC was also done by performing the f-test and t-test which was found that there was very small variation in the final results. The linearity of propyphenazone and ketoprofen was studied separately. The drug concentrations were plotted against area under the curve. In the case of propyphenazone the curve was found to be linear in the range of 0-120 µg/ml and that of the ketoprofen in the range of 0-40 µg/ml. The limit of detection for ketoprofen and propyphenazone was found to be 10 ng/ml.

A new simple method was described for the rapid and quantitative estimation of propyphenazone and ketoprofen in drug preparations by HPLC using C-18 bonding column and a UV detector operated at 254 nm. Phenobarbitone was used as an internal standard. The % of drug/capsule was calculated using area under the curve. The chromatograms in fig. 26 shows well resolved peaks of ketoprofen, phenobarbitone and propyphenazone at a retention times of 0.93, 2.06 and 3.33 minutes respectively. Detector response showed good linearity to the concentration of each drug over the analytical concentration range. The coefficient of variation (%) was 0.695 in case of propyphenazone and 1.403 in case of ketoprofen. Mean recoveries of each drug from the solutions were more than 99.0 % in case of propyphenazone and ketoprofen. Common excipients like starch, lactose and talc do not interfere with the proposed method. Synthetic drug mixture and their commercial formulations were analysed to ensure the validity and applicability of the proposed method can be conveniently adopted for routine quality control analysis.

(4.4)

SIMULTANEOUS DETERMINATION OF MEFENAMIC ACID AND PARACETAMOL IN DRUG PREPARATIONS BY UV-ABSORPTION SPECTROSCOPY

Instrument : UV-Visible Jasco autoscan spectrophotometer model 7800 equipped with two 1 cm matched quartz cells.

STANDARD PREPARATION

Stock solutions of pure mefenamic acid and paracetamol were prepared separately by dissolving 50 mg and 45 mg of pure drugs in 100 ml of methanol and separately with deareted 0.1N NaOH. Thereafter 1.0 ml each of the two stock solutions were further diluted to 50 ml with methanol and 0.1N NaOH respectively thus giving a concentration of 10 µg/ml for mefenamic acid and 9.0 µg/ml for paracetamol. The two solutions were scanned on a spectrophotometer and their absorption was determined in wavelength range of 240-300 nm in methanol as blank (Table 35 & 36) and wavelength range of 215-290 nm with deareted 0.1N NaOH as blank (Table 38 & 39). The absorption maximum for mefenamic acid estimation using methanol as solvent was found at 284 nm and for paracetamol at 248 nm. Similarly the absorption maximum for mefenamic acid in the estimation using 0.1N NaOH as the solvent was found at 219 nm and paracetamol at 256 nm. In the same manner a mixture of pure drug was prepared by dissolving 50 mg of mefenamic acid and 45 mg of paracetamol in 100 ml of methanol and 0.1N NaOH separately. From the two stock solutions 1.0 ml was again diluted to 50 ml with methanol and 0.1N NaOH respectively. The absorption data for the two separate estimations in separate solvents was again measured at the aforesaid two wavelengths without any interferences from the two drug constituents.

ASSAY PREPARATION

Twenty tablets were weighed and powdered. An average weight of the tablet containing both the drugs mefenamic acid and paracetamol in the ratio of 1:0.9 and the amounts of 50 mg and 45 mg was dissolved in 50 ml of methanol and 0.1N NaOH separately. The solutions were vigorously shaken for 10 minutes and centrifuged. 1.0 ml of each of the two solutions for two separate estimations was diluted to 100 ml with methanol and 0.1N NaOH respectively. The absorbance of the final solutions of concentration 10 µg/ml of mefenamic acid and 9.0 µg/ml of paracetamol were then determined in the wavelength range of 240-300 nm with methanol as blank (Table 37) and for range 215-290 nm with deareted 0.1N NaOH as blank (Table 40). The absorption maxima for the two drugs were again located at 284 nm and 248 nm with methanol as the solvent and 219 nm and 256 nm with 0.1N NaOH as the solvent without any

interference from the two individual drug components. The results of estimation and statistical analysis are shown in table 41-47.

RECOVERY PROCEDURE

In order to establish the suitability of this method, known amounts of the pure drug was added to previously analysed formulation and the mixture was again analysed by the assay method. The difference between the two absorbance readings was noted which showed the quantity of the drug added. It was calulated from the standard curve and is reported under results.

Results

Table 35

Absorption maximum for Mefenamic acid in methanol

S No.	Wavelength (nm)	Absorbance			
		Trial 1	Trial 2	Trial 3	Mean
1	240	0.370	0.371	0.372	0.371
2	244	0.305	0.308	0.311	0.308
3	248	0.258	0.255	0.261	0.258
4	252	0.243	0.248	0.244	0.245
5	256	0.234	0.238	0.242	0.238
6	260	0.237	0.241	0.242	0.240
7	264	0.264	0.262	0.263	0.263
8	268	0.285	0.286	0.287	0.286
9	272	0.345	0.343	0.341	0.343
10	276	0.367	0.372	0.371	0.370
11	280	0.385	0.383	0.384	0.384
12	284°	0.412	0.410	0.414	0.412
13	288	0.406	0.409	0.403	0.406
14	292	0.390	0.392	0.388	0.390
15	296	0.367	0.371	0.372	0.370
16	300	0.332	0.327	0.331	0.330

Table 36

Absorption maximum for Paracetamol in methanol

S No.	Wavelength (nm)	Absorbance			
		Trial 1	Trial 2	Trial 3	Mean
1	240	0.650	0.652	0.651	0.651
2	244	0.727	0.729	0.728	0.728
3	248*	0.922	0.920	0.918	0.920
4	252	0.704	0.708	0.709	0.707
5	256	0.641	0.644	0.647	0.644
6	260	0.503	0.505	0.505	0.504
7	264	0.377	0.375	0.376	0.378
8	268	0.263	0.268	0.267	0.266
9	272	0.212	0.214	0.210	0.212
10	276	0.193	0.188	0.186	0.189
11	280	0.184	0.183	0.182	0.183
12	284	0.170	0.166	0.174	0.170
13	288	0.111	0.113	0.112	0.112
14	292	0.092	0.091	0.090	0.091
15	296	0.066	0.070	0.074	0.070
16	300	0.049	0.050	0.048	0.049

Table 37

Absorption maxima for mixture containing Paracetamol and Mefenamic acid in methanol

S No.	Wavelength (nm)	Absorbance			
		Trial 1	Trial 2	Trial 3	Mean
1	240	1.001	1.002	1.003	1.002
2	244	1.092	1.094	1.090	1.092
3	248*	1.153	1.155	1.151	1.153
4	252	1.123	1.127	1.125	1.125
5	256	1.078	1.079	1.077	1.078
6	260	0.923	0.925	0.924	0.924
7	264	0.755	0.756	0.757	0.756
8	268	0.658	0.659	0.657	0.658
9	272	0.646	0.645	0.641	0.644
10	276	0.651	0.652	0.650	0.651
11	280	0.671	0.672	0.673	0.672
12	284*	0.570	0.568	0.572	0.570
13	288	0.540	0.539	0.541	0.540
14	292	0.490	0.488	0.492	0.490
15	296	0.475	0.473	0.477	0.475
16	300	0.438	0.436	0.434	0.437

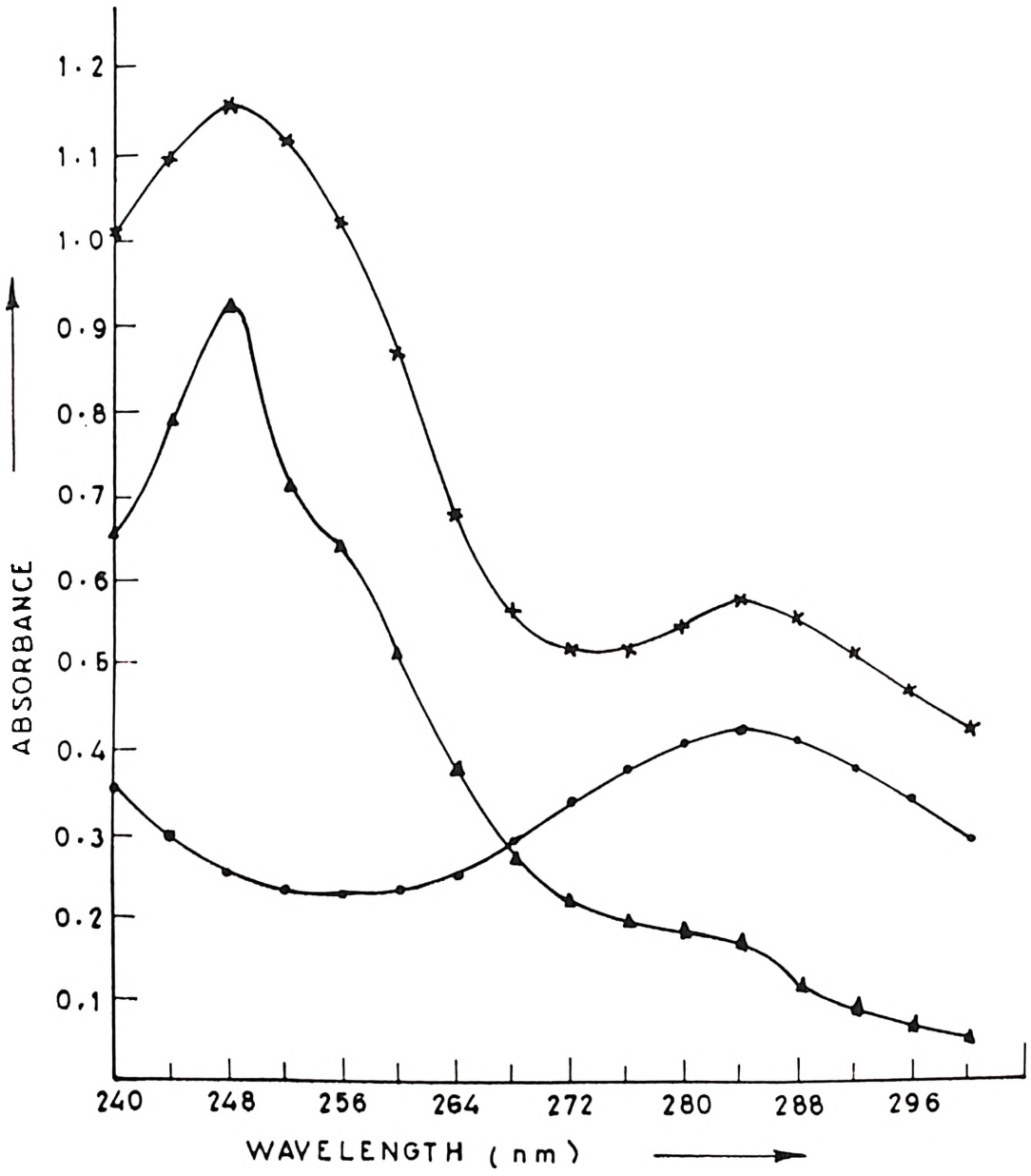


Fig. 29 ABSORPTION SPECTRA FOR ●—● MEFENAMIC ACID ▲—▲ PARACETAMOL AND ✕—✕ MIXTURE (1:1) IN METHANOL

Table 38

Absorption maximum for Mefenamic acid in Sodium hydroxide

S No.	Wavelength (nm)	Absorbance			
		Trial 1	Trial 2	Trial 3	Mean
1	215	0.628	0.630	0.632	0.630
2	219*	1.038	1.036	1.040	1.038
3	220	1.016	1.020	1.018	1.018
4	225	0.777	0.781	0.782	0.780
5	230	0.627	0.632	0.631	0.630
6	235	0.477	0.482	0.481	0.480
7	240	0.499	0.501	0.500	0.500
8	245	0.387	0.392	0.391	0.390
9	250	0.335	0.338	0.338	0.337
10	255	0.319	0.316	0.310	0.315
11	260	0.324	0.329	0.328	0.327
12	265	0.352	0.351	0.353	0.352
13	270	0.379	0.379	0.376	0.378
14	275	0.417	0.421	0.422	0.420
15	280	0.448	0.450	0.452	0.450
16	285	0.496	0.496	0.493	0.495
17	290	0.478	0.480	0.482	0.480

Table 39

Absorption maximum for Paracetamol in Sodium hydroxide

S No.	Wavelength (nm)	Absorbance			
		Trial 1	Trial 2	Trial 3	Mean
1	220	0.395	0.396	0.397	0.396
2	226	0.328	0.331	0.331	0.330
3	232	0.368	0.363	0.364	0.365
4	238	0.447	0.451	0.452	0.450
5	244	0.579	0.582	0.580	0.580
6	250	0.650	0.652	0.648	0.650
7	256*	0.703	0.704	0.702	0.703
8	262	0.639	0.641	0.640	0.640
9	268	0.560	0.561	0.559	0.560
10	274	0.477	0.480	0.483	0.480
11	280	0.419	0.421	0.420	0.420

Table 40

Absorption maxima for Mixture containing Paracetamol and Mefenamic acid in 0.1N Sodium hydroxide

S No.	Wavelength (nm)	Absorbance			
		Trial 1	Trial 2	Trial 3	Mean
1	215	1.017	1.021	1.022	1.020
2	219°	1.452	1.455	1.450	1.4525
3	220	1.407	1.406	1.408	1.407
4	225	1.258	1.262	1.260	1.260
5	230	0.979	0.981	0.980	0.980
6	235	0.871	0.870	0.869	0.870
7	240	0.883	0.885	0.887	0.885
8	245	0.930	0.931	0.929	0.930
9	250	0.967	0.965	0.969	0.967
10	255	1.025	1.028	1.031	1.028
11	256°	1.040	1.042	1.039	1.0405
12	260	0.987	0.991	0.992	0.990
13	265	0.959	0.960	0.961	0.960
14	270	0.897	0.901	0.902	0.900
15	275	0.837	0.842	0.841	0.840
16	280	0.787	0.790	0.793	0.790
17	285	0.699	0.701	0.700	0.700
18	290	0.640	0.639	0.641	0.640

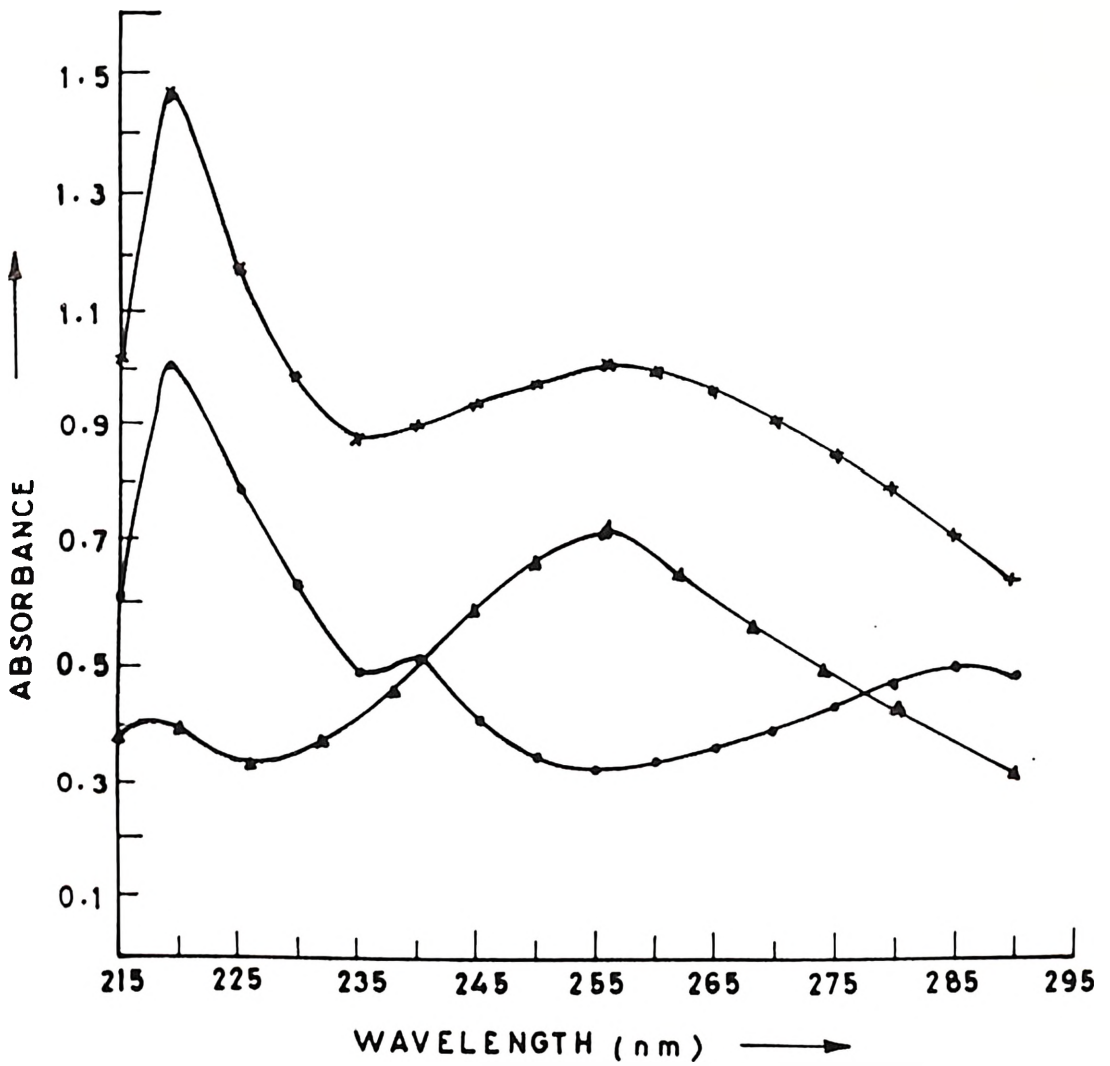


Fig. 30 ABSORTION SPECTRUM FOR ▲—▲ PARACETAMOL ●—● MEFENANIC ACID AND ✕—✕ MIXTURE (1:0·5) IN 0.1 SODIUM HYDROXIDE

Table 41

For combination of paracetamol and mefenamic acid in 0.1N sodium hydroxide

	λmax (256 nm)	λmax (219 nm)
Paracetamol	0.703	0.400
Mefenamic acid	0.316	1.038
Mixture	1.0405	1.4524

$1.0405 = 0.703 \text{ X } 111.11\ x + 0.316 \text{ X } 100\ y$

$1.4525 = 0.400 \text{ X } 111.11\ x + 1.038\ x\ 100\ y$

$1.0405 = 78.1103\ x + 31.6\ y$

$1.4525 = 44.444\ x + 103.8\ y$

Solving for x and y we get,

$$x = 9.265 \text{ X } 10^{-3} \text{ X } \frac{1}{9.2} = 100.70\ \%$$

$$y = 10.026 \text{ X } 10^{-3} \text{ X } \frac{1}{10.0} = 100.26\ \%$$

Table 42

For combination of paracetamol and mefenamic acid in 0.1N sodium hydroxide

	Trial No.	λmax (256 nm)	λmax (219 nm)
Paracetamol	1.	0.7027	0.396
Mefenamic acid		0.318	1.095
Formulation		0.9854	1.4445
	2.	0.703	0.396
		0.318	1.089
		0.9854	1.4445
	3.	0.7027	0.396
		0.318	1.120
		0.9854	1.4445
	4.	0.705	0.399
		0.321	1.101
		0.988	1.447
	5.	0.7027	0.396
		0.318	1.105
		0.9854	1.4445

Table 43

Recovery data for the assay by simultaneous UV-Absorption in 0.1N sodium hydroxide

Formulation	Trial No.	Amount found (mg)	%Recovery
Paracetamol	1	440.216	97.83
Mefenamic acid		493.209	98.64
	2	436.26	96.95
		493.82	98.76
	3	457.475	101.66
		492.95	98.59
	4	444.24	98.72
		495.67	99.13
	5	446.98	99.33
		492.97	98.59

Table 44

For combination of paracetamol and mefenamic acid in methanol

	λmax (248 nm)	λmax (284 nm)
Paracetamol	0.920	0.170
Mefenamic acid	0.258	0.412
Mixture	1.153	0.570

$1.153 = 0.258 \times 100\ x + 0.920 \times 100\ y$

$0.570 = 0.412 \times 100\ x + 0.170 \times 100\ y$

$1.153 = 25.8\ x + 92.0\ y$

$0.570 = 41.2\ x + 17.0\ y$

Solving for x and y we get,

$$x = 9.797 \times 10^{3} \times \frac{1}{9.8} = 99.969\ \%\ \text{(Mefenamic acid)}$$

$$y = 9.785 \times 10^{3} \times \frac{1}{9.8} = 99.84\ \%\ \text{(Paracetamol)}$$

Table 45

For combination of paracetamol and mefenamic acid in methanol

	Trial No.	λmax (248 nm)	λmax (284 nm)
Paracetamol	1.	0.754	0.130
Mefenamic acid		0.252	0.5012
Formulation		1.190	0.725
	2.	0.751	0.130
		0.250	0.499
		1.160	0.724
	3.	0.752	0.132
		0.250	0.499
		1.140	0.725
	4.	0.751	0.130
		0.250	0.499
		1.145	0.724
	5	0.752	0.130
		0.250	0.501
		1.135	0.725

Table 45

For combination of paracetamol and mefenamic acid in methanol

	Trial No.	λmax (248 nm)	λmax (284 nm)
Paracetamol	1.	0.754	0.130
Mefenamic acid		0.252	0.5012
Formulation		1.190	0.725
	2.	0.751	0.130
		0.250	0.499
		1.160	0.724
	3.	0.752	0.132
		0.250	0.499
		1.140	0.725
	4.	0.751	0.130
		0.250	0.499
		1.145	0.724
	5	0.752	0.130
		0.250	0.501
		1.135	0.725

Table 46

Recovery data for the assay by simultaneous UV-Absorption in methanol

Formulation	Trial No.	Amount found (mg)	%Recovery
Paracetamol	1	455.59	101.24
Mefenamic acid		498.20	99.64
	2	443.43	98.54
		498.41	99.68
	3	451.94	100.43
		495.94	99.19
	4	443.93	98.65
		498.81	99.76
	5	440.76	97.94
		502.68	100.54

Table 47

Statistical Analysis

Formulation	Labelled amount (mg)	Amount found* (mg)	%Recovery	SD**	CV***
In Methanol					
Paracetamol	450	447.13	99.36	6.308	6.348
Mefenamic acid	500	498.81	99.76	2.435	2.445
In 0.1N sodium hydroxide					
Paracetamol	450	445.03	98.89	8.050	8.140
Mefenamic acid	500	493.72	98.74	1.140	1.160

*Average of five determinations

**Standard deviation

***Coefficient of variation (%)

DISCUSSION

The literature describes various methods for the analysis of mefenamic acid and paracetamol as individual drug products, but there are no reports of analysis which describe their determinations simultaneously by means of UV spectroscopy. The determination was carried out in two different solvents namely methanol and 0.1N sodium hydroxide. The absorption maximum for mefenamic acid in methanol was found at 284 nm and for the paracetamol at 248 nm. The absorption maximum for mefenamic acid in 0.1N sodium hydroxide was found at 219 nm and for paracetamol at 256 nm. There were no interferences in their estimations.

Solutions containing both x and y cannot be analysed by measuring an absorbance value at any one wavelength because this value represents the absorbance due to both of the components in the solution. However, if x doesnot react with y and each component follows Beer's law, a binary mixture containing both components can be easily analysed by measuring absorbances at two wavelengths. Binary mixtures cannot be analysed unless: (a) spectral data for the pure components are available (b) the absorptivity values for the components can be easily and accurately determined (c) the absorptivity values for the components are sufficiently different at the chosen wavelength to permit an accurate solution (d) the absorbance values for the mixture are accurately determined.

After the absorption maximum for the individual pure drugs had been determined and those were found to be very far apart from each other, it was therefore felt that the spectral data could also be obtained by combining them in the amounts of their present combinations. Our spectral data did not show any kind of interference when the two drugs were combined in the ratios as prescribed in the formulation. It was decided to set up two simultaneous equations as :

$$X = A_{\lambda_1}\, 1\, C_a + A_{\lambda_1}\, 1\, C_b$$

$$Y = A_{\lambda_2}\, 1\, C_a + A_{\lambda_2}\, 1\, C_b$$

Where A_{λ_1} and A_{λ_2} are the optical densities or absorbances of a and b at λ_1 ; those at λ_2 are A_{λ_1} and A_{λ_2} ; C_a and C_b are the molar concentrations of the components in the solutions. The UV absorption spectrum for the pure drugs and their mixture is shown in fig. 29 & 30.

When the absorption maximum for the individual pure drugs had been determined and as these were found to be very far apart from each other, it was therefore felt that the spectral data could also be obtained by combining them in the amounts of their present formulations. The spectral data did not show any type of interferences when the two drugs were combined in the ratios as prescribed in the formulation. However, the pure mefenamic acid in 0.1N sodium hydroxide has showed two peaks, one at 219 nm and second at 285 nm but in combined mixture along with paracetamol the peak at 285 nm was not clear whereas the peak at 219 nm was distinct. Hence the absorption maximum at 219 nm was chosen for mefenamic acid rather than the absorption maximum at 285 nm.

The contents of the individual drugs as present in the commercial formulations were then calculated and are found to be within the prescribed limits. The results of estimation were evaluted by the standard deviation and coefficient of variation .

As the results obtained with the pure sample preparation conformed with the USP requirements, therefore we decided to apply to the commercial formulations which were also found to conform to the label claim specifications.

The study on these drug combinations has proved that a lot of time can be saved by adopting the assay devised. The method is accurate, sensitive and reproducible.

(4.5)

SIMULTANEOUS AND SELECTIVE DETERMINATION OF DICLOFENAC SODIUM AND PARACETAMOL IN TABLET PREPARATIONS BY TITRIMETRIC METHOD

PROCEDURE

STANDARD PREPARATION FOR DICLOFENAC SODIUM

Pure drug powder (50 mg) was accurately weighed and dissolved in 25 ml of 4N HCl. The free acid which precipitated was extracted 3 times with 25 ml portions of diethylether. The ether layers were combined, dried and evaporated. The powder obtained was dissolved in 25 ml of ethanol (96%) [previously neutralised using 0.1N Na_2CO_3 solution] and titrated with 0.1N Na2CO3 using phenol red as an indicator. The end point was the appearance of pale permanent pink colour. Four such titrations were performed and results are shown in table 48. There is no official method of estimation reported for Diclofenac sodium in IP, BP and USP. Recently it has been included in BP 1993 addendum 1994.

STANDARD PREPARATION FOR PARACETAMOL

Paracetamol powder (250 mg) was accurately weighed and transferred into 100 ml of 4N HCl solution and refluxed for 1 and 1/2 hour on a steam bath. The solution was cooled below 10°C and titrated against 0.1M $NaNO_2$ using starch-iodide paper as an external indicator. The end point was indicated by the presence of blue color. Four such estimations were performed and results are shown in table 49 . The official assay results for the pure sample consists of the spectrophotometric analysis which was also performed.

TABLET PREPARATION

Twenty tablets were weighed and powdered. The powder equivalent to one tablet was treated with 100 ml of 4N HCl and extracted 5 times with 20 ml portions of diethyl ether. The Diclofenac sodium was neutralised from its salt and the free liberated acid was taken in diethyl ether. The ether layers were combined, dried on water-bath and residual drug product obtained was then titrated with 0.1N Sodium carbonate as in standard preparation. Five such titrations were performed and the content of Diclofenac sodium was calculated.

Each ml of 0.1N Na_2CO_3 ≡≡ 0.0295 gms of Diclofenac

The remaining solution containing Paracetamol in 4N HCl was refluxed for 1 and 1/2 hr as in standard preparation. The solution was cooled below 10° C and titrated against 0.1M NaNO2 using starch-iodide paper as an external indicator. Five such determinations were performed and the content of Paracetamol was calculated. The results of estimation are shown in table 50 & 51.

Each ml of 0.1N $NaNO_2$ ≡ 0.015116 gms of Paracetamol

Standard preparation of paracetamol by uv absorption

The powder equivalent to 150 mg of paracetamol was weighed, 50 ml of 0.1N sodium hydroxide was added and diluted to 100 ml with water. The mixture was shaken for 15 minutes and quantity sufficient 200 ml of water was added. The solution was mixed, filtered and 10 ml of the filterate was diluted to 100 ml with water. Ten (10) ml of this solution was added to 10 ml of 0.1N sodium hydroxide and diluted to 100 ml with water. The extinction of a 1.0 cm layer of the solution was measured at 256 nm.Similar procedure was followed for tablets containing paracetamoland the content of paracetamol was calculated by comparing with standard preparation.

RESULTS

Table 48

Diclofenac verses 0.1N Sodium carbonate

S No.	Weight of Diclofenac (gms)	Initial volume (ml)	Final volume (ml)	Volume run down (ml)	% Recovery
1	0.0466	0.0	1.6	1.6	101.99
2	0.0473	1.7	3.3	1.6	100.48
3	0.0450	3.3	4.9	1.6	099.02

Indicator = Phenol red

End point = Appearance of pale pink colour

Each ml of 0.1N Sodium carbonate = 0.0295 gms of Diclofenac

46.5 mg of Diclofenac = 50.0 mg of Diclofenac sodium

Normality of Sodium carbonate = 0.1007N

Table 49

Paracetamol verses 0.1N Sodium nitrite

S No.	Weight of Paracetamol (gms)	Initial volume (ml)	Final volume (ml)	Volume run down (ml)	% Recovery
1	0.5002	0.0	33.2	33.2	100.63
2	0.5005	0.0	33.6	33.6	101.78
3	0.5001	0.0	33.2	33.2	100.65

Indicator = Starch iodide paper

End point = Appearance of blue colour

Each ml of 0.1N Sodium nitrite = 0.015116 gms of Paracetamol

Normality of Sodium nitrite = 0.1003N

Table 50

For Combination of Paracetamol and Diclofenac sodium

Commercial preparation

Brand A

Paracetamol IP = 500 mg

Diclofenac sodium = 50 mg

Average weight of the tablet = 0.6350 gms

Formulation	Trial No.	Label Claim (mg)	Weight of Powder taken (gms)	Volume run down (ml)	Amount Found (mg)	% Recovery
Paracetamol	1	500	0.6352	33.2	503.17	100.63
Diclofenac Na		50	0.0476	1.6	46.565 (as Diclofenac)	100.14
Paracetamol	2	500	0.6355	33.6	509.01	101.80
Diclofenac Na		50	0.0469	1.6	47.123 (as Diclofenac)	101.34
Paracetamol	3	500	0.6355	33.5	507.50	101.50
Diclofenac Na		50	0.0470	1.6	47.021 (as Diclofenac)	101.12

Brand B

Paracetamol IP = 500 mg

Diclofenac sodium = 50 mg

Average weight of the tablet = 0.6579 gms

Formulation	Trial No.	Label Claim (mg)	Weight of Powder taken (gms)	Volume run down (ml)	Amount Found (mg)	% Recovery
Paracetamol	1	500	0.6592	33.0	499.33	099.86
Diclofenac Na		50	0.0477*	1.6	46.332	099.64
Paracetamol	2	500	0.6570	33.2	504.04	100.80
Diclofenac Na		50	0.0469*	1.6	47.123	101.34
Paracetamol	3	500	0.6585	33.3	504.41	100.88
Diclofenac Na		50	0.0468*	1.6	47.225	101.56

* Calculated as Diclofenac

Brand C

Paracetamol IP = 325 mg

Diclofenac sodium = 50 mg

Average weight of the tablet = 0.5026 gms

Formulation	Trial No.	Label Claim (mg)	Weight of Powder taken (gms)	Volume run down (ml)	Amount Found (mg)	% Recovery
Paracetamol	1	325	0.5037	21.7	328.28	101.01
Diclofenac Na		50	0.0476*	1.6	46.43	099.85
Paracetamol	2	325	0.5021	21.6	327.81	100.86
Diclofenac Na		50	0.0468*	1.6	47.225	101.56
Paracetamol	3	325	0.5035	21.7	328.41	101.05
Diclofenac Na		50	0.0469*	1.6	47.123	101.34

* Calculated as Diclofenac

Table 51
Statistical Analysis

Formulation	Labelled amount (mg)	Amount found (mg)	%Recovery	SD*	CV**
Brand A					
Paracetamol	500	506.56	101.13	2.546	2.518
Diclofenac sodium	50	50.46	100.92	0.259	0.257
Brand B					
Paracetamol	500	502.59	100..51	2.832	2.817
Diclofenac sodium	50	50.40	100.81	0.545	0.540
Brand C					
Paracetamol	325	328.16	100.97	0.316	0.313
Diclofenac sodium	50	50.43	100.87	2.036	2.019

For paracetamol by UV-spectroscopy

The λmax for pure paracetamol and the its content in formulation was found to be 256 nm.

At 256 nm the reading for pure paracetamol = 0.6311

At 256 nm the reading for formulation containing paracetamol = 0.6248

Hence,

$$\frac{\text{Sample reading}}{\text{Standard reading}} \times \frac{\text{Standard weight}}{\text{Sample weight}} \times \text{Average weight} = \text{content of paracetamol}$$

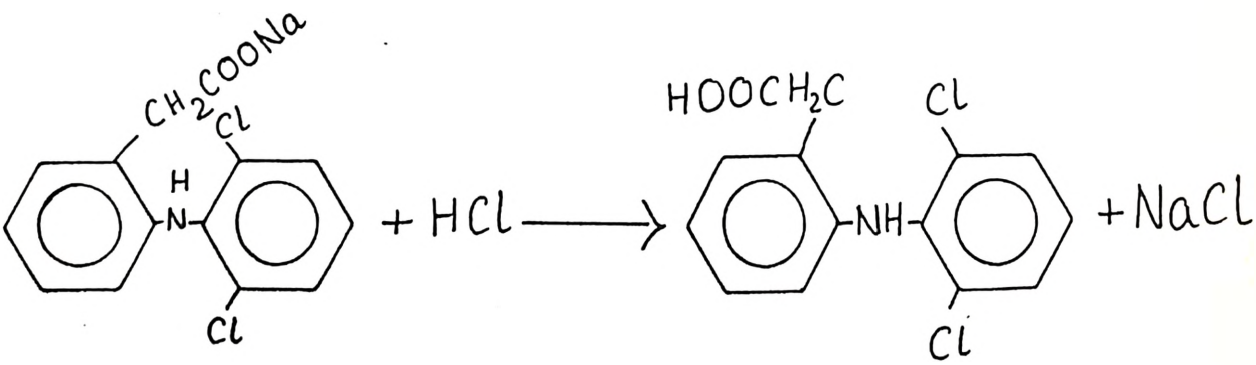
$$\frac{0.6248}{0.6311} \times \frac{0.1501}{0.1861} \times 0.6210 = 496.19 \text{ mg}$$

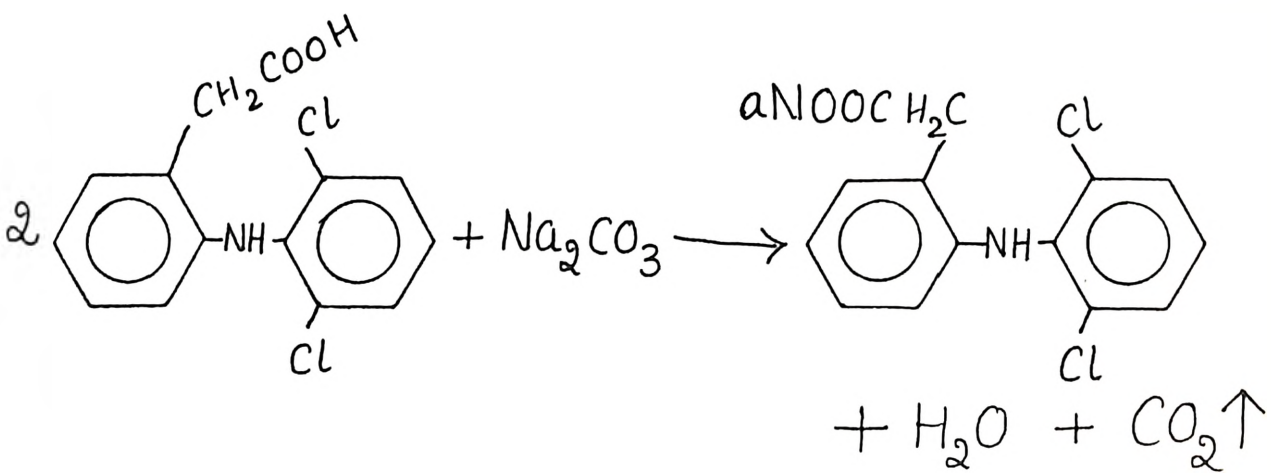
% Purity

$$\frac{496.19}{500.00} \times 100 = 99.23\ \%$$

DISCUSSION

Although several methods of analysis are available for individual constituents of the present combination but since no work has been described on the analysis of both the constituents simultaneously therefore, this study was undertaken. Diclofenac sodium is chemically 2[(2,6,dichloro phenyl) amino] benzene acetic acid mono sodium salt. Diclofenac sodium has been converted to diclofenac by treating with hydrochloric acid. The -COOH group present in diclofenac dissolves readily in solutions of alkali carbonates with evolution of CO_2 and formation of nonmetallic salts by displacement of H of the carboxyl group. The drugs which are free bases or free acids in character are assayed by acidimetry and alkalimetry. Titrations of a free base with a standard acid is called acidimetry and titration of a free acid with a standard base is called alkalimetry. These are also called neutralisation titrations as these reactions involve the combination of hydrogen and hydroxyl ions to form water. The choice of suitable indicator for acidimetry and alkalimetry is made on the basis of the pH at the end point. In this above titration phenol red is used as an indicator.





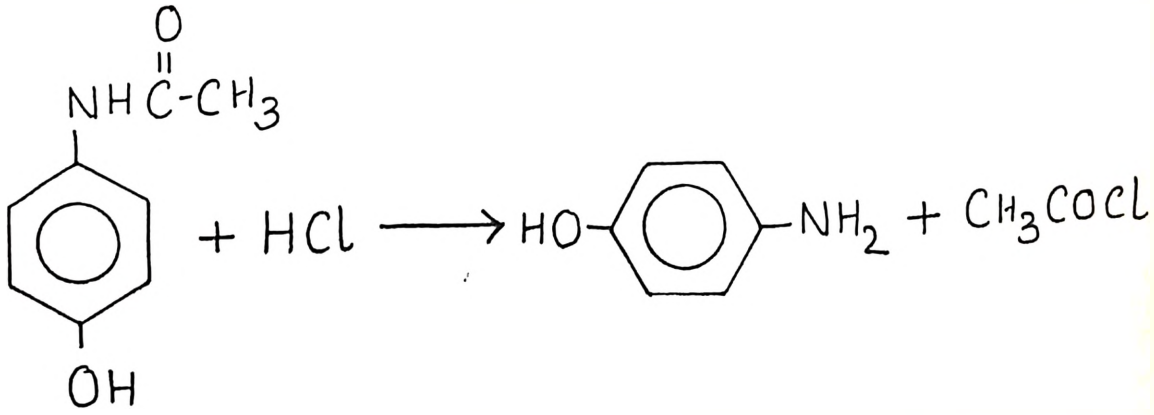
Paracetamol is N-acetyl p-aminophenol and contains phenolic -OH. It does not react with metallic carbonates. On the contrary it dissolves freely in solutions of NaOH or KOH forming corresponding phenoxide. Diazotisation is an important method employed in pharmaceutical analysis of drugs such as aromatic amines. Aromatic primary amines differ markedly from aliphatic amines in their reaction with nitrous acid to give mainly the corresponding primary alcohol, p-aminophenol hydrolysed product of paracetamol under similar conditions gives diazonium compound.The diazonium compounds are usually very soluble in water and cannot be readily isolated. Since on warming their aqueous solutions decomposition occurs with formation of phenol. In preparing an aqueous solution of a diazonium salt such as diazonium compound, it is usual to dissolve the amine in a slight excess of dil.HCl and then an aqueous solution of a metallic nitrite is added. Nitrous acid is thus generated in situ and reacts with the amine salt to give the diazonium compound, for a successful preparation of an aqueous solution of the diazonium salt, however two conditions must always be observed :

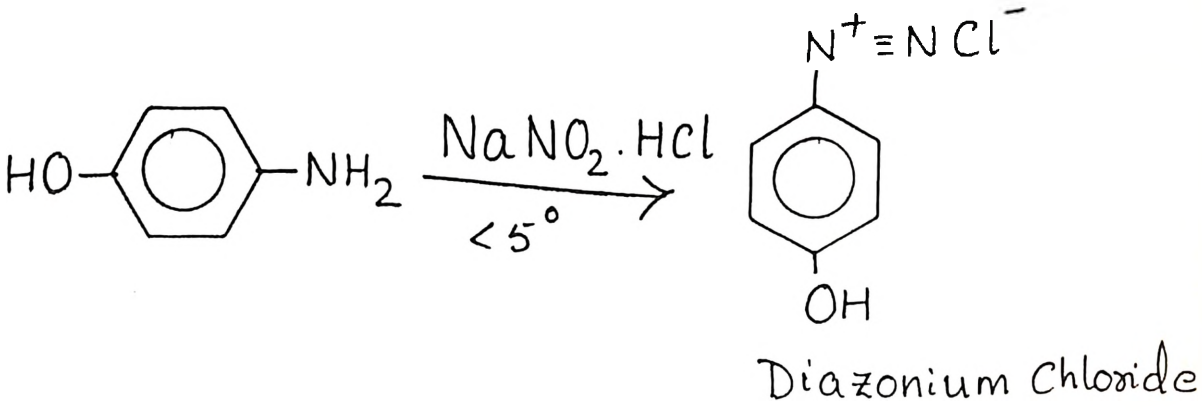
(1) The solution of the p-aminophenol HCl should be cooled to 5 °C and the temperature maintained throughout the addition of the sodium nitrite solution. External cooling has to be maintained otherwise the heat of the reaction would cause temperature to rise with the consequent decomposition of diazotisation chloride and the production of phenol. But if reduced to about 0°C diazotisation becomes extremely slow and unchanged nitrous acid may remain in the solution for an unpredictably long time.

(2) Sufficient sodium nitrite must be added to diazotise all the aryl primary amine present (p-aminophenol) otherwise the unchanged p-aminophenol will react with the diazonium chloride to give diazoaminobenzene.To ensure the presence of a slight excess of nitrous acid, KI-starch paper is sometimes used as external indicator, a drop of the solution being removed from time to time during the addition of the sodium nitrite and then dropped on the paper. When an excess of nitrous acid is present iodine is liberated and gives the familiar blue colour with starch.

When a mixture of paracetamol and diclofenac were treated with excess of sodium hydroxide, then the hydrogen atom of both acidic -COOH and phenolic -OH groups are displaced by the metal. Therefore, one can use sodium carbonate instead of sodium hydroxide to estimate the quatity of diclofenac alone with out any interference from paracetamol. For paracetamol, the acetamide group -$NHCOCH_3$,is hydrolysed in dilute HCl by refluxing to liberate p-aminophenol which behaves like a primary amine. The latter is diazotised and titrated against Sodium nitrite with out any interference from diclofenac. When a mixture of paracetamol and diclofenac is treated with excess of sodium hydroxide, then the hydrogen atom of both acidic -COOH and phenolic -OH groups are displaced by the metal. Therefore 0.1N sodium carbonate is used instead of 1N sodium hydroxide to estimate the quantity of diclofenac alone without any interference from paracetamol.

The titrimetric method devised is very suitable for the estimation of this particular combination simultaneouly and the results are reasonably good. Further the method is quite quick, inexpensive,reproducibleand does not involve any sophisticated instrument. The results of estimation were evaluated by the standard deviation and coefficient of variation.





Diazonium Chloride

PART-B

DIURETICS

(4.6)

SIMULTANEOUS DETERMINATIONS OF SPIRONOLACTONE IN COMBINATION WITH FRUSEMIDE AND SPIRONOLACTONE IN COMBINATION WITH HYDROFLUMETHIAZIDE IN DRUG FORMULATIONS BY UV ABSORPTION SPECTROSCOPY

Instrument : UV-Visible Jasco model 7800 (MHT-344) autoscan spectrophotometer.

PROCEDURE

STANDARD PREPARATION

FOR SPIRONOLACTONE AND HYDROFLUMETHIAZIDE

Stock solutions of spironolactone and hydroflumethiazide were prepared separately by dissolving 50 mg of pure drugs in 100 ml of methanol in 100 ml volumetric flasks. Thereafter, 1.0 ml of each of the two stock solutions was further diluted to 50 ml with methanol in 50 ml volumetric flask, thus giving them a concentration of 10 µg/ml. These solutions were scanned on a spectrophotometer and their absorption was determined in the wavelength range of 210-300 nm (Table 52 & 53). The absorption maximum for spironolactone was found at 238 nm and separately the absorption maximum for hydroflumethiazide was found to be 273 nm respectively. In a similar manner,a mixture of the pure drugs were prepared by dissolving 50 mg of spironolactone and 50 mg hydroflumethiazide in 100 ml of methanol and from this solution 1 ml was again diluted to 50 ml with methanol. The absorption data was again read at the aforesaid two wavelengths without any interferences from each other (Table 55).

FOR SPIRONOLACTONE AND FRUSEMIDE

Stock solutions of spironolactone and frusemide were prepared separately by dissolving 50 mg and 20 mg of pure drugs in 100 ml of methanol in 100 ml volumetric flasks. Thereafter, 1.0 ml each of the two stock solutions was further diluted to 50 ml with methanol in 50 ml volumetric flask. Thus giving them a concentration of 10 µg/ml for spironolactone and 4.0 µg/ml for frusemide. These solutions were scanned on a spectrophotometer and their absorption was determined in the wavelength range of 220-290 nm (Table 52 & 54). The absorption maximum for spironolactone was located at 238 nm and separately the absorption maximum for frusemide was found to be 276 nm respectively. In the similar manner a mixture of the pure drugs was prepared by dissolving 50 mg of spironolactone and 20 mg of frusemide in 100 ml of methanol and from this solution 1 ml was again diluted to 50 ml with methanol in a volumetric flasks. The absorption data was again read at the aforesaid two wavelengths without any interferences from each other (Table 56).

SAMPLE PREPARATION

FOR SPIRONOLACTONE & HYDROFLUMETHIAZIDE

Twenty tablets were weighed and powdered. An average weight of the tablet containing both the drugs, spironolactone & hydroflumethiazide in equal amounts was dissolved in 50 ml of methanol (i.e., 25 mg of spironolactone & 25 mg of hydroflumethiazide) in a volumetric flask which was vigorously shaken for 10 minutes and centrifuged. One millilitre (1.0 ml) of this solution was then diluted to 50 ml with methanol in a volumetric flask. The absorbance of this final solution of concentration 10 µg/ml of spironolactone and 10 µg/ml of hydroflumethiazide was then measured in the wavelength range of 210-300 nm. The absorption maximum for the two drugs were again located at 238 nm and 273 nm respectively without any interferences. The results of estimation are shown in table 57.

FOR SPIRONOLACTONE AND FRUSEMIDE

Twenty tablets were weighed and powdered. An average weight of the tablet containing both the drugs spironolactone and frusemide in the amounts of 50 mg and 20 mg was dissolved in 50 ml of methanol in a volumetric flask, which was vigorously shaken for 10 minutes and centrifuged. One millilitre (1ml) of this solution was then diluted to 100 ml with methanol in a volumetric flask. The absorbance of this final solution of concentration 10 µg/ml of spironolactone and 4 µg/ml of frusemide were then determined in the wavelength range of 220-290 nm. The absorption maximum for the two drugs were again located at 238 nm and 276 nm respectively without any inteferences. The results of estimation are shown in table 58.

RECOVERY PROCEDURE

In order to establish the suitability of this method, known amounts of the pure drug was added to previously analysed formulation and the mixture was again analysed by the assay method. The difference between the two absorbance readings was noted which showed the quantity of the drug added. It was calculated from the standard curve and is reported under results.

RESULTS

Table 52

Absorption maximum for Spironolactone in methanol

SNo.	Wavelength (nm)	Absorbance			
		Trial 1	Trial 2	Trial 3	Mean
1.	222	0.263	0.252	0.265	0.260
2.	230	0.403	0.399	0.398	0.400
3.	238	0.491	0.490	0.492	0.491
4.	246	0.408	0.415	0.407	0.410
5.	254	0.258	0.259	0.263	0.260
6.	262	0.147	0.153	0.150	0.150
7.	270	0.072	0.073	0.080	0.075
8.	278	0.030	0.022	0.023	0.025
9.	286	0.020	0.018	0.022	0.020
10.	294	0.019	0.020	0.015	0.018

Table 53

Absorption maximum for Hydroflumethiazide in methanol

S No.	Wavelength (nm)	Absorbance			Mean
		Trial 1	Trial 2	Trial 3	
1.	243	0.103	0.105	0.107	0.105
2.	253	0.192	0.197	0.199	0.196
3.	263	0.447	0.449	0.448	0.448
4.	273*	0.590	0.587	0.593	0.590
5.	283	0.289	0.294	0.299	0.294
6.	293	0.086	0.084	0.082	0.084
7.	303	0.076	0.075	0.080	0.077

Table 54

Absorption maxima for Frusemide in methanol

S No.	Wavelength (nm)	Absorbance			Mean
		Trial 1	Trial 2	Trial 3	
1.	220	0.310	0.300	0.290	0.300
2.	224*	0.405	0.400	0.395	0.400
3.	228	0.329	0.333	0.328	0.330
4.	232	0.265	0.260	0.255	0.260
5.	236	0.178	0.182	0.180	0.180
6.	240	0.120	0.125	0.130	0.125
7.	244	0.071	0.076	0.078	0.075
8.	248	0.045	0.050	0.051	0.048
9.	252	0.035	0.040	0.045	0.040
10.	256	0.022	0.026	0.027	0.025
11.	260	0.024	0.028	0.026	0.026
12.	264	0.025	0.027	0.029	0.027
13.	268	0.038	0.035	0.032	0.035
14.	272	0.090	0.091	0.089	0.090
15.	276*	0.215	0.217	0.213	0.215
16.	280	0.128	0.132	0.130	0.130
17.	284	0.045	0.049	0.053	0.049
18.	288	0.002	0.003	0.004	0.003

Table 55

Absorption maxima for Mixture containing Spironolactone and Hydroflumethiazide in methanol

S No.	Wavelength (nm)	Absorbance			Mean
		Trial 1	Trial 2	Trial 3	
1.	228	0.150	0.152	0.154	0.152
2.	233	0.200	0.202	0.204	0.202
3.	238*	0.252	0.251	0.247	0.250
4.	243	0.227	0.230	0.233	0.230
5.	248	0.202	0.200	0.204	0.202
6.	253	0.197	0.199	0.198	0.198
7.	258	0.204	0.203	0.202	0.203
8.	263	0.223	0.228	0.230	0.227
9.	268	0.282	0.281	0.283	0.282
10.	273*	0.302	0.301	0.303	0.302
11.	278	0.198	0.200	0.202	0.200
12.	283	0.119	0.122	0.125	0.122
13.	288	0.039	0.039	0.042	0.040

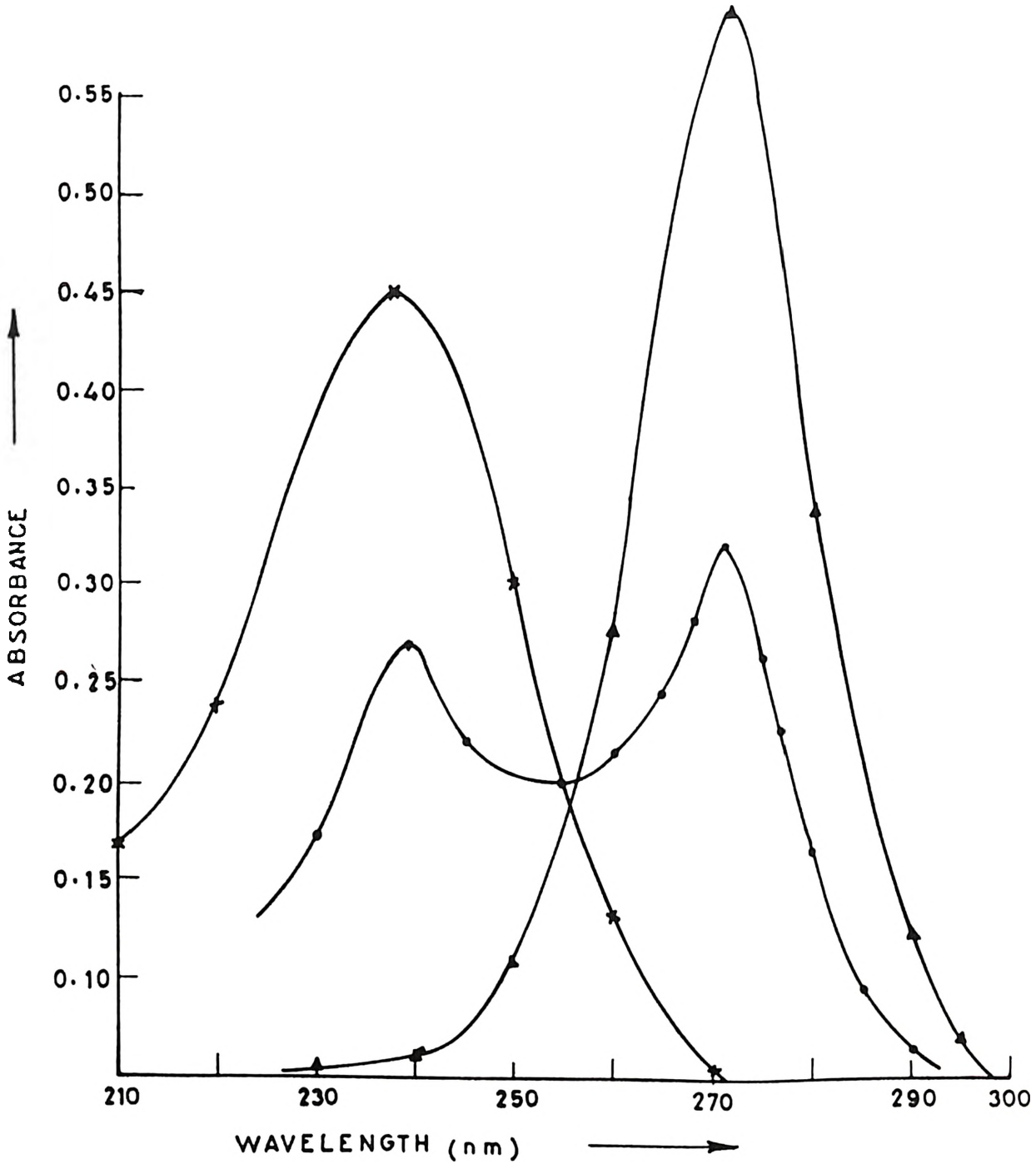


Fig.31 ABSORPTION SPECTRUM FOR ×—× SPIRONOLACTONE, ▲—▲ HYDROFLUMETHIAZIDE AND •—• MIXTURE (1:1) IN METHANOL

Table 56

Absorption maxima for Mixture containing Spironolactone and Frusemide in methanol

S No.	Wavelength (nm)	Absorbance			Mean
		Trial 1	Trial 2	Trial 3	
1.	230	0.670	0.673	0. 667	0.670
2.	232	0.710	0.715	0.720	0.715
3.	234	0.753	0.755	0.757	0.755
4.	236	0.817	0.819	0.824	0.820
5.	238*	0.862	0.867	0.866	0.865
6.	240	0.782	0.787	0.786	0.785
7.	242	0.762	0.765	0.768	0.765
8.	244	0.694	0.695	0.696	0.695
9.	246	0.652	0.650	0.648	0.650
10.	248	0.587	0.585	0.583	0.585
11.	250	0.545	0.540	0.535	0.540
12.	252	0.482	0.480	0.478	0.480
13.	254	0.421	0.420	0.419	0.420
14.	256	0.388	0.372	0.370	0.370
15.	258	0.321	0.320	0.319	0.320
16.	260	0.266	0.270	0.274	0.270
17.	262	0.228	0.232	0.230	0.230
18.	264	0.202	0.201	0.203	0.202
19.	266	0.173	0.175	0.177	0.175
20.	268	0.159	0.160	0.161	0.160
21.	270	0.153	0.155	0.157	0.155
22.	272	0.174	0.175	0.176	0.175
23.	274	0.213	0.215	0.217	0.215
24.	276*	0.251	0.249	0.247	0.249
25.	278	0.201	0.202	0.197	0.200

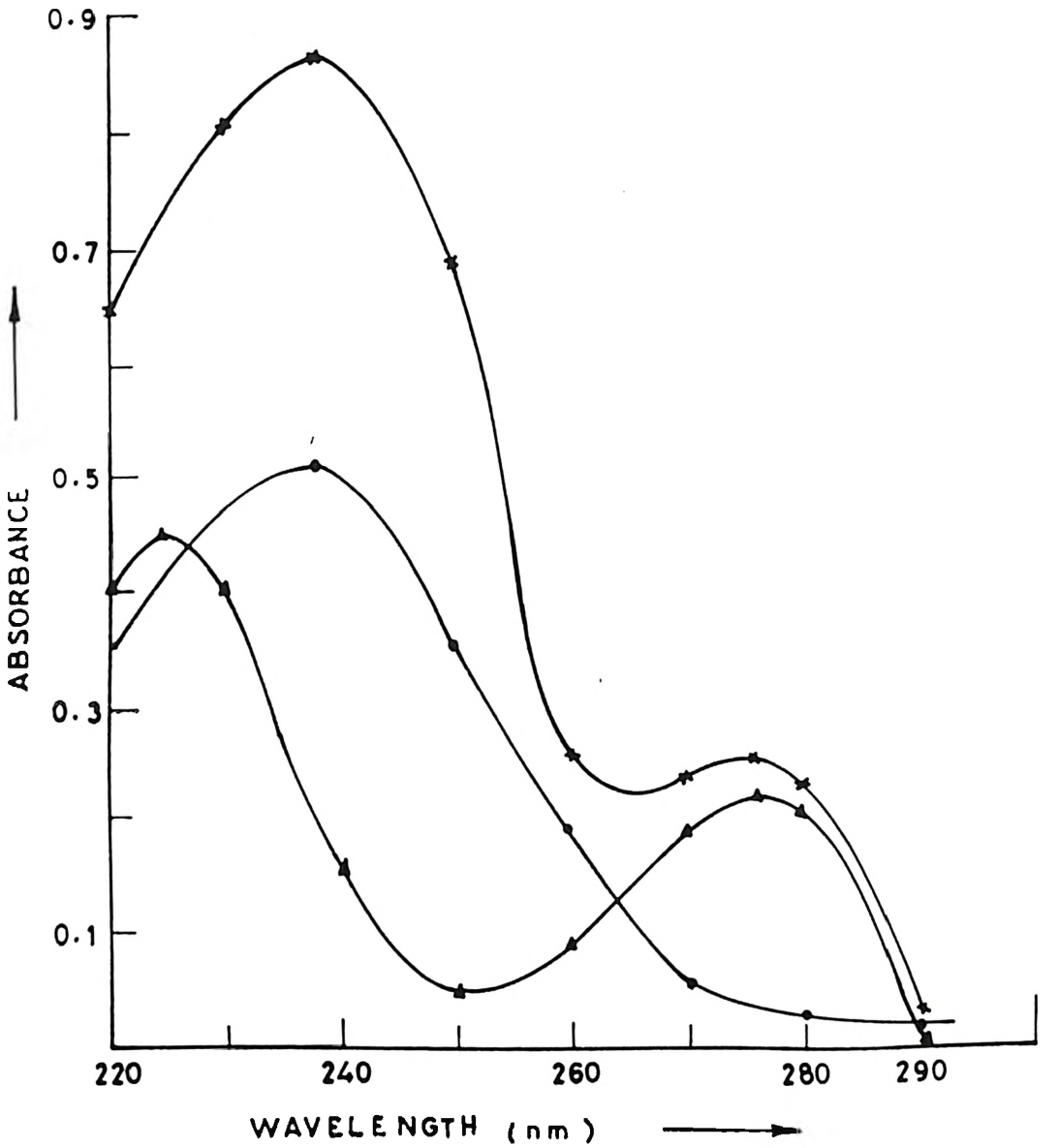


Fig. 32 ABSORPTION SPECTRA FOR ●—● SPIRONOLACTONE ▲—▲ FRUSEMIDE AND ×—× MIXTURE (1:0·4) IN METHANOL

Table 57

For combination of spironolactone and hydroflumethiazide in methanol

	λmax (238 nm)	λmax (273 nm)
Spironolactone	0.491	0.015
Hydroflumethiazide	0.008	0.590
Mixture	0.250	0.302

Using the following equation,

$0.250 = 0.008 \text{ X } 100\,x + 0.491 \text{ X } 100\,y$

$0.302 = 0.590 \text{ X } 100\,x + 0.015 \text{ X } 100\,y$

OR

$0.250 = 0.8\,x + 49.1\,y$

$0.302 = 59.0\,x + 1.5\,y$

Solving for x and y we have,

$x = 4.9916 \text{ X } 10^{-3}$ gm/lt

$= 4.9916$ µg/ml

hence calculating the % purity for hydroflumethiazide we get,

$$-\frac{4.9916}{5.0000} \text{ X } 100 = 99.83\ \%$$

similarly $y = 5.0103 \text{ X } 10^{-3}$ gm/lt

$= 5.0103$ µg/ml

the % purity for spironolactone is,

$$\frac{5.0103}{5.0000} \text{ X } 100 = 100.20\ \%$$

Table 58

For combination of spironolactone and frusemide in methanol

	λmax (238 nm)	λmax (276 nm)
Spironolactone	0.506	0.041
Frusemide	0.352	0.215
Mixture	0.865	0.249

Using the following equation,

$0.865 = 0.352 \text{ X } 250 \text{ x} + 0.506 \text{ X } 100 \text{ y}$

$0.256 = 0.215 \text{ X } 250 \text{ x} + 0.041 \text{ X } 100 \text{ y}$

OR

$0.865 = 88 \text{ x} + 50.6 \text{ y}$

$0.256 = 53.75 \text{ x} + 4.1 \text{ y}$

Solving for x and y we have,

$x = 3.9878 \text{ X } 10^{-3}$ gm/lt

$= 3.9878$ µg/ml

hence calculating the % purity for frusemide we get,

$$\frac{3.9878}{4.0000} \text{ X } 100 = 99.69 \%$$

similarly $y = 9.9615 \text{ X } 10^{-3}$ gm/lt

$= 9.9615$ µg/ml

the % purity for spironolactone is,

$$\frac{9.9615}{10.000} \text{ X } 100 = 99.61 \%$$

Table 59

Recovery data & Statistical Analysis for the assay by simultaneous UV-Absorption

Formulation	Labelled Amount (mg/tab)	Amount Found* (mg)	% Recovery	SD**	CV***
Brand A					
Spironolactone	25.00	24.88	99.52	0.046	0.0462
Hydroflumethiazide	25.00	24.70	98.80	0.033	0.0334
Brand B					
Spironolactone	50.00	50.24	100.48	0.049	0.0488
Frusemide	20.00	20.12	100.60	0.046	0.0457

* Average of five determinations

** Standard Deviation

*** Coefficient of Variation (%)

DISCUSSION

The literature describes various types of analytical procedures for the individual drug products, but none are available which determine them simultaneously in the form of their present formulation.

Solutions containing both x and y cannot be analysed by measuring an absorbance value at any one wavelength because this value represents the absorbance due to both of the components in the solution. However, if x doesnot react with y and each component follows Beer's law, a binary mixture containing both components can be easily analysed by measuring absorbances at two wavelengths. Binary mixtures cannot be analysed unless: (a) spectral data for the pure components are available (b) the absorptivity values for the components can be easily and accurately determined (c) the absorptivity values for the components are sufficiently different at the chosen wavelength to permit an accurate solution (d) the absorbance values for the mixture are accurately determined. After the absorption maximum for the individual pure drugs had been determined and those were found to be very far apart from each other, it was therefore felt that the spectral data could also be obtained by combining them in the amounts of their present combinations. Our spectral data did not show any kind of interference when the two drugs were combined in the ratios as prescribed in the formulation. It was decided to set up two simultaneous equations as :

$$X = A_{1a}\,l\,C_a + A_{1b}\,l\,C_b$$

$$Y = A_{2a}\,l\,C_a + A_{2b}\,l\,C_b$$

Where A_{1a} and A_{1b} are the optical densities or absorbances of a and b at λ_1 ; those at λ_2 are A_{2a} and A_{2b} ; C_a and C_b are the molar concentrations of the components in the solutions. Fig. 31 show that the spironolactone has absorbance at 238 nm, hydroflumethiazide at 273 nm and its mixture at 238 and 273 nm. Similarly the fig. 32 shows the spironolactone has absorbance at 238 nm, frusemide at 276 nm and its mixture at 238 and 276 nm.An analytical procedure for the simultaneous determination of spironolactone and

hydroflumethiazide/spironolactone and frusemide in commercial formulations has been established by using UV spectroscopy.

The contents of the individual drugs as present in the commercial formulations were then calculated and are shown in table no. 59 under results.As the results obtained with the pure sample preparation conformed with the USP requirements, therefore it was decided to apply to the commercial formulations which were found to conform to the label claim specifications.

The study on these drug combinations has proved that a lot of time can be saved by adopting the assay devised. The method is accurate, sensitive and reproducible.

(4.7)

DETERMINATION OF SPIRONOLACTONE BY SPECTROPHOTOMETRY

Instrument : UV-Visible Jasco model 7800 (MHT-344) autoscan spectrophotometer equipped with two 1 cm matched quartz cells.

PROCEDURE

STANDARD PREPARATION

A stock solution of 5 mg of pure drug was prepared in 25 ml of methanol giving a concentration of 200 µg/ml. A series of dilutions ranging in concentration from 2 µg/ml to 10 µg/ml (i.e. 0.10, 0.20, 0.30, 0.40 and 0.50 ml) of stock solution was transferred into 10 ml volumetric flask. To each of these solutions 5 ml of 0.05 % anthrone in concentrated sulphuric acid was added slowly and volume was made upto 10 ml with methanol. The solutions were allowed to stand for one hour for the completion of the coupling reaction whereby a greenish-yellow coloured solutions were obtained. After allowing them to stand for one hour the flasks were slowly shaken with cooling. Each of the solution was then transferred into a 10 mm glass cell and the absorbances measured in the range of 350-700 nm using a mixture of 5 ml of methanol and 5 ml of 0.05 % anthrone in concentrated sulphuric acid as the blank. The absorption maximum was found to be at 486 nm (Fig.38). The calibration curve was drawn and is shown in the fig. 39.

TABLET PREPARATION

Twenty tablets were weighed and the contents finely powdered. The average weight of the tablet equivalent to about 5 mg of spironolactone was accurately weighed and dissolved in 25 ml of methanol giving concentrations of 3.0, 5.0 and 7.0 µg/ml. To each of these solutions 5 ml of anthrone reagent was added and the volume was made upto 10 ml with methanol. The solutions were allowed to stand for one hour until the chromogenic species was formed. The rest of the procedure was similar to as described for the standard preparations. The results obtained from the assays are summarised in the table 60-62 under results.

RECOVERY PROCEDURE

In order to establish the suitability of this method, known amounts of the pure drug was added to previously analysed formulation and the mixture was again analysed by the assay method. The difference between the two absorbance readings was noted which showed the quantity of the drug added. It was calculated from the standard curve and is reported under results.

RESULTS

Table 60

Calibration curve for Spironolactone

Concentration (µg/ml)	Trial 1	Trial 2	Trial 3	Mean	SD*	CV**
2	0.096	0.095	0.094	0.095	0.0010	1.0528
4	0.197	0.194	0.194	0.195	0.0017	0.8869
6	0.298	0.294	0.296	0.296	0.0020	0.6764
8	0.396	0.396	0.393	0.395	0.0017	0.4370
10	0.494	0.493	0.495	0.494	0.0010	0.2067

*Standard deviation

**Coefficient of variation (%)

Covariance = 0.6653335

Correlation coefficient = 0.9999924

Regression = 0.999985

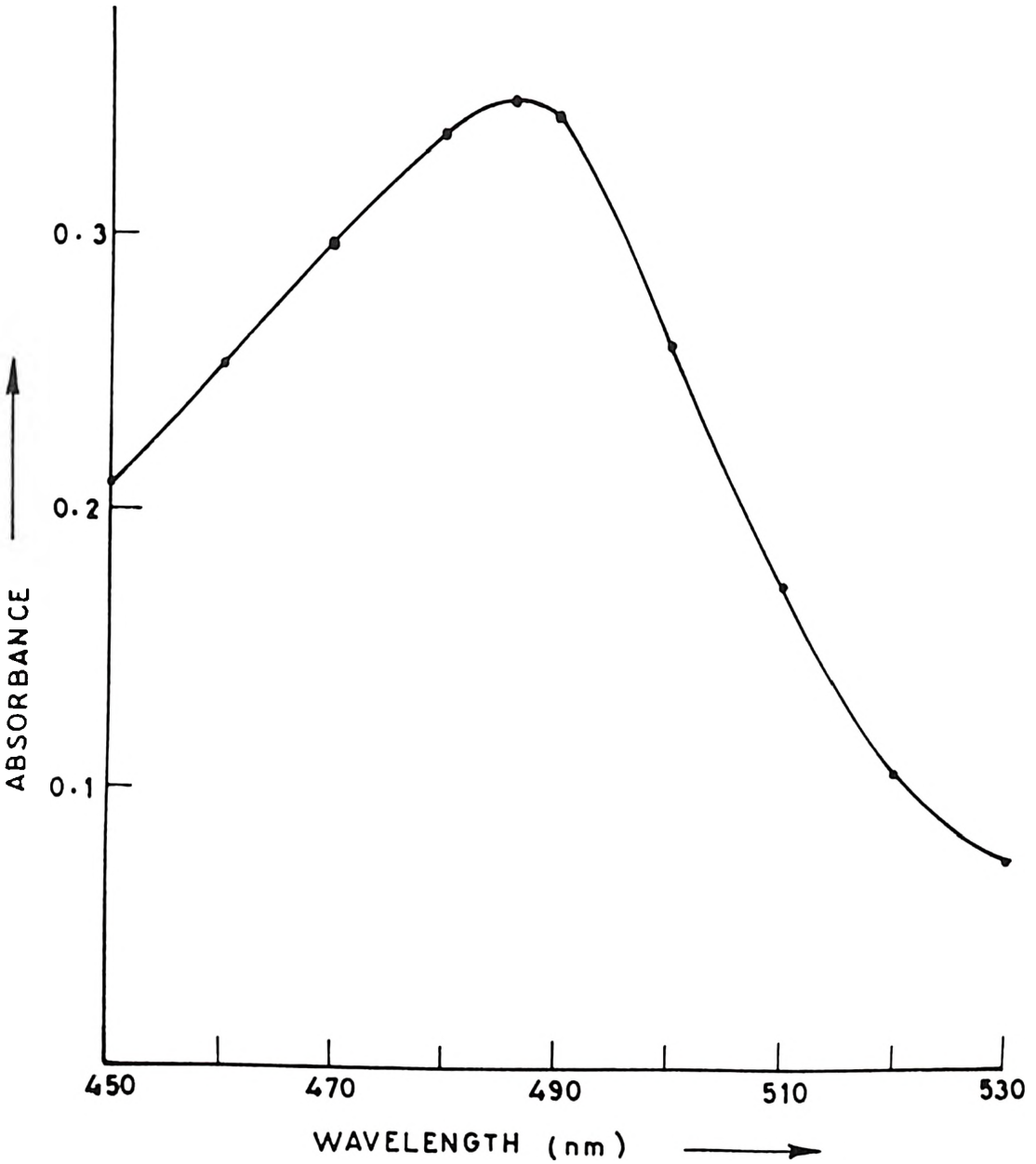


Fig. 33 ABSORPTION SPECTRUM FOR SPIRONOLACTONE

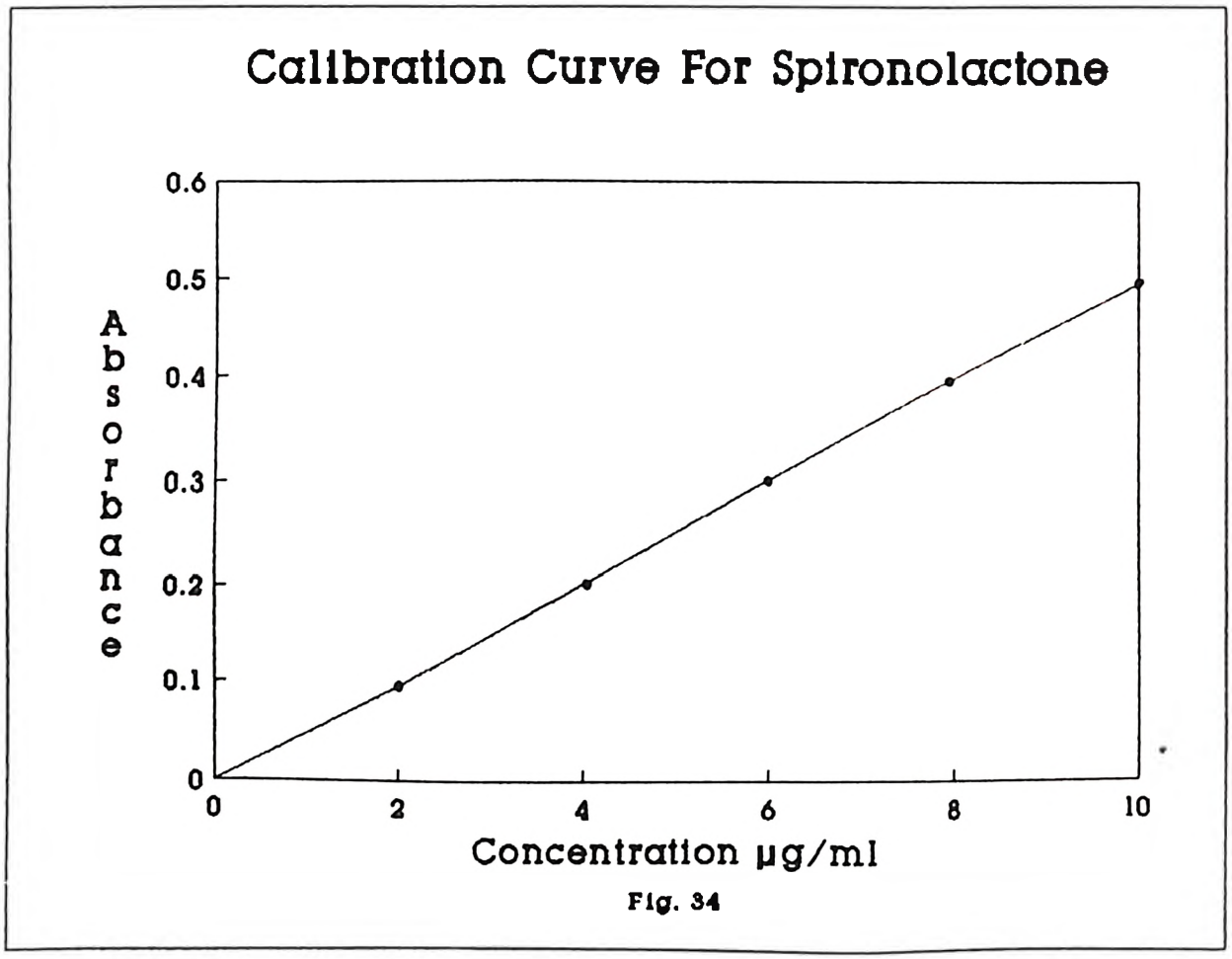

Calibration Curve For Spironolactone
Absorbance
0.6
0.5
0.4
0.3
0.2
0.1
0
0
2
4
6
8
10
Concentration µg/ml

Fig. 34

Table 61

Recovery data for Spironolactone in drug formulation

Commercial formulation

Each tablet contains

Spironolactone IP = 25 mg

Formulation	Trial No.	Absorbance	Concentration from std graph (µg/ml)	Amount found (mg)	% Recovery
Brand A					
Spironolactone	1	0.300	6.08	25.30	101.20
	2	0.199	4.08	25.17	100.68
	3	0.298	6.04	24.80	99.20
	4	0.305	6.18	25.26	101.04
	5	0.203	4.16	25.09	100.36

Average result

$$\frac{25.30 + 25.17 + 24.80 + 25.26 + 25.09}{5} = 25.12 \text{ mg (23.75-26.25 mg)}$$

% Purity

$$\frac{25.12}{25.00} \times 100 = 100.48\ \%$$

Table 62

Statistical Analysis

Formulation	Label claim (mg)	Amount Found* (mg)	SD**	CV***	t-test
Brand A					
Spironolactone	25.00	25.12	0.1986	0.7903	282.923

* Average of five determinations

** Standard deviation

*** Coefficient of variation (%)

DISCUSSION

A new colorimetric assay procedure has been developed for the quantitative analysis of spironolactone. The method is based on the chemical reaction with 0.05 % anthrone reagent in Conc.H_2SO_4 to produce a greenish yellow coloured chromogenic species and was stable until the solutions are shaken before use. The Beers-Lambert law was adhered over a range from 2.0-10.0 µg/ml. The absorption maximum was found to be 486 nm. The recovery studies performed on the tablet preparations in the concentrations range examined were reproducible. As the method is easy to perform, precise and is reproducible, it will serve as an alternative method of estimation for spironolactone.The spectral absorption curves which are produced by the interaction of anthrone with spironolactone or the free alcohol show maxima at 486 and 415 nm. Anthrone itself absorbs considerably at 415 nm and does not interfere with the absorption spectra of spironolactone employing the interaction of the steroid with the anthrone reagent. Concentration varing from 2-10 µg/ml of spironolactone may be quantitatively determined by the method described. Spironolactone produces a chromogen when allowed to react with the anthrone reagent. No chromogen formation was obtained when using sulphuric acid alone. The production of the colour when spironolactone is treated with the anthrone reagent is the basis for the method presented.

(4.8)

FLOURIMETRIC DETERMINATION OF SPIRONOLACTONE IN PRESENCE OF FRUSEMIDE AND HYDROFLUMETHIAZIDE IN DRUG FORMULATIONS

Instrument : Jasco model FP-777 spectrofluorimeter

PROCEDURE

STANDARD CURVE

FOR SPIRONOLACTONE

A stock solution of pure spironolactone was prepared by dissolving 5 mg of the pure drug in 50 ml of absolute alcohol giving a concentration of 100 µg/ml.Solutions of 1.0, 2.0, 3.0, 4.0 and 5.0 ml were transferred from the stock solution into five test-tubes and were diluted to 6ml with absolute alcohol. An equal amount (6ml) of 62% H_2SO_4 was then added slowly with cooling into each of the test-tubes to give final concentrations of 8.33, 16.66, 25.0, 33.33 and 41.66 µg/ml respectively. All the five test-tubes were then allowed to stand for 1.0 hr for the completion of the reaction and production of green fluorescence. Each of these solutions were transferred into a 10 mm glass cell and their fluorescence intensities were measured. A blank determination consisting of 62% H_2SO_4 in absolute alcohol was also performed. The calibration curve is shown in fig.40 and Table 63 and statistical analysis in Table 64 under results.

FOR SPIRONOLACTONE AND HYDROFLUMETHIAZIDE

A stock solution of a mixture of pure spironolactone & hydroflumethiazide of concentration of 100 µg/ml was prepared by dissolving 5 mg each of the two drugs in 50ml of absolute alcohol. Solutions of 1.0, 2.0, 3.0, 4.0 and 5.0 ml from the stock solution were transferred into five test-tubes and diluted to 6ml with absolute alcohol. An equal amount of 62% H_2SO_4 was then slowly added with cooling to all the five test-tubes giving concentrations of 8.33, 16.66, 25.0, 33.33 and 41.66 µg/ml respectively. The solutions were allowed to stand at room temperature for 1.0 hour for the completion of the reaction and generation of green fluorescence. The rest of the procedure is same as above. The calibration curve is shown in Table 65 & Fig. 37 & statistical analysis in Table 66 under results.

FOR SPIRONOLACTONE AND FRUSEMIDE

A stock solution of a mixture of pure spironolactone and frusemide of concentration of 100 µg/ml and 40 µg/ml were prepared by dissolving 5 mg and 2 mg each of the two drugs in 50 ml of absolute alcohol. Solutions of 1.0, 2.0, 3.0, 4.0 and 5.0 ml from the stock were

transferred into five test-tubes and diluted to 6 ml with absolute alcohol. An equal amount of 62% H_2SO_4 was then slowly added with cooling to all test-tubes giving concentrations of 8.33, 16.66, 25.0, 33.33 and 41.66 µg/ml of spironolactone respectively.The rest of the procedure is similar to as described under spironolactone. The calibration curve is shown in Table 67 & Fig. 38 & statistical analysis in Table 68 under results.

ASSAY PREPARATION

Twenty tablets of different brands consisting of above combinations were weighed accurately and the average weight was calculated. The samples were ground to a fine powder and the quantity corresponding to the average tablet weight was transferred to a 50 ml volumetric flask and the powder was dissolved in absolute alcohol. The solution was filtered to obtain a clear solution. Further dilutions were performed to obtain a stock solution of 100 µg/ml for spironolactone from tablet preparations. From the stock solution, 3.0 ml was taken and diluted upto 6.0 ml with absolute alcohol and then 6.0 ml of 62% H_2SO_4 was slowly added with cooling to bring the final concentration to 25 µg/ml. This solution was transferred into a 10 mm glass cell and its fluorescence intensity measured similarly as done in standard solutions.The results of estimation are shown in table 69 & 70.

RECOVERY PROCEDURE

In order to establish the suitability of this method, known amounts of the pure drug was added to previously analysed formulation and the mixture was again analysed by the assay method. The difference between the two absorbance readings was noted which showed the quantity of the drug added. It was calculated from the standard curve and is reported under results.

RESULTS

Excitation = 324 nm

Emission = 530 nm

Response = 0.1 sec

PMT gain = Very low

Ex SBW = 10 nm

Em SBW = 10 nm

Table 63

Calibration curve for Spironolactone

Concentration (µg/ml)	Fluorescence intensity				
	Trial 1	Trial 2	Trial 3	Trial 4	Mean
8.33	66.48	63.40	65.20	64.20	64.82
16.66	140.20	142.30	140.10	142.60	141.30
25.00	185.50	187.10	186.30	185.90	186.20
33.33	257.40	258.70	257.60	259.10	258.20
41.66	317.30	320.10	319.60	317.00	318.50

Table 64
Statistical Analysis

Concentration (µg/ml)	Mean	SD*	CV**	t-test
8.33	064.82	1.3293	2.0507	97.5281
16.66	141.30	1.3366	0.9459	211.4344
25.00	186.20	0.6770	0.3636	550.0713
33.33	258.20	0.8229	0.3187	627.5744
41.66	318.50	1.5745	0.4944	404.5634

* Standard deviation

** Coefficient of variation (%)

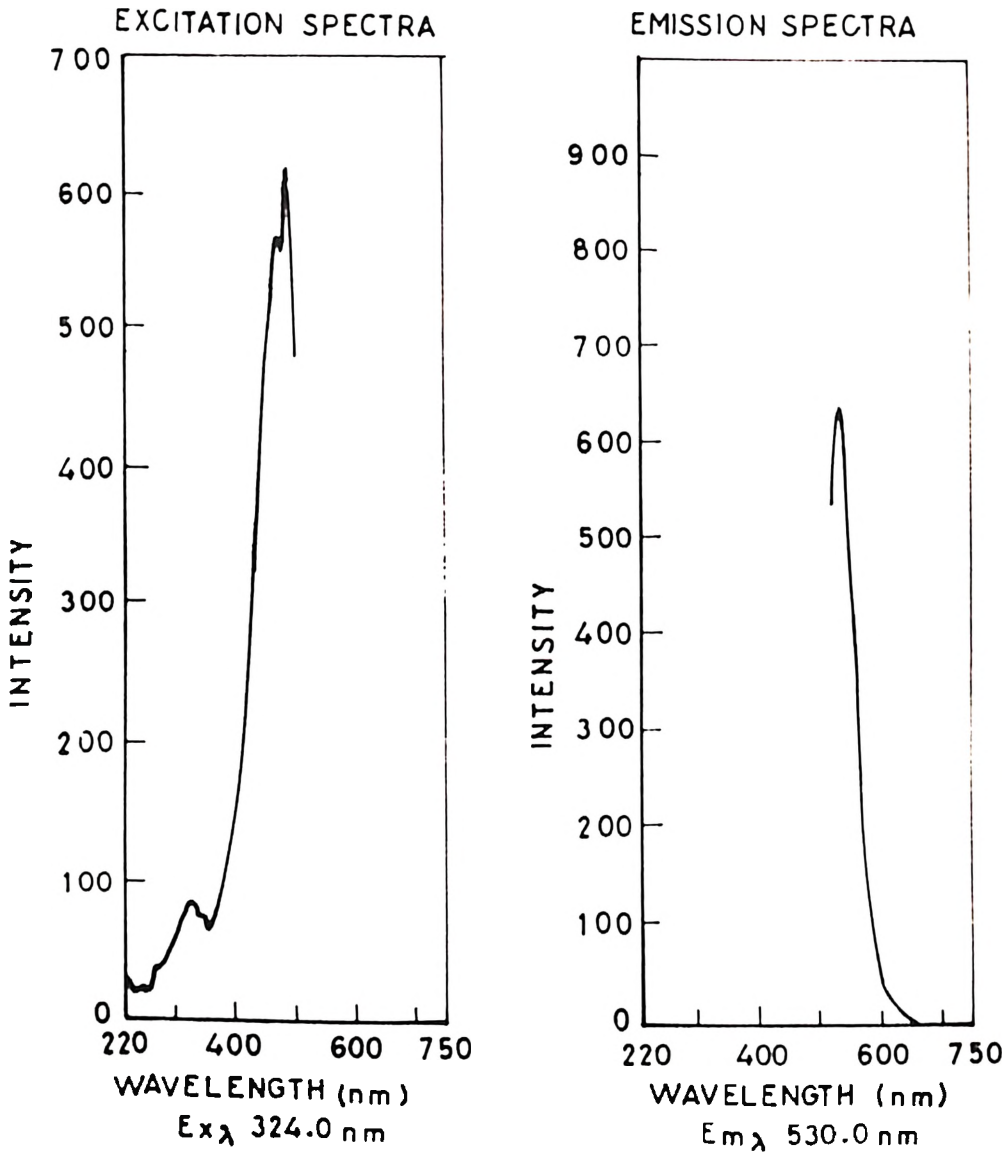


Fig.35 FLOURIMETRIC DETERMINATION

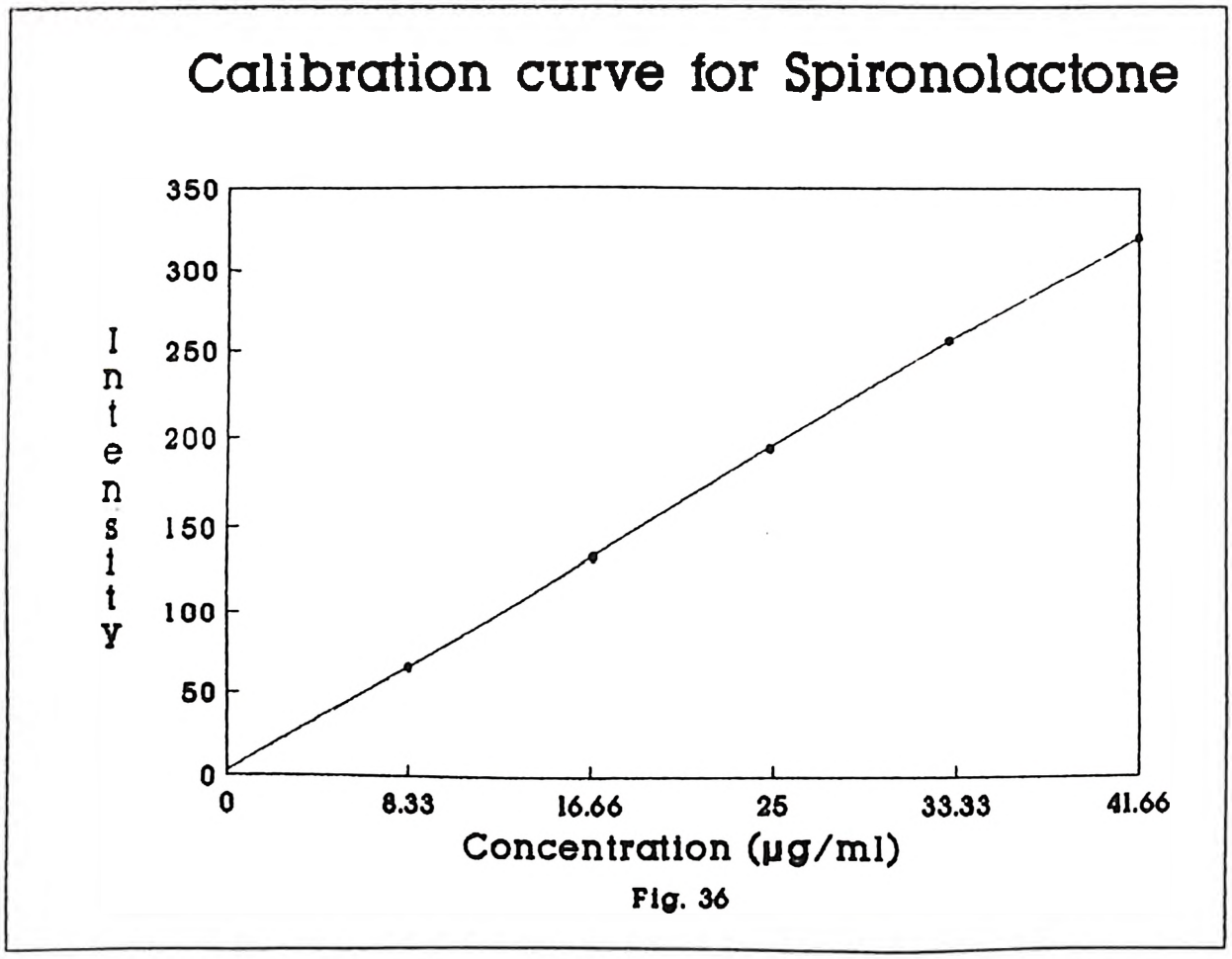

Calibration curve for Spironolactone
Intensity
350
300
250
200
150
100
50
0
0
8.33
16.66
25
33.33
41.66
Concentration (µg/ml)

Fig. 36

Covariance = 1733.968

Correlation coefficient = 0.9977224

Regression = 0.9954501

Table *65*

Calibration Curve for Spironolactone and containing Hydroflumethiazide

Concentration (µg/ml)	Fluorescence Intensity			
	Trial 1	Trial 2	Trial 3	Mean
8.33	57.60	57.20	56.20	57.00
16.66	119.20	120.20	120.60	120.00
25.00	181.30	180.10	179.20	180.20
33.33	244.80	244.70	242.50	244.00
41.66	298.60	297.10	298.30	298.00

Table 66

Statistical Analysis

Concentration (µg/ml)	Mean	SD*	CV**	t-test
8.33	57.00	1.0065	1.7601	113.6307
16.66	120.00	1.2108	1.0090	198.2087
25.00	180.20	0.9354	0.5191	385.2838
33.33	244.00	2.6091	1.0693	187.0392
41.66	298.00	2.2707	0.7619	262.4698

*Standard deviation

**Coefficient of variation (%)

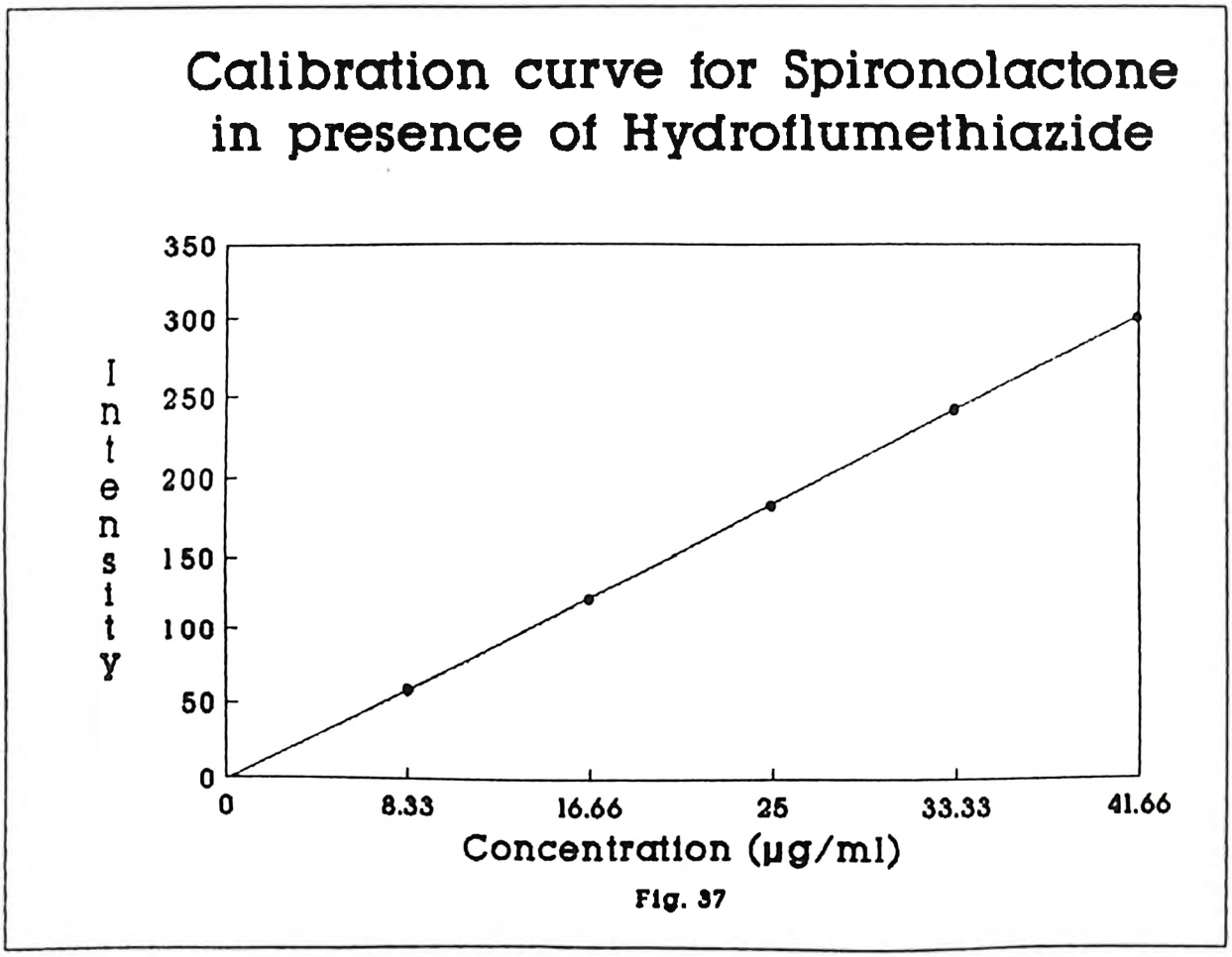

Calibration curve for Spironolactone in presence of Hydroflumethiazide
Intensity
350
300
250
200
150
100
50
0
0
8.33
16.66
25
33.33
41.66
Concentration (µg/ml)

Fig. 37

Covariance = 1688.27

Correlation coefficient = 0.999657

Regression = 0.9993148

Table 67

Calibration curve for Spironolactone containing Frusemide

Concentration (µg/ml)	Fluorescence intensity			
	Trial 1	Trial 2	Trial 3	Mean
8.33	53.60	53.80	52.60	53.00
16.66	111.60	111.80	112.60	112.00
25.00	170.60	170.20	171.60	171.00
33.33	225.10	226.40	226.50	226.00
41.66	284.50	285.30	287.10	285.30

Table 68

Statistical Analysis

Concentration (µg/ml)	Mean	SD*	CV**	t-test
8.33	53.00	1.0675	2.0142	99.2934
16.66	112.00	1.4515	1.2959	154.3261
25.00	171.00	1.2194	0.7131	280.4618
33.33	226.00	1.0232	0.4527	441.7648
41.66	285.30	1.5778	0.5530	361.6333

*Standard deviation

**Coefficient of variation (%)

Covariance = 1607.16

Correlation coefficient = 0.9999283

Regression = 0.9998566

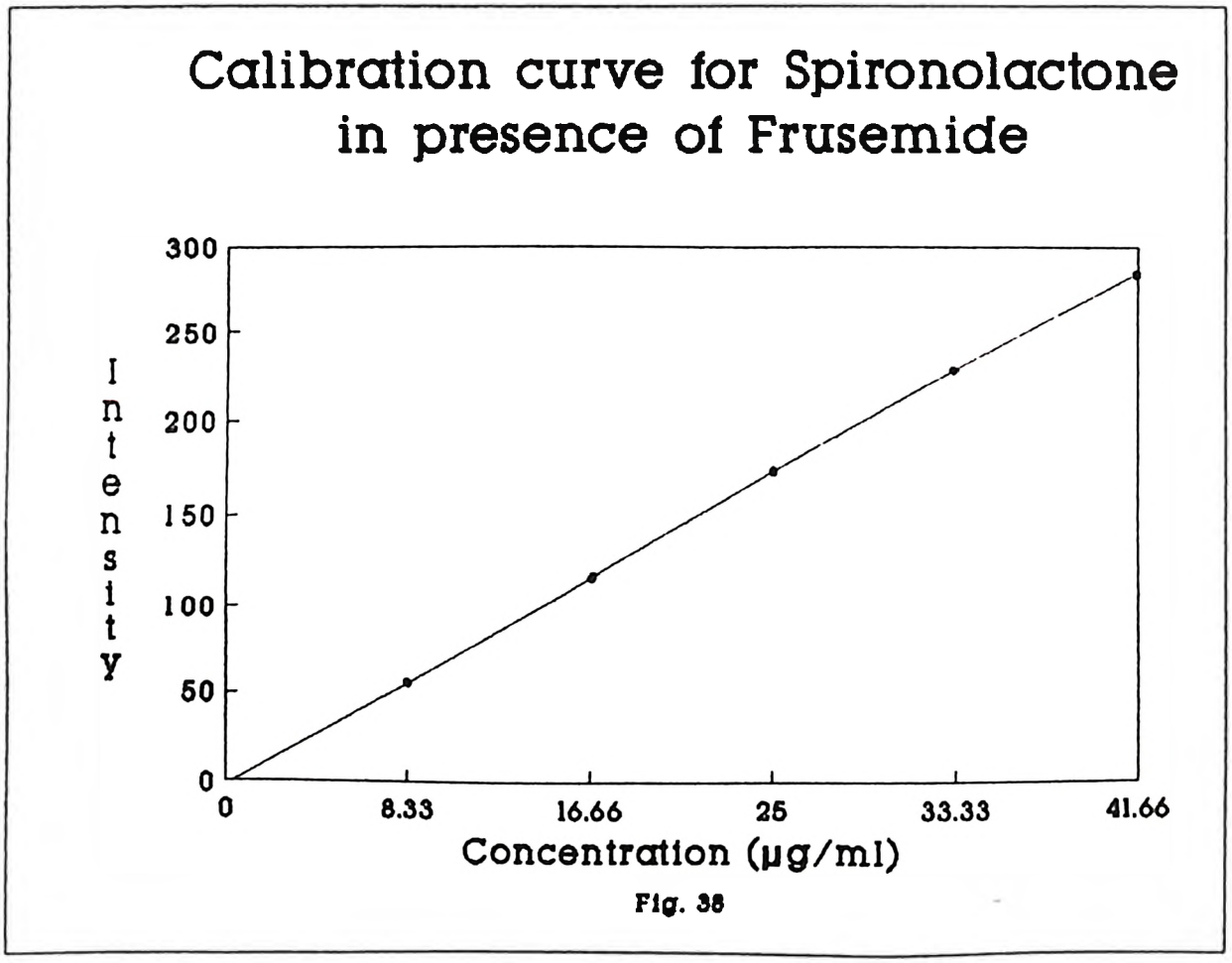

Calibration curve for Spironolactone in presence of Frusemide
Intensity
300
250
200
150
100
50
0
0
8.33
16.66
25
33.33
41.66
Concentration (µg/ml)

Fig. 38

Table 69

Recovery assay data for spironolactone in drug formulations

Formulation	Trial No.	Fluorescence intensity (µg/ml)	Concentration from std graph	Amount in each sample(mg)	% Recovery
Brand A					
Spironolactone	1	186.79	25.08	25.08	100.32
	2	191.63	25.73	25.73	102.92
	3	192.45	25.84	25.84	103.36
	4	193.49	25.98	25.98	103.92
Brand B					
Spironolactone	1	180.27	25.01	25.01	100.04
Hydroflumethiazide		-	-	-	-
	2	185.24	25.70	25.70	102.80
		-	-	-	-
	3	183.87	25.51	25.51	102.04
		-	-	-	-
	4	180.63	25.06	25.06	100.24
Brand C					
Spironolactone	1	165.93	24.26	48.52	097.04
Frusemide		-	-	-	-
	2	167.71	24.52	49.04	098.08
		-	-	-	-
	3	172.50	25.22	50.44	100.88
		-	-	-	-
	4	169.76	24.82	49.64	099.28
		-	-	-	-

Table 70

Statistical Analysis

Formulation	Label Claim (mg)	Amount found* (mg)	SD**	CV***	t-test
Brand A					
Spironolactone	25	25.40	0.4435	1.7457	114.5649
Brand B					
Spironolactone	25	25.32	0.3389	1.3383	149.4414
Hydroflumethiazide	25	-	-	-	-
Brand C					
Spironolactone	50	49.41	0.8252	1.6702	119.7498
Frusemide	20	-	-	-	-

*Average of four determinations

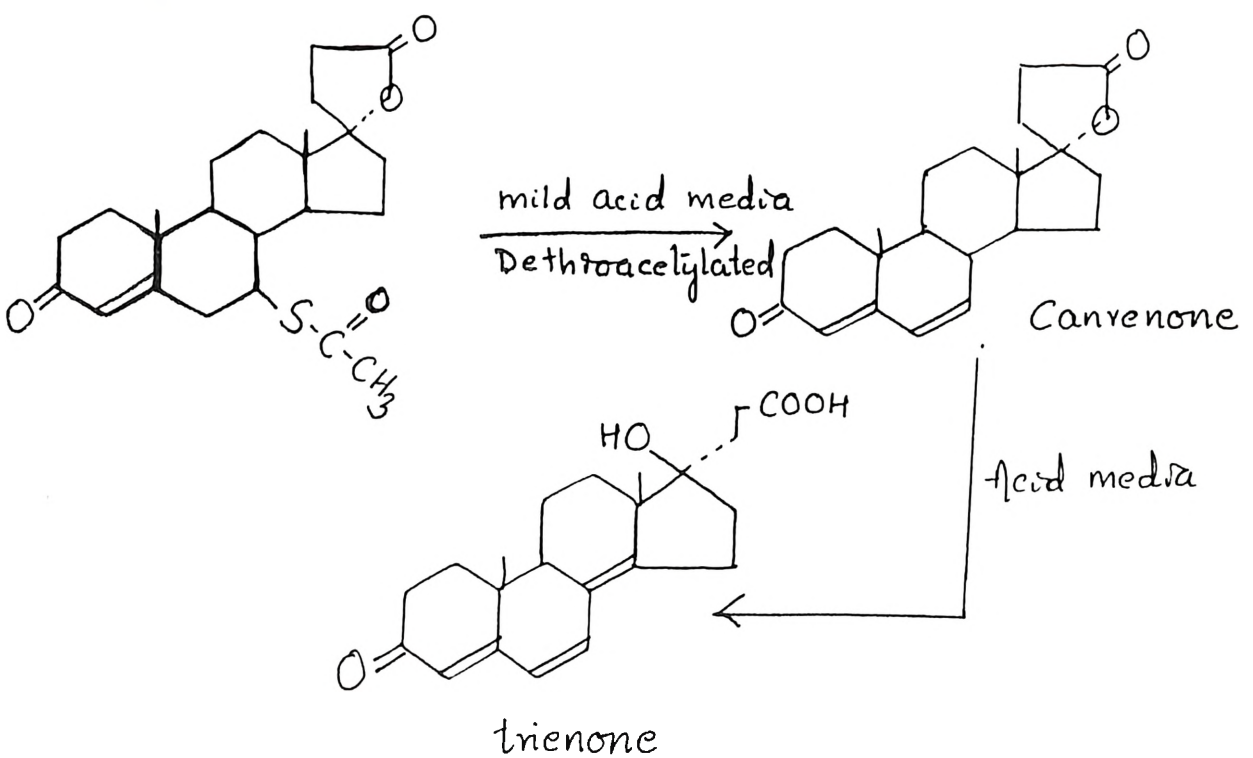
** Standard deviation

*** Coefficient of variation (%)

DISCUSSION

In our ongoing effort to develop new analytical procedures for drug substances, our attention was drawn to spironolactone and its combination formulation available in the market. While there was report in the previous literature of the presence of fluorescence in spironolactone observed during its metabolism studies, it became curious to examine this property and see if it could be utilised for drug combinations which has not been reported so far in the literature. Therefore our initial studies comprised the determinations of the excitation-emission spectra as well as limit of detection for spironolactone. The excitation spectra for spironolactone at wavelength of 324 nm and the emission spectra fluorescence at 530 nm. The fluorophore was obtained by irradiating an acidic methanolic solution of the drug with UV light. The linear relationship between fluorescence and concentration existed over the concentration range of 8.33-41.66 µg/ml. The fluorescence reaction of a broad variety of steroids in sulphuric acid is widely used for quantitative analysis.

Spironolactone yields an intense fluorescence in 62 % sulphuric acid. The limit of detection for spironolactone was found to be 8.33 µg/ml.Spironolactone may be dethioacetylated under mild acid\alkaline conditions yielding corresponding 4,6 dienone, canrenone which in 62 % sulphuric acid is converted to fluorescent trienone. The fluorescence is useful forquantitation of canrenone in plasma over a range of 210–260 µg/ml.



The above study was extended and fluorescence determinations were performed to see if any quenching took place in the presence of hydroflumethiazide under various concentrations including those in the amount of its combination. The study showed that no quenching of fluorescence of spironolactone took place in the presence of hydroflumethiazide. However, further investigation involved the addition of various amounts of frusemide to spironolactone and show that quenching of fluorescence due to spironolactone did occur in higher concentrations but when as the dilutions were performed to establish the detection limits, it was found that the presence of frusemide did not affect the fluorescence intensity of spironolactone.

In order to confirm the above studies it became imperative to see the effect of pH changes on the fluorescence of spironolactone. Our studies on variations of pH has shown that the maximum fluorescence for spironolactone is obtained at pH 1–2 while it decreases at higher pH.

The work proves that fluorimetric method can be easily adopted for the simultaneous determination of spironolactone in its drug combinations with hydroflumethiazide and frusemide with precision and without any interferences. The results of estimation were evaluated by the standard deviation and the coefficient of variation and the amounts of the spironolactone contained in the aforesaid formulation conformed to the label claim specifications. The standard graphs were found to be linear and obeyed Lambert-Beer's law.

CHAPTER-5

CONCLUSIONS AND SUMMARY

The use and misuse of drugs for various purpose has been on the increase and this requires a constant updating in the methods of analysis. These methods may be used in quality assurance during the process of manufacturing and formulating drugs or in the analysis of drugs in forensic and clinical samples. While official pharmacopoeial methods have been described to cater to the former, forensic and clinical analysis requires a constant updating in the techniques in order to match the ingenuity of the criminal. The pharmaceutical methods have come to be supplemented by other alternative methods due to the complexity of problems encountered. Therefore,there is a constant need for the development of methods which are simple, sensitive, inexpensive, rapid and specific. The methods have to be precise, accurate and reproducible in order to be analytically viable.

Since the official methods of analysis are not available for drug preparations containing two or more constituents simultaneously,the methods developed in this study will serve as new and alternative method of analysis. In the present study, new analytical methods were developed by using uv-visible spectrophotometry, flourimetry, thin layer chromatography, high performance thin layer chromatography and liquid chromatography. The new methods developed in this research work are stated below :

(a) Chromatographic techniques performed for the determinations of drug formulations containing :

(i) Oxyphenbutazone and Ibuprofen in presence of Paracetamol and Dextropropoxyphene hydrochloride. The method involved the determination of

(a) oxyphenbutazone, paracetamol and dextropropoxyphene hydrochloride and
(b) ibuprofen, dextropropoxyphene hydrochloride and paracetamol in combination.

Since there were no methods of analysis available for these drug combinations containing these constituents simultaneously, the proposed method has been developed which is precise and reproducible.

(ii) Paracetamol, Dicycloamine hydrochloride and Dextropropoxyphene hydrochloride.

The method comprised of separating the three constituents by TLC technique, scraping, extraction and measuring the absorption in the UV and visible region. As dicycloamine hydrochloride can not be determined in UV region . Hence spectrophotometry in UV region and titrimetry were not tried.

(iii) Ketoprofen and Propyphenazone.

Methods like quatitative thin layer chromatography, high performance thin layer chromatography, high performance liquid chromatography and spectrophotometry have been attempted, in the analysis of propyphenazone and ketoprofen. Spectrophotometric method was not successful since absorption maxima were found to be very close for both the drugs. Although literature is available for the estimation of propyphenazone and ketoprofen as individual drugs but no work had been reported for their analysis in combination. In view of convenience required for routine analysis, rapid thin layer chromatography, high performance thin layer chromatography and high performance liquid chromatography methods were developed in this study for their simultaneous determination.

(b) A simple, sensitive and selective new analytical method for the simultaneous determination of paracetamol and mefenamic acid by UV-absorption spectroscopy was developed. It will serve as the most useful method of analysis reported for the first time in this study.

(c) A sensitive and selective method for the analysis of a combination containing paracetamol and diclofenac sodium was established. Diclofenac sodium was converted to free acid and estimated by alkalimetry while paracetamol was converted to free amine, estimated by diazotisation method in a selective and simultaneous manner.

(d) For the first time in this study a simple and sensitive fluorimetric procedure for the analysis of spironolactone in presence of hydroflumethiazide and frusemide in the form of combination preparations has been established.

(e) A new analytical procedure for the simultaneous determination of spironolactone in combination with frusemide and spironolactone in combination with hydroflumethiazide by UV-Absorption spectroscopy has been worked out in this study. As no official pharmacopoeial method was available for their simultaneous estimation, for the first time a UV absorption method was establised which was found to be free from interferences.

(f) A simple spectrophotometric method was also developed for the analysis of spironolactone in single dosage form. A unique and a selective coupling agent has been utilised to react with the steroidal compound to generate a stable chromophore which was measured. The method is quick, precise and reproducible.

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LIST OF PUBLICATIONS

Published / Accepted / Communicated

1. Estimation of Oxyphenbutazone and Ibuprofen in presence of Paracetamol and Dextropropoxyphene hydrochloride in dosage forms by Quantitative Thin Layer Chromatography.

   Indian Drugs 1994,31(4), 139-143

2. Determination of Paracetamol,Dextropropoxyphene hydrochloride and Dicycloamine hydrochloride in pharmaceutical formulations by Quantitative Thin Layer Chromatography.

   Indian Drugs 1994,31(5), 211-214

3. Determination of Propyphenazone and Ketoprofen by Quantitative Thin Layer Chromato -graphy and High Performance Thin Layer Chromatography.

   Indian J. Pharmaceutical Sciences 1995, 57(1), 31-34

4. Fluorimetric determination of Spironolactone in presence of Frusemide and Hydroflumethia-zide in drug formulations.

   (Accepted in Indian Drugs)

5. Simultaneous determinations of Spironolactone with Frusemide and Spironolactone with Hydroflumethiazide in combination preparations using UV-Absorption Spectroscopy.

   Indian J. Pharmaceutical Sciences, 1995, 57(3), 126-129.

6. Simultaneous determination of Mefenamic Acid and Paracetamol by UV-Absorption Spectroscopy.

   (Accepted in Indian Drugs)

7. Simultaneous and selective determination of Paracetamol and Diclofenac sodium in dosage forms by Titrimetry.

   (Accepted in Indian Drugs)

8. Determination of Spironolactone by Colourimetry.

   Eastern Pharmacist, 1995 (May), Vol XXXVIII, 449, 137-138.

9. Determination of Propyphenazone and Ketoprofen in dosage forms by High Performance Liquid Chromatography.

   (Communicated to J. Chromat. & Biomedical analysis)

Other Work

1. Hydrogen bonding studies in Menthol and Methyl Salicylate in drug formulations by Vibrational Spectroscopy.

   (Accepted in Asian Journal of Chemistry)

2. Determination of Paracetamol, Pseudoephedrine hydrochloride, and Chlorpheniramine maleate in dosage forms by Quantitative Thin Layer Chromatography.

   (Communicated to Indian Drugs)

3. Simultaneous determination of Nandrolone phenyl propionate and Desoxycortone phenyl propionate in drug formulations by Quantitative Thin Layer Chromatography and High Performance Thin Layer Chromatography.

   (Communicated to Indian Drugs)

4. Two simple spectrophotometric methods for the determination of citrizine hydrochloride in dosage forms.

   (Under preparation)