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FACULTY OF PHARMACY AND PHARMACEUTICAL SCIENCES

DEPARTMENT OF PHARMACEUTICAL CHEMISTRY

THE USE OF SURROGATE REFERENCE STANDARD IN QUANTITATIVE HPLC: A CASE STUDY OF ALBENDAZOLE AND QUININE SULPHATE TABLETS

BY

JAMES WORLANYO OBIMPEH

A THESIS SUBMITTED IN PARTIAL FULLFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF

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OF SURROGATE REFERENCE STANDARDS

QUANTITATIVE HPLC

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MASTER OF PHILOSOPHY

In the

Department of Pharmaceutical Chemistry,

Faculty of Pharmacy and Pharmaceutical Sciences (KNUST)

By

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OCTOBER, 2014

DECLARATION

I James Worlanyo Obimpeh, declare that this work was wholly done by me at the Department of Pharmaceutical Chemistry, KNUST, and any reference material consulted has been duly acknowledged. This work has not been submitted anywhere for the award of any degree.

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DEDICATION

I dedicate this work to my loving wife Mrs. Sylvia Ethel Obimpeh and my mother Mad. Margaret Briku.

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ABSTRACT

A simple, fast and a sensitive Reverse-phase HPLC method was developed for the quantitative analysis of **Albendazole** and **Quinine Sulphate** tablets using Aspirin, Amoxicillin Trihydrate and Metronidazole as surrogate reference standards.

For the assay of Albendazole tablets, a C-18 waters 4.6 x 150mm column (stationary phase) and a mobile phase made of Methanol-Water (60:40 ^{V}/v) at pH of 5.40±0.02 was used. The analysis was carried out at 245nm and flow rate of 1ml/min. The mean retention time of Albendazole, Aspirin, Amoxicillin Trihydrate and Metronidazole were; **4.21±0.036min, 1.74±0.021min, 2.53±0.018min and 2.28±0.027min** respectively. When Aspirin was used as a surrogate reference standard, the constant 'K' obtained was **2.297±0.004** and the average percentage content of Albendazole in the Alben, Wormplex 400 and the Zeben tablets after five determinations were; **98.22±0.66%, 101.63±0.587% and 98.82±0.535%** respectively. When Amoxicillin Trihydrate was used as a surrogate reference standard, the 'K' value obtained was **2.693±0.009** and the average percentage content of Albendazole in the Alben, Wormplex 400 and the Zeben tablets after five determinations were**; 99.55±0.675%, 101.02±0.639%** and **99.79±0.824%** respectively. When Metronidazole was used as a surrogate reference standard, the 'K' obtained was **1.827±0.003** and the average percentage content of Albendazole in the Alben, Wormplex 400 and Zeben tablets were **99.37±0.84, 101.13±0.500** and **99.82±0.577** respectively.

A C-18 waters 4.6 x 150mm column, mobile phase combination of Methanol-Water (60:40 ^{V}/v) with the pH adjusted to 5.20±0.02 were employed for the analysis of Quinine Sulphate tablets, wavelength of detection was 245nm, and flow rate was 1.5ml/min. Quinine Sulphate, Aspirin, Amoxicillin Trihydrate and Metronidazole had average retention time **of**

6.65±0.015min, 2.78±0.03min, 3.02±0.017min and **2.02±0.007** min respectively. When Aspirin was used as a surrogate reference standard, the 'K' value obtained was **2.549±0.007** and the percentage content of Quinine Sulphate in the Actavis, Co-Pharma and the Wockhardt tablets were; **98.57±0.89%, 102.74±0.64%** and **98.18±0.75%** respectively. When Amoxicillin Trihydrate was used as a surrogate reference standard, the 'K' value obtained was **3.893±0.008** and the average percentage content of Quinine Sulphate in the Actavis, Co-Pharma and the Wockhardt tablets were **99.99±0.43%, 101.84±0.44%** and **97.004±0.62%** respectively. When Metronidazole was used as a surrogate reference standard, the 'K' value obtained was 1.034±0.006 and the average percentage content of Quinine Sulphate in the Actavis, Co-Pharma and the Wockhardt tablets were **99.87±0.42%, 103.22±0.38%** and **100.17±0.399%** respectively.

The results obtained from the developed method were statistically comparable to results obtained from the standard methods. The developed method showed good precision, was robust and linear within the working range, thus making the developed methods very suitable for quantification of Albendazole and Quinine Sulphate tablets.

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LIST OF ABBREVATIONS

HPLC: High Performance Liquid Chromatography.

UV: Ultra Violet.

ALB: Albendazole.

ASP: Aspirin.

QS: Quinine Sulphate.

AMOX: Amoxicillin Trihydrate.

BP: British Pharmacopoeia.

USP: United States Pharmacopoeia

CHAPTER ONE

1.1 INTRODUCTION

The quality of a drug incorporates its safety, efficacy, efficiency and its economic value. These parameters are examined based on the principles of quality assurance and quality control. The principles of quality assurance is the totality of the arrangements made with the objective of ensuring that pharmaceutical products are consistent with the quality appropriate to their intended use (1).

Quality control is that part of Good manufacturing practice concerned with sampling, specifications, and testing and with the organization, documentation and release procedures which ensures that the necessary and relevant tests are carried out and materials are not released for use or products release for sale or supply, until their quality has been judged to be satisfactory (1; 2).

Specifications are a set of properly selected standards with associated methods of analysis that may be used to assess the integrity of drugs and raw materials (1). Specifications can also be defined as a list of test and analytical procedures with proposed acceptance criteria (3).

The current Good Manufacturing Practice regulation requires that test methods which are used for assessing compliance of pharmaceutical articles with established specifications must meet proper standards of accuracy and reliability through validation (3).

Validation refers to established documented evidence that a process or a system when operated within established parameters can perform effectively and reproducibly to produce a pre-determined specification and quality attributes. Some validation parameters are accuracy, precision, reproducibility, reliability, simplicity, robustness, selectively (specificity) and

sensitivity. Validation studies are essential part of Good Manufacturing Practices (GMP) and should be conducted in accordance .with pre-defined protocols. The important steps involved in setting up a validation program are; determination of the critical variables, establishing acceptable ranges, and continuous control of variables. The process of validation is obtained through collection and evaluation of data (3; 1).

High Performance Liquid Chromatography (HPLC) is a mode of chromatography that is employed as an analytical technique in the chemical laboratory. Chromatographic process is defined as a separation technique involving mass-transfer between stationary and a mobile phase (4).

HPLC utilizes a liquid mobile phase to separate the components of a mixture. The stationary phase can be a liquid or a solid. The components are first dissolved in a solvent and then forced through a chromatographic column under high pressure. In the column, the mixture separates into its components. The amount of resolution is important and is dependent on the extent of interaction between the solute components and the stationary phase.

The interaction of the solute with the mobile and the stationary phases can be manipulated through different choices of solvents and stationary phases. As a result HPLC acquires a degree of versatility than the other chromatographic modes. Also HPLC has the ability to easily separate wide variety of chemical mixtures (5).

HPLC has been used in various analyses such as pharmaceutical products, food, cosmetics, environmental matrices forensic samples and industrial chemicals.

In the pharmaceutical industry, HPLC based techniques are well accepted. It is a powerful technology that allows complex mixtures to be transformed into separated components. HPLC

as compared with the classical liquid chromatographic technique is characterized by; high resolution, small diameter, stainless steel, glass or titanium columns with very small particles (3. 5 and 10 μm).It has a relatively high inlet pressure, controlled flow of the mobile phase, continuous flow detector capable of handling small flow rates and detecting very small amounts as well as rapid analysis (6).

There are many ways to classify liquid column chromatography. If the classification is based on the nature of the stationary phase and the separation process, then three modes can be specified. These are; Adsorption chromatography, Ion-exchange chromatography, and Size exclusion chromatography.

In adsorption chromatography, the stationary phase is an adsorbent such as silica gel or any other silica based packing .The separation is based on repeated adsorption-desorption steps

In Ion-exchange chromatography, the stationary bed has an ionic charged surface of opposite charge to the sample ions. This technique is used to analyze or separate ionic or ionizable samples. The greater the charge on the sample, the stronger the attraction to the ionic surface and thus the longer it will take to elute. The mobile phase is an aqueous buffer where both pH and ionic strength are used to control the elution.

In size exclusion chromatography, the column is filled with materials having precisely controlled pore sizes and the sample is simply screened or filtered according to its solvated molecular size. Larger molecules are rapidly washed through the column, whiles smaller molecules penetrate the pores of the packing and later elutes. This technique is also called gel-permeation or gel-filtration (1).

Adsorption chromatography is classified into two types, these are; Normal-phase and the Reversed-phase chromatography.

The Normal-phase chromatography uses a strongly polar stationary (e.g. Silica), and a non-polar mobile phase (e.g. hexane). Polar samples are thus retained on the polar surface of the column packing longer than less polar materials.

In the Reversed-phase chromatography, the stationary phase is non-polar in nature whilst the mobile phase is a polar liquid (e.g. a mixture of water, methanol and or acetonitrile). In this, non-polar substances are retained longer. Eluent polarity thus plays a major role in all types of HPLC. There are two elution types. These are; Isocratic elution and Gradient elution. In the isocratic elution, only one mobile phase composition is pumped through the column during the entire analysis. Gradient elution on the other hand employs more than one solvent composition during an analysis (5).

Though several solvents are employed in HPLC analysis, there are certain common properties such as; purity, detector compatibility, ability to dissolve the sample completely, low viscosity and chemical inertness which a solvent must possessed to be used in HPLC analysis (7).

The HPLC separation and analysis of samples is achieved by injecting the sample (in solution) into a stream of mobile phase being pumped into a column packed with a solid separating material. The interaction is a liquid-solid separation. The separation occurs when a mixture of compounds dissolved in a solvent can either stay in the solvent or adhere to the packing material in the column.

The detector measures the concentration of sample bands as they leave the column and pass through the detector flow cell. When no band passes through the detector, a constant signal

called the Baseline of the chromatogram is recorded. When a sample band reaches the detector, the detector responds to the difference in the mobile phase properties caused by the presence of the sample compound. This gives rise to a change in detector signal, seen as a peak. The result is that, components with the highest affinity for the column packing adhere more strongly and is washed out last. This differential washout or elution of compounds is the basis for the use of HPLC in separation. The peak data can be used either to quantitate with standard calibration, the amount of each material present or to control the collection of purified material in a fraction.

1.2 JUSTIFICATION

There are two approaches to the application of UV in quantification of pharmaceutical products. One of the approaches is to make a five point calibration curve for the reference standard, determine the absorbance of the test sample (analyte) and then deduce the concentration of the of the test sample from the calibration curve. The second approach is to determine the Specific Absorbance, A (1%, 1cm) of the pure reference substance from literature. The absorbance of the test sample (analyte) is measured and its percentage content calculated. It is obvious the use of the A (1%, 1cm) decreases the volume of data and consequently the time required to analyze a sample.

Likewise in quantitative pharmaceutical analysis of drugs using HPLC, a pure reference standard of the analyte is needed to help in preparing controls and calibration curves for the analyses. This approach is time consuming.

Most often, the reference standards are not available locally, and are also very expensive. The relative cost of **Albendazole** and **Quinine Sulphate**, reference standards are detailed in the table below.

Table 1.1: Cost of drug samples

Drug	Quantity (mg)	Price ($)	Catalogue No
Albendazole	200	215	HIK275
Quinine Sulphate	500	215	JOK293

Source: USP daily reference catalog

Importation of reference standards into Ghana for use by institutions, industry and regulatory agencies usually takes between three to six months, thus making it difficult for routine analysis to be carried out successfully within time. In view of these challenges, it has become very essential to research into the possibility of using compounds other than the pure compound as a reference standard. Substances that are physic-chemically related were selected to be used as a surrogate for this investigation. Research has been carried out for Prednisolone, Diazepam, Indometacin, Paracetamol, Aspirin, Diclofenac Sodium, Metformin Hydrochloride, Chlorpheniramine Maleate, Piroxicam, Naproxen, Promethazine Hydrochloride, Ciprofloxacin, Cetirizine Hydrochloride and Glybenaclamide have been successfully completed at the Department of Pharmaceutical Chemistry, KNUST. This research therefore seeks to extend the search for appropriate surrogate reference standards by using the analysis of **Albendazole** and **Quinine Sulphate** as a case study.

1.3 MAIN OBJECTIVE

This project seeks to investigate the possibility of using various compounds as surrogate reference standards for the analysis of **Albendazole** tablets and **Quinine Sulphate** tablets in quantitative HPLC analysis.

1.4 SPECIFIC OBJECTIVES

The specific objectives of this research included;

1. To establish conditions for an HPLC assay procedure for Albendazole tablets and Quinine Sulphate tablets using surrogate reference compounds.
2. To validate the method developed by using validation parameters such as Specificity, Selectivity, Linearity, Precision, Accuracy, Limit of detection (LOD), Limit of quantification (LOQ)
3. To determine a constant, K that can effectively be used for quantitative analysis.
4. To determine the percentage content of Albendazole and Quinine Sulphate in their various tablet forms for different brands using the method developed.
5. To compare the results obtained from the method developed with a standard method in the British Pharmacopoeia and the United States Pharmacopoeia.

1.5 HYPOTHESIS OF STUDY

The UV detectors used in HPLC generate electrical signals whose magnitude is determined by the concentration of the analyte. Consequently, the area under a peak 'A' is proportional to the concentration 'C' of the analyte injected.

This implies;

$$A \alpha C,$$

Introducing a constant, X

$$A = XC$$

Therefore, $X = A/C$

For the same compound, the constant X remains constant.

That is;

$$(A_{analyte} / C_{analyte}) = (A_{standard} / C_{standard})$$

Where, the standard is the pure form of the analyte.

In using surrogate compounds (substances) as a standard

$$(A_{analyte} / C_{analyte}) \neq (A_{standard} / C_{standard})$$

Introducing a constant, K

$$(A_{analyte} / C_{analyte}) = K (A_{standard} / C_{standard}) \quad (8).$$

Where, K is a constant that is dependent upon the nature of the surrogate compound.

$A_{analyte}$ is the peak area of the analyte.

$A_{standard}$ is the peak area of the standard

$C_{standard}$ is the concentration of the standard.

$C_{analyte}$ is the concentration of the analyte.

Once K is obtained from the developed method, the $A_{analyte}$ and $A_{standard}$ are deduced from the chromatograph, $C_{analyte}$ can be calculated.

$$C_{analyte} = (A_{analyte} \times C_{standard}) / (A_{standard} \times K)$$

Percentage content = (Actual concentration/ Nominal concentration) X 100%.

CHAPTER TWO

LITERATURE REVIEW

2.1 PROFILE OF DRUG SUBSTANCES

2.1.1. Albendazole

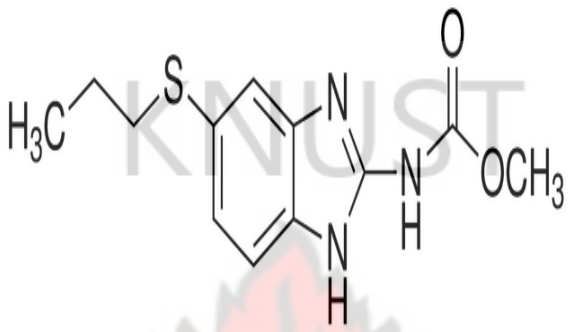

Fig 2.1: Chemical Structure of Albendazole

Albendazole is a white or almost white powder. It has an IUPAC name of Methyl-5-(propylthio)-2-benzimidazole carbamate. It has a molecular weight of 265.3 g/mol. Albendazole is an Anthelminthic and melts at around 210^{o}C with decomposition. It is practically insoluble in water, but soluble in glacial acetic acid, slightly soluble in acetone and very slightly soluble in ethanol. Its chemical formular is $C_{12}H_{15}N_3O_2S$ (9).

Albendazole is used to treat neurocysticercosis (infection caused by the pork tapeworm in the muscles, brain, and eyes that may cause seizures, brain swelling, and vision problems). Albendazole is also used along with surgery to treat cystic hydatid disease (infection caused by the dog tapeworm in the liver, lung, and lining of the abdomen that may damage these organs). Albendazole is in a class of medications called anthelmintic (10).

2.1.2 Quinine Sulphate

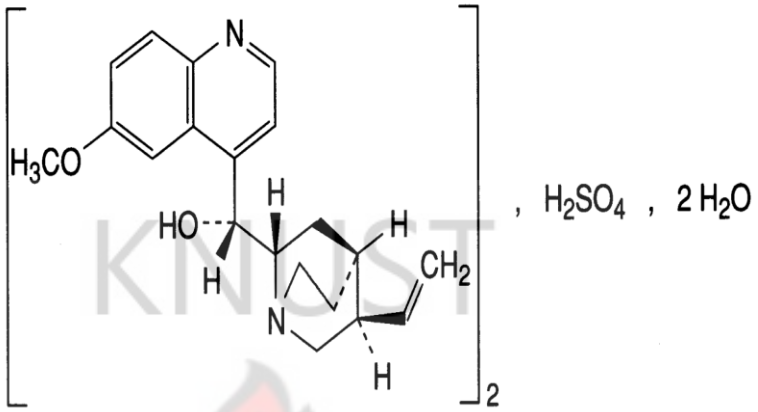

Fig 2.2: Chemical Structure of Quinine Sulphate

Quinine Sulphate is a white or almost white crystalline powder or fine, colorless needles, slightly soluble in water, sparingly soluble in boiling water and in alcohol. Its IUPAC name is [(R)-[(2S, 4S, 5R)-5-ethenyl-1-azabicyclo [2.2.2] oct-2-yl] (6-methoxyquinolin-4-yl) methanol] Sulphate. It has a chemical formular of $(C_{20}H_{24}N_2O_2)_2$, H_2SO_4, H_2O, and a molecular weight of 783g/mol (9).

Quinine Sulphate is a type of medicine called an antimalarial. Its main use is in treating established malarial infections, though it can also be used for treating leg cramps. Quinine works by attacking the parasites once they have entered the red blood cells. It kills the parasites and prevents them from multiplying further. It is not fully understood how it kills the parasites.

Quinine is used to treat falciparum malaria (the most serious kind, caused by a type of malaria parasite called Plasmodium falciparum). It is also used to treat malaria where the type of

infecting organism is unknown or caused by mixed types of Plasmodium. It is not suitable for the prevention of malaria (11).

2.1.3 Aspirin

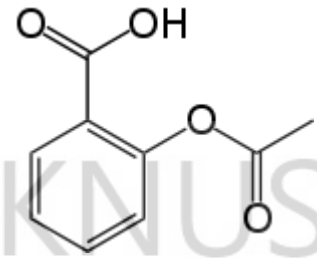

Fig 2.3: Chemical structure of aspirin (Acetylsalicylic acid) (12).

Aspirin is an odorless, colorless or a white crystalline powder with a molecular weight of about 180.15g/mol. It also has melting and boiling points of about 135^{o}C. It has its IUPAC name as 2-(acetyloxy) benzoic acid (13).

Aspirin, one of the first drugs to come into common usage, is still widely used in the world. Aspirin is prepared by chemical synthesis from salicylic acid, by acetylation with acetic anhydride. Aspirin is analgesic, anti-inflammatory, and antipyretic and is an inhibitor of platelet aggregation. It inhibits fatty acid cyclo-oxygenase by acetylation of the active site of enzyme and the pharmacological effects of aspirin are due to the inhibition of the formation of cyclo-oxygenase products including prostaglandins, thromboxane and prostacyclin.

The problem with aspirin is that it upsets the user's stomach fairly badly. In fact, some people had bleeding in their digestive tracts from the high doses of aspirin needed to control pain and swelling.

2.1.4 Metronidazole

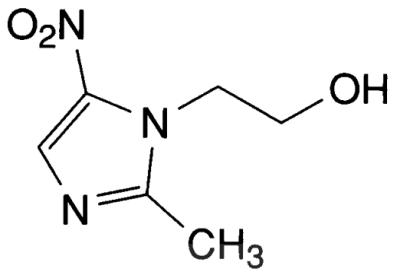

Fig 2.4: The Chemical structure of Metronidazole (14).

Metronidazole has an IUPAC name as 2-(2-Methyl-5-nitro-1H-imidazol-1-yl) ethanol and an empirical formular of $C_6H_9N_3O_3$. Its molecular mass is 171.2g/mol. It appears as white or yellowish crystalline powder. It is slightly soluble in water, acetone, and alcohol and methylene chloride (14; 15).

Metronidazole is a nitroimidazole antibiotic medication used particularly for anaerobic bacteria and protozoa. Metronidazole is an antibiotic, amoebicide, and antiprotozoal. It is the drug of choice for first episodes of mild-to-moderate Clostridium difficile infection. It is marketed mostly under the trade name Flagyl. Metronidazole is also used as a gel preparation in the treatment of the dermatological conditions such as rosacea (16)

2.1.5 Amoxicillin

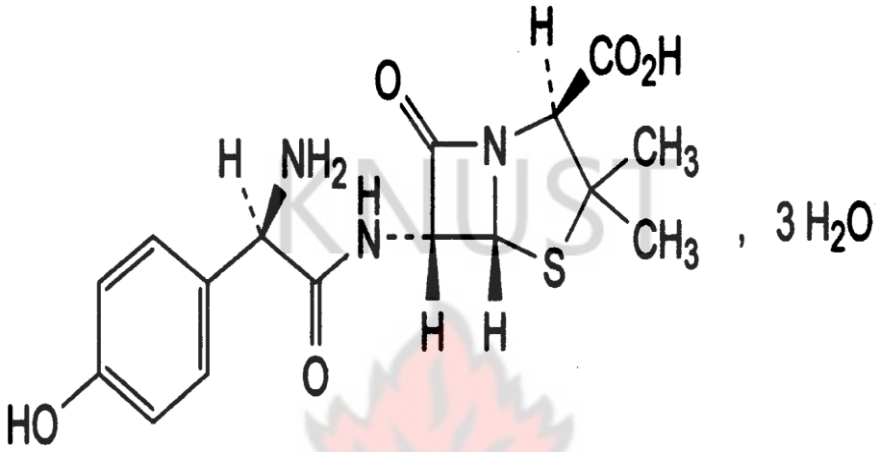

Fig 2.5: Chemical structure of Amoxicillin Trihydrate (14).

Amoxicillin has its IUPAC name as; (2S, 5R, 6R)-6-{[(2R)-2-amino -2-(4-hydroxylphenyl) acetyl] amino)-3, 3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0] heptane-2-carboxylic acid. It has a chemical formular of $C_{16}H_{19}N_3O_5S$, $3H_2O$ and a molecular formular of 419.4g/mol. It is a white or almost white crystalline powder. It is slightly soluble water, very slightly soluble in ethanol, practically in fatty acids. It also dissolves in acids and weak solutions of alkali (14).

Amoxicillin, is a moderate-spectrum, bacteriolytic, β-lactam antibiotic in the amino penicillin family used to treat bacterial infections caused by susceptible microorganisms. It is usually the drug of choice within the class because it is better-absorbed, following oral administration, than other β-lactam antibiotics.

Amoxicillin is used to treat certain infections caused by bacteria, such as pneumonia; bronchitis; gonorrhea; and infections of the ears, nose, throat, urinary tract, and skin. It is also

used in combination with other medications to eliminate H. pylori, bacteria that cause ulcers. Amoxicillin is in a class of medications called penicillin-like antibiotics. It works by stopping the growth of bacteria. Amoxicillin also is used sometimes to prevent anthrax infection after exposure and to treat anthrax infection of the skin and chlamydia infections during pregnancy (17).

2.2 THEORY OF ANALYTICAL TECHNIQUES

2.2.1 Chromatography

The birth of chromatography is linked to Michael Tswett, a botanist who in the year 1903, isolated plant pigments on chalk columns (18). It is the separation of components by the distribution between two or more immiscible phases. Chromatography is one of the most widely used techniques for the separation and purification of components. It has seen tremendous growth over the past four decades owing to its ease of operation, speed, relatively low cost and wide applicability. It can be used to separate mixtures of similar components such as proteins with great precision. Chromatography may be used to purify delicate compounds and even volatile substances. It is commonly used to isolate new compounds formed during chemical synthesis. In the pharmaceutical industry, chromatography is used in the quality control during drug production to monitor the purity of drugs. Chromatography is also used in the qualitative and quantitative applications. Separation is brought about by the different affinity of components to the stationary phase. There is repeated adsorption and desorption as the components moves over the stationary phase and this determines the rate of separation (19). There are different types of chromatography based on the physical and chemical nature of the stationary phase, the purpose of the chromatographic experiment, the

polarity of the stationary and mobile phases, the principle of separation among others. The different types of chromatography include:

- Thin layer chromatography
- Paper chromatography
- Column chromatography
- Gas chromatography
- Liquid chromatography
- Supercritical Fluid chromatography

While it may be possible to make use of more than one of the techniques in analysis, the choice of a particular chromatographic technique is dependent on the

- Availability and cost of equipment
- Ease and speed of the technique
- Chemical and physical characteristics of the compounds to be separated
- Complexity of sample
- Resolution required

2.2.1.1 High Performance Liquid Chromatography

Advancement of research and the quest for further improvements in speed and efficiency of analysis led to the discovery and development of high performance liquid chromatography (HPLC). HPLC is one mode of the chromatographic systems which is widely used in the fields of clinical research, biochemical research, industrial quality control etc. This is because HPLC has a high degree of versatility not found in other chromatographic systems. Its applications include detection, analysis, quantification and derivation of molecules from

mixtures of biological and plants of medical importance by preparative HPLC (20). This separation technique involves differential interaction of the sample between the stationary and mobile phases. HPLC utilizes a liquid mobile phase to separate the components of a mixture. The stationary phase may be liquid or solid. The components are first dissolved in a suitable solvent, and then forced through a chromatographic column containing stationary phase of small particle size under high pressure. The mixture separates into its components on the column. Retention is based on adsorption and also partitioning between the sample particles and the stationary phase. The extent of interaction between the sample particles and the stationary phase affects resolution of sample components (21). The weaker the interaction with the mobile phase the longer the sample is retained on the stationary phase.

2.2.1.1.1 Stationary phase

The stationary phase is the immobile packing materials within the column. There are several types of matrices for support of the stationary phase. These include silica, polymers, alumina, and zirconia. Silica is the dominant support material used in HPLC columns (2). This is because silica matrices are;

- Robust
- Easily derivatized
- Do not tend to compress under pressure
- Chemically stable to most organic solvents and to low pH systems.

Porous polymer support materials such as polyether have been used mostly in ion exchange chromatography. Even though their performance has improved over the years, they still lag behind silica in terms of efficiency. Zirconia support materials are stable from pH 1 to 14 and at elevated temperatures. It can be derivatized for reversed - phase applications (22).

The particles of the silica support come in a variety of sizes and this defines the quality, back-pressure and efficiency of the column. Particle sizes range from 2 - 5 μm for analytical columns and above 10 μm for preparative columns. Smaller particle size provides larger surface area resulting in a greater number of theoretical plates or increased separation efficiency.

Most support materials are porous thereby providing a larger surface area to maximize interaction between the sample and the stationary phase. Pore sizes range from 60 - 200 A°. In general, the nature of stationary phase has the greatest effect on capacity factor, selectivity, efficiency and elution (7).

2.2.1.1.2 Mobile phase

This is the liquid phase that moves the analyte across the stationary phase. The mobile phase interacts with the analyte and the stationary phase hence influencing retention and separation. An ideal mobile phase or solvent should:

- Have the ability to interact with the stationary phase and the analyte to bring about the desired separation.
- Be highly pure
- Be relatively inexpensive
- Be compatible with the HPLC system and the detection system
- Have low UV cut - off if UV detector is used
- Have low viscosity
- Be safe for use i.e. low flammability and toxicity
- Highly inert

Eluent polarity plays the major role in HPLC. Different proportions of different solvents are used to adjust the polarity of the mobile phase. Binary mixtures are most commonly used however ternary mixtures are also used. Methanol and acetonitrile are frequently used solvents in reversed phase chromatography and hexane is used in normal phase chromatography.

Mobile phase additives are used to control retention, enhance separation and peak shapes as well as reduce peak tailing. Buffers are added to control the pH of the mobile phase. They are most effective within ± 1.5 units of their pKa. Phosphate buffer is commonly used with reversed phase HPLC. Acid modifiers such as Trifluoroacetic acid, phosphoric acid and Acetic acid are commonly used to lower the pH of mobile phases to suppress ionization of weakly acidic analyte. Ion pairing reagents can be added to the mobile phase to provide retention for analyte (4). Examples are heptanesulphonate and heptafluorobutyric acid. There are two types of elution, these are;

2.2.1.1.2.1 Isocratic Elution

It is the simplest form of elution. It employs a mobile phase of constant composition throughout the analysis. It is achieved by either pumping the mixed mobile phase prepared prior to analysis through a single reservoir or by the delivery of a constant ratio of solvents by the binary pumps (2). It is the preferred form of elution to maximize the loading capacity. The system as well as the column are equilibrated at all times and don't suffer from fast chemical changes.

2.2.1.1.2.2 Gradient Elution

The general problem of elution common with complex mixtures characterized by poor separation of components is reduced with this form of elution. It offers complete separation of the components with good peak resolution in reasonable time. It can be used in the separation of mixtures containing compounds of a wide range of polarities in shorter time without compromising resolution. It involves a continuous or steady alteration in mobile phase composition to increase the strength of the mobile phase. It enables the faster elution of strongly retained components while having the weakly retained components well resolved (20).

Gradient elution separations generally provide faster and more efficient separations with improved limits of detection and less tailing for most compounds present in the sample even though chromatographers mostly avoid its use because of "ghost" peaks, baseline noise associated with it. Gradient elution requires more complex and expensive equipment and is difficult to maintain at a constant flow rate (23).

2.2.1.2 Separation mechanisms of HPLC

Liquid chromatography is further classified according to the type of the interactions between the analyte and the stationary phase surface and according to the relative polarity of the stationary and mobile phases. The classes are;

2.2.1.2.1 Adsorption Chromatography

It is the oldest of the chromatographic separation techniques. The two most common adsorbents are silica gel and alumina, with silica being by far the most popular. The separation is based on repeated adsorption and desorption between the analyte and the

adsorbent. Adsorptive forces such as hydrophobic, dipole-dipole, ionic interactions are involved in this technique. The binding of the analyte to the stationary phase is proportional to the contact surface area around the analyte and the adsorbent (24).

2.2.1.2.2 Partition Chromatography

Partition chromatography, also known as liquid - liquid chromatography involves the distribution of solute between two immiscible solvents. The difference in partition coefficients of the solutes between the thin film of solvent on the stationary phase, which is usually the more polar solvent and the mobile phase accounts for the separation (19).

2.2.1.2.3 Ion-Exchange Chromatography

This technique is used almost exclusively with ionic or ionizable samples. The stationary phase consists of insoluble matrix to which charged groups have been covalently bound. Typical stationary phases are cationic exchange (sulfonate) or anionic exchange (quaternary ammonium) groups bonded to silica or polymeric materials. The mobile phase consists of aqueous buffers, where pH and ionic strength are used to control elution time. Separation in ion exchange chromatography is achieved by the reversible adsorption of ionic analyte to immobilized ion exchange sites or counter-ions of opposite charge on the ion exchanger (25).

Ion exchange is probably the most frequently used chromatographic technique for the separation and purification of proteins, polypeptides, nucleic acids, polynucleotides, and other charged biomolecules. This is due to its widespread applicability, high resolving power, high capacity, simplicity and controllability of the method (26).

2.2.1.2.4 Size Exclusion Chromatography

Separation in size exclusion chromatography is on the basis of molecular size. The columns contain porous particles with precisely controlled pore sizes. The pore diameter defines the exclusion limit of the gel. Particles too large to enter the pores are excluded and have access to only the void volume and rapidly elute together in a single peak. Molecules that are smaller than the pore size enter the particles and their separation is determined by the pore size distribution within the pore volume (4).

2.2.1.2.5 Chiral chromatography

Chiral chromatography predominantly used in biomedical and pharmaceutical analysis involves the use of chemically bound chiral stationary phases. These include polysaccharide derivatives, cyclodextrins, pirkle type, proteins among others. The stationary phase interacts with the analyte enantiomers to form short - lived diastereometric association. Intermolecular interactions including hydrogen bonding and ionic interactions occur between the analyte and the stationary phase material. It is a useful technique in the detection, separation and quantitation of optically active impurities in chiral active pharmaceutical ingredients and drug products (27).

2.2.1.2.6 Normal phase chromatography

Normal phase chromatography is a technique that employs polar stationary phase material in combination with a non - polar or moderately polar mobile phase to separate components of mixtures. There are a number of stationary phase materials or adsorbents but silica is the most common and provides very high selectivity for many applications. Non - polar solvents such as hexane and heptane are usually used.

Retention of solutes is primarily a function of the relative polarity of the solutes. Polar samples are thus retained on the polar surface of the column packing longer than less polar materials. Non - polar solvents such as hexane with the addition of polar modifiers allow for control of retention of analyte to the stationary phase. Compounds that are not water soluble or that may decompose in water are better analyzed using normal phase chromatography. It is also useful for the separation of isomers and compounds that differ in the number or character of functional groups (20).

2.2.1.2.7 Reversed phase chromatography

Reversed - phase chromatography also called bonded phase is widely used in pharmaceutical analysis. The packing materials employed in this mode of chromatography are usually porous materials with hydrophobic surfaces. The separation mechanism depends on the hydrophobic interaction between the solute molecules and the immobilized hydrophobic ligands on the stationary phase. Majority of packing materials or adsorbents are chemically modified porous silica (1). The mobile phase is usually a mixture of aqueous and organic solvents. Organic solvents most frequently used are methanol and acetonitrile. In this, the more non - polar the material is, the longer it is retained.

The hydrophobicity of an analyte can be influenced by pH. For this reason, buffers are used in most HPLC methods however covalently bounded silane ligands of bonded-phase packing are hydrolyzed and the silica support is dissolved by aqueous mobile phases above pH 8. This often leads to premature column failure limiting the use of silica-based columns for applications requiring high pH (28). Reverse phase chromatography has found both analytical and preparative applications in biochemical separation and purification.

2.2.1.3 Instrumentation

2.2.1.3.1 Pump

The high-pressure pumping system is an important part of the liquid chromatograph. It provides the high pressure required to propel the mobile phase and analyte through the densely packed column. Its performance directly affects the retention time and reproducibility. The flow is monitored by computer controlled devices. Most conventional pumping systems provide pressure up to 6000 psi (27).

The pumping system must be able to provide constant and reproducible pressure up to 6000 psi, pulseless output and flow rates ranging from 0.1 to 10 ml/min. The reciprocating piston pumps, displacement type pumps and the pneumatic or the constant pressure pump are the types of pumps encountered (1).

2.2.1.3.2 Column

The column is an essential part of the HPLC. It holds the stationary bed which provides differential retention of components. It is usually a stainless steel tube filled with the packing material, often at ambient temperature. The stainless steel can be replaced with titanium or polyetheretherketone (PEEK) for more corrosion resistance in ion chromatography. Typical analytical columns are 50 - 250 mm long and 2.0 - 4.6 mm in diameter. Larger columns exist for preparative work. Shorter columns and smaller internal diameter analytical columns offer higher sensitivity, lower solvent usage and reduced analysis time (7). Columns are further categorized according to the different chromatographic modes of separation. Normal phase, reverse phase, size exclusion, ion exchange columns as well as specialized chiral columns may be used depending on the nature of the mixture to be separated. A typical column lifetime is about three to twenty four months.

Care of the column is the key to obtaining reliable results in analysis and to the life span of the column. A guard column which is of the same nature as the main analytical column can be used to trap impurities or particles from the samples especially environmental samples where sample clean up may not be feasible. This is because impurities block adsorption sites, change the selectivity of the column and cause peak splitting in the chromatogram. Buffers need to be thoroughly washed off by conditioning the column when they are used. Highly pure HPLC grade solvents can be used. Columns should be stored in the appropriate solvent after use.

2.2.1.3.3 Injector

Samples are injected into the HPLC via an injection port. The injection port of an HPLC consists of an injection valve and a sample loop. The sample dissolved in an appropriate solvent drawn into a syringe is injected into the loop via the injection valve. The rotation of the valve rotor closes the injection valve and opens the sample loop in order to inject the sample into the stream of the mobile phase. The loop volume ranges from 10 µl to over 500 µl (29). In more sophisticated liquid chromatographic systems, automatic sampling devices are incorporated where the sample is introduced with the help of auto samplers. This is very useful in multiple analyses. It is always best to remove particles from the sample by filtering or by centrifuging to prevent blockage in the injection device (4).

2.2.1.3.4 Detectors

The detector is the component of the HPLC instrument that emits response due to the eluting compound, by monitoring properties inherent in them. Positioned immediately posterior to the column, it translates the changes in the chemical composition of the analyte at the column exit into an electrical signal. This signal is subsequently processed and recorded as a peak which gives information about the analyte (7).

2.2.2 Theory of Thin Layer Chromatography

In thin layer chromatography, a solid phase (adsorbent) is coated onto a solid support as a thin layer (about 0.25 mm thick). In many cases, a small amount of a binder such as plaster of Paris is mixed with the absorbent to facilitate the coating. Many different solid supports are employed, including thin sheets of glass, plastic, and aluminum. The mixture to be separated is dissolved in a solvent and the resulting solution is spotted onto the thin layer plate near the bottom. A solvent, or mixture of solvents, called the eluent, is allowed to flow up the plate by capillary action. At all times, the solid will adsorb a certain fraction of each component of the mixture and the remainder will be in solution. Thus, the more weakly a substance is adsorbed, the farther up the plate it will move. The more strongly a substance is adsorbed, the closer it will stay near the origin. Several factors determine the efficiency of a chromatographic separation. The adsorbent should show a maximum of selectivity toward the substances being separated so that the differences in rate of elution will be large (30).

2.2.4 Ultraviolet-Visible Spectroscopy

Ultraviolet-visible spectroscopy (UV-Vis) refers to absorption spectroscopy in the ultraviolet-visible spectral region. This means it uses light in the visible and adjacent (near-UV and near-infrared (NIR)) ranges. The absorption in the visible range directly affects the perceived color of the chemicals involved. In this region of the electromagnetic spectrum, molecules undergo electronic transitions. This technique is complementary to fluorescence spectroscopy, in that fluorescence deals with transitions from the excited state to the ground state, while absorption measures transitions from the ground state to the excited state (31).

UV/Vis spectroscopy is routinely used in the quantitative determination of solutions of transition metal ions and highly conjugated organic compounds.

Organic compounds, especially those with a high degree of conjugation, also absorb light in the UV or visible regions of the electromagnetic spectrum. The solvents for these determinations are often water for water soluble compounds, or ethanol for organic-soluble compounds. Organic solvents may have significant UV absorption; not all solvents are suitable for use in UV spectroscopy. Ethanol absorbs very weakly at most wavelengths. Solvent polarity and pH can affect the absorption spectrum of an organic compound. Tyrosine, for example, increases in absorption maxima and molar extinction coefficient when pH increases from 6 to 13 or when solvent polarity decreases. While charge transfer complexes also give rise to colours, the colours are often too intense to be used for quantitative measurement (32)

The Beer-Lambert law states that the absorbance of a solution is directly proportional to the concentration of the absorbing species in the solution and the path length. Thus, for a fixed path length, UV/Vis spectroscopy can be used to determine the concentration of the absorber in a solution.

A UV/Vis spectrophotometer may be used as a detector for HPLC. The presence of an analyte gives a response assumed to be proportional to the concentration. For accurate results, the instrument's response to the analyte in the unknown should be compared with the response to a standard; this is very similar to the use of calibration curves. The response (e.g., peak height) for a particular concentration is known as the response factor (33).

The nature of the solvent, the pH of the solution, temperature, high electrolyte concentrations, and the presence of interfering substances can influence the absorption spectrum. Experimental variations such as the slit width (effective bandwidth) of the spectrophotometer

will also alter the spectrum. To apply UV/Vis spectroscopy to analysis, these variables must be controlled or accounted for in order to identify the substances present. UV/Vis spectroscopy is most often used in a quantitative analysis to determine concentrations of an absorbing species in solution, using the Beer-Lambert law: $A = - \log_{10} (I/I_0) = \varepsilon cL$

Where A is the measured absorbance, I_0 is the intensity of the incident light at a given wavelength, I is the transmitted intensity, L the pathlength through the sample, and c the concentration of the absorbing species. For each species and wavelength, ε is a constant known as the molar absorptivity or extinction coefficient. This constant is a fundamental molecular property in a given solvent, at a particular temperature and pressure.

2.2.5 Nuclear Magnetic Resonance Spectroscopy

Over the past fifty years, nuclear magnetic resonance spectroscopy, commonly referred to as NMR, has become the preeminent technique for determining the structure of organic compounds. Of all the spectroscopic methods, it is the only one for which a complete analysis and interpretation of the entire spectrum is normally expected. Although larger amounts of sample are needed than for mass spectroscopy, NMR is non-destructive, and with modern instruments good data may be obtained from samples weighing less than a milligram.

Many types of information can be obtained from an NMR spectrum. Much like using infrared spectroscopy (IR) to identify functional groups, analysis of a NMR spectrum provides information on the number and type of chemical entities in a molecule. However, NMR provides much more information than IR.

The impact of NMR spectroscopy on the natural sciences has been substantial. It can, among other things, be used to study mixtures of analytes, to understand dynamic effects such as

change in temperature and reaction mechanisms, and is an invaluable tool in understanding protein and nucleic acid structure and function. It can be applied to a wide variety of samples, both in the solution and the solid state (6).

The NMR sample is prepared in a thin-walled glass tube. When placed in a magnetic field, NMR active nuclei (such as 1H or 13C) absorb at a frequency characteristic of the isotope. The resonance frequency, energy of the absorption and the intensity of the signal are proportional to the strength of the magnetic field. In the Earth's magnetic field the same nuclei resonate at audio frequencies. This effect is used in Earth's field NMR spectrometers and other instruments (34) .

2.2.5.1 Chemical Shift

Unlike infrared and UV-Visible spectroscopy, where absorption peaks are uniquely located by a frequency or wavelength, the location of different NMR resonance signals is dependent on both the external magnetic field strength and the radio frequency. Since no two magnets will have exactly the same field, resonance frequencies will vary accordingly and an alternative method for characterizing and specifying the location of NMR signals is required (34).

2.2.5.2 Signal Strength

The magnitude or intensity of NMR resonance signals is displayed along the vertical axis of a spectrum, and is proportional to the molar concentration of the sample. Thus, a small or dilute sample will give a weak signal, and doubling or tripling the sample concentration increases the signal strength proportionally. Considering the NMR spectrum of equal molar amounts of benzene and cyclohexane in carbon tetrachloride solution, the resonance signal from cyclohexane will be twice as intense as that from benzene because cyclohexane has twice as

many hydrogens per molecule. This is an important relationship when samples incorporating two or more different sets of hydrogen atoms are examined, since it allows the ratio of hydrogen atoms in each distinct set to be determined (35).

2.2.6 Infrared Spectroscopy

Infrared (IR) spectroscopy is one of the most common spectroscopic techniques used by organic and inorganic chemists. It is the absorption measurement of different IR frequencies by a sample positioned in the path of an IR beam. The main application of IR spectroscopic analysis is to determine the functional groups in the sample. Different functional groups absorb characteristic frequencies of IR radiation. Using various sampling accessories, IR spectrometers can accept a wide range of sample types such as gases, liquids, and solids. IR spectroscopy is an important and popular tool for structural elucidation and compound identification. Infrared radiation spans a section of the electromagnetic spectrum having wavenumbers from about 13,000 to 10 cm^{-1}, or wavelengths from 0.78 to 1000 μm. It is boarded by the red end of the visible region at high frequencies and the microwave region at low frequencies. IR absorption positions are generally presented as either wavenumbers ($\bar{\upsilon}$) or wavelengths (l).

Wavenumber defines the number of waves per unit length. Thus, wavenumbers are directly proportional to frequency, as well as the energy of the IR absorption. The wavenumber unit ($cm–^{1}$, reciprocal centimeter) is more commonly used in modern IR instruments that are linear in the cm^{-1} scale. In the contrast, wavelengths are inversely proportional to frequencies and their associated energy. At present, the recommended unit of wavelength is μm (micrometers). IR absorption information is generally presented in the form of a spectrum with wavelength or wavenumber as the x-axis and absorption intensity or percentage

transmittance as the y-axis Transmittance, T, is the ratio of radiant power transmitted by the sample (I) to the radiant power incident on the sample (I0). Absorbance (A) is the logarithm to the base 10 of the reciprocal of the transmittance (T). The transmittance spectra provide better contrast between intensities of strong and weak bands because transmittance ranges from 0 to 100% T whereas absorbance ranges from infinity to zero (36).

The IR region is commonly divided into three smaller areas: near IR, mid IR, and far IR. The most frequently used is the mid IR region, between 4000 and 400 cm^{-1} (2.5 to 25 μm). The far IR requires the use of specialized optical materials and sources. It is used for analysis of organic, inorganic, and organometallic compounds involving heavy atoms (mass number over 19). It provides useful information to structural studies such as conformation and lattice dynamics of samples. Near IR spectroscopy needs minimal or no sample preparation. It offers high-speed quantitative analysis without consumption or destruction of the sample. Its instruments can often be combined with UV-visible spectrometer and coupled with fiber optic devices for remote analysis. Near IR spectroscopy has gained increased interest, especially in process control applications (37).

The combination of the fundamental vibrations or rotations of various functional groups and the subtle interactions of these functional groups with other atoms of the molecule results in the unique, generally complex IR spectrum for each individual compound. IR spectroscopy is mainly used in two ways: structural elucidation and compound identification (38).

IR spectroscopy was generally considered to be able to provide only qualitative and semi-quantitative analyses of common samples, especially when the data were acquired using the conventional dispersive instruments. However, the development of reliable FTIR

instrumentation and strong computerized data-processing capabilities has greatly improved the performance of quantitative IR work. Thus, modern infrared spectroscopy has gained acceptance as a reliable tool for quantitative analysis. The basis for quantitative analysis of absorption spectrometry is the Bouguer–Beer–Lambert law, commonly called Beer‘s law (39).

2.2.7 Titrimetric and Chemical Methods of Analysis

Titrimetry, also known as titration, is a common laboratory method of quantitative chemical analysis that is used to determine the unknown concentration of an identified analyte. Since volume measurements play a key role in titration, it is also known as volumetric analysis (40).

2.2.7.1 Non aqueous titrations

Non aqueous titration is a titrimetric procedure used in pharmacopoeial assays for the titration of substances, normally very weak acids and very weak bases dissolved in non- aqueous solvents. The most commonly used procedure is the titration of organic bases e.g. pyridine with perchloric acid in anhydrous acetic acid (40).

Water, being amphoteric, behaves as both a weak acid and a weak base. In an aqueous environment, it can compete effectively with very weak acids and very weak bases with regard to proton donation and acceptance. The effect of this competition is that the inflection in the titration curves for very weak acids and very weak bases is small, thus making endpoint detection relatively more difficult (29).

A general rule is that bases with pKb < 7 or acids with pKa > 7 cannot be determined accurately in aqueous solution.

Substances which are either too weakly basic or too weakly acidic to give sharp endpoints in aqueous solution can often be titrated in non- aqueous solvents. The reactions which occur during many non-aqueous titrations can be explained by means of the concepts of the Brønsted-Lowry theory (29; 1; 29)

2.2.7.2 Acid base titration

This is a titration that involves acids and bases regardless of their strength. It is the most common form of titration that is employed in most chemical methods of analysis. It involves the determination of the concentration of an acid or base by exactly neutralizing the acid/base with an acid or base of known concentration. This allows for quantitative analysis of the concentration of an unknown acid or base solution. It may be a direct titration or a back titration. They are sometimes called alkalimetric titrations (40).

2.2.7.3 Potentiometric titration

This is probably the most frequently used electrochemical technique in pharmaceutical analysis especially in very dilute or colored solutions where the detection of the endpoint by visual indicators may be inaccurate. It is based on the relationship between the potentials of electrochemical cells and the concentrations or activities of the chemical species in the cells. The equipment required is a reference electrode, an indicator or working electrode and a potential-measuring device, for example pH meter. The other apparatus consist of a burette, beaker and a stirrer. The indicator electrode must be suitable for the particular type of titration (i.e. a glass electrode for acid/base reactions and a platinum electrode for redox titrations). The electrodes are immersed in the solution to be titrated and the potential difference between the electrodes is measured. Measured volumes of titrant are added, with thorough stirring, and the corresponding values of e.m.f. or pH recorded. The endpoint is noted graphically by the

burette reading corresponding to the maximum change of e.m.f. or pH per unit change of volume (41).

2.2.7.4 Redox titration

Redox titrations are based on an oxidation-reduction reaction between an analyte and a titrant. There must be a sufficiently large difference between the oxidizing and reducing capabilities of these agents for the reaction to go to completion and give a sharp end-point. Oxidation of a substance simultaneously results in the reduction of the oxidant. The most common oxidizing agents in such determinations are Iodine, Potassium Iodate or Bromate, Ceric Ammonium Sulphate, Potassium Permanganate and Potassium dichromate. Titanium Chloride, Amalgamated Zinc and Iodine ion are used as reducing agents. The endpoint is detected with the use of a redox indicator or by potentiometry; however, with coloured reagents such as potassium permanganate and iodine, the reagent itself may act as an indicator (41).

2.2.7.5 Complexometric titration

Complexometric titration is a form of volumetric analysis in which the formation of a coloured complex is used to indicate the end point of a titration. Complexometric titrations are based on the reaction between Lewis acids (usually metal cations) and Lewis bases. Complexometric titrations are particularly useful for the determination of a mixture of different metal ions in solution. An indicator capable of producing an unambiguous colour change is usually used to detect the end-point of the titration (40).

A special subset of ligands are those that contain more than one binding site on the molecule; these are called chelating agents. Chelating agents form particularly strong complexes called chelates with Lewis acids. By far the most common Complexometric titrant is

ethylenediaminetetraacetic acid, EDTA. This is a hexadentate chelating ligand; this means there are six ligand binding sites on EDTA molecule. EDTA titrations are very versatile: they can be used for the analysis of all the metal cations except the alkali metals, and can even be used (through back-titration and similar methods) for the analysis of many anions. EDTA titrations are also fairly sensitive, capable of detecting concentrations of some metals at levels of approximately 10 ppm (i.e., 10 mg/L) (29).

2.2.7.6 Precipitation Titration

In a precipitation titration, the stoichiometric reaction is a reaction which produces in solution a slightly soluble salt that precipitates out. For example, to determine the concentration of chloride ion in a particular solution, an analyst could titrate the solution with a solution of a silver nitrate, whose concentration is known. A white precipitate of AgCl is deposited on the bottom of the flask during the course of the titration. Since the chemical reaction is one Ag^+ to one Cl^-, we know that the amount of Ag^+ used to the equivalence point equals the amount of Cl^- originally present (40).

CHAPTER THREE

MATERIALS AND METHODS

3.1 REAGENTS

- Glacial Acetic acid (BDH, Analar grade)
- Sulphuric acid (BDH)
- Acetic Anhydride (BDH)
- Perchloric acid (BDH)
- Methanol (general purpose grade)
- Hydrochloric acid (BDH)
- Sodium Hydroxide pellets (BDH)
- Chloroform (BDH)
- Ammonia (BDH)
- Ethanol
- Ether (BDH)
- Potassium Hydrogen phthalate (BDH)
- Sodium Bicarbonate (BDH)
- Formaldehyde (BDH)
- Ferric Chloride
- Potassium Iodobismuthate

3.2 INSTRUMENTS AND MATERIALS

- Eutech Instruments Cyberscan pH meter
- Stuart Melting Point SMP 10 Apparatus
- Cecil Ce 2041 200 Series-UV Spectrophotometer

- Shimadzu HPLC
  - SPD – 20A Prominence UV- Detector
  - DGU- $20A_3$ Prominence Degasser
  - LC – $20A_B$ Prominence Liquid Chromatograph
- ODS C-18 (4.6 x 150mm) waters column
- Chromato-Vue C-70 UV view system(UVP Inc) 254nm short wave; 365nm long wave
- PG Instruments T90 +UV/Vis Spectrophotometer
- Buchi Rotary Evaporator
- Adam –analytical weighing balance, WA 210; 210/0.0001g
- FS 28H Fischer Scientific Sonicator

Other materials

- Whatman Filter paper 11.0cm
- Melting point capillary tubes
- Pre-coated TLC plate

3.2.1 Acquisition of pure samples

The pure samples were obtained from Ernest Chemist and Amponsah-Effah pharmaceutical Ltd.

Table 3.1: Profile of the pure samples

Drug	Batch Number	Manufacturing date	Expiring Date	Assay
Albendazole	61411338	25/11/2011	25/11/2015	99.51%
Quinine Sulphate	341187	17/06/2013	15/06/2016	99.74%
Aspirin	12018	07/02/2013	07/02/2016	99.4%
Amoxicillin Trihydrate	1305026118	04/05/2011	04/05/2017	98.3%
Metronidazole	201103292	15/03/2011	15/03/2015	99.4%

3.2.2 Acquisition of Commercial Tablets of Albendazole and Quinine Sulphate

Three different brands each of Albendazole and Quinine Sulphate were purchased from pharmacies around Ayeduase and Tech Junction in the Kumasi Metropolis.

Table 3.2: Profile of Albendazole tablets

Brand	Manufacturer	Strength	Batch number	Manufacturing Date	Expiring Date
Alben	Smithkline Beecham Pharmaceuticals	400mg,USP	330065A	05/01/2013	05/12/2016
Wormplex 400	Micro Labs Ltd	400mg, USP	WPTP0014	20/06/2013	30/05/2017
Zeben	Medreich (MD)	200mg, USP	610252	21/05/2011	20/05/2016

Table 3.3: Profile of Quinine Sulphate tablets

Brand	Manufacturer	Strength	Batch number	Manufacturing Date	Expiring Date
Quinine Sulphate	Actavis,Barstaple, UK	300mg,BP	QM106	07/2013	07/2016
Quinine Sulphate	Co-Pharma Ltd, UK	300mg,BP	7215644	11/2012	12/14
Quinine Sulphate	Wockhardt UK Ltd	300mg,BP	LN10575	03/2013	02/2015

3.3 IDENTIFICATION TESTS

The following identification tests were carried out to ascertain the identity of all the pure samples used in the study.

3.3.1 Melting Point Determination

The dry pure powders of each of the pure sample were introduced into separate capillary tubes sealed at one end. The filled capillary tubes were tapped on a hard surface to ensure that the powders are tightly packed. The tubes were then inserted into the melting point and the various melting points determined (14).

3.3.2.1 Identification Test for Albendazole

3.3.2.1.1 Colour Test

0.010g of the pure Albendazole powder was added to 3ml of Sulphuric acid in a test tube. The mixture was sonicated for five minutes to dissolve. 1ml of Potassium Iodobismuthate was added (42).

3.3.2.1.2 UV-Visible Spectroscopy

0.01g of the pure Albendazole sample was dissolved in 100ml of 0.1M HCl. 10ml portion of the solution was diluted with the 0.1M HCl to 100ml. The resulting solution was examined between 200nm and 400nm (2).

3.3.2.2 Identification Test for Quinine Sulphate

3.3.2.2.1 Colour Identification

0.01g of the pure Quinine Sulphate sample was dissolved in 3ml of 0.1M Sulphuric acid. The mixture was topped up using the 0.1M Sulphuric acid to make 100ml and then observed under UV light at 365nm. 1ml of 0.1M HCl was added (14).

3.3.2.2.2 UV-Visible Spectroscopy

0.01g of the pure Quinine Sulphate sample was dissolved in Methanol and Sonicated at room temperature. The resulting solution was examined between 200nm and 400nm (9).

3.3.2.3 Identification Test for Metronidazole

0.02g of the pure Metronidazole sample was dissolved in 0.1M HCl to make 100ml. 5.0ml portion of the solution was diluted with the same diluent to 100ml. The resulting solution was examined between 200nm and 400nm (14).

3.3.2.4 Identification Test for Aspirin

3.3.2.4.1 Colour Test

0.05g of the pure Aspirin granules was dissolved in 50ml of water and boiled for fifteen (15) minutes. The solution was cooled and two drops of Ferric Chloride added (9).

3.3.2.4.2 UV- Spectroscopy

0.03g of the pure Aspirin granules was dissolved in 20ml of 0.1M HCl. The solution was topped up with the diluent to make 100ml. 5ml portion of the solution was diluted to100ml using the diluent (0.1M HCl). The resulting solution was examined between 200nm and 400nm (2).

3.3.2.5 Identification Test for Amoxicillin Trihydrate

3.3.2.5.1 Colour Test

0.02g of the pure Amoxicillin Trihydrate was placed in a 50ml beaker. 0.5ml of distilled water was added. 20ml of 0.1M Sulphuric acid-Formaldehyde (2ml of Formaldehyde solution mixed with 100ml of 96% ^{w}/v Sulphuric acid) was added. The beaker was swirled to allow for uniform mixing. The solution was placed in water bath for about two minutes (9).

3.3.2.5.2 UV – Spectroscopy

0.01g of the pure Amoxicillin Trihydrate was dissolved in 20ml of 0.1M Sodium Hydroxide. The solution was topped up using the same 0.1M Sodium Hydroxide to make 100ml. 5ml portion of the solution was diluted to 50ml. The resulting was examined between 200nm and 400nm (2)

3.3.3 Thin Layer Chromatography

3.3.3.1 Albendazole

A quantity of the powdered tablets of the three different brands each equivalent to 0.01g of Albendazole were each weighed into a 25ml volumetric flask. Glacial acetic acid was added to dissolve to make 10ml. 0.001g of the pure Albendazole powder was also dissolved in the same solvent to make 10ml. 10µl of the solutions were applied to a thin layer chromatographic plate of about 15cm x 5cm coated with 0.25mm thick Silica gel. The spots were allowed to dry. The chromatogram was developed in a solvent system consisting Glacial acetic acid, Chloroform and Ether in the ratio 60:10:10.

The plates were removed when the solvent front had moved to about 70% of the length of the plate, the solvent front was marked and the plate dried using hand drier. The spots were located and examined under UV light at 254nm. The retardation factor was calculated. The retardation factor (Rf) of the tablets were compared with that of the pure Albendazole (43).

3.3.3.2 Quinine Sulphate

A quantity of the powdered Quinine Sulphate tablets of each of the three brands equivalent to 0.005mg of Quinine Sulphate were weighed and dissolved in a solution made up of 2:1 Chloroform Ethanol to make 10ml. 0.005g of the pure Quinine Sulphate was also dissolved in the same solvent mixture to make 10ml.10µl of the solutions were separately applied to the TLC plate (15cmx5cm coated with 0.25mm layer of Silica gel. The spots were allowed to dry. The chromatogram was developed in a solvent system consisting of Methanol: Ammonia (60:40). The plate was removed after the solvent front had moved about 70% of the length of the plate. The solvent front was marked and air dried. The spots were observed at 365nm. The

retardation factor (Rf) was calculated and the Rf values for the pure Quinine Sulphate was compared to the Rf values of the tablets (9).

3.4 PREPARATION OF SOLUTION

3.4.1 Preparation of 1M HCl

About 9ml of the stock hydrochloric acid was measured with a measuring cylinder and transferred in a thin stream into a beaker containing about 50ml of distilled water. The mixture was stirred and allowed to cool after which it was quantitatively transferred into 100ml volumetric flask and made to the mark with distilled water. The flask was stoppered, shaken and labelled appropriately.

3.4.2 Preparation of 1M Sodium Hydroxide (NaOH)

4.0500g of sodium Hydroxide pellets was weighed in a small beaker using an analytical balance. The pellets were then transferred quantitatively with distilled water into a bigger beaker containing about 50ml of water. The solution was stirred with a stirrer to ensure complete dissolution and allowed to cool. The resulting solution was then transferred quantitatively into a 100 ml volumetric flask and adjusted to the mark with distilled water. The flask was stoppered, shaken and labelled appropriately.

3.4.3 Standardization of 0.1M Perchloric Acid ($HClO_4$) using Potassium Hydrogen Phthalate ($C_8H_5KO_4$)

0.5g of potassium hydrogen phthalate was weighed into 100ml conical flask; 25 ml of glacial acetic acid was added and warmed to dissolve. The solution was allowed to cool and then titrated with 0.1M perchloric acid using two drops of oracet blue as an indicator. The end point was indicated by change in the blue colour.

3.4.4 Standardization of HCl using Sodium Bicarbonate (Na_2CO_3)

6.6920g of Na_2CO_3 analar grade was weighed into a 250ml volumetric flask.100ml of distilled water was added to dissolve the Na_2CO_3, and then topped up with distilled water to the 250ml mark. 25ml portion of the solution was titrated with 0.1M HCl using methyl orange as the indicator.

3.4.5 Standardization of 0.5M NaoH using 0.5M HCl

The standardized HCl was used to standardize the NaOH. The HCl was titrated with NaOH using methyl orange as indicator until the first definite orange colour was observed.

3.5.1 Uniformity of Weight of Albendazole tablets

Twenty (20) tablets each of the three brands of the Albendazole tablets were weighed individually and their weights recorded. The average weight was determined for each of the brands. Weight deviations and percentage weight deviations were calculated for each brand (14).

3.5.2 Uniformity of Weight of Quinine Sulphate tablets

Twenty (20) tablets of each of the three brands of Quinine Sulphate tablets were individually weighed and the weights recorded. The average weights were calculated for each of the three brands. The weight deviation and percentage weight deviation were calculated for the three brands (14).

3.6 Assay of Pure Samples

3.6.1 Aspirin

0.401g of the pure Aspirin granules was weighed into a beaker. 50ml of 0.5M Sodium Hydroxide was added and boiled gently for ten minutes. The excess Sodium Hydroxide was titrated with 0.5M.HCl using phenolphthalein as an indicator. Blank titration was performed to determine the actual volume of Sodium Hydroxide that reacted with the Aspirin. Each ml of Sodium Hydroxide is equivalent to 0.04504g of Aspirin (9).

3.6.2 Metronidazole

0.104g of the pure Metronidazole powder was dissolved in 50ml of Anhydrous Acetic acid and titrated with 0.1M Perchloric acid. The end point was determined potentiometrically. One ml of the 0.1M Perchloric acid is equivalent to 0.01712g of Metronidazole (14).

3.6.3 Albendazole

0.1502g of the pure Albendazole powder was dissolved in glacial acetic acid and sonicated for five minutes at room temperature. The resulting solution was titrated with 0.1M Perchloric acid. The end point was determined potentiometrically. Each ml of Perchloric acid is equivalent to 0.02653g of Albendazole (9).

3.6.4 Quinine Sulphate

0.200g of the pure Quinine Sulphate powder was dissolved in 100ml of acetic anhydride and sonicated at room temperature for five minutes. The resulting solution was titrated with 0.1M Perchloric acid. The end point was determined potentiometrically. Each ml of the Perchloric acid is equivalent to 0.0261g of Quinine Sulphate (14).

3.6.5 Amoxicillin Trihydrate

0.0019g of the pure Amoxicillin Trihydrate was dissolved in 0.1M HCl and sonicated at room temperature for five minutes. The absorbance of the solution was measured at 272nm against 0.1M HCl as a blank. The absorbance was recorded and the percentage determined using the Beer's law (2).

3.7 ASSAY OF THE COMMERCIAL TABLETS USING STANDARD METHODS

3.7.1 Albendazole tablets

Twenty tablets were weighed and powdered. A quantity of the powdered tablets equivalent to 0.200g of Albendazole was weighed and dissolved in 100ml of Glacial acetic acid and sonicated at room temperature for five minutes. The resulting solution was titrated with 0.1M Perchloric acid and the end point determined potentiometrically. Each ml of the 0.1M Perchloric acid is equivalent to 0.02653g 0f Albendazole ($C_{12}H_{15}N_3O_2S$) (9).

3.7.2 Quinine Sulphate

Twenty tablets were weighed and powdered. A quantity of the powdered tablets equivalent to 0.200g of Quinine Sulphate was weighed into a beaker 100ml of acetic anhydride and sonicated at room temperature for five minutes. The resulting solution was titrated with 0.1M Perchloric acid. The end point determined potentiometrically. Each ml of the 0.1M Perchloric acid is equivalent to 0.0261g of Quinine Sulphate (14).

3.8 HPLC Method Development

The choice of the mobile phase combination was made after intensive reading of publications on the HPLC assay of the drugs of interest and their corresponding surrogates. Solubility, stability and ionizability of the drugs and their surrogates were also considered.

Different proportions of the solvents making up the mobile phase were tried at different flow rates to investigate their effect on resolution and the shape of the peaks. The following conditions were finally settled on.

3.8.1 Chromatographic Conditions for Albendazole and its Surrogate reference standards

Column: ODS C-18 waters (4.6 x 150mm, 5µm particle size)

Mobile Phase: Methanol: Water (60:40 %v/v)

pH: 5.40± 0.02

Flow Rate: 1ml/min

Detector: UV-Visible

Wavelength of detection: 245nm

Injector volume: 20µL

Mode of Elution: Isocratic

3.8.2 Chromatographic Conditions for Quinine Sulphate and its Surrogate reference standards

Column: ODS C-18 waters (4.6 x 150mm, 5µm particle size)

Mobile Phase: Methanol: Water (60:40 % v/v)

pH: 5.20± 0.02

Flow Rate: 1.5ml/min

Detector: UV-Visible

Wavelength of Detection: 245nm

Injector volume: 20µL

Mode of Elution: Isocratic

3.8.3 Column Conditioning and Equilibration

Before samples were injected, Methanol was pumped through the Column for about an hour followed by water: methanol in the ratio 50:50 % v/v also for an hour. The mobile phase was also pumped for about an hour. This was repeated each time before work to ensure good results were obtained.

3.9 METHOD VALIDATION

3.9.1 Linearity

A stock solution of 0.01% $^{w}/_{v}$ and 0.005% $^{w}/_{v}$ of the target analytes and the surrogate reference standards respectively were prepared and serially diluted to different concentrations using the mobile phase. The solutions were filtered and injected. The peak Areas were plotted against their respective concentrations to obtain their calibration curves. The coefficients of correlation (R^2), the limit of detection (LOD), the limit of quantification (LOQ) were all calculated from the calibration curves (44).

3.9.2 Specificity and Selectivity

A quantity of 0.005% $^{w}/_{v}$ of the pure Albendazole and Quinine Sulphate were also prepared and separately injected three times each and their retention times noted. 0.005% $^{w}/_{v}$ of Albendazole and the Quinine Sulphate tablets were also and each injected three times. Their retention times were noted. The resolutions and the retention time of the pure samples were compared to that of the tablets (44).

3.9.3 Precision

0.005% $^{w}/_{v}$ of all the pure samples were each injected six times whilst observing a ten minute interval. The corresponding peak areas were recorded. The standard deviation and the relative standard deviation were calculated (44).

3.9.4 Repeatability

A mixture of the Albendazole tablets (0.02%w/v), and the surrogate reference standards (0.01%w/v) were prepared, filtered and injected three times observing an interval of one hour. The peak areas were recorded and the percentage content of Albendazole in the tablets determined. Statistical analysis was carried out to verify whether any difference exist between the results (44).

The procedure was repeated for Quinine Sulphate tablets and the surrogate reference standards.

3.9.5 Reproducibility (Inter-day)

A mixture of the Albendazole tablets (0.02%w/v) and the surrogate reference standards (0.01%w/v) were separately prepared, filtered, mixed together and injected. The percentage content was determined. The procedure was repeated on two other days using freshly prepared solutions and mobile phase. Statistical analysis was performed to verify the reproducibility of the results. The procedure was repeated for Quinine Sulphate and the surrogate reference standards (44).

3.9.6 Robustness

Analysis was carried out varying one parameter at a time whilst keeping the others constant. The parameters varied were flow rate and wavelength (3).

3.10 Stability Studies on Samples

A known concentration of each of the drug samples used was prepared. Each sample solution was injected six times at an interval of twenty (20) minutes. The peak areas were recorded and a graph of peak area against time plotted.

3.11 DETERMINATION OF THE CONSTANT "K" USING THE SURROGATE REFERENCE STANDARDS

Stock solution of 0.02% $^{w}/_{v}$ of the target analyte and 0.01%$^{w}/_{v}$ of the surrogate reference standards were prepared. Five different concentrations of the surrogate reference standards as well as the target analytes were prepared. 5ml of the surrogate reference standards was mixed with 5ml of the target analyte. The resulting solution was injected and the peak area recorded electronically. The constant "K" for each surrogate reference standard in relation to the target analyte was calculated using the relation stated in the Hypothesis for the constant K.

3.12 ASSAY OF ALBENDAZOLE AND QUININE SULPHATE TABLETS

3.12.1 Albendazole tablets

Twenty (20) tablets of each of the three brands of Albendazole were powdered. An amount of the powder corresponding to 0.020g of Albendazole was weighed and dissolved in 100ml of the mobile phase and sonicated at room temperature for five minutes and filtered. 0.01% $^{w}/_{v}$ of the surrogate reference standards was also prepared. 5ml portion each of the analyte and the surrogate reference standards were mixed together, injected and the peak area recorded. The percentage content of Albendazole in the tablets was determined.

3.12.2 Quinine Sulphate tablets

Twenty tablets of each of the three brands of Quinine Sulphate were powdered. An amount equivalent to 0.020g of Quinine Sulphate was weighed into a 100ml volumetric flask,50ml of the mobile phase was added then topped up with the mobile phase to the mark and sonicated at room temperature for five minutes. 0.01% $^{w}/_{v}$ of the surrogate reference standards was prepared. 5ml portion each of the analyte and the surrogate reference standards were mixed together. The resulting solution was injected, the peak area electronically recorded and the percentage content determined.

CHAPTER FOUR

RESULTS AND CALCULATIONS

4.1 IDENTIFICATION OF PURE SAMPLE

4.1.1 Colour and UV test

Table 4.1: Results for colour and UV test

Sample	Result	Inference
Albendazole	1.A reddish -brown precipitate developed 2.The solution showed absorption maxima at 292nm	Positive Positive
Quinine Sulphate	1.Blue colour developed but disappeared upon addition of 1ml of 0.1M HCl 2.The solution showed absorption maxima at 250nm and 346nm	Positive Positive
Aspirin	1.A violet colour developed 2.The solution showed absorption maxima at 230nm and 278nm	Positive Positive
Amoxicillin Trihydrate	1.A dark yellow colour developed after heating 2. The solution showed absorption maxima at 247nm and 291nm	Positive Positive
Metronidazole	The solution showed absorption maxima at 277nm and 240nm	Positive

4.1.2 Melting Point

Table 4.2: Result for melting point determination

Sample	Experimental values (^oC)	Literature values(^oC)	Inference
Albendazole	208-210	209-211	Positive
Quinine Sulphate	225-227	225-233	Positive
Aspirin	135-137	135-136	Positive
Amoxicillin Trihydrate	194-196	194	Positive
Metronidazole	160-163	159-163	Positive

4.1.3 Thin Layer Chromatography (TLC)

4.1.3.1 Albendazole tablets

Fig 4.1: TLC for the three brands of Albendazole and the reference sample

Sample calculation

Rf Value = Distance travelled by component from the origin
Distance travelled by the solvent front from the origin

For the pure Albendazole reference standard, the Rf was calculated as follows

Rf = 9/10

= 0.90

The calculation was repeated for the three brands of Albendazole tablets and the Rf values recorded.

4.1.3.2 Quinine Sulphate tablets

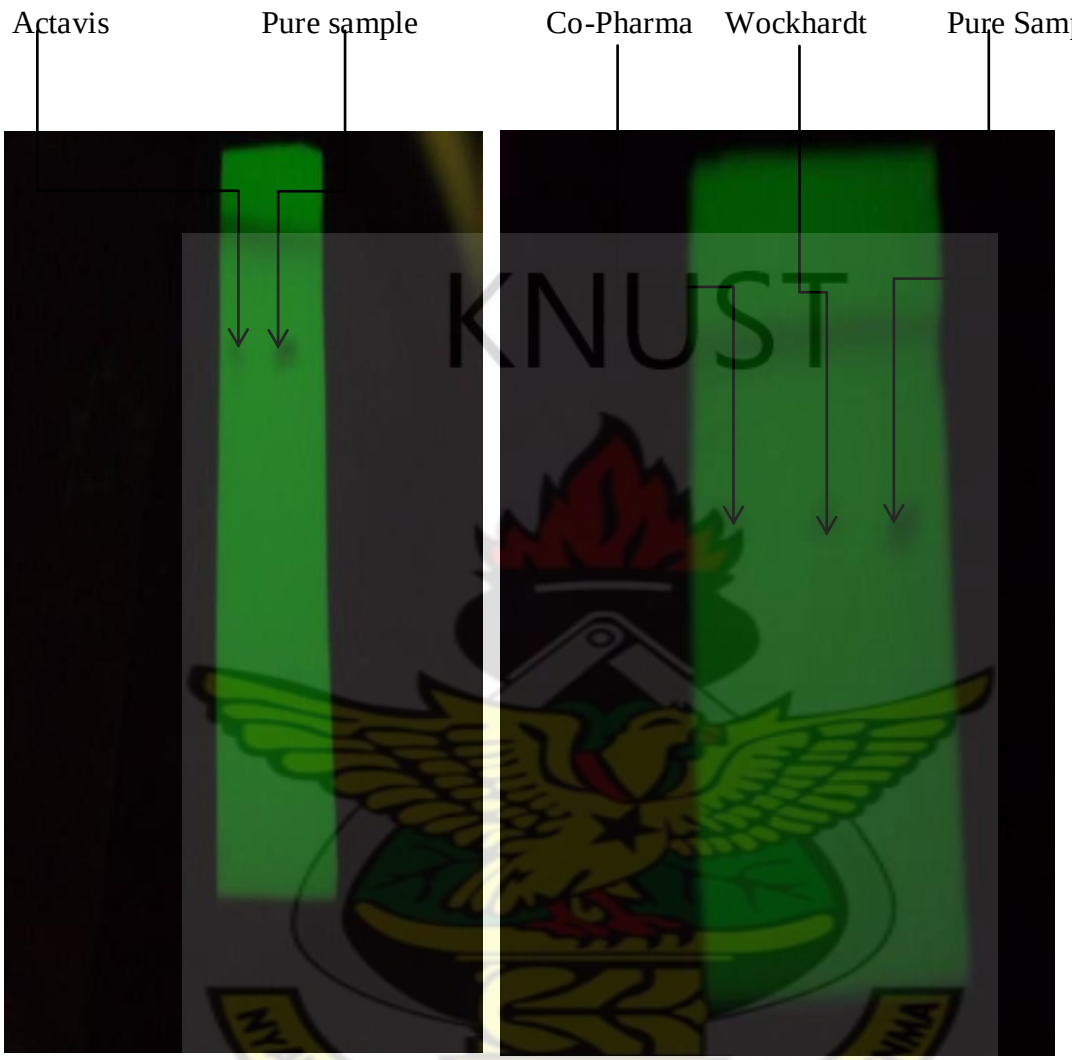

Fig 4.2: TLC of the three brands of Quinine Sulphate and the pure sample

For the pure Quinine Sulphate sample the Rf was calculated as follows

Rf = 4.5/8.5

= 0.53

The Rf values for the three brands of the Quinine Sulphate were determined and recorded.

Table 4.3: Rf values for the three brands of Albendazole

Brands	Rf values of the pure sample	Rf values of tablets
Alben	0.90	0.90
Wormplex 400	0.90	0.90
Zeben	0.90	0.90

Table 4.4: Rf values for the three brands of Quinine Sulphate

Brands	Rf values of the pure sample	Rf values of tablets
Actavis	0.51	0.51
Co-Pharma	0.53	0.53
Wockhardt	0.53	0.53

4.2 PREPARATION OF SOLUTION

4.2.1 Preparation of 1M HCl

36.4500g in 1000ml ≡ 1M HCl

3.6450g in 100ml ≡ 1M HCl

Assay = 36%

36% = 3.6450g

100% = (100 × 3.6450g)/36

= 10.1250g

Specific gravity = 1.18g/ml

1.18g = 1ml

10.1250g = (10.1250g × 1ml)/1.18g

= 8.58 ml

4.2.2 Preparation of 1M NaOH

40.0000g in 1000ml ≡ 1M NaOH

4.0000g in 100ml ≡ 1M NaOH

% purity of NaOH = 99%

99% = 4.0000g

100% = (100× 4.0000g)/99

= 4.0404g

Hence 4.0404g of NaOH was dissolved in 100ml of distilled water.

4.2.3 Standardization of 0.1M Perchloric Acid ($HClO_4$) using Potassium Hydrogen Phthalate ($C_8H_5KO_4$)

1M ($C_8H_5KO_4$) ≡ 1M $HClO_4$

204.14g of ($C_8H_5KO_4$) ≡ 1000 ml of 1M $HClO_4$

20.41g ≡ 1000ml of 0.1M $HClO_4$

Therefore 0.020414g of ($C_8H_5KO_4$)≡ I ml of 0.1M ClO_4

Table: 4.5: Titration results for Perchloric acid

Burette reading (ml)	1ST Determination	2ND Determination	3RD Determination	Blank
Final reading	24.60	24.50	24.60	20.10
Initial reading	0.00	0.00	0.00	20.00
Titre volume	24.60	24.50	24.60	0.010

Average titre = (24.60 +24.50 + 24.60) ml/3

= 24.57 ml

Actual titre = average titre -blank

= (24.57- 0.10) ml

= 24.47 ml

Expected weight of ($C_8H_5KO_4$): 0.500g

Actual weight of ($C_8H_5KO_4$): 0.507

Factor of ($C_8H_5KO_4$): Actual weight/Expected weight

= 0.507g/0.500g

= 1.014

Fa× Va ×Ca = Fb× Cb× Vb

Where, Fa is the factor of $HClO_4$; Va is the volume of $HClO_4$; Ca is the concentration of $HClO_4$; Fb is the factor of ($C_8H_5KO_4$); Vb is the volume of ($C_8H_5KO_4$) and Cb is the concentration of ($C_8H_5KO_4$).

Fa ×24.47ml × 0.1M = 1.014×25ml×0.1M

Fa= 1.036

Therefore the factor of $HClO_4$ is 1.036

4.2.4 Standardization of HCl using Sodium Bicarbonate (Na_2CO_3)

Molar mass of Na_2CO_3 ≡ 106g/mol

That is 106g of Na_2CO_3 in 1000 ≡ 2M HCl

53g of Na_2CO_3 in 1000ml ≡ 1M HCl

26.5gof Na_2CO_3 in 1000ml ≡ 0.5M HCl

6.625g of Na_2CO_3 in 250ml ≡ 0.5M HCl

0.0265g of Na_2CO_3 in 25ml ≡ 0.5M HCl

Table 4.6: Titration results for Na_2CO_3

Burette reading(ml)	1st determination	2nd determination	3rd determination
Final reading	25.10	25.10	25.20
Initial reading	0.00	0.00	0.00
Titre volume	25.10	25.10	25.20

Average titre = (25.10 +25.10 +25.20) ml/3 = 25.13ml

Percentage purity of Na_2CO_3 = 99%

If 99% of Na_2CO_3 = 6.6250g, then

100% = (100/99) × 6.6250g = 6.6919g

Actual weight = 6.692g

Factor of Na_2CO_3 = 6.692g/6.6919g = 1.000

$F_{Na2CO3} \times V_{Na2CO3} = F_{HCl} \times V_{HCl}$

F_{HCl} = (1.00 x 25 ml)/25.13ml = 0.995

4.2.5 Standardization of 0.5M NaoH using 0.5M HCl

Molecular weight (NaOH) = 40g

40g of NaOH in 1000ml = 1M HCl

20g of NaOH in 1000ml = 0.5M HCl

5g of NaOH in 250ml = 0.5M HCl.

Table 4.7: Titration results for HCl

Burette Reading (ml)	1st Determination	2nd Determination	3rd Determination
Final reading	24.90	24.80	24.80
Initial reading	0.00	0.00	0.00
Titre volume	24.90	24.80	24.80

Average titre = (24.90 + 24.80 +24.80) ml/3 = 24.83ml

$F_{HCl} \times V_{HCl} = F_{NaOH} \times V_{NaOH}$

$F_{NaOH} = (F_{HCl} \times V_{HCl)/} V_{NaOH}$

= (0.99x 25ml) /24.83ml = 1.00

Therefore the factor of NaOH is 1.00

4.3 ASSAY OF PURE SAMPLES

4.3.1 Aspirin

Table 4.8: Titration results for pure aspirin

Burette reading (ml)	1st Determination	2nd Determination	3rd Determination	Blank
Final reading	20.50	20.40	20.50	29.30
Initial reading	0.00	0.00	0.00	0.00
Titre	20.50	20.40	20.50	29.30

Average titre = (20.50+20.40+20.50) ml /3 = 20.467 ml

Actual volume of NaOH that reacted with Aspirin = Average titre - Blank = 8.833ml

Each ml of NaOH = 0.0 450g of Aspirin

Factor of NaOH = 1.0

Therefore the weight of Aspirin that reacted with NaOH is given as

(8.833ml x 1.00) x 0.04504g/ml

=0.3986g

Weight of Aspirin dissolved in NaOH (expected weight) is 0.401g

Percentage content of Aspirin = (actual weight/expected weight) x 100

= (0.3986g /0.401g) x 100

= 99.4%

Therefore Aspirin granules contain 99.4% of pure Aspirin.

4.3.2 Metronidazole

Table 4.9: Titration results for Pure Metronidazole

Burette reading(ml)	1st Determination	2nd Determination	3rd Determination	Blank Determination
Final reading	7.10	7.00	7.00	1.20
Initial reading	0.00	0.00	0.00	0.00
Titre	7.10	7.00	7.00	1.20

Average titre = (7.10+7.00+7.00) ml/3 = 7.033ml

Actual volume of $HClO_4$ that reacted =Average titre –blank

= (7.033ml - 1.20) ml = 5.833ml

1ml $HClO_4$ is equivalent to 0.01712g of metronidazole

Factor of $HClO_4$ = 1.036

Weight of metronidazole that reacted = (5.833ml x 1.036) x 0.01712g/ml

= 0.01035g

Percentage content = (Actual weight/Expected weight) × 100

= (0.1035g/0.1040g) × 100

= 99.5%

Therefore, the percentage content Metronidazole is 99.50%

4.3.3 Albendazole

Table 4.10: Titration results for Pure Albendazole

Burette reading	1st	2nd	3rd	Blank
Final reading	6.60	6.70	6.60	1.20
Initial reading	0./00	0.00	0.00	0.00
Titre volume	6.60	6.70	6.60	1.20

Average titre = (6.60 +6.70 + 6.60) ml/3 = 6.633ml

Titre = average titre - blank = (6.633-1.20) = 5.433ml

Each ml of perchloric acid ($HClO_4$) consumes 0.02653g of Albendazole
Hence total amount of Albendazole that reacted with $HClO_4$ =(5.433 × 1.036) × 0.02653g/ml

= 0.1493g.

Percentage content of Albendazole = (Actual weight/Expected weight) × 100

= (0.1443/ 0.1502) ×100

= 99.4%

Therefore the percentage content of Albendazole in the Albendazole powder is 99.40%.

4.3.4 Quinine Sulphate

Table 4.11: Titration results for pure Quinine Sulphate powder

Burette reading (ml)	1st Determination	2nd Determination	3rd Determination	Blank
Final reading	7.80	15.80	7.90	8.10
Initial reading	0.00	8.00	0.00	0.00
Titre	7.80	7.80	7.90	0.10

Average titre = (7.80 + 7.80 +7.90) ml/3 = 7.833 ml

Titre= Average titre- Blank

= (7.833 – 0.10) ml = 7.733ml

Actual volume of $HClO_4$ that reacted with Quinine Sulphate = 7.733ml × 1.036

= 8.011388ml

Each ml of $HClO_4$ Consumes 0.0249g of the pure Quinine Sulphate,

Therefore, 8.011388ml will consume 8.011388ml × 0.0249g/ml

= 0.19948356g

Expected weight = 0.200g

Percentage content = (Actual weight/Expected weight) ×100

= (0.19948356g/0.200g) × 100

= 99.74%

Therefore the percentage content of Quinine Sulphate in the Quinine Sulphate reference powder is 99.74%.

4.3.5 Amoxicillin Trihydrate

Absorbance = 0.433

A(1%, 1cm)= 225

From Beer's law,

Absorbance= abc

Where, a= A (1%, 1cm)

B= pathlength, c= concentration.

Therefore, C= A/ab

C= 0.433/225

= 0.001923%$^{W}/_{V}$

Percentage content = (Expected weight/Actual weight) x 100

= (0.00189$^{W}/_{V}$/0.001923$^{W}/_{V}$) x 100

= 98.30%

Therefore the percentage content of Amoxicillin Trihydrate in the pure sample is 98.30%

4.4 ASSAY OF COMMERCIAL TABLETS USING STANDARD METHODS

4.4.1 Albendazole

Refer to appendix V for titre values and sample calculations

Table 4.12: Average percentage content of **Albendazole** in three different brands of Albendazole tablets, USP. (n=5).

Determinations	Alben	Wormplex 400	Zeben
1	98.95	101.39	99.33
2	99.10	101.91	99.26
3	98.49	101.24	99.15
4	98.85	101.74	99.40
5	98.44	101.64	99.70
Average	98.77 ±0.29	101.58±0.27	99.37±0.21

4.4.2 Quinine Sulphate

Refer to appendix V for titre values and calculations.

Table 4.13: Average percentage content of Quinine Sulphate in three different brands of Quinine tablets, BP 2007. (n=5)

Determinations	Actavis	Co-Pharma	Wockhardt
1	99.14	102.17	97.65
2	99.69	102.46	97.72
3	99.20	101.66	97.34
4	99.64	103.49	97.55
5	99.74	101.46	97.33
Average	99.5±0.29	102.25±0.80	97.52±0.18

4.5 HPLC METHOD DEVELOPMENT

4.5.1 Chromatograms

4.5.1.1 Chromatograms of Albendazole and surrogate standards

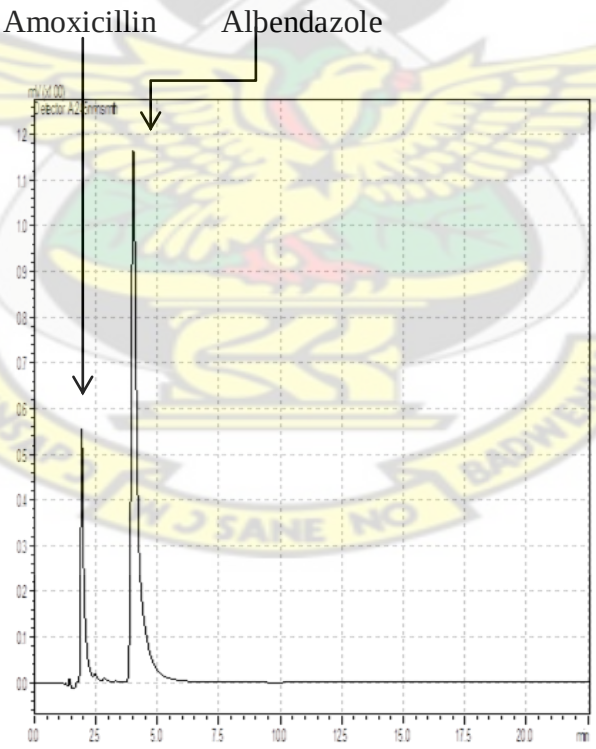

Fig 4.3: Chromatogram of Amoxicillin Trihydrate and Albendazole

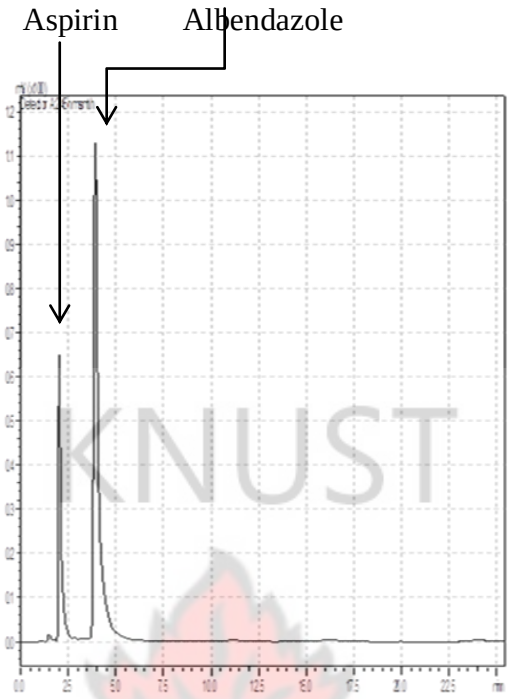

Fig 4.4: Chromatogram of Aspirin and Albendazole

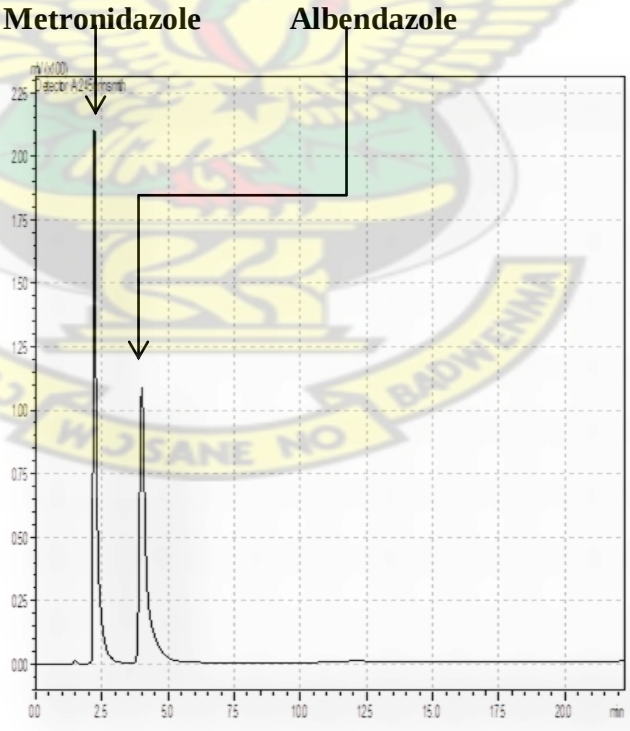

Fig 4.5: Chromatogram of Metronidazole and Albendazole

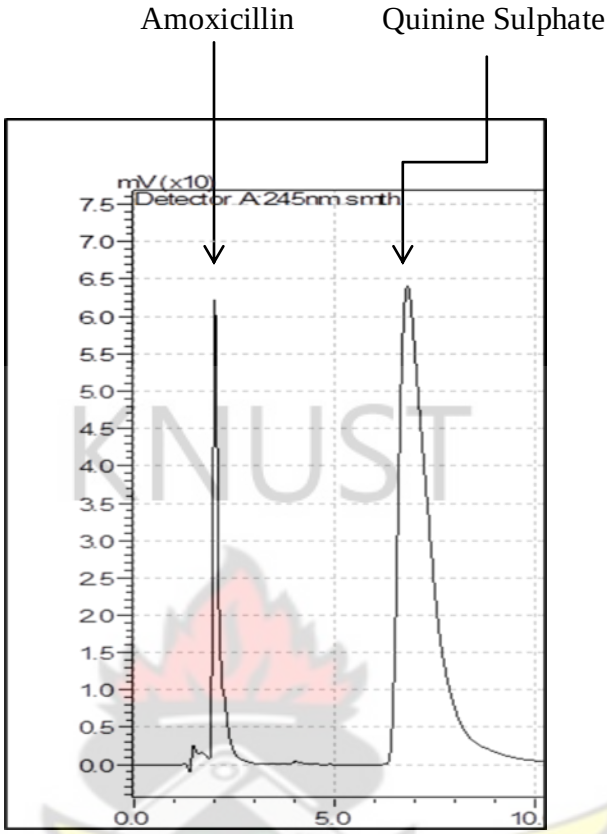

Fig 4.6 Chromatogram of Amoxicillin Trihydrate and Quinine Sulphate

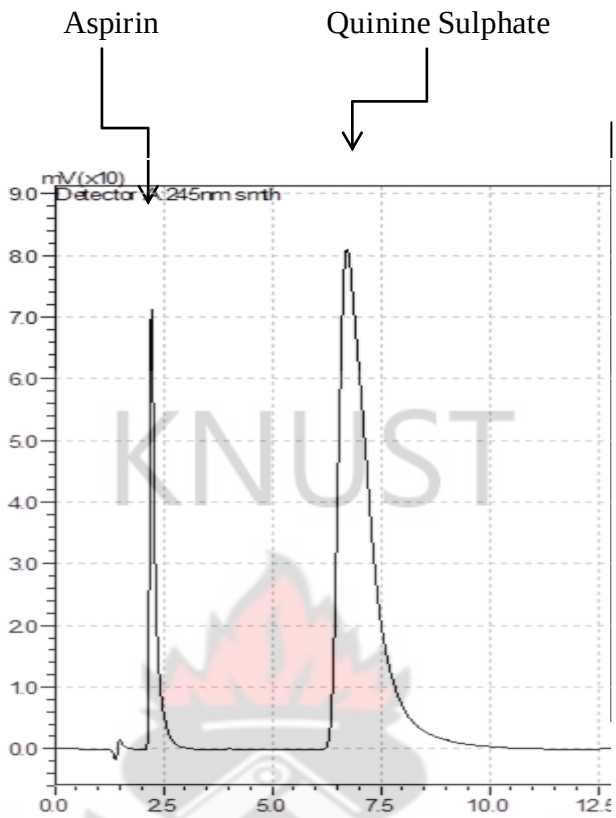

Fig 4.7: Chromatogram of Aspirin and Quinine Sulphate

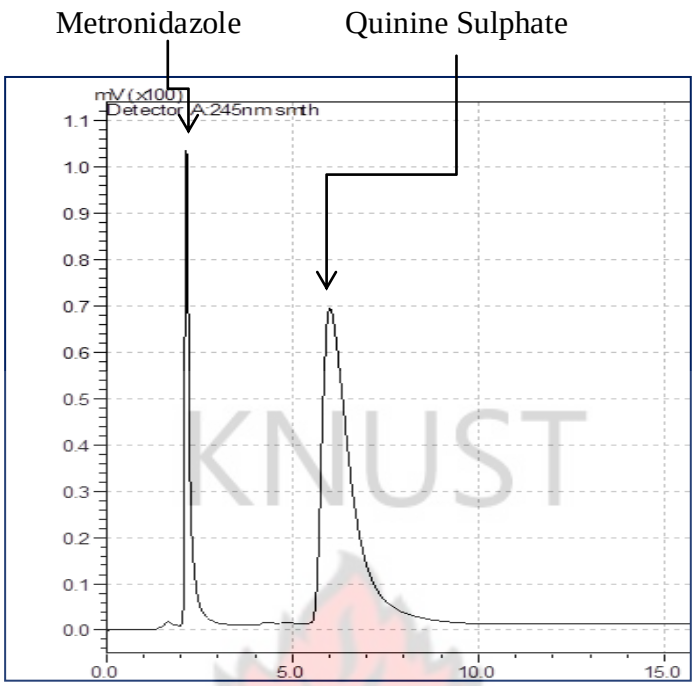

Fig 4.8: Chromatogram of Metronidazole and Quinine Sulphate

Table 4.14: Retention time of Albendazole and the Surrogate reference standards

Drugs	Albendazole	Aspirin	Amoxicillin Trihydrate	Metronidazole
Retention time	4.21±0.036	1.74±0.021	2.53±0.018	2.28±0.029
Tailing factor	1.04			

Table 4.15: Retention time of Quinine Sulphate and the Surrogate reference standards

Drugs	Quinine Sulphate	Aspirin	Amoxicillin Trihydrate	Metronidazole
Retention time	6.65± 0.015	2.78±0.030	3.02± 0.017	2.02±0.007
Tailing factor	1.38			

4.5.1 Linearity

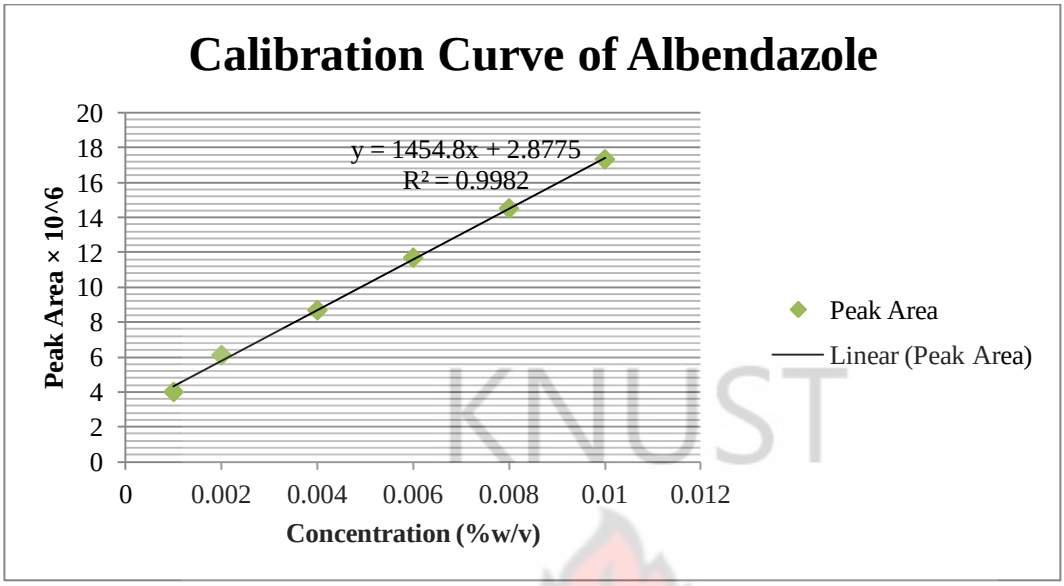

Fig 4.9: Calibration curve of Albendazole

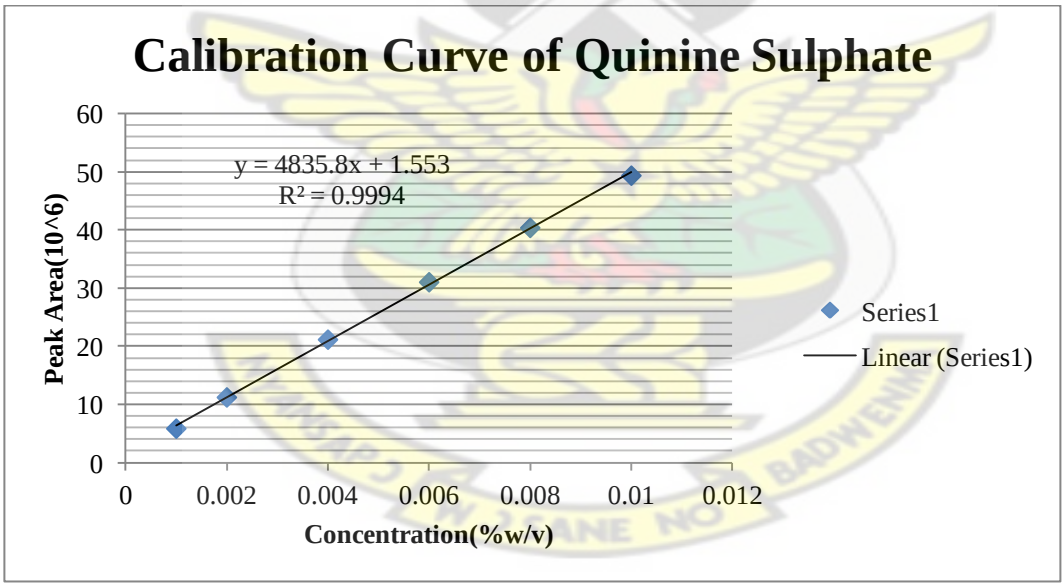

Fig 4.10: Calibration curve of Quinine Sulphate

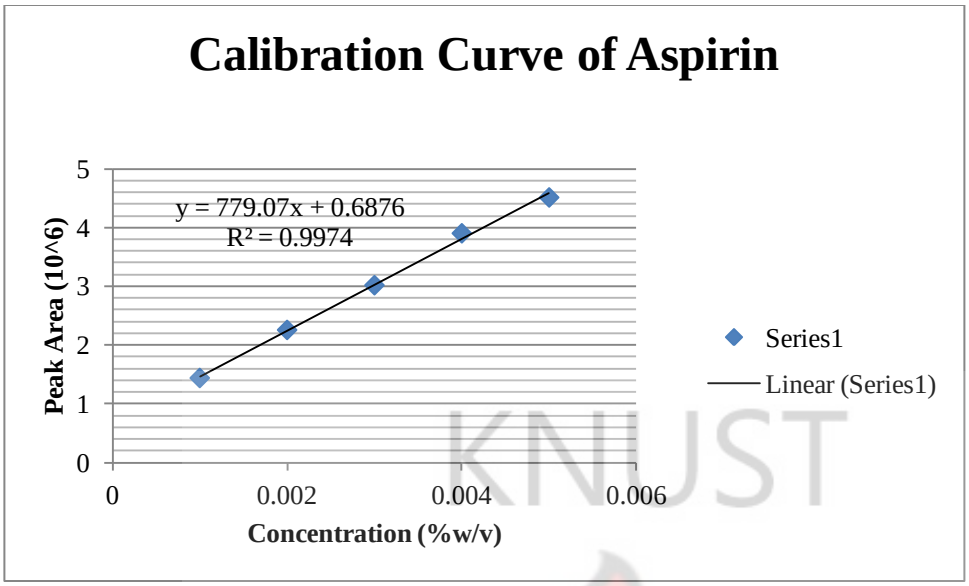

Fig 4.11: Calibration curve of Aspirin

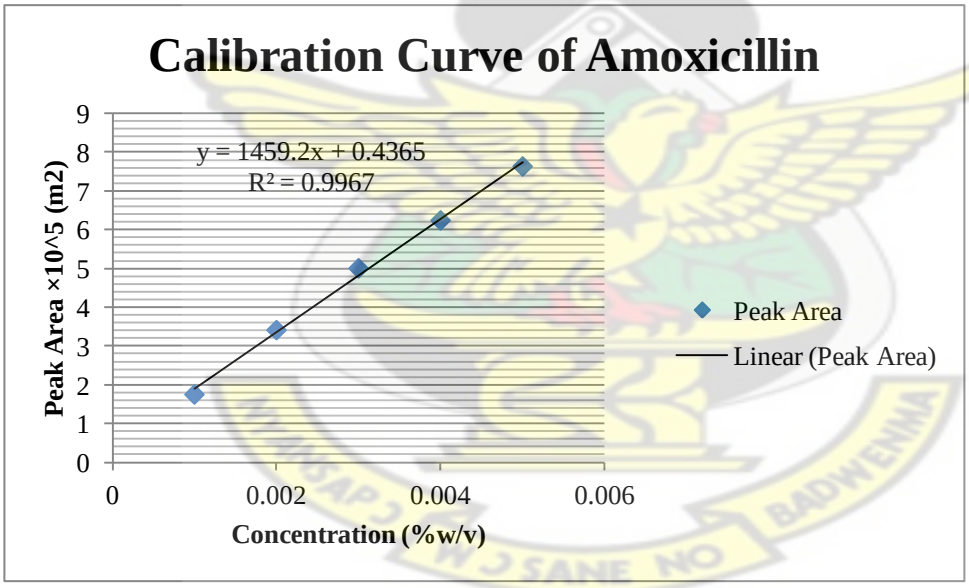

Fig 4.12: Calibration curve of Amoxicillin Trihydrate

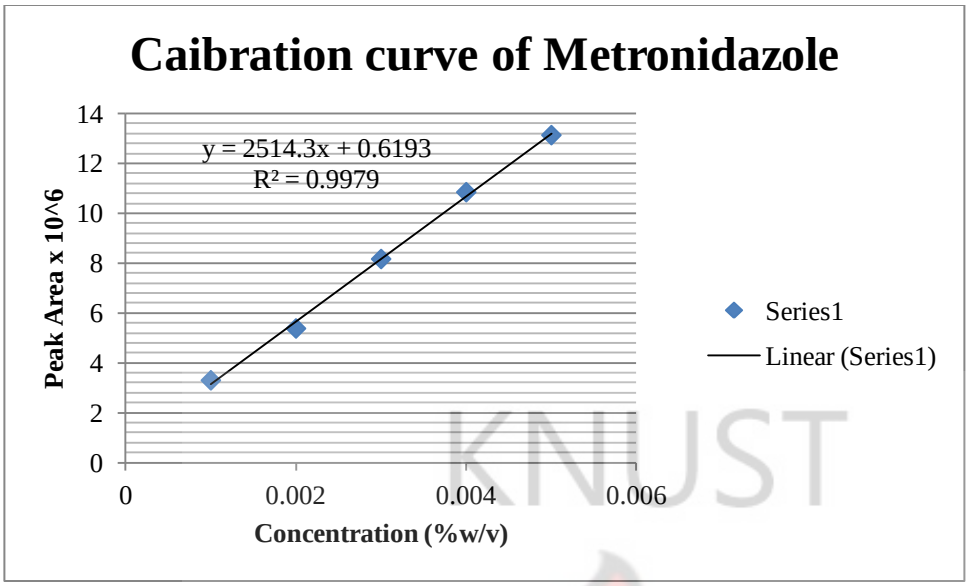

Fig 4.13: Calibration curve of Metronidazole

Table 4.16: Coefficient of Correlation (R^2) and equations of line for Albendazole and its surrogate reference standards

Drug	R^2	Slope	Intercept
Albendazole	0.9982	1454.8	2.8775
Aspirin	0.9967	1459.2	0.4365
Amoxicillin	0.9974	777.07	0.6876
Metronidazole	0.9979	2514.3	0.6193

Table 4.17: Coefficient of Correlation (R^2) and equations of line for Quinine Sulphate and its surrogate reference standards

Drugs	R^2	Slope	Intercept
Quinine Sulphate	0.9994	4835.8	1.553
Aspirin	0.998	1305.4	0.4489
Amoxicillin	0.9986	1283	1.1172
Metronidazole	0.9979	3835	2.9256

4.5.2 Sensitivity

Sample calculation of Limit of Detection (LOD) and Limit of Quantification (LOQ)

Determination of LOD and LOQ for Albendazole

LOD = 3.3σ/S

LOQ = 10σ/S

Where,

σ = Residual standard deviation,

That is $\sigma = \{\Sigma(Y\text{-}Yest)/n\text{-}1\}^2$

Y = y values (peak area) from the calibration curve

Yest = y values calculated using the equation of line; y = mx+c,

n = number of determinations,

n-1 = number of degrees of freedom,

S = the slope of the equation of line from the calibration curve.

Table 4.18: Determination of LOD and LOQ for Albendazole

Concentration	Peak Area $\times 10^6$ (Y)	Yest	Y-Yest
0.001	4.005733	4.3323	0.326567
0.002	6.113174	5.7871	0.326074
0.004	8.688254	8.6967	0.008446
0.006	11.69916	11.6063	0.09286
0.008	14.52232	14.5159	0.00642
0.010	17.33610	17.4255	0.08940
			Σ(Y-Yest) = 0.849767

Equation of line: y = 1454.8x+2.8775

S = 1454.8, n = 6, n-1 = 5

$\sigma = \{\Sigma(Y\text{-}Yest)/n\text{-}1\}^2$

$= (0.849767/5)^2$

$= 0.028884$

But LOD = 3.3σ/S

$3.3(0.028884/1454.8)$

Therefore LOD = $6.552\times10^{-5}\%^{w}/_{v}$

LOQ = 10σ/S

=10(0.028884/1454.8)

Therefore LOQ = $1.985\times10^{-4}\%^{W}/_{V}$

Table 4.19: LOD and LOQ of Albendazole and the surrogate reference standards

Drugs	Limit of Detection($\%^{w}/_{v}$)	Limit of Quantification ($\%^{w}/_{v}$)
Albendazole	6.552×10^{-5}	1.985×10^{-4}
Aspirin	1.231×10^{-5}	3.731×10^{-5}
Amoxicillin	4.059×10^{-5}	1.230×10-4
Metronidazole	3.580×10^{-5}	1.085×10^{-4}

Table 4.20: LOD and LOQ of Quinine Sulphate and the surrogate reference standards

Drugs	Limit of Detection($\%^{w}/_{v}$)	Limit of Quantification($\%^{w}/_{v}$)
Quinine Sulphate	1.1156×10^{-4}	3.381×10^{-4}
Aspirin	1.887×10^{-6}	5.718×10^{-6}
Amoxicillin	1.844×10^{-5}	5.587×10^{-5}
Metronidazole	5.604×10^{-5}	1.698×10^{-4}

4.5.3 Precision

Refer to appendix VI for the RSD of the analytes and their surrogate reference standards.

4.5.4 Robustness

Table 4.21: Percentage content of Albendazole in the Alben tablet using Metronidazole as a surrogate reference standard (varying Conditions)

Determination	Varying Wavelengths		Varying Flow rate	
	245nm	240nm	1ml/min	1.2ml/min
1	99.43	99.32	99.43	100.12
2	99.35	98.91	99.35	99.98
3	99.6	100.12	99.6	98.99
4	100.7	99.72	100.7	100.32
5	100.42	99.48	100.42	100.73
t-test	1.141		0.3205	

Table 4.22: Percentage content of Quinine Sulphate in Quinine Sulphate tablet (Actavis) using Aspirin as a surrogate reference standard (varying conditions)

Determinations	Varying Wavelength		Varying Flow rate	
	245nm	250nm	1.5ml/min	1.8ml/min
1	97.54	98.34	97.54	98.02
2	98.45	98.12	98.45	98.89
3	99.3	99.06	99.3	99.23
4	99.52	98.79	99.52	99.76
5	97.73	99.22	97.73	98.34
t-test	0.4389		0.6720	

4.5.5 Repeatability

Table 4.23: Percentage of Albendazole in Wormplex 400 tablet using Amoxicillin Trihydrate as a surrogate reference standard

Determinations	Intra-day variation		Inter-day variation	
	Test I	Test II	Day I	Day II
1	102.07	101.99	102.07	102.76
2	102.29	101.46	102.29	103.04
3	101.14	100.98	101.14	102.32
4	102.62	102.01	102.62	101.23
5	101.54	102.87	101.54	101.33
t-test	0.1699		0.4499	

Table 4.24: Percentage content of Quinine Sulphate in Quinine Sulphate tablet (Co-Pharma) using Metronidazole as a surrogate reference standard

Determinations	Intra-day variation		Inter-day variation	
	Test I	Test II	Day I	Day II
1	103.17	102.42	103.17	103.43
2	103.02	101.56	103.02	103.08
3	102.12	103.71	102.12	102.11
4	103.13	100.89	103.13	101.32
5	103.74	102.21	103.74	103.61
t-test	1.629		0.6444	

4.6 Stability profile of samples

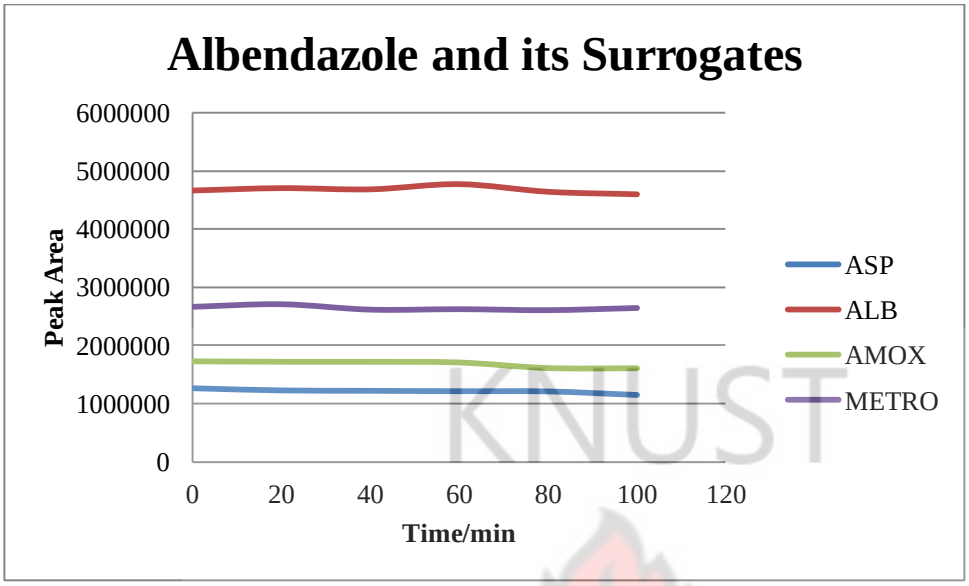

Fig 4.14: Stability profile of Albendazole and surrogate standards

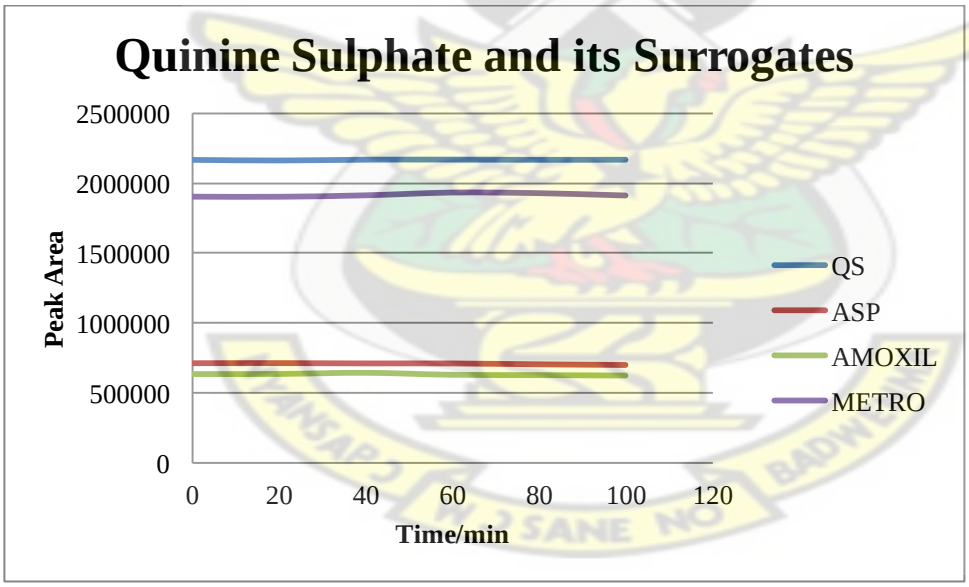

Fig 4.15: Stability of Quinine Sulphate and surrogate standards

4.7 DETERMINATION OF THE CONSTANT "K"

Sample calculation

K= (Peak Area of ALB/Conc of ALB) ÷ (Peak Area of AMOXIL/Conc of AMOXIL)
= (9323965/0.010) ÷ (1726953/0.005)
= 932396500/345390600
=2.6998

Table 4.25: K values for Albendazole and Amoxicillin Trihydrate.

Conc of ALB ($\%^{W}/_{V}$)	Peak Area of ALB (m^2)	Conc of AMOXIL ($\%\ ^{W}/_{V}$)	Peak Area of AMOXIL ($\%^{W}/_{V}$)	K
0.010	9323965	0.005	1726953	2.700
0.008	6954855	0.004	1292661	2.690
0.006	5826267	0.003	1088537	2.688
0.004	3712524	0.002	686760	2.703
0.002	2444197	0.001	453648	2.694

Table 4.26: K values for Albendazole and the Surrogate reference standards

Surrogates/Days	1	2	3	4	5	Average
AMOXIL	2.700	2.690	2.676	2.703	2.694	2.693±0.009
ASP	2.301	2.298	2.290	2.300	2.296	2.297±0.004
METRO	1.830	1.821	1.826	1.826	1.830	1.827±0.003

Table 4.27: K values for Quinine Sulphate and the Surrogate reference standards

Surrogates/Days	1	2	3	4	5	Average
AMOXIL	3.900	3.885	3.901	3.900	3.885	3.893±0.008
ASP	2.543	2.550	2.541	2.558	2.551	2.549±0.007
METRO	1.0380	1.037	1.038	1.039	1.038	1.034±0.0006

4.8 DETERMINATION OF PERCENTAGE CONTENT OF ALBENDAZOLE AND QUININE SULPHATE TABLETS USING THE DEVELOPED METHOD USING 'K'

4.8.1 Alben (Albendazole)

Sample calculation

Average weight of tablet= 1.032375g

Weight equivalent to 0.02%$^{w}/_{v}$ = 0.05162g

Surrogate reference standard= metronidazole

Peak Area of Alben = 4848171

Peak Area of Surrogate= 1335578

Concentration of surrogate= 0.005%$^{w}/_{v}$

Concentration of Alben = 0.01%$^{w}/_{v}$

K of metronidazole= 1.827

From the hypothesis,

Concentration of analyte = (Area of analyte x Concentration of surrogate standard) / (k value x Area of surrogate standard)

= (4848171 x 0.005)/ (1.827 x 1335578)

= 0.009934%$^{w}/_{v}$

Percentage content= (actual weight/expected weight) x 100%

= (0.009934/0.01) x 100%

= 99.35%

Therefore the Alben tablet contains 99.35% of the stated amount of Albendazole.

Table 4.28: Mean percentage content of the three brands of Albendazole tablets using the surrogate reference standards (n=5)

Surrogate Standards	Mean Percentage Content (n=5)		
	Alben	Wormplex 400	Zeben
Aspirin	98.22± 0.66	101.63± 0.587	98.82± 0.535
Amoxicillin	99.55± 0.675	101.02± 0.639	99.79± 0.824
Metronidazole	99.37± 0.84	101.13± 0.500	99.822± 0.577

Table 4.29: Mean percentage content of the three brands of Quinine Sulphate tablets using the surrogate reference standards

Surrogate Standards	Mean Percentage Content (n= 5)		
	Actavis	Co-Pharma	Wockhardt
Aspirin	98.51± 0.89	102.74± 0.64	98.180± 0.75
Amoxicillin	99.99± 0.43	101.842± 0.44	97.004± 0.62
Metronidazole	99.87± 0.421	103.22± 0.380	100.17± 0.399

4.9 COMPARING THE ACCURACY OF THE DEVELOPED METHOD TO THE STANDARD METHOD USING T-TEST

Table 4.30: t-Test for Alben tablets

Determinations	Standard method	Aspirin	Amoxicillin Trihydrate	Metronidazole
1	98.95	98.97	99.28	99.43
2	99.10	98.74	98.70	99.35
3	98.49	98.29	99.40	99.6
4	98.85	97.65	100.5	98.07
5	98.44	97.46	99.87	100.42
Average	98.80± 0.289	98.22± 0.66	99.55±0.675	99.37± 0.84
t-test		2.329	2.0987	1.3259

Table 4.31: t-Test for Wormplex 400 tablets

Determinations	Standard Method	Aspirin	Amoxicillin Trihydrate	Metronidazole
1	101.39	100.85	101.07	101.17
2	101.91	101.68	100.29	100.67
3	101.24	101.67	101.14	101.49
4	101.74	101.47	100.62	100.59
5	101.64	102.49	101.98	101.73
Average	101.58± 0.269	101.63± 0.587	101.02± 0.639	101.13± 0.500
t- test		0.1873	1.590	1.4545

Table 4.32: t-Test for Zeben tablets

Determinations	Standard method	Aspirin	Amoxicillin Trihydrate	Metronidazole
1	99.33	98.56	98.75	99.09
2	99.26	98.80	100.26	100.44
3	99.15	98.48	100.54	99.34
4	99.40	99.75	100.34	100.08
5	99.70	98.51	99.05	100.16
Average	99.37± 0.207	98.82± 0.535	99.79± 0.82	99.822± 0.577
t- test		2.157	0.977	1.9104

Table 4.33: t-Test for Actavis tablets

Determinations	Standard method	Aspirin	Amoxicillin Trihydrate	Metronidazole
1	99.14	97.54	100.70	100.26
2	99.69	98.45	99.75	99.55
3	99.20	99.30	99.65	99.50
4	99.64	99.52	99.78	100.38
5	99.74	97.73	100.05	99.64
Average	99.48± 0.278	98.51± 0.894	99.99± 0.426	99.87± 0.42
t- test		2.356	1.894	1.578

Table 4.34: t-Test for Co-Pharma tablets

Determinations	Standard method	Aspirin	Amoxicillin Trihydrate	Metronidazole
1	102.17	102.77	101.72	103.17
2	102.46	103.79	101.18	103.07
3	101.66	1022.44	102.13	102.72
4	103.49	102.59	101.84	103.42
5	101.46	102.09	102.34	103.74
Average	102.25± 0.80	102.74± 0.64	101.84± 0.443	103.22± 0.383
t- test		1.315	0.8345	2.547

Table 4.35: t-Test for Wockhardt tablets

Determinations	Standard Method	Aspirin	Amoxicillin Trihydrate	Metronidazole
1	97.65	97.49	98.14	97.89
2	97.72	97.36	97.34	97.49
3	97.34	96.05	98.21	96.67
4	97.55	96.60	99.37	96.72
5	97.33	97.52	97.84	98.97
Average	97.52± 0.18	97.00± 0.65	98.18± 0.75	97.348±0.623
t- test		1.9176	1.864	0.6180

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 DISCUSSIONS

5.1.1 Identification Test

In a chemical laboratory, it is always important to establish the true identity of any substance that one works with. Some compounds could bear superficial resemblance to the sample being worked with or intended to work with and it is only tests of identity that can discriminate against these unwanted substances. One sure way of doing this is by subjecting the samples to identification tests to confirm their identities so that any conclusions drawn from results with such samples will be valid and authentic. Identification tests could take any form ranging from measurement of physical properties of compound, chemical reactions or the use of equipment like the IR spectrophotometer and the HPLC. HPLC is also able to detect adulteration with chemically related substances that can affect laboratory results significantly.

5.1.1.1 Albendazole

For the colour test for the identification of Albendazole, a reddish-brown precipitate was observed after the addition of 1ml of Potassium Iodobismuthate to the sample in Sulphuric acid. (45) Albendazole in 0.01M HCl showed maximum absorbance at 292nm with specific absorbance of 370. These values agree with the values stated in the Clark's analysis of drugs and poisons. The melting range of the sample was $208^{O}C$-$210^{O}C$. The experimental melting range falls within the stated range in the BP 2007. The assay value of the pure Albendazole sample was 99.40%. This falls within the 98.0%-102% stated in the BP 2007. Considering these factors, the sample was confirmed to be Albendazole and was sufficiently pure for the analysis.

5.1.1.2 Quinine Sulphate

A blue colour was observed under UV light at 365nm. The blue colour disappeared on addition of 1ml of 0.01MHCl (14). The melting range of the Quinine Sulphate sample was 225^{O}C-227^{O}C. This agrees with 225^{O}C-233^{O}C stated in the USP. In Methanol, the Quinine Sulphate sample showed maximum absorbance at 346nm. This conforms to the stated value in the BP 2007. The percentage content of Quinine Sulphate in the sample was 99.74%. The BP 2007 states 98.0%-102.0%. With the above results, the sample was pure Quinine Sulphate and sufficiently pure for the analysis.

5.1.1.3 Aspirin

The violet colour that developed after two drops of Ferric Chloride was added to the sample boiled in water indicated the presence of Aspirin (14). The melting range of the Aspirin sample was found to be 135^{O}C-136^{O}C compared to the 135^{O}C-136^{O}C. In 0.1M HCl, the sample showed maximum absorption at 230nm and 278nm. The percentage content of Aspirin in the sample was found to be 99.52% which falls within 99.50%-101.0% stated in the BP 2007. The above factors confirmed that the sample is pure Aspirin and enough for the analysis.

5.1.1.4 Amoxicillin Trihydrate

The dark yellow colour that developed after heating indicated the sample was Amoxicillin Trihydrate. The melting range of the sample was determined to be 194^{O}C-196^{O}C compared to the 194^{O}C (2). The percentage of Amoxicillin Trihydrate in the sample was found to be 99.87% which falls within the 95.0%-102.0% stated in the BP 2007. The sample therefore is pure Amoxicillin Trihydrate and pure enough for the analysis.

5.1.1.5 Metronidazole

The Metronidazole sample in 0.1M HCl showed maximum absorbance at 277nm and the specific absorbance was 365 (14). The melting range of the sample was found to be 160^{O}C-163^{O}C compared to 159^{O}C-163^{O}C stated in the BP 2007. The percentage content of Metronidazole in the sample was determined to be 99.50% which falls within the 99.0%-101.0% stated in the BP 2007.The sample is therefore pure Metronidazole and pure enough for the analysis.

5.1.1.6 Thin Layer Chromatography (TLC)

TLC was carried out to verify whether the commercial tablets contain the stated active ingredients. The tablets gave Rf values comparable to the Rf values of the reference standards.

For the Albendazole tablets, Rf values of 0.90, 0.899, and 0.90 were obtained for Alben, Wormplex 400 and Zeben tablets respectively whilst 0.90 was obtained for the Albendazole reference standard. Hence the Albendazole commercial tablets contain Albendazole as the active ingredient.

For Quinine Sulphate tablets, Rf values of 0.49, 0.50 and 0.50 were obtained for Actavis, Co-Pharma and Wockhardt tablets respectively as compared to 0.49, 0.50 and 0.50 for the Quinine Sulphate reference standard BP 2007. Therefore, the tablets contain Quinine Sulphate as the active ingredient.

5.1.2 UNIFORMITY OF WEIGHT

According to the BP 2007, for a tablet, not more than two of individual weights should deviate by figure provided for the respective weight in table 5.1, and none of the weights should deviate by more than twice that percentage (14).

Table 5.1: Uniformity of weight of tablets (coated and uncoated)

Average weight of tablets	Percentage deviation allowed	Number of tablets
80mg	± 10	20
More than 80mg and less than 250mg	± 7.5	20
250mg or more	± 5	20

Source: Bp 2007.

In effect not more than two tablets should deviate by 5%, and none of the tablets should deviate by more than 10%.

The average weight of Alben, Wormplex 400 and Zeben (Albendazole tablets) were 1.0324g, 0.739g and 0.557g respectively. The highest percentage deviation for Alben, Wormplex 400 and the Zeben tablets were 1.489%, 1.364% and 2.889% respectively. Since none of the tablets deviated by more than 5% , the three brands of Albendazole tablets used in the analysis passed the uniformity of weight test.

The average weight of the three brands of Quinine Sulphate tablets manufactured by Actavis, Co-Pharma and Wockhardt were 0.445g, 0.595g and 0.667g respectively. The highest percentage deviations for the tablets were 2.279%, 2.23% and 3.964 respectively.

Since none of the tablets from the three brands of Quinine Sulphate deviated by 5%, all the three brands passed the uniformity of weight test.

5.1.3 DETERMINATION OF PERCENTAGE CONTENT THE COMMERCIAL TABLETS USING STANDARD METHODS

The commercial tablets were analyzed and their respective percentage contents determined prior to the method development. This was done so as to have a fair idea of the tablets being analyzed.

5.1.3.1 Albendazole

The percentage content of Albendazole in the three commercial brands was determined five times for each of the brands. The average percentage content of Albendazole in the Alben, Wormplex 400 and Zeben tablets were 98.77± 0.29%, 101.58± 0.27 and 99.37± 0.21% respectively. Albendazole tablet is to contain not less than 90.0% and not more than 110% of the stated amount of Albendazole (9). Therefore the three brands of Albendazole tablets passed the assay test.

5.1.3.2 Quinine Sulphate

The percentage content of Quinine Sulphate in the three brands of Quinine Sulphate tablets was determined five times each. The average percentage content of Quinine Sulphate in the tablets manufactured by Actavis, Co-Pharma and Wockhardt are ; 99.50± 0.29%, 102.25± 0.80% and 97.52± 0.18%. Quinine Sulphate tablets should contain not less than 95.0% and not more than 105.0% of the stated amount of Quinine Sulphate. (14) . Therefore the three brands passed the assay test.

5.1.4 STABILITY STUDIES ON THE DRUG SAMPLES IN SOLUTION

Injections were done six times for each of the drug samples at an interval of twenty (20) minutes. The peak areas were plotted against time. The graph showed linear plot for all the drug samples. This showed that all the drug samples were stable in solution.

5.1.5 HPLC METHOD DEVELOPMENT

The aim of this study was to develop a simple, fast and cost effective method. The molecular weight, stability, ionic character, stability and polarity of the samples being analyzed were considered prior to the method development. A C_{18} column (stationary phase) was used because a very simple mobile phase can be applied to alter the retention and selectivity.

UV detector was employed because all the samples involved in the analysis possess enough chromophores and hence absorb UV light.

Cost, availability and the ability to dissolve the samples were the factors considered in the choice of the mobile phase. Methanol-Water combination was used as the mobile phase for the study. This is because water (distilled) is readily available and cheap, and methanol (redistilled) is also readily available and cheaper than HPLC grade methanol.

The pH of the mobile phase was given serious consideration because pH has great effect on the elution of ionizable samples. It affects the retention time and the resolution of the peaks.

For Albendazole and its surrogate reference standards, a mobile phase made up of Methanol-Water (60:40%v/v) with the pH adjusted to 5.40±0.02 was used. 1.0ml/min flow rate was used; the wavelength of measurement was 245nm. These conditions gave well resolved peaks and average retention times of 4.21± 0.36min, 1.74± 0.21min 2.53± 0.018min and 2.28± 0.29min for Albendazole, Aspirin, Amoxicillin Trihydrate and Metronidazole respectively.

For Quinine Sulphate and its surrogate reference standards, mobile-phase made up of Methanol-Water (60:40% v/v) with the pH adjusted to5.20± 0.02 was used. 1.5ml/min flow rate 245nm wavelength of measurement was used. The above factors gave well resolved peaks devoid of tailing. The average retention times were 6.65±0.015min, 2.78± 0.03min, 3.02± 0.017 and 2.02± 0.007mins for Quinine Sulphate, Aspirin, Amoxicillin Trihydrate and Metronidazole respectively

5.1.6 METHOD VALIDATION

Method validation is a way of ensuring through studies carried out in the laboratory that an analytical method's performance characteristics meet the requirements for the intended application. Method validation is necessary to ensure that a particular procedure when followed with a particular instrument or equipment by different analysts in the same or different laboratories will give reliable and reproducible results or data (44).

5.1.6.1 Linearity

It is the ability of an analytical method or procedure to produce results or responses that are directly proportional to the analyte in a sample within a given concentration range (44). As can be observed from tables 4.16 and 4.17, all the samples analyzed gave linear responses over a concentration range of 0.001%$^{w}/_{v}$ -0.01%$^{w}/_{v}$ for the analytes and 0.001%$^{w}/_{v}$ -0.005%$^{w}/_{v}$ for the surrogate reference standards. This is because the correlation coefficient (R^2) all fell within the stipulated values in literature (44).

5.1.6.2 Sensitivity (LOD and LOQ)

The limit of detection (LOD) of an analytical method is the lowest amount of an analyte in a sample that can be detected, but not necessarily quantified as an exact value (44). The

calculated LOD of the various samples are recorded in tables 4.18 and 4.19. This implies that concentrations below those recorded in the tables stated above would not give signals or may not be detected using the chromatographic conditions developed.

The limit of detection (LOD) is the lowest amount of the analyte that can be quantitatively determined with defined level of precision (44). The LOQ calculated are recorded in tables 4.18 and 4.19. Concentration of analytes lower than these values cannot be quantified.

5.1.6.3 Robustness

Robustness is the ability of an analytical method to give reproducible results for the analyte under varying conditions (44). The robustness of the method developed was put to test by varying the flow rate (1ml/min and 1.2ml/min for Albendazole and 1.5ml/min and 1.8ml/min for Quinine Sulphate) and the wavelength of detection (245nm and 240nm for Albendazole and 245nm and 250nm for Quinine Sulphate).

The percentage contents of Albendazole and Quinine Sulphate in their respective tablets were determined under the varying conditions. The percentage contents obtained were not significantly different at 95% confidence level (using Graphpad prism version 5). This means that the developed method is robust. However, a slight variation in the retention times was observed when the flow rates were varied.

5.1.6.4 Precision

Precision of an analytical method expresses the closeness of agreement (degree of deviation) between a series of measurements obtained from multiple sampling of the same homogenous sample under a given condition.

In order to demonstrate the precision of the method, the relative standard deviation (RSD) was calculated for the various samples. The RSD of Albendazole, Aspirin, Amoxicillin Trihydrate and Metronidazole are; 1.314, 1.503, 0.560 and 1.281 respectively. And the RSD for Quinine Sulphate and its surrogate reference standards (Aspirin, Amoxicillin and Metronidazole) are; 0.1744, 1.101, 0.1985 and 0.6524 respectively. The method is precise since all the calculated RSD are below the critical literature value (44).

5.1.6.5 Repeatability (Intra-day)

Three different analyses were done at different times within the same day. The percentage content of Albendazole and Quinine Sulphate in their respective tablets was determined. There was no significant difference between the results at 95% confidence level (Graphpad prism version 5 was used for analysis). This indicates that the method developed was repeatable.

5.1.6.6 Reproducibility

An analysis was carried out on two different days using freshly prepared solutions and mobile phase. The percentage content of the analytes was determined. The results were subjected to statistical analysis using graphpad prism version 5.The results showed that there was no significant difference between the results at 95% confidence level. Therefore the developed method is reproducible.

5.1.6.7 Selectivity and Specificity

Based on ICH guidelines, an investigation of specificity should be conducted during the validation of identification tests, the determination of impurities, and the assay. The procedure

was shown to be specific and selective for the analyte showing distinctly the component of interest from excipients with no interference. Refer to fig 4.3 to fig 4.8

5.1.7 DETERMINATION OF THE CONSTANT 'K'

K is the constant that relates the concentration of the analytes to their respective peak areas. The various concentrations and their corresponding peak areas of the analytes and the surrogate reference standards were substituted into the formular for the calculation of K stated in the hypothesis. The K values were expected to be the same but there were slight variations which were due to random error. The variations are statistically insignificant. The K values calculated were independent of the concentration. The K values for Aspirin, Amoxicillin Trihydrate and Metronidazole in respect of Albendazole were 2.297± 0.004, 2.693± 0.009 and 1.827± 0.003 respectively. The K values for Aspirin, Amoxicillin Trihydrate and Metronidazole in respect of Quinine Sulphate were 2.549± 0.007, 3.893± 0.008 and 1.034± 0.006 respectively.

5.1.8 DETERMINATION OF PERCENTAGE CONTENT USING THE CONSTANT 'K'

Three brands each of Albendazole and Quinine Sulphate were analyzed using the developed method. The average percentage contents are recorded in the tables 4.27 and 4.28. The percentage content of the three brands of Albendazole tablets fell within the 90% to 110% stated in the USP. The percentage content of the three brands of Quinine Sulphate fell within the 95% to 105% stipulated by the BP. The percentage contents obtained for the brands of the analytes using the developed method fell within the accepted range.

5.1.9 COMPARISON OF THE STANDARD METHOD TO THE DEVELOPED METHOD

Results from the developed methods and the standard methods (BP and USP) were compared statistically using graphpad prism version 5 to determine whether a significant difference exist between the results.

Random errors make it impossible for experimental values to be exactly the same as the standard value. To verify whether differences in the results from two procedures is solely as a result of random error requires statistical analysis using t-test. If the experimental t value (t_{exp}) is smaller than the critical value of t ($t_{critical}$), then the null hypothesis is retained. In that case, there is no evidence of systematic error. Graphpad prism version5 was used for the statistical analysis.

5.1.9.1 Albendazole

The t_{exp} for Albendazole tablet Alben were 2.329, 2.0987 and 1.3259 using Aspirin, Amoxicillin Trihydrate and Metronidazole as surrogate reference standards respectively. The t_{exp} for Wormplex 400 were 0.1873, 1.590 and 1.4545 when Aspirin, Amoxicillin Trihydrate and Metronidazole were used as surrogate reference standards respectively. The t_{exp} values for Zeben were 2.157, 0.977 and 1.9104 when Aspirin, Amoxicillin Trihydrate and Metronidazole were used as surrogate reference standards respectively. Since all the t_{exp} are less than the $t_{critical}$ value (2.78) , the null hypothesis is retained at 95% confidence level. Hence there is no significant difference between the developed and the standard methods.

5.1.9.2 Quinine Sulphate

The t_{exp} for Quinine Sulphate tablet Actavis were 2.356, 1.894 and 1.518 when Aspirin, Amoxicillin Trihydrate and Metronidazole were used as surrogate reference standards respectively. The t_{exp} for the Co-Pharma tablets were 1.315, 0.835 and 2.547 when Aspirin, Amoxicillin Trihydrate and Metronidazole were used as surrogate reference standards respectively. The t_{exp} for Wockhardt tablets were 1.918, 1.864 and 0.618 when Aspirin, Amoxicillin Trihydrate and Metronidazole were used as surrogate reference standards respectively.

It is observed that all the t_{exp} values are less than the $t_{critical}$ value (2.78). This indicates that there is no significant difference between the methods at 95% confidence level. Hence the null hypothesis is retained.

5.2 CONCLUSION

The possibility of using surrogate reference standards in the analysis of Albendazole and Quinine Sulphate tablets were investigated. The identity and the purity of the pure samples were verified prior to their use in the analysis. The results showed that the samples were adequately pure for the analysis. TLC was carried out to confirm the presence of the active ingredients in the commercial tablets. The results revealed that all commercial tablets contained the stated active ingredient.

A simple, fast and cost effective HPLC method was developed for the quantification of both Albendazole and Quinine Sulphate using Aspirin, Amoxicillin Trihydrate and Metronidazole as surrogate reference standards. The optimum condition found for Albendazole and the surrogate reference standards was mobile phase made up of Methanol: Water ($60:40\ ^{V}/_{V}$) with

the pH adjusted to 5.40±0.02, 245nm wavelength, and a flow rate of 1ml/min. The conditions for Quinine Sulphate and the surrogate reference standards was Methanol: Water (60:40 $^{V}/_{V}$) with the pH adjusted to 5.20±0.02 as the mobile phase, 245nm wavelength and a flow rate of 1.5ml/min. The developed methods were validated under validation parameters such as linearity, robustness, repeatability, reproducibility, sensitivity, specificity and selectivity.

Three brands of tablets each of Albendazole and Quinine Sulphate were analyzed using the developed method. The brands of Albendazole and Quinine Sulphate were analyzed using standard methods in the BP and USP respectively. The results from the methods were subjected to statistical analysis. There was no statistical difference between the standard method and the developed method.

The developed method is simple, fast and cheaper than existing methods. The developed method can therefore effectively replace the standard methods.

5.3 RECOMMENDATIONS

Surrogate reference standards should be found for the analysis of other pharmaceutical preparations so as to reduce the burden on manufacturing companies, regulatory agencies and academic institutions.

The possibility of using surrogate reference standards in the quantification of plant extracts and herbal preparations should be investigated.

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APPENDICES

APPENDIX I PREPARATION OF SOLUTIONS

AP1.1 Preparation of 1M HCl

36.4500g in 1000ml ≡ 1M HCl

3.6450g in 100ml ≡ 1M HCl

Assay = 36%

36% = 3.6450g

100% = (100 × 3.6450g)/36

= 10.1250g

Specific gravity = 1.18g/ml

1.18g = 1ml

10.1250g = (10.1250g × 1ml)/1.18g

= 8.58 ml

AP1.2 Preparation of 1M NaOH

40.0000g in 1000ml ≡ 1M NaOH

4.0000g in 100ml ≡ 1M NaOH

% purity of NaOH = 99%

99% = 4.0000g

100% = (100× 4.0000g)/99

= 4.0404g

Hence 4.0404g of NaOH was dissolved in 100ml of distilled water.

AP 1.3 Standardization of 0.1M Perchloric Acid ($HClO_4$) using Potassium Hydrogen Phthalate ($C_8H_5KO_4$)

1M KHphthatale ≡ 1M $HClO_4$

204.14g of KHphthalate ≡ 1000 ml of 1M $HClO_4$

20.41g ≡ 1000ml of 0.1M $HClO_4$

Therefore 0.020414g KHphthalate ≡ I ml of 0.1M $HClO_4$

Table AP 1: Titration results of $HClO_4$

Burette reading (ml)	1ST Determination	2ND Determination	3RD Determination	Blank
Final reading	24.60	24.50	24.60	20.10
Initial reading	0.00	0.00	0.00	20.00
Titre volume	24.60	24.50	24.60	0.010

Average titre = (24.60 +24.50 + 24.60) ml/3

= 24.57 ml

Actual titre = average titre -blank

= (24.57- 0.10) ml

= 24.47 ml

Expected weight of KHphthalate: 0.500g

Actual weight of KHphthalate: 0.507

Factor of KHphthalate: Actual weight/Expected weight

= 0.507g/0.500g

= 1.014

Fa× Va ×Ca = Fb× Cb× Vb

Where, Fa is the factor of $HClO_4$; Va is the volume of $HClO_4$; Ca is the concentration of $HClO_4$; Fb is the factor of KHphthalate; Vb is the volume of KHphthalate and Cb is the concentration of KHphthalate.

Fa ×24.47ml × 0.1M = 1.014×25ml×0.1M

Fa= 1.036

Therefore the factor of $HClO_4$ is 1.036

AP 1.4 Standardization of HCl using Sodium Bicarbonate (Na_2CO_3)

Molar mass of Na_2CO_3 ≡ 106g/mol

That is 106g of Na_2CO_3 in 1000 ≡ 2M HCl

53g of Na_2CO_3 in 1000ml ≡ 1M HCl

26.5gof Na_2CO_3 in 1000ml ≡ 0.5M HCl

6.625g of Na_2CO_3 in 250ml ≡ 0.5M HCl

0.0265g of Na_2CO_3 in 25ml ≡ 0.5M HCl

Table AP 2: Titration results of Na_2CO_3

Burette reading(ml)	1st determination	2nd determination	3rd determination
Final reading	25.10	25.10	25.20
Initial reading	0.00	0.00	0.00
Titre volume	25.10	25.10	25.20

Average titre = (25.10 +25.10 +25.20) ml/3 = 25.13ml

Percentage purity of Na_2CO_3 = 99%

If 99% of Na_2CO_3 = 6.6250g, then

100% = (100/99) × 6.6250g = 6.6919g

Actual weight = 6.692g

Factor of Na_2CO_3 = 6.692g/6.6919g = 1.000

$F_{Na2CO3} \times V_{Na2CO3} = F_{HCl} \times V_{HCl}$

F_{HCl} = (1.00 x 25 ml)/25.13ml = 0.995

AP 1.5 Standardization of 0.5M NaoH using 0.5M HCl

Molecular weight (NaOH) = 40g

40g of NaOH in 1000ml = 1M HCl

20g of NaOH in 1000ml = 0.5M HCl

5g of NaOH in 1ml = 0.5M HCl.

Table AP 3: Titration results of HCl

Burette Reading (ml)	1st Determination	2nd Determination	3rd Determination
Final reading	24.90	24.80	24.80
Initial reading	0.00	0.00	0.00
Titre volume	24.90	24.80	24.80

Average titre = (24.90 + 24.80 +24.80) ml/3 = 24.83ml

$F_{HCl} \times V_{HCl} = F_{NaOH} \times V_{NaOH}$

$F_{NaOH} = (F_{HCl} \times V_{HCl}) / V_{NaOH}$

= (0.99x 25ml) /24.83ml = 1.00

Therefore the factor of NaOH is 1.00

APPENDIX II ASSAY OF PURE SAMPLES

AP 2.1 Aspirin

Table AP 4: Titration results of Aspirin

Burette reading (ml)	1st Determination	2nd Determination	3rd Determination	Blank
Final reading	20.50	20.40	20.50	29.30
Initial reading	0.00	0.00	0.00	0.00
Titre	20.50	20.40	20.50	29.30

Average titre = (20.50+20.40+20.50) ml /3 = 20.467 ml

Actual volume of NaOH that reacted with Aspirin = Average titre - Blank = 8.833ml

Each ml of NaOH = 0.0 450g of Aspirin

Factor of NaOH = 1.0

Therefore the weight of Aspirin that reacted with NaOH is given as

(8.833ml x 1.00) x 0.04504g/ml

=0.3986g

Weight of Aspirin dissolved in NaOH (expected weight) is 0.401g

Percentage content of Aspirin = (actual weight/expected weight) x 100

= (0.3986g /0.401g) x 100

= 99.4%

Therefore Aspirin granules contain 99.4% of pure Aspirin.

AP 2.2 Metronidazole

Table AP 5: Titration results of Metronidazole

Burette reading(ml)	1st Determination	2nd Determination	3rd Determination	Blank Determination
Final reading	7.10	7.00	7.00	1.20
Initial reading	0.00	0.00	0.00	0.00
Titre	7.10	7.00	7.00	1.20

Average titre = (7.10+7.00+7.00) ml/3 = 7.033ml

Actual volume of $HClO_4$ that reacted =Average titre –blank

= (7.033ml - 1.20) ml = 5.833ml

1ml $HClO_4$ is equivalent to 0.01712g of metronidazole

Factor of $HClO_4$ = 1.036

Weight of metronidazole that reacted = (5.833ml x 1.036) x 0.01712g/ml

= 0.01035g

Percentage content = (Actual weight/Expected weight) × 100

= (0.1035g/0.1040g) × 100

= 99.5%

Therefore, the percentage content of Metronidazole is 99.50%

AP 2.3 Albendazole

Table AP 6: Titration results of pure Albendazole

Burette reading	1st	2nd	3rd	Blank
Final reading	6.60	6.70	6.60	1.20
Initial reading	0./00	0.00	0.00	0.00
Titre volume	6.60	6.70	6.60	1.20

Average titre = (6.60 +6.70 + 6.60) ml/3 = 6.633ml

Titre = average titre - blank = (6.633-1.20) = 5.433ml

Each ml of perchloric acid ($HClO_4$) consumes 0.02653g of Albendazole
Hence total amount of Albendazole that reacted with $HClO_4$ =(5.433 × 1.036) × 0.02653g/ml

= 0.1493g.

Percentage content of Albendazole = (Actual weight/Expected weight) × 100

= (0.1443/ 0.1502) ×100

= 99.4%

Therefore the percentage content of Albendazole in the Albendazole powder is 99.40%.

AP 2.4 Quinine Sulphate

Table AP 7: Titration results of pure Quinine Sulphate

Burette reading (ml)	1st Determination	2nd Determination	3rd Determination	Blank
Final reading	7.80	15.80	7.90	8.10
Initial reading	0.00	8.00	0.00	0.00
Titre	7.80	7.80	7.90	0.10

Average titre = (7.80 + 7.80 +7.90) ml/3 = 7.833 ml

Titre= Average titre- Blank

= (7.833 – 0.10) ml = 7.733ml

Actual volume of $HClO_4$ that reacted with Quinine Sulphate = 7.733ml × 1.036

= 8.011388ml

Each ml of $HClO_4$ Consumes 0.0249g of the pure Quinine Sulphate,

Therefore, 8.011388ml will consume 8.011388ml × 0.0249g/ml

= 0.19948356g

Expected weight = 0.200g

Percentage content = (Actual weight/Expected weight) ×100

= (0.19948356g/0.200g) × 100

= 99.74%

Therefore the percentage content of Quinine Sulphate in the Quinine Sulphate reference powder is 99.74%.

APPENDIX III UV SPECTRA OF PURE SAMPLES

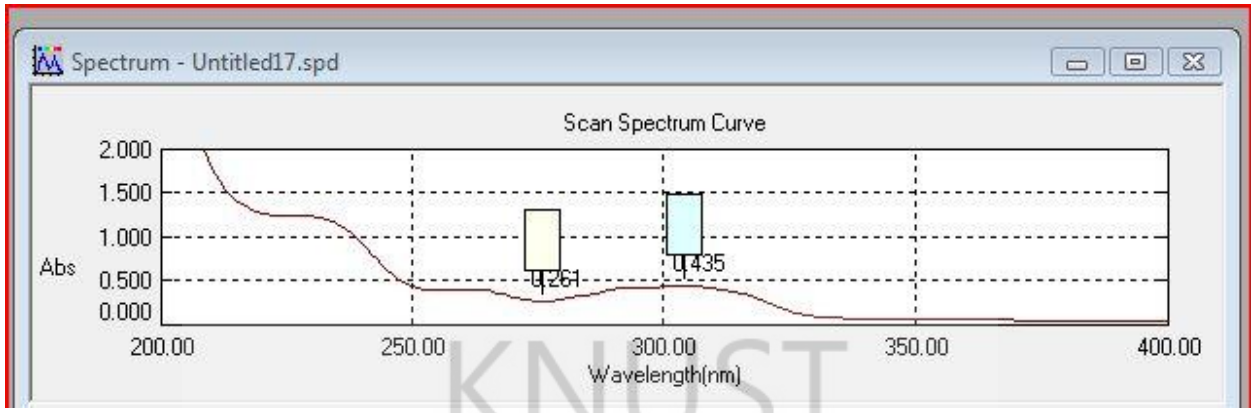


Fig AP 3A: UV Spectrum of pure Albendazole

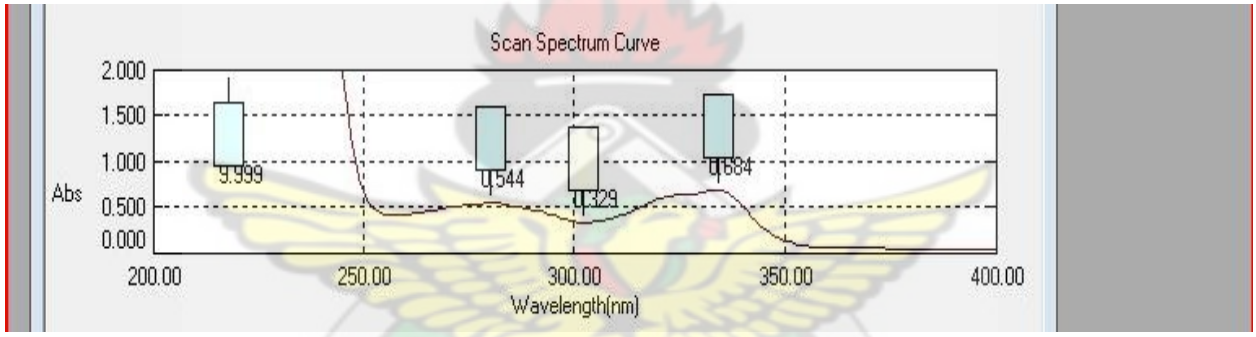


Fig AP 3B: UV Spectrum of pure Quinine Sulphate

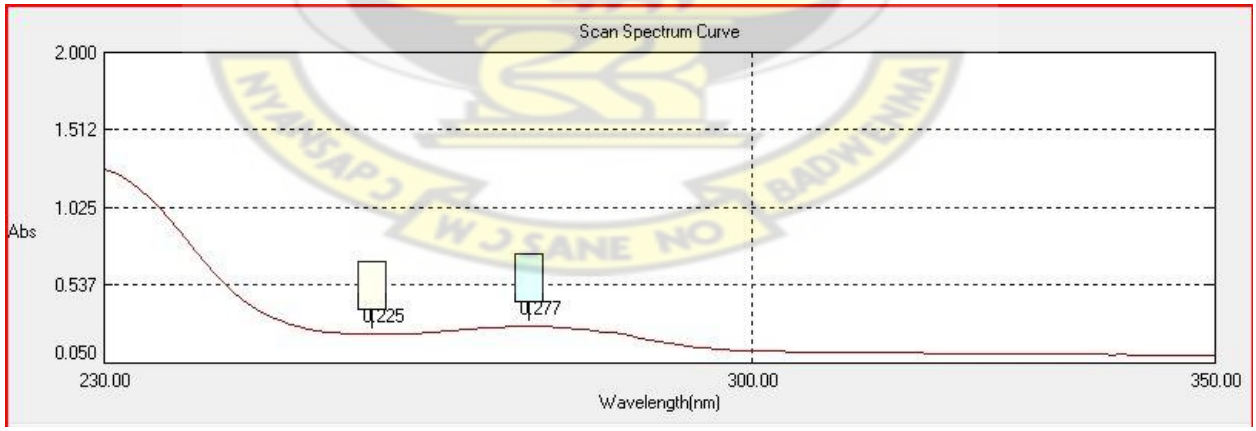
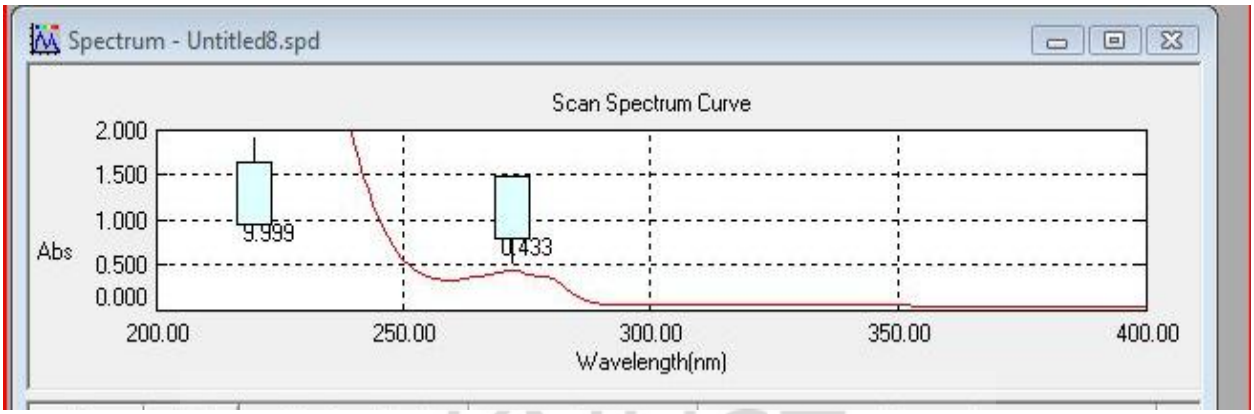


Fig AP 3C: UV Spectrum of pure Aspirin



AP 3D: UV Spectrum of pure Amoxicillin Trihydrate

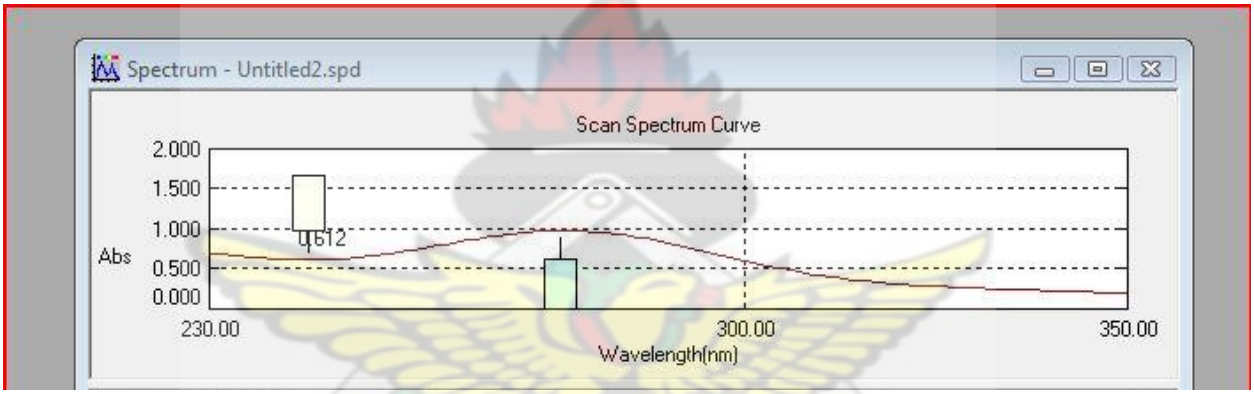


Fig AP 3E: UV Spectrum of Metronidazole

APPENDIX IV UNIFORMITY OF WEIGHT

AP 4.1 Uniformity of weight for Alben

No	Weight (x)g	Deviation (x-t)g	% Deviation (x-t)/t x 100
1	1.0376	0.005225	0.506115
2	1.0304	-0.00198	-0.19131
3	1.0384	0.006025	0.583606
4	1.0334	0.001025	0.099286
5	1.0172	-0.01517	-1.46991
6	1.0402	0.007825	0.757961
7	1.026	-0.00638	-0.61751
8	1.035	0.002625	0.254268
9	1.0331	0.000725	0.070226
10	1.0404	0.008025	0.777334
11	1.0402	0.007825	0.757961
12	1.017	-0.01538	-1.48928
13	1.035	0.002625	0.254268
14	1.0301	-0.00228	-0.22037
15	1.0402	0.007825	0.757961
16	1.0175	-0.01488	-1.44085
17	1.0377	0.005325	0.515801
18	1.03	-0.00238	-0.23005
19	1.035	0.002625	0.254268
20	1.0331	0.000725	0.070226
Average (t)			1.0323

AP 4.2 Uniformity of weight for Wormplex 400

No	Weight (x)g	Deviation (x-t)g	% Deviation (x-t)/t x100
1	0.7488	1.364522	1.364522
2	0.7292	-1.28872	-1.28872
3	0.7435	0.647065	0.647065
4	0.7378	-0.12454	-0.12454
5	0.7385	-0.02978	-0.02978
6	0.7354	-0.44943	-0.44943
7	0.7374	-0.17869	-0.17869
8	0.7386	-0.01624	-0.01624
9	0.7366	-0.28698	-0.28698
10	0.7362	-0.34113	-0.34113
11	0.7291	-1.30225	-1.30225
12	0.7367	-0.27345	-0.27345
13	0.7385	-0.02978	-0.02978
14	0.7377	-0.13808	-0.13808
15	0.7432	0.606454	0.606454
16	0.7481	1.269764	1.269764
17	0.736	-0.3682	-0.3682
18	0.7367	-0.27345	-0.27345
19	0.7433	0.619991	0.619991
20	0.7431	0.592917	0.592917
Average (t)	0.73872		

AP 4.3 Uniformity of Weight for Zeben

No	Weight (x)g	Deviation (x-t)g	% Deviation (x-t)/t x 100
1	0.5687	0.011885	2.134461
2	0.5729	0.016085	2.888751
3	0.5658	0.008985	1.613642
4	0.5477	-0.00911	-1.63699
5	0.5552	-0.00161	-0.29004
6	0.5407	-0.01612	-2.89414
7	0.541	-0.01581	-2.84026
8	0.5389	-0.01791	-3.21741
9	0.5549	-0.00192	-0.34392
10	0.5621	0.005285	0.949148
11	0.564	0.007185	1.290375
12	0.5583	0.001485	0.266695
13	0.554	-0.00281	-0.50555
14	0.5475	-0.00931	-1.67291
15	0.561	0.004185	0.751596
16	0.5508	-0.00601	-1.08025
17	0.571	0.014185	2.547525
18	0.5518	-0.00501	-0.90066
19	0.5721	0.015285	2.745077
20	0.5579	0.001085	0.194858
Average (t)		0.556815	

PS 4.4 Uniformity of Weight for Actavis

No	Weight (x)g	Deviation (x-t)g	% Deviation (x-t)/t x 100
1	0.4368	-0.00814	-1.82946
2	0.4393	-0.00564	-1.26759
3	0.4456	0.00066	0.148335
4	0.4507	0.00576	1.294557
5	0.4411	-0.00384	-0.86304
6	0.4425	-0.00244	-0.54839
7	0.4388	-0.00614	-1.37996
8	0.4397	-0.00524	-1.17769
9	0.4423	-0.00264	-0.59334
10	0.4449	-0.0004	-0.00899
11	0.4536	0.00866	1.94633
12	0.4462	0.00126	0.283184
13	0.4507	0.00576	1.294557
14	0.45	0.00506	1.137232
15	0.4525	0.00756	1.699105
16	0.4458	0.00086	0.193284
17	0.451	0.00606	1.361981
18	0.4469	0.00196	0.440509
19	0.4456	0.00066	0.148335
20	0.4348	-0.01014	-2.27896
Average (t)	0.44494		

PS 4.5 Uniformity of Weight for Co-Pharma

No	Weight (x)g	Deviation (x-t)g	% Deviation (x-t)/t x100
1	0.5952	0.000125	0.021006
2	0.5818	-0.01328	-2.23081
3	0.5969	0.001825	0.306684
4	0.5968	0.001725	0.289879
5	0.5961	0.001025	0.172247
6	0.6	0.004925	0.827627
7	0.589	-0.00608	-1.02088
8	0.5992	0.004125	0.69319
9	0.5981	0.003025	0.508339
10	0.5949	-0.00018	-0.02941
11	0.598	0.002925	0.491535
12	0.6023	0.007225	1.214133
13	0.5958	0.000725	0.121833
14	0.6003	0.005225	0.878041
15	0.595	-7.5E-05	-0.0126
16	0.5909	-0.00418	-0.70159
17	0.6032	0.008125	1.365374
18	0.5858	-0.00928	-1.55863
19	0.5819	-0.01318	-2.21401
20	0.6003	0.005225	0.878041
Average (t)	0.595075		

PS 4.6 Uniformity of Weight for Wockhardt

No	Weight (x)g	Deviation (x-t)g	% Deviation (x-t)t x100
1	0.6477	-0.0192	-2.88288
2	0.6718	0.0049	0.735736
3	0.6534	-0.0135	-2.02703
4	0.6698	0.0029	0.435435
5	0.6614	-0.0055	-0.82583
6	0.6806	0.0137	2.057057
7	0.6826	0.0157	2.357357
8	0.6735	0.0066	0.990991
9	0.6621	-0.0048	-0.72072
10	0.6689	0.002	0.3003
11	0.662	-0.0049	-0.73574
12	0.6734	0.0065	0.975976
13	0.67	0.0031	0.465465
14	0.6606	-0.0063	-0.94595
15	0.6503	-0.0166	-2.49249
16	0.675	0.0081	1.216216
17	0.6679	0.001	0.15015
18	0.6581	-0.0088	-1.32132
19	0.6514	-0.0155	-2.32733
20	0.6933	0.0264	3.963964
Average (t)			0.6667

APPENDIX V ASSAY OF THE COMMERCIAL PRODUCTS (TABLETS)

AP 4.1 Albendazole

Twenty tablets of each brand of the Albendazole tablets were weighed and powdered. An amount (weight) of the powdered tablets equivalent to 0.200g of pure Albendazole was weighed and dissolved 100ml of glacial acetic acid and sonicated for five minute then titrated with Perchloric acid. The end point was determined potentiometrically. Blank determination was done and the necessary corrections made.

Each ml of 0.1M Perchloric acid ($HClO_4$) is equivalent to 0.02653g of Albendazole ($C_{12}H_{15}N_3O_2S$)

Table AP4AA1: Titration results for the Alben (day one)

Expected weight of Alben powder = 0.5166, Actual weight of Alben powder taken = 0.5161

Burette reading(ml)	1st Determination	2nd Determination	3rd Determination	Blank Determination
Final reading	8.20	17.30	8.10	10.00
Initial reading	0.00	9.00	0.00	9.00
Titre volume	8.20	8.30	8.10	1.00

SAMPLE CALCULATION

Average titre = (8.20 + 8.30 +8.10) ml/3 = 8.20 ml

Volume of $HClO_4$ that reacted = (8.20- 1.00) ml = 7.20ml

Actual volume of $HClO_4$ that reacted = (Volume of $HClO_4$ that reacted × factor)

= 7.20 × 1.036

= 7.4592ml

Each ml of $HClO_4$ is equivalent to 0.02653g of Albendazole

Hence 7.4592ml of $HClO_4$ ≡ 7.4592ml × 0.02653g/ml

=0.19789g

Percentage content = (actual weight/expected weight) × 100

= (0.19789g/0.200g) × 100

= 98.95%

Therefore Alben tablet contains 98.95% of the stated amount of Albendazole.

Table AP4AA2: Titration results for the Alben (day two)

Burette reading (ml)	1st Determination	2nd Determination	3rd Determination	Blank Determination
Final reading	8.20	17.20	8.20	10.00
Initial reading	0.00	9.00	0.00	9.00
Titre volume	8.20	8.20	8.30	1.00

Table AP4AA3: Titration results for the Alben (day three)

Burette reading (ml)	1st Determination	2nd Determination	3rd Determination	Blank Determination
Final reading	8.10	17 .20	8.20	10.00
Initial reading	0.00	9.00	0.00	0.00
Titre volume	8.10	8.20	8.20	1.00

Table AP4AA4: Titration results for the Alben (day four)

Burette reading (ml)	1st Determination	2nd Determination	3rd Determination	Blank Determination
Final reading	8.20	17 20	8.20	10.00
Initial reading	0.00	0.00	0.00	9.00
Titre volume	8.20	8.20	8.20	1.00

Table AP4AA5: Titration results for Alben (day five)

Burette reading(ml)	1st Determination	2nd Determination	3rd Determination	Blank Determination
Final reading	8.20	17.20	8.10	10.00
Initial reading	0.00	9.00	0.00	9.00
Titre volume	8.20	8.20	8.10	1.00

Table AP4AW1: Titration results for Wormplex 400 tablet (day one)

Burette reading (ml)	1st Determination	2nd Determination	3rd Determination	Blank Determination
Final reading	8.40	17.30	8.50	10.00
Initial reading	0.00	9.00	0.00	9.00
Titre volume	8.40	8.30	8.50	1.00

Table AP4AW2: Titration results for Wormplex 400 tablet (day two)

Burette reading (ml)	1st Determination	2nd Determination	3rd Determination	Blank Determination
Final reading	8.30	17.20	8.30	10.00
Initial reading	0.00	9.00	0.00	9.00
Titre volume	8.30	8.20	8.30	1.00

Table AP4AW3: Titration results for Wormplex 400 tablet (day three)

Burette reading(ml)	1st Determination	2nd Determination	3rd Determination	Blank Determination
Final reading	8.40	17.40	8.30	10.00
Initial reading	0.00	0.90	0.00	0.90
Titre volume	8.40	8.40	8.30	1.00

Table AP4AW4: Titration results for Wormplex 400 tablet (day four)

Burette reading(ml)	1st Determination	2nd Determination	3rd Determination	Blank Determination
Final reading	8.40	17.40	8.50	10.00
Initial reading	0.00	9.00	0.00	9.00
Titre volume	8.40	8.40	8.50	1.00

Table AP4AW4: Titration results for Wormplex 400 tablet (day five)

Burette reading (ml)	1st Determination	2nd Determination	3rd Determination	Blank Determination
Final reading	8.40	8.50	8.50	1.00
Initial reading	0.00	9.00	0.00	9.00
Titre volume	8.40	8.50	8.50	1.00

Table AP4AZ1: Titration results for Zeben (day one)

Burette reading (ml)	1st Determination	2nd Determination	3rd Determination	Blank Determination
Final reading	8.30	17. 30	8.30	10.00
Initial reading	0.00	9.00	0.00	9.00
Titre volume	8.30	8.30	8.30	1.00

Table AP4AZ2: Titration results for Zeben (day two)

Burette reading (ml)	1st Determination	2nd Determination	3rd Determination	Blank Determination
Final reading	8.40	17 40	8.30	10.00
Initial reading	0.00	9.00	0.00	9.00
Titre volume	8.40	8.40	8.30	1.00

Table AP4AZ3: Titration results for Zeben (day three)

Burette reading (ml)	1st Determination	2nd Determination	3rd Determination	Blank Determination
Final reading	8.50	17.40	8.40	10.00
Initial reading	0.00	9.00	0.00	9.00
Titre volume	8.50	8.40	8.40	1.00

Table AP4AZ4: Titration results for Zeben (day four)

Burette reading	1st Determination	2nd Determination	3rd Determination	Blank Determination
Final reading	8.20	17.20	8.20	10.00
Initial reading	0.00	9.00	0.00	9.00
Titre volume	8.20	8.20	8.30	1.00

Table AP4AZ5: Titration results for Zeben (day five)

Burette reading (ml)	1st Determination	2nd Determination	3rd Determination	Blank Determination
Final reading	8.30	17 30	8.20	10.00
Initial reading	0.00	9.00	0.00	9.00
Titre volume	8.30	8.30	8.20	1.00

AP 4.2 Quinine Sulphate

Twenty tablets of each of the three brands of the Quinine Sulphate tablets were weighed and powdered.

An amount of the powdered tablet corresponding to 0.200g of the pure Quinine Sulphate was weighed in each case, dissolved in 100ml acetic anhydride and sonicated for five minutes and titrated against 1M perchloric acid ($HClO_4$). The end point was determined potentiometrically. Each ml of the perchloric acid is equivalent to 0.0261g of Quinine Sulphate.

Table AP4QSA1: Titration results for Actavis (day one)
Expected weight: 0.2966g; Actual weight: 0.2966g; Factor: 1.00

Burette reading(ml)	1st Determination	2nd Determination	3rd Determination	Blank Determination
Final reading	7.40	7.50	7.40	8.10
Initial reading	0.00	0.00	0.00	8.00
Titre	7.40	7.50	7.40	0.10

SAMPLE CALCULATION

Average titre = (7.40 +7.50 +7.40) ml/3 = 7.433ml

Volume of $HClO_4$ that reacted = (7.433 -0.10) ml

=7.433ml

Actual volume of $HClO_4$ that reacted = 7.333ml x 1.036

= 7.596988ml

Each ml of $HClO_4$ is equivalent to 0.0261g 0f Quinine Sulphate

Hence 7.59688ml $HClO_4$ = 7.59698ml x 0.026g/ml

=0. 198281g of Quinine Sulphate

Percentage content = (0.198281g /0.200g) x 100

= 99.14%

Therefore the Actavis tablet contains 99.14% of the stated amount of Quinine Sulphate

Table AP4QSA2: Titration results for Actavis (day two)

Burette reading (ml)	1st Determination	2nd Determination	3rd Determination	Blank Determination
Final reading	7.40	15.40	7.40	8.10
Initial reading	0.00	8.00	0.00	8.00
Titre	7.40	7.40	7.40	0.10

Table AP4QSA3: Titration results for Actavis (day three)

Burette reading(ml)	1st Determination	2nd Determination	3rd Determination	Blank Determination
Final reading	7.50	15.40	7.40	8.10
Initial reading	0.00	8.00	0.00	8.00
Titre	7.50	7.40	7.40	0.10

Table AP4QSA4: Titration results for Actavis (day four)

Burette reading (ml)	1st Determination	2nd Determination	3rd Determination	Blank Determination
Final reading	7.40	15.50	7.50	8.10
Initial reading	0.00	8.00	0.00	8.00
Titre	7.40	7.50	7.50	0.10

Table AP4QSA5: Titration results for Actavis (day five)

Burette reading(ml)	1st Determination	2nd Determination	3rd Determination	Blank Determination
Final reading	7.30	15.30	7.40	8.10
Initial reading	0.00	8.00	0.00	8.00
Titre volume	7.30	7.30	7.40	0.10

Table AP4QSC1: Titration results for Co-Pharma (day one)

Burette reading(ml)	1st Determination	2nd Determination	3rd Determination	Blank Determination
Final reading	7.80	15.80	7.70	8.10
Initial reading	0.00	8.00	0.00	8.00
Titre	7.80	7.80	7.70	0.10

Table AP4QSC2: Titration results for Co-Pharma (day two)

Burette reading (ml)	1st Determination	2nd Determination	3rd Determination	Blank Determination
Final reading	7.80	15.80	7.90	8.10
Initial reading	0.00	8.00	0.00	8.00
Titre	7.80	7.80	7.90	0.10

Table AP4QSC3: Titration results for Co-Pharma (day three)

Burette reading(ml)	1st Determination	2nd Determination	3rd Determination	Blank Determination
Final reading	7.80	15.70	7.80	8.10
Initial reading	0.00	8.00	0.00	8.00
Titre	7.80	7.70	7.80	0.10

Table AP4QSC4: Titration results for Co-Pharma (day four)

Burette reading(ml)	1st Determination	2nd Determination	3rd Determination	Blank Determination
Final reading	7.80	15.80	7.70	8.10
Initial reading	0.00	8.00	0.00	8.00
Titre	7.80	7.80	7.70	0.10

Table AP4QSC5: Titration results for Co-Pharma (day five)

Burette reading (ml)	1st Determination	2nd Determination	3rd Determination	Blank Determination
Final reading	7.90	15.80	7.80	8.10
Initial reading	0.00	8.00	0.00	8.00
Titre volume	7.90	7.80	7.80	0.10

Table AP4QSW1: Titration results for Wockhardt (day one)

Burette reading(ml)	1st Determination	2nd Determination	3rd Determination	Blank Determination
Final reading	7.40	15.30	7.30	8.30
Initial reading(ml)	0.00	8.00	0.00	8.00
Titre volume	7.40	7.30	7.30	0.10

Table AP4QSW2: Titration results for Wockhardt (day two)

Burette reading (ml)	1st Determination	2nd Determination	3rd Determination	Blank Determination
Final Reading	7.40	15.50	7.30	8.10
Initial reading	0.00	8.00	0.00	8.00
Titre volume	7.40	7.50	7.30	0.10

Table AP4QSW3: Titration results for Wockhardt (day three)

Burette reading(ml)	1st Determination	2nd Determination	3rd Determination	Blank Determination
Final reading	7.30	15.30	7.30	8.10
Initial reading	0.00	8.00	0.00	8.00
Titre	7.30	7.30	7.30	0.10

Table AP4QSW4: Titration results for Wockhardt (day four)

Burette reading(ml)	1st Determination	2nd Determination	3rd Determination	Blank Determination
Final reading	7.30	15.40	7.30	8.10
Initial reading	0.00	8.00	0.00	8.00
Titre reading	7.30	7.40	7.30	0.10

Table AP4QSW5: Titration results for Wockhardt (day five)

Burette reading(ml)	1st Determination	2nd Determination	3rd Determination	Blank Determination
Final reading	7.40	15.20	7.30	8.10
Initial reading	0.00	8.00	0.00	8.00
Titre	7.40	7.20	7.30	0.10

APPENDIX VI RELATIVE STANDARD DEVIATION

RELATIVE STANDARD DEVIATION OF ALBENDAZOLE

SAMPLE CALCULATION.

0.005%$^{w}/_{v}$ of the pure Albendazole reference standard was injected six times (20µL) at an interval of ten (10) minutes. The peak Areas was recorded

Table APRSA1: RSD of Albendazole.

No times	Peak Area
1	4661983
2	4702715
3	4682174
4	4669821
5	4540886
6	4598185
Average	4642637
Standard deviation	61016.4

RSD= (Standard deviation/Average Peak Area) x 100

= (61016.4/4642637) x 100

Therefore RSD of Albendazole is 1.314

Table APRSA2: RSD of Aspirin

No of Times	Peak Area
1	1770120
2	1758210
3	1724629
4	1719182
5	1718702
6	1707317
Average	1731800.33
Standard deviation	26028.251
RSD	1.503

Table APRSA3: RSD of Amoxicillin Trihydrate

No of times	Peak Area
1	1726953
2	1719821
3	1717918
4	1706256
5	1714248
6	1700456
Average	1714275.333
Standard deviation	9591.99
RSD	0.560

Table APRSA4: RSD of Metronidazole

No of Times	Peak Area
1	2661290
2	2700841
3	2617824
4	2624856
5	2610027
6	2642988
Average	2642971
Standard deviation	33853.670
RSD	1.281

Table APRSQ1: RSD OF Quinine Sulphate

No of Times	Peak Area
1	2167483
2	2160628
3	2170042
4	2171041
5	2168435
6	2169827
Average	2167909.333
Standard Deviation	3781.771
RSD	0.1744

Table APRSQ2: RSD of Amoxicillin Trihydrate.

No of Times	Peak Area
1	630608
2	631482
3	639280
4	625528
5	623082
6	620024
Average	628334
Standard Deviation	6919.67
RSD	1.101

Table APRSQ4: RSD of Metronidazole

No of Times	Peak Area
1	2201566
2	2200820
3	2212731
4	2235261
5	2230281
6	2211214
Average	2215312.167
Standard Deviation	14453.092
RSD	0.6524

Table APRQ3: RSD of Aspirin

No of Times	Peak Area
1	630608
2	631482
3	639280
4	625528
5	623082
6	620024
Average	628334
Standard Deviation	6919.67
RSD	1.101

APPENDIX VII DETERMINATION OF THE CONSTANT 'K'

Table AKA1: K values for Albendazole and Amoxicillin Trihydrate.

Conc of ALB (%$^{W}/_{V}$)	Peak Area of ALB (m^2)	Conc of AMOXIL (% $^{W}/_{V}$)	Peak Area of AMOXIL (%$^{W}/_{V}$)	K
0.010	9323965	0.005	1726953	2.69954
0.008	6954855	0.004	1292661	2.690131
0.006	5826267	0.003	1088537	2.67619
0.004	3712524	0.002	686760	2.702927
0.002	2444197	0.001	453648	2.693936

Average K=2.693± 0.0104

Table AKA2: K values for Albendazole and Aspirin

Conc of ALB (%$^{w}/_{v}$)	Peak Area (m^2)	Conc of ASP (%$^{w}/_{v}$)	Peak Area of ASP (m^2)	K
0.010	8144766	0.005	1770120	2.30063
0.008	7081348	0.004	1540880	2.29786
0.006	5298983	0.003	1156999	2.29933
0.004	3873017	0.003	842266	2,29933
0.004	3873017	0.002	599445	2.2999414

Average K == 2.297± 0.004

Table AKA3: K values for Albendazole and Metronidazole.

Conc of ALB (%$^{w}/_{v}$)	Peak Area of ALB (m^2)	Conc of METRO (%$^{w}/_{v}$)	Peak Area of METRO (m^2)	K
0.010	97390058	0.005	2661290	1.82976
0.008	7150480	0.04	1963354	1.82099
0.006	6015103	0.003	1647014	1.826063
0.004	3703890	0.002	1014333	1.825776
0.002	2780713	0.001	759650	1.8302559

Average K = 1.827± 0.004

Table AKQ1: K for Quinine Sulphate and Amoxicillin Trihydrate

Conc of QS (%$^{w}/_{v}$)	Peak Area of QS (m^2)	Conc of AMOXIL (%$^{w}/_{v}$)	Peak Area of AMOXIL (m^2)	K
0.010	4913917	0.005	630608	3.89617
0.008	4616601	0.004	594209	3.88466
0.006	3257995	0.003	417605	3.90081
0.004	2445908	0.002	313548	3.90037
0.002	1527301	0.001	196562	3.88504

Average K = 3.893± 0.

Table AKQ2: K values for Quinine Sulphate and Aspirin

Conc of QS ($\%^{w}/_{v}$)	Peak Area of QS (m_2)	Conc of ASP ($\%^{w}/_{v}$)	Peak Area of ASP (m^2)	K
0.010	3617539	0.005	711257	2.543060
0.008	3020330	0.004	592183	2.550166
0.006	2890464	0.003	568776	2.540951
0.004	2048321	0.002	400389	2.55790
0.002	1417369	0.001	238616	2.550891

Average K = 2.5497 ±0.007

Table AKQ3: K values for Quinine Sulphate and Metronidazole

Conc of QS ($\%^{w}/_{v}$)	Peak Area of QS (m^2)	Conc of METRO ($\%^{w}/_{v}$)	Peak Area of METRO (m^2)	K
0.010	4570451	0.005	2201566	1.037999
0.008	3931863	0.004	1895606	1.037099
0.006	3005533	0.003	1448031	1.0377995
0.004	1789381	0.002	861383	1.038668
0.002	1475220	0.001	710403	1.038298

Average K = 1.034± 0.0006

Table ATKA: Average values of the Constant 'K' of Albendazole and its Surrogates

Surrogates/Days	1	2	3	4	5	Average
AMOXIL	2.69954	2.690131	2.67619	2.702927	2.693936	2.693±0.0104
ASP	2.30063	2.29786	2.28997	2.29933	2.299414	2.297±0.004
METRO	1.82976	1.82099	1.826063	1.825776	1.830259	1.827±0.004

Table ATKQ: Average values of the Constant K of Quinine Sulphate and its Surrogates

Surrogates/Days	1	2	3	4	5	Average
AMOXIL	3.89617	3.88466	3.90081	3.90037	3.88504	3.893±0.008
ASP	2.543060	2.550166	2.540951	2.55790	2.550891	2.549±0.007
METRO	1.037999	1.037099	1.0377995	1.038668	1.038298	1.034±0.0006

APPENDIX VIII DETERMINATION OF PERCENTAGE CONTENT USING THE DEVELOPED METHOD

Table APCA1: Percentage content of Alben using Aspirin as a surrogate standard

Cs	As	Aa	Aa×Cs	K×As	Aa×Cs/K×As	Percentage content
0.005	1012675	4655508	23277.5	2326114.5	0.009974	99.74
0.004	817664	4662107	18648.4	1878174.2	0.009929	99.29
0.003	616551	4672098	14016.3	1416217.6	0.009897	98.97
0.002	407327	4598145	9196.3	935630.1	0.009829	98.29
0.001	203285	4648921	4648.9	466945.6	0.009956	99.56

Table APCA2: Percentage content of Alben using Amoxicillin Trihydrate a surrogate standard

Cs	As	Aa	Aa×Cs	K×As	Aa×Cs/K×As	Percentage Content
0.005	915325	4867659	24338.3	2464970.2	0.009873	98.73
0.004	734331	4867248	1946.0	1977553.4	0.009845	98.45
0.003	549047	4866992	14601.0	1474584.0	0.009875	98.75
0.002	365151	4870218	9740.4	983352	0.009905	99.05
0.001	183600	4859297	4859.3	494435	0.009828	98.28

Table APCA3: Percentage content of Alben using Metronidazole as a surrogate standard

Cs	As	Aa	Aa× Cs	K×As	Aa×Cs/K×As	Percentage content
0.005	1335578	4848171	24240.9	244008.6	0.009934	99.35
0.004	1054775	4792151	19168.6	1927074.0	0.009947	99.47
0.003	801631	4890243	14670.7	1464580.0	0.010017	100.17
0.002	514187	4672187	9344.4	939420.1	0.009947	99.47
0.001	264415	4799453	4799.5	483086.2	0.009937	99.76

Table APCA4: Percentage content of Wormplex 400 using Aspirin as a surrogate standard

Cs	As	Aa	Aa×Cs	K×As	Aa×Cs/K×As	Percentage content
0.005	1000935	4675897	23379.5	2299147.7	0.010169	101.68
0.004	786354	4552693	18210.8	1806255.1	0.010082	100.72
0.003	614897	4709472	14128.4	1412418.4	0.010029	99.93
0.002	397408	4662481	9324.9	912846.2	0.010215	102.05
0.001	194339	4534975	4535.0	446396.7	0.010159	101.49

Table APCA5: Percentage content of Wormplex 400 using Amoxicillin Trihydrate as a surrogate standard

Cs	As	Aa	Aa×Cs	K×As	Aa×Cs/K×As	Percentage content
0.005	840911	4542426	22712.1	2264573.0	0.010029	100.29
0.004	666274	4503192	18012.8	1794275.9	0.010039	99.45
0.003	502722	4492417	13477.3	1353830.3	0.009955	100.58
0.002	330712	4483356	8966.7	890607.4	0.010068	100.19
0.001	166381	4519211	4519.2	448064.0	0.010086	100.76

Table APCA6: Percentage content of Wormplex 400 using Metronidazole as a surrogate standard

Cs	As	Aa	Aa×Cs	K×As	Aa×Cs/K×As	Percentage content
0.005	1153617	4240352	21201.8	2107658.3	0.010059	100.67
0.004	919705	4210021	16840.0	1680301.0	0.010022	100.12
0.003	708340	4309021	12927.1	1294137.2	0.009989	99.79
0.002	454342	4199873	8399.7	830082.8	0.010119	101.09
0.001	230524	4237821	4237.8	421167.3	0.010062	100.52

Table APCA7: Percentage content of Zeben using Aspirin as surrogate standard

Cs	As	Aa	Aa×Cs	K×As	Aa×Cs/K×As	Percentage content
0.005	703171	3223842	16119.2	1615183.8	0.00998	99.80
0.004	566234	3225623	12902.5	1300639.5	0.00992	99.40
0.003	418186	3198713	9596.1	960573.0	0.00999	100.20
0.002	291120	3300456	6600.9	668702.6	0.00987	98.91
0.001	139782	3218779	3218.8	321079.3	0.010025	100.45

Table APCA8: Percentage content of Zeben using Amoxicillin Trihydrate as a surrogate standard

Cs	As	Aa	Aa×Cs	K×As	Aa×Cs/K×As	Percentage content
0.005	872351	4663567	23317.8	2349241.1	0.009926	99.26
0.004	693201	4623183	18492.7	1866790.3	0.009906	99.57
0.003	531399	4709218	14127.7	14310575	0.009872	98.98
0.002	348552	4672128	9344.3	938650.5	0.009955	99.75
0.001	169828	4509081	4509.1	457346.8	0.00986	98.79

Table APCA9: Percentage content of Zeben using Metronidazole as a surrogate standard

Cs	As	Aa	Aa×Cs	K×As	Aa×Cs/K×As	Percentage content
0.005	703171	3223842	16119.2	1615183.8	0.00998	99.80
0.004	566234	3225623	12902.5	1300639.5	0.00992	99.40
0.003	418186	3198713	9596.1	960573.0	0.00999	100.20
0.002	291120	3300456	6600.9	668702.6	0.00987	98.91
0.001	139782	3218779	3218.8	321079.3	0.010025	100.45

Table APCQS1: Percentage content of Actavis using Aspirin as a surrogate standard

Cs	As	Aa	Aa×Cs	K×As	Aa×Cs/K×As	Percentage content
0.005	1066686	5408087	27040.4	2718982.6	0.009945	99.45
0.004	852188	5414269	21657.1	2172227.2	0.009969	99.70
0.003	640641	5399750	16199.3	1632993.9	0.009920	99.20
0.002	426928	5381363	10762.7	1088239.5	0.00989	98.90
0.001	210839	5371582	5371.6	537428.6	0.009995	99.95

Table APCQS2: Percentage content of Actavis using Amoxicillin Trihydrate as a surrogate standard

Cs	As	Aa	Aa×Cs	K×As	Aa×Cs/K×As	Percentage content
0.005	728706	5628054	28140.3	2836852.5	0.009919	99.19
0.004	585241	5639471	22557.9	2278343.2	0.009901	99.01
0.003	442058	5700284	17100.9	1720931.8	0.009937	99.37
0.002	294018	5661242	11322.5	1144612.1	0.009892	98.92
0.001	145418	5619817	5619.8	566112.3	0.009927	99.27

Table APCQS3: Percentage content of Actavis using Metronidazole as a surrogate standard

Cs	As	Aa	Aa×Cs	K×As	Aa×Cs/K×As	Percentage content
0.005	2799785	5764128	28820.6	2894977.7	0.009955	99.55
0.004	2220168	5704703	22818.8	2295653.7	0.009940	99.40
0.003	1637501	5624178	16872.5	1693176.0	0.009965	99.65
0.002	1127975	5772138	11544.3	1166326.2	0.009898	98.98
0.001	552349	5699284	5699.3	571128.9	0.009979	99.79

Table APCQS4: Percentage content of Quinine Sulphate in Co-Pharma tablet using Aspirin as a surrogate standard

Cs	As	Aa	Aa×Cs	K×As	Aa×Cs/K×As	Percentage content
0.005	799459	4191386	20956.9	2037821	0.010028	101.79
0.004	638406	4182472	16729.9	1627297	0.010281	101.08
0.003	484440	4190124	12570.4	1234838	0.010798	100.79
0.002	322861	4200437	8400.9	822973	0.010208	102.08
0.001	159022	4172187	4172.2	405347	0.010293	101.91

Table APCQS5: Percentage content of Quinine Sulphate in Co-Pharma tablet using Amoxicillin Trihydrate as a surrogate standard

Cs	As	Aa	Aa×Cs	K×As	Aa×Cs/K×As	Percentage content
0.005	695073	5511538	27557.7	2705591.9	0.010184	101.84
0.004	556275	5534812	22139.2	2165579.1	0.010223	101.22
0.003	410287	5498934	16496.8	1597247.3	0.010238	102.26
0.002	274524	5501217	11002.4	1068721.9	0.010295	101.93
0.001	137754	5511158	5511.2	536276.3	0.010277	101.75

Table APCQS6: Percentage content of Quinine Sulphate in Co-Pharma tablet using Metronidazole as a surrogate reference standard

Cs	As	Aa	Aa×Cs	K×As	Aa×Cs/K×As	Percentage content
0.005	2731720	5824183	29120.9	2824598.5	0.01031	102.07
0.004	2184106	5801017	23204.1	2258365.6	0.01027	101.73
0.003	1660407	5919413	17758.2	1716860.8	0.01034	102.41
0.002	1097226	5822218	11644.4	1134531.7	0.01026	101.62
0.001	541546	5799816	5799.8	559958.6	0.01036	102.55

Table APCQS7: Percentage content of Quinine Sulphate in Wockhardt tablet using Aspirin as a surrogate standard

Cs	As	Aa	Aa×Cs	K×As	Aa×Cs/K×As	Percentage content
0.005	920186	4616449	23082.2	2345554.0	0.00984	98.21
0.004	733077	4608324	18433.3	1868613.1	0.00986	98.45
0.003	553849	4620017	13860.1	1411761.2	0.009818	97.98
0.002	364330	4598723	9197.4	928677.1	0.00990	98.84
0.001	182962	4580495	4580.5	466370.1	0.00982	98.02

Table APCQS8: Percentage content of Quinine Sulphate in Wockhardt tablet using Amoxicillin Trihydrate as a surrogate standard

Cs	As	Aa	Aa×Cs	K×As	Aa×Cs/K×As	Percentage content
0.005	505549	3836302	19181.5	1968102.3	0.00975	97.36
0.004	404224	3830024	15320.1	1573644.0	0.00974	97.16
0.003	300740	3814997	11445.0	1170780.8	0.009776	97.56
0.002	202280	3863178	7726.4	787476.0	0.009812	97.92
0.001	99919	3798987	3799.0	388984.7	0.009766	97.47

Table APCQS9: Percentage content of Quinine Sulphate in Wockhardt tablet using Metronidazole as a surrogate standard

Cs	As	Aa	Aa×Cs	K×As	Aa×Cs/K×As	Percentage content
0.005	2640519	5344656	26723.3	2730296.6	0.009788	97.67
0.004	2025505	5102639	20410.6	2094372.2	0.009745	97.26
0.003	1568194	5311873	15935.6	1621512.6	0.009828	98.08
0.002	1042234	5301378	10602.8	1077670.0	0.009839	98.19
0.001	526339	5298873	5298.9	544234.5	0.009740	97.17