KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI
COLLEGE OF HEALTH SCIENCES
FACULTY OF PHARMACY AND PHARMACEUTICAL SCIENCES
DEPARTMENT OF PHARMACEUTICAL CHEMISTRY

THE USE OF SURROGATE REFERENCE STANDARDS IN QUANTITATIVE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY; A CASE STUDY OF THE ANALYSIS OF CHLORPHENIRAMINE MALEATE TABLETS AND METFORMIN HYDROCHLORIDE TABLETS

BY

TETTEH, CHRISTIANA (MISS)

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DECLARATION

I hereby declare that this research and all the experimental work described herein were solely carried out by me at the Department of Pharmaceutical Chemistry, Kwame Nkrumah University of Science and Technology, Kumasi, under the supervision of Professor J.K. Kwakye. As such, no previous submission for a degree has been made here or elsewhere. References cited herein were duly acknowledged.

HEAD OF DEPARTMENT SUPERVISOR

……

……..

PROF. N. N. A. OKINE PROF. J. K. KWAKYE

CANDIDATE

……

TETTEH CHRISTIANA

PG 3788609

DEDICATION

This thesis is dedicated to my mother, Mrs. Beatrice Amiyoe Tetteh who inspired me by her words: “hard work does not kill, but rather toughens” and to my late father, Mr. Joseph Anyanumeh Tetteh who by ensuring my success in all that I do, cost him his life.

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ABSTRACT

The study sought to investigate the use of compounds that were physico-chemically related to the analytes; (Chlorpheniramine Maleate and Metformin Hydrochloride) as surrogate reference standards for the assay of the analytes using High Performance Liquid Chromatography. The surrogate reference standards for Chlorpheniramine Maleate were Piroxicam, Ascorbic Acid and Caffeine while those for Metformin Hydrochloride were Metronidazole and Paracetamol. A reversed-phase isocratic HPLC method with UV detection was developed and validated. Phosphate buffer and Acetate buffer were used to effectively control the pH of the mobile phase. Phosphate buffer (0.025M) and Methanol in a ratio of 50:50 and detection at 266nm eluted well resolved peaks of Chlorpheniramine Maleate and its surrogate reference standards; Piroxicam, Ascorbic Acid and Caffeine within the pH range of 6.37 ± 0.02, while Acetate buffer and Methanol in a ratio of 70:30 within the pH range of 5.46 ± 0.02 was used for Metformin Hydrochloride and its surrogate reference standards; Metronidazole and Paracetamol and detection at 254nm. Both were carried out on a C18 Phenomenex, 250x4.6mm, 5µ column. The mean retention times obtained were as follows; Chlorpheniramine Maleate 2.6 ± 0.09min, Metformin Hydrochloride 3.4 ± 0.03min, Ascorbic acid 3.2 ± 0.02min, Piroxicam 6.5 ± 0.02min, Caffeine 5.9 ± 0.02min, Metronidazole 5.3 ± 0.20min and Paracetamol 4.7 ± 0.02min. The peak areas obtained from the chromatograms and the specific concentrations of the solutions analysed were used to find the surrogate constant K for Chlorpheniramine Maleate and the values obtained as follows: Piroxicam 0.8095 ± 0.003, Caffeine 0.2224 ± 0.006, and Ascorbic acid 0.1560 ± 0.002 and that of Metformin Hydrochloride are Metronidazole 1.3262 ± 0.02 and Paracetamol 0.8623 ± 0.02. The

K values were found to be influenced by the molecular weight ratio of analyte to surrogate where the lower the ratio, the higher the K value. However, the K values were not affected by changes in concentration of both analyte and surrogate. The K values were then inserted into the hypothetical formular and the percentage content of Chlorpheniramine Maleate in four different brands of Chlorpheniramine Maleate tablets and Metformin Hydrochloride in four different brands of Metformin Hydrochloride tablets was found. Although the percentage contents were within the pharmacopoeial limits, statistical tests i.e. t-Test and F-test were carried out to compare the results obtained from the new method to the results obtained from the standard method in the British Pharmacopoeia and it was found out that there was no significant difference between the two methods, though some brands deviated. Similarity in physico-chemical parameters between analyte and surrogate is found to be favourable as observed in all the brands of Metformin Hydrochloride analyzed when Paracetamol and Metronidazole are used as surrogate reference standards, the two methods did not differ significantly in their precision because of the similarity in their solubility. Similar trend was observed in the analyses of Chlorpheniramine Maleate where the two methods did not differ significantly in their precision for all the brands when Piroxicam was used as the surrogate reference standard due to closeness of the wavelength of maximum absorption. With the experimental conditions maintained, Chlorpheniramine Maleate and Metformin Hydrochloride can therefore be analyzed using their respective surrogate reference standards in place of the their pure reference standards.

CHAPTER ONE

1.0 Introduction

The quality of a drug incorporates its safety, efficacy, efficiency and its economic value. These parameters of the drug are determined from the raw material, through the manufacturing processes to the finished product before it gets to the consumer. This is very important because it determines the level of pharmaceutical action of the drug when administered to the consumer. These parameters are examined based on the principle of drug quality assurance and quality control. The principle of drug quality assurance is the totality of the arrangements made with the objective and intention of ensuring that pharmaceutical products manufactured in general are of consistent quality appropriate to their intended use [1].

Quality assurance is a wide ranging concept covering all matters that individually or collectively influence the quality of a product. It is the totality of the arrangements made with the objective of ensuring that pharmaceutical products are of the quality required for their intended use [1, 2].

Quality control is that part of Good Manufacturing Practices (GMP) concerned with sampling, specifications, and testing and with the organization, documentation and release procedures which ensure that the necessary and relevant tests are carried out and that materials are not released for use, nor products released for sale or supply, until their quality has being judged to be satisfactory [1].

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. They are guides (applicable to raw materials, manufacturing processes and final products) which provide limits within which the properties of raw materials should fall, or a process run or finished product perform, in order to ensure consistent batch-to-batch quality in a particular medicinal product [1]. Specification can also be defined as a list of tests and analytical procedures with proposed acceptance criteria [2]. Specifications are an important element in a drug quality assurance system and they form a basis for the laboratory examination of drugs and their dosage forms. This ensures that a given batch of a drug and its dosage forms are efficacious and safe, and therefore the desired quality can be achieved by strict adherence to these specifications [1].

An integrated effort, involving the role of an analyst with regard to the chemical purity of pharmaceutical substances and drugs that are manufactured, and finally the dosage forms that are usually available for direct patient's usage; has become not only extremely crucial but also equally important and vital. As on date product, safety has to be an integral part of all product research in pharmaceutical substances. However, the risk-benefit-ratio has got to be pegged to a bare minimum level. Therefore, it has become absolutely necessary to lay emphasis on product safety research and development which is very crucial in all the developmental stages of new secondary pharmaceutical products [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 [4].

Validation refers to establishing documented evidence that a process or system, when operated within established parameters, can perform effectively and reproducibly to produce pre-determined specifications and quality attributes. Some validation parameters are accuracy, precision, reproducibility, reliability, simplicity, robustness, selectivity (specificity) and sensitivity. Validation studies are essential part of GMP and should be conducted in accordance with pre-defined protocols. The important steps involved in setting up a validation programme are determination of the critical variables, establishing acceptable ranges and continuous control of variables. The proof of validation is obtained through collection and evaluation of data [1].

High-performance liquid chromatography or high-pressure liquid chromatography, HPLC, is a chromatographic technique that can separate a mixture of compounds and is used to identify, quantify and purify the individual components of the mixture [5]. The present popularity of HPLC results from its convenient separation of a wide range of sample types, exceptional resolving power, and speed and nanomolar detection levels. It is presently used in pharmaceutical research and development to assay active ingredients, impurities, degradation products and in dissolution assay, and in pharmacodynamic and pharmacokinetic studies [6].

The following parameters must also be considered before embarking on developing a method for the analysis of a particular drug or a mixture of drugs; selection of the HPLC column that eventually contains the stationary phase appropriate for the analyte, the mobile phase combination in their right ratios, pH of the analyte, detector selection, wavelength detection range, selection of chromatographic mode and flow rate of the analyte [7]. When all these parameters are carried out and the right validation tools are used to validate the method and are found to meet specifications, then a method can be said to have been developed for the analysis of that particular analyte. These and other relevant information and findings during the analysis must be documented. Good Manufacturing Practices (GMP) outlined by the World Health Organization (WHO) requires that every non-compendia analytical method (or modified compendia method) must be validated and the validation result should be documented [1].

HPLC method development for the analysis of mixtures of substances is a task that usually requires much expertise, is time consuming and is still based on critical 'trial and error' [13]. One must first of all, define the method and separation goals, obtain adequate information about the analyte and finally validate the method developed [7].

HPLC is the most widely used of all the analytical techniques owing to its sensitivity, its ready adaptability to accurate quantitative and qualitative determination, its suitability to separating non-volatile species and/or thermally fragile ones and its widespread application to substances such as drugs, proteins, amino acids, carbohydrates, pesticides and a variety of inorganic substances that are of prime interest to industry, many fields of science and to the public [1].

An HPLC instrument has at least the following elements; solvent reservoir, transfer line with frit, high-pressure pump, sample injection device, column, detector, and data recorder, usually together with data evaluation [8].

HPLC typically utilizes different types of stationary phases, a pump that moves the mobile phase and an analyte through the column, and a detector that provides a characteristic retention time for the analyte. The pump provides the higher pressure required to propel the mobile phase and analyte through the densely packed column. The increased density arises from smaller particle sizes. This allows for a better separation on columns of shorter length when compared to ordinary column chromatography [5].

The stationary phase is either solid long chain hydrocarbons attached to silica, porous, surface-active material in small-particle form or a thin film of liquid coated on a solid support which is the column wall. This is usually selected to be in opposite polarity to the substance(s) or analyte, i.e. if the analyte is polar, the stationary phase should be non-polar. The mobile phase which is a liquid or gas usually has the same polarity as the analyte; thus the analyte should be soluble in the mobile phase [8]. In this case, there will be a strong attraction between the polar solvent and polar molecules in the analyte being passed through the column and less attraction between the hydrocarbon chains attached to the silica (the stationary phase) and the polar molecules in the solution. Polar molecules in the mixture will therefore spend most of their time moving with the solvent i.e. the mobile phase before they are eluted [9]. This is termed analyte retention time or simply retention time, which is the time in minutes required for elution of the sample [1]. Analyte retention time varies depending on the strength of its interactions with the

stationary phase, the ratio/composition of solvent(s) used, column length and column diameter and the flow rate of the mobile phase [5].

During the HPLC analysis, the analyte is dissolved in suitable solvent(s) which serves as the mobile phase and then forced to flow through a chromatographic column under high pressure by the pump. The pump forces the mobile phase from the solvent reservoir to the column where separation of the components of the analyte occurs. The detector captures the components in the injected sample as they are eluted to produce signals. These signals are known as peaks and the whole entity is the chromatogram [9].

The peaks give both qualitative and quantitative information on the mixture in the analyte. The qualitative determination is by the retention time of a component which is always constant under identical chromatographic conditions. The retention time is the period that elapses between sample injection and the recording of the signal maximum. The column dimensions, type of stationary phase, mobile phase composition and flow rate, sample size and temperature provide the chromatographic conditions. Hence, a peak can be used to identify a particular analyte by injecting the relevant substance and then comparing retention times [8, 10].

In the quantitative determination, both the area and height of a peak are proportional to the amount of a compound injected. A calibration graph can be derived from peak areas or heights obtained for various solutions of precisely known concentrations and a peak-size comparison can then be used to determine the concentration of an unknown sample [8].

1.1 Justification

In most quantitative pharmaceutical analysis of drugs using High Performance Liquid Chromatography, a pure reference standard of the analyte is needed to help in preparing controls, calibration curves and system suitability tests for the analyses**.** In the British Pharmacopoeia, it stated that where the letters *BPCRS* appear after the name of a substance in a test or assay, the British Pharmacopoeia Chemical Reference Substance is to be used. Where the letters *CRS* or *EPCRS* appear, the Chemical Reference Substance issued by the European Pharmacopoeia Commission is to be used and, where the letters *BRP* or *EPBRP* appear, the Biological Reference Preparation issued by the European Pharmacopoeia Commission is to be used [11].

These pure reference standards are not readily available locally for the analysis and are also very expensive. The relative cost of Chlorpheniramine Maleate (125mg) and Metformin Hydrochloride reference standard as at 13th May, 2011 is detailed in the table below;

Table 1.0 Cost of Reference Standard

Catalog #	**Product Description**	**Current Lot**	**Previous Lot**	**Unit Price**
1123000	Chlorpheniramine Maleate (125mg)	N0G316	M0B020 (03/09)	$158.00 EACH
1396309	Metformin Hydrochloride (200mg)	I0H236	H0E136 (09/09)	$233.00 EACH

Source: USP Daily Reference Standards Catalog

Importation of these reference standards into Ghana for example, to be used by institutions, industries, regulatory bodies and other agencies usually takes a very long time, e.g. between three to six months or even a year, thus making it difficult for such

routine analysis to be carried out successfully and within time. In view of this challenge, it has become very essential to research into the possibility of using compounds other than the pure compound as a reference standard. Substances that were physico-chemically related were selected to be used as the surrogate standards to investigate the effect of this relation of the surrogate standard on the analyte. Such research has been carried out for Prednisolone, Diazepam, Indometacin, Paracetamol, Aspirin and Diclofenac Sodium and has successfully been completed at the Department of Pharmaceutical Chemistry, KNUST. This thesis research therefore seeks to extend the search for appropriate surrogate reference standards by using the analysis of Chlorpheniramine Maleate tablet and Metformin Hydrochloride tablets as another case study.

1.2 Main Objective

This project seeks to consider the possibility of using surrogate reference standards for the analyses of Chlorpheniramine Maleate tablet and Metformin Hydrochloride tablets in quantitative HPLC.

1.3 Specific Objectives

The specific objectives of this research are;

1. To develop an HPLC assay procedure for Chlorpheniramine Maleate tablet and Metformin Hydrochloride tablet using surrogate reference compounds.

2. To validate the method developed by using validation parameters such as Specificity and 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 Chlorpheniramine Maleate and Metformin Hydrochloride in their respective tablets for various brands using the method developed.
5. To compare the results obtained from the method developed with a standard method in the British Pharmacopoeia.

1.4 Hypothesis of Study

In the quantitative analysis of a drug sample using HPLC,

$$\frac{A_{analyte}}{C_{analyte}} = \frac{A_{standard}}{C_{standard}}$$

Where;

$A_{analyte}$ = peak area of the analyte

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

$C_{analyte}$ = concentration of the analyte

$C_{standard}$ = concentration of the standard

However, using surrogate compounds as the standard,

$$\frac{A_{analyte}}{C_{analyte}} \neq \frac{A_{standard}}{C_{standard}}$$

But rather,

$$\frac{A_{analyte}}{C_{analyte}} \alpha \frac{A_{standard}}{C_{standard}}$$

Therefore,

$$\frac{A_{analyte}}{C_{analyte}} = K \frac{A_{standard}}{C_{standard}} \quad \ldots\ldots\ldots\ldots\ldots\ldots(1)$$

Therefore, $$K = \frac{A_{analyte} \times C_{standard}}{C_{analyte} \times A_{standard}}$$

Once K, $A_{analyte}$ and $C_{standard}$ are known for a particular system, $C_{analyte}$ can be calculated.

Hence percentage content = (Actual concentration / Nominal concentration) X 100 %

CHAPTER TWO

2.0 Literature review

Counterfeit, adulterated and substandard medicines are a global menace. Efforts to safeguard the quality of medicines usually involved the application of instrumental methods for both qualitative and quantitative analyses of active pharmaceutical ingredients in bulk and formulations. The HPLC technique has been extensively used and is recommended for both in vitro and in vivo quality monitoring of medicines. [12]. However, most HPLC applications require the use of chemical reference standards for identification and/or quantitation. This notwithstanding, accessibility, cost of reference standards and shipments often make it difficult for the full utilization of the capacity of HPLC. It is therefore imperative to find alternative means to carry out such analyses to achieve comparable results.

According to S. Asare-Nkansah *et al,* surrogate reference standards; Aspirin, Benzoic acid and Phenacetin have successfully been applied to the assay of Paracetamol tablets and it has shown the potential for use in routine quantitative HPLC applications once the HPLC method is evolved and the surrogate constant (S_a) is determined. T. Tuani also affirmed that it is possible to assay Aspirin tablets with the use of Benzoic acid, Phenacetin and Sulphadoxine as surrogate reference standards and Diclofenac Sodium tablets using Caffeine, Phenacetin and Paracetamol as surrogate reference standards.

No work has so far been done on the HPLC assay of Chlorpheniramine Maleate and Metformin Hydrochloride tablets using surrogate reference standards. This study therefore seeks to investigate this possibility since Chlorpheniramine Maleate tablet is most widely used as an antihistamine and Metformin Hydrochloride as a hypoglycaemic and their quality ought to be assessed through alternative means without the use of their pure chemical reference standards.

2.0.1 Ultraviolet – visible absorption spectroscopy

This analytical technique is one of the most frequently employed in pharmaceutical analysis. The ultraviolet (UV) and visible region of the electromagnetic spectrum covers the wavelength range from about 100nm to about 800nm. The vacuum ultraviolet region, which has the shortest wavelengths and highest energies (100-200nm), is difficult to make measurements in this range and is of little use in analytical procedures. Ultraviolet – visible absorption spectroscopy involves the measurement of the amount of ultraviolet (190 – 380nm) or visible (380 – 800nm) radiation absorbed by a substance in solution. Instruments which measure the ratio or a function of the ratio of the intensity of two beams of light in the ultraviolet-visible region are called ultraviolet-visible spectrophotometers. Absorption of light in both the ultraviolet and visible regions of the electromagnetic spectrum occurs when the energy of the light matches that required to induce in the molecule an electronic transition and its associated vibrational and rotational transition [13].

Two empirical laws made by Lambert and Beer govern the phenomenon of absorption of light by molecules and form the basis for the quantitative analysis of drugs. Lamberts law relates the total absorption to the optical path length at constant concentration:

Absorbance (A) = $\log_{10}(I_o/I) = kl$

where:

I_o = incident light

I = transmitted light

l = path length

k = proportionality constant

While Beer's law relates absorption to the concentration of the absorbing solute, C, in the solution at constant pathlength:

$\log_{10}(Io/I) = kC$

A combination of these two laws, Beer-Lambert law, defines the absorbance of a solution of a substance as being related to the path length of a solution through which light passes and to its concentration; i.e.

$\varepsilon = A/cl$

Where ε = absorptivity [1].

Another form of the Beer-Lambert proportionality constant is the specific absorbance, which is the absorbance of a specified concentration in a cell of specified pathlength. The most common form in pharmaceutical analysis is the A(1%, 1cm), which is the

absorbance of a 1g/100ml (1% w/v) solution in a 1cm cell. The Beer-Lambert equation therefore takes the form

$$A = A_{1\ cm}^{1\ per\ cent} bc$$

Where c is in g/100ml

b is in cm, and

A(1%, 1cm) is in $dlg^{-1}cm^{-1}$. [5]

Ultraviolet and Visible Spectrophotometry finds its primary application in quantitative analysis. The scope of absorption spectroscopy can be significantly extended by the use of colour reactions, often with a concomitant increase in sensitivity and/or selectivity. However, spectral selectivity, and in some cases detection selectivity, can be significantly enhanced by various chemical and instrumental techniques. Such method must be validated by applying the conventional analytical criteria of accuracy and independence from interfering substances. [6].

2.0.1.1 Single component analyses

For samples within which only one component absorbs significantly, a wavelength must be chosen and this wavelength must be the wavelength of maximum absorption in the spectrum in order to minimize wavelength-setting errors. Stray light errors may occur if wavelength at the extreme ends of the ultraviolet and visible ranges is used. The specific absorbance can be used for the calculation of sample concentration.

2.0.1.2 Multicomponent analyses

An overlap of the absorption spectra of two or more drugs of interest may occur. There may also be some interference from impurities in manufacturing, decomposition products and formulation excipients. These irrelevant absorptions, if not removed can cause systematic error to the assay of the sample component. The basis of all the spectrophotometric techniques for multicomponent samples is the property that at all wavelengths:

a. the absorbance of a solution is the sum of absorbance of the individual components; or

b. the measured absorbance is the difference between the total absorbance of the solution in the sample cell and that of the solution in the reference cell [14].

2.0.2 Nuclear magnetic resonance spectroscopy (NMR)

This is an analytical technique that permits the exploration of a molecule at the level of the individual atom and affords information concerning the environment of that particular atom. [13].

It has become one of the foremost methods for molecular identification, for evaluating detailed molecular structures, for understanding conformations and for probing molecular dynamics [6].

NMR is concerned with the magnetic properties of certain atomic nuclei, especially the nucleus of the hydrogen atom (proton magnetic resonance- PMR) and that of the Carbon-13 isotope (^{13}C NMR). It is similar to other types of absorption spectroscopy in that

absorption of electromagnetic energy in the radio-frequency region provides analytical information, but differs by its requirement of the presence of an external magnetic field and that the phenomenon concerns atomic nuclei rather than electrons. The frequency at which energy is absorbed depends on the magnetic properties of the nucleus, the electronic environment and the kind of neighboring atoms. NMR is thus the most powerful technique for structure determination of organic molecules, being the only method that explores the molecule at the level of the individual atoms. In pharmaceutical analysis, it is employed in the area of structural elucidation, identification of drugs, differentiation of closely related compounds, such as a drug and its metabolites or decomposition products, and multi-component analysis [1, 15].

When a nucleus is placed in a static uniform external magnetic field, there will be an interaction between the nuclear magnet and the external magnetic field. The nucleus will try to align itself with the direction of the applied magnetic field, but because the nucleus is spinning, it is unable to do so. Instead, it proceeds about the direction of the applied magnetic field. If the precessional nucleus is irradiated with an electromagnetic radiation, then the precessional motion will be disturbed and there will be resonance. Thus the NMR experiment consist of radiating a precessing nucleus and this can be regarded as inducing transitions in the nuclear magnetic energy level [14].

2.0.3 Infra Red Spectroscopy

Infra–red (IR) spectroscopy is the study of the scattering, reflection, absorption or transmission of infrared radiation in the spectral range 800 nm to 1 000 000 nm (0.8 to 1000 μm), that is light with a longer wavelength and lower frequency than visible light [10]. It deals with the measurement of absorption of electromagnetic radiation by molecules due to vibrational energy inherent in them. The vibration energy in a molecule is within the infrared range of the electromagnetic spectrum. Thus when infrared radiation of an appropriate frequency interacts with a molecule, the energy is absorbed, leading to an increase in the vibrational energy of that bond. The infrared spectrophotometer measures the energy absorbed by the bonds in the molecule at different wavelengths or wavenumbers to give an infrared spectrum [1].

The infrared portion of the electromagnetic spectrum is usually divided into three regions; the near-, mid- and far- infrared, named for their relation to the visible spectrum. The higher energy near-IR, approximately 14000–4000 cm^{-1} (0.8–2.5 μm wavelength) can excite overtone or harmonic vibrations. The mid-infrared, approximately 4000–400 cm^{-1} (2.5–25 μm) may be used to study the fundamental vibrations and associated rotational-vibrational structure. The far-infrared, approximately 400–10 cm^{-1} (25–1000 μm), lying adjacent to the microwave region, has low energy and may be used for rotational spectroscopy [16].

The main goal of IR spectroscopic analysis is to determine the chemical 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. Thus, IR spectroscopy is an important and popular tool for structural elucidation and compound identification. It is also used in forensic analysis, drug metabolism as well as the determination of water content in a drug [6].

A common laboratory instrument that uses this technique is a Fourier Transform Infrared (FTIR) Spectrometer [14].

2.0.4 Mass Spectrometry

Mass spectrometry is an analytical tool used for measuring the molecular mass of a sample [17]. It is an analytical technique that measures the mass-to-charge ratio of charged particles. It is used for determining masses of particles, for determining the elemental composition of a molecule, and for elucidating their chemical structures. Structural information can be generated using certain types of mass spectrometers, usually those with multiple analyzers which are known as tandem mass spectrometers. This is achieved by fragmenting the sample inside the instrument and analyzing the products generated. This procedure is useful for the structural elucidation of organic compounds and other chemical compounds [18]. It also provides information about the qualitative (identification) and quantitative status of compounds and also gives isotopic ratios of atoms in a molecule [1]. In a typical MS procedure, ions are formed by the ion source and detected by the detector, when a sample is vaporized by impacting them with an electron beam and are separated by the mass analyzer according to their mass-to-charge ratio by electromagnetic fields. The ion signal is processed into mass spectra.

Mass Spectrometry is now in very common use in analytical laboratories that study physical, chemical, or biological properties of a great variety of compounds, isotope ratio, isotope dating and tracking, trace gas analysis, atom probe, pharmacokinetics, protein characterization, glycan analysis, space exploration, respired gas monitor etc. [18].

2.0.5 Thin Layer Chromatography

Chromatographic separations are based on the principle that different substances are partitioned differently between two phases: a mobile phase and a stationary phase.

Thin Layer Chromatography (TLC) is a widely used chromatographic technique for the separation and identification of drugs. In TLC, a spot of the analyte is put onto a TLC plate and is separated by partitioning [19].

A TLC plate is a sheet of glass, metal, or plastic which is coated with a thin layer of a solid adsorbent, about 0.25mm thick, usually silica or alumina. . In many cases, a small amount of a binder such as plaster of Paris is mixed with the absorbent to facilitate the coating. A small amount of the mixture to be analyzed/separated is dissolved in a solvent and spotted near the bottom of this plate. The TLC plate is then placed in a shallow pool of a solvent in a developing chamber so that only the very bottom of the plate is in the liquid. This liquid, or the eluent, is the mobile phase, and it slowly rises up the TLC plate by capillary action [20].

As the solvent moves past the spot that was applied, equilibrium is established for each component of the mixture between the molecules of that component which are adsorbed on the solid and the molecules which are in solution. In principle, the components will differ in solubility and in the strength of their adsorption to the adsorbent and some components will be carried farther up the plate than others. A substance that is strongly adsorbed will have a greater fraction of its molecules adsorbed at a time, whilst a weakly adsorbed substance will have a smaller fraction of its molecules adsorbed at a time. Thus, the more weakly a substance is adsorbed, the farther up the plate it will move and the more strongly a substance is adsorbed, the nearer it will stay to the origin [21].

When the solvent has reached the top of the plate, the separated components appear as spots on the plate. The plate is removed from the developing chamber, dried, and the separated components of the mixture are visualized if they are colored, if not then a UV lamp is used to visualize the spots on the plates. These are then used for the identification. The identification is done when the retention factor, R_f of a compound in the mixture is compared with the R_f of a known compound (preferably both run on the same TLC plate). The R_f value is determined by dividing the distance traveled by the product by the total distance traveled by the solvent (the solvent front). These values depend on the solvent used, and the type of TLC plate, and are not physical constants.

$$R_f = \frac{\text{Distance the product travels from the origin}}{\text{Distance the solvent front travels from the origin}}$$ [19].

Several factors determine the efficiency of a chromatographic separation. The adsorbent should be as selective as possible towards the components of the mixture so that the

differences in rate of elution will be large. For the separation of any given mixture, some adsorbents may be too strongly adsorbing or too weakly adsorbing. The eluting solvent should also show a maximum of selectivity in its ability to dissolve or the substances being separated. The fact that one substance is relatively soluble in a solvent can result in its being eluted faster than another substance. However, a more important property of the solvent is the ability of itself to be adsorbed on the adsorbent. If the solvent is more strongly adsorbed than the substances being separated, it can take their place on the adsorbent and all the substances will flow together. If the solvent is less strongly adsorbed than any of the components of the mixture, its contribution to different rates of elution will be only through its difference in solvent power toward them. If, however, it is more strongly adsorbed than some components of the mixture and less strongly than others, it will greatly speed the elution of those substances that it can replace on the absorbent, without speeding the elution of the others. [21]

2.0.6 Titrimetric and Chemical Methods of Analysis

Some titrimetric and chemical methods of analyses are;

2.0.6.1 Non aqueous titrations

Non aqueous titration is mostly 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 [22].

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 effective competition is that the inflection in the titration curves for very weak acids and very weak bases is small, because they approach the pH limits in water of 14 or 0 respectively, thus making endpoint detection relatively more difficult [22].

A general rule is that bases with $pK_b < 7$ or acids with $pK_a > 7$ 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 [1, 22].

2.0.6.2 Acid base titration

This is a titration that involves acids and bases regardless of the strength. It is the most common and simple 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.

Commonly used standard substances and reagents in acid/ base titrations are Hydrochloric Acid, Sodium Hydroxide, Sodium Carbonate, Borax and Potassium Hydrogen Phthalate.

Acid/ base titrations employ the use of indicators and the type of indicator used depends on several factors. One of them is the equivalence point pH. Depending on the titrated substance and titrant used this can vary, usually between 4 and 10.

Acid-base titrations can also be used to find percent purity of chemicals [1, 23].

2.0.6.3 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 indicator 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 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 [1, 13].

2.0.6.4 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. Titanous Chloride, Amalgamated Zinc and Iodine ion are used as reducing agents.

The endpoint is commonly 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 [1, 24].

2.0.6.5 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 [25].

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, meaning that 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) [22].

2.0.6.5.1 Reactions for Complexometric Titration

In theory, any complexation reaction can be used as a volumetric technique provided that:

1. The reaction reaches equilibrium rapidly after each portion of titrant is added.
2. Interfering situations do not arise. For instance, the stepwise formation of several different complexes of the metal ion with the titrant, resulting in the presence of more than one complex in solution during the titration process.
3. A complexometric indicator capable of locating equivalence point with fair accuracy is available.

In practice, the use of ethylenediaminetetraacetic acid, EDTA as a titrant is well established.

To carry out metal cation titrations using EDTA, it is almost always necessary to use a complexometric indicator to determine when the end point has been reached. Common

indicators are organic dyes such as Fast Sulphon Black, Eriochrome Black T, Eriochrome Red B or Murexide. A color change in the solution being titrated indicates that all of the dye has been displaced from the metal cations in solution, and that the endpoint has been reached. Thus, the free indicator (rather than the metal complex) serves as the endpoint indicator [1, 25].

2.0.6.6 Precipitation Titrations

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 this solution with a solution of a silver salt, say 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. Since n = cV, the number of moles of either Ag^+ or Cl^- can be calculated from the number of moles of the other, and the molar concentration or the volume of added solution can be calculated for either ion if the other is known [26].

2.0.7 High Performance Liquid Chromatography

High performance liquid chromatography, HPLC is a chromatographic technique that can separate a mixture of compound to identify, quantify and purify the individual components of the mixture. There are two main types of HPLC based on the type of stationary phase being used for the separation and on the physical and chemical

properties of the chemical to be separated. These are the normal phase and the reversed phase chromatography [1, 22].

2.0.7.1 Normal-phase HPLC

This method separates analytes based on adsorption to a stationary surface chemistry and by polarity. It uses a polar stationary phase and a relatively non-polar, non-aqueous mobile phase, and works effectively for separating analytes readily soluble in non-polar solvents. The column is filled with tiny silica particles, and the solvent is non-polar - hexane, for example, which constitutes the mobile phase. The analyte associates with and is retained by the polar stationary phase. Adsorption strengths increase with increased analyte polarity, and the interaction between the polar analyte and the polar stationary phase increases the retention time. The interaction strength depends on localization is a measure of the ability of the solvent molecules to interact with the adsorbent which is used as stationary phase, the functional groups in the analyte molecule and also on steric factors [5, 9].

2.0.7.2 Reversed-phase chromatography (RPC)

This has a non-polar stationary phase and an aqueous, moderately polar mobile phase. One common stationary phase is silica which has been treated with RMe_2SiCl, where R is a straight chain alkyl group such as $C_{18}H_{37}$ or C_8H_{17}. With these stationary phases, retention time is longer for molecules which are more non-polar, while polar molecules elute more readily. The retention can be decreased by adding a less polar solvent (Methanol, Acetonitrile) into the mobile phase to reduce the surface tension of water.

Gradient elution uses this effect by automatically reducing the polarity and the surface tension of the aqueous mobile phase during the course of the entire analysis [9].

Structural properties of the analyte molecule play an important role in its retention characteristics. In general, an analyte with a larger hydrophobic surface area (C-H, C-C, and generally non-polar atomic bonds, such as S-S and others) results in a longer retention time because it increases the molecule's non-polar surface area, which is non-interacting with the water structure. On the other hand, polar groups, such as -OH, $-NH_2$, COO^- or $-NH_3^+$ reduce retention as they are well integrated into water. Very large molecules, however, can result in an incomplete interaction between the large analyte surface and the ligand's alkyl chains and can have problems entering the pores of the stationary phase. The pharmaceutical industry regularly employs reversed-phase chromatography to qualify drugs before their release. [8]

2.0.8 Buffer Solutions

Another important component is the influence of the pH of the analyte since this can change the hydrophobicity of the analyte. For this reason most methods use a buffering agent, such as Sodium Phosphate to control the pH. The buffers serve multiple purposes: they control pH, neutralize the charge on any residual exposed silica on the stationary phase and act as ion pairing agents to neutralize charge on the analyte. If acids or bases are present in the sample, the mobile phase should be buffered in most cases; a buffer is not required for non-ionizable samples [9, 27].

2.0.9 Isocratic flow and gradient elution

A separation in which the mobile phase composition remains constant throughout the procedure is termed isocratic and a separation in which the mobile phase composition is changed during the separation process is described as a gradient elution [8, 27].

2.0.10 General components of the High Performance Liquid Chromatography Instrument

A complete High Performance Liquid Chromatography Instrument should have at least the following components:

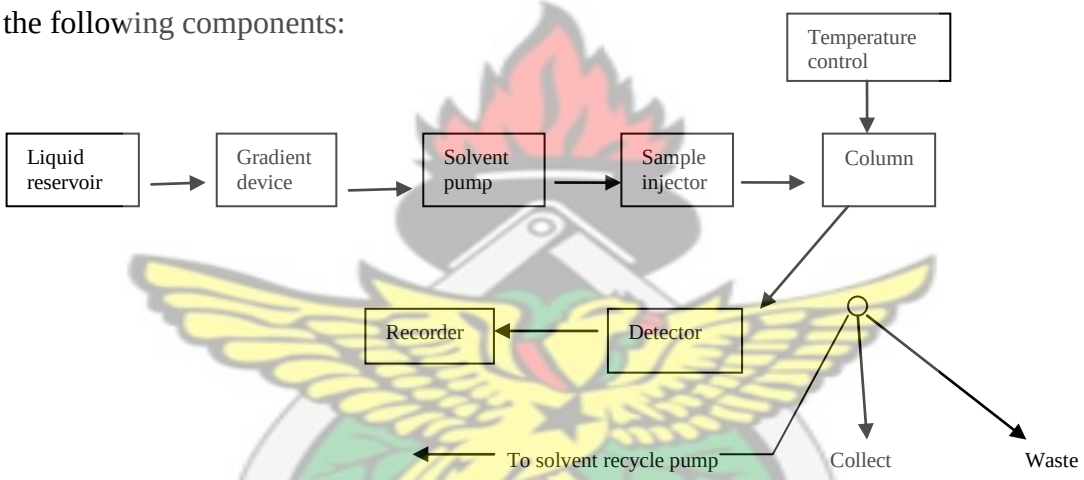


Fig.2.0 Schematic diagram of the HPLC equipment

2.0.10.1 Solvent Reservoir

This is the container that carries the mobile phase and is made of glass or stainless steel equipped with a means of removing dissolved gases e.g. degassers and a means of filtering of dust and particulate matter from solvents. [27]

2.0.10.2 Solvent Pumps

These contain corrosive-resistance components and are used to force the mobile phase from the enclosed solvent reservoir to the column [27]. Pumps vary in pressure capacity, but their performance is measured on their ability to yield a consistent and reproducible flow rate. Pressure may reach as high as 40 MPa (6000 lbf/in^2), or about 400 atmospheres. Modern HPLC systems have been improved to work at much higher pressures, and therefore are able to use much smaller particle sizes in the columns (<2 μm) [1, 27].

2.0.10.3 Sample Injector

This is used to introduce the analyte into the column. They are made of graduated glass or plastic measuring cylinders with a syringe equipped with a special flat tip needle, fitting especially to the HPLC injection valve. They are made to provide sample sizes from 5μ to 500μ. The introduction of samples onto the column packing must be reproducible in order not to affect the precision of liquid chromatographic measurements [1, 28].

2.0.10.4 Column

These are made of stainless steel (to cope with high pressure) containing the packing material of either polar or non-polar substances. This serves as the stationary phase of which the analyte interacts with and cause the separation. The sample to be analyzed is introduced in small volume to the stream of mobile phase. The solution movement through the column is slowed by specific chemical or physical interactions with the

stationary phase present within the column. The velocity of which the solution moves depends on the nature of the sample and on the compositions of the stationary (column) phase. The time at which a specific sample elutes (comes out of the end of the column) is called the retention time; the retention time under particular conditions is considered an identifying characteristic of a given sample [1, 22].

2.0.10.4.1 Caring for the column

- Reversed phase columns should not be used with aqueous bases as these will destroy the underlying silica particle and should not be exposed to the acid for too long, as it can corrode the metal parts of the HPLC equipment.
- Highly purified HPLC grade solvent should be used to prevent particles from adhering to the column surface thereby blocking the pores and rendering the column ineffective.
- The use of guard column is also encouraged as this will absorb particles likely to block the column before the solvent enters the column.
- RP-HPLC columns should be flushed with clean solvent after use to remove residual acids or buffers, and stored in an appropriate composition of solvent.

2.0.10.5 Detector

The detector for an HPLC is the component that offers continuous monitoring at the column exit and emits a response due to the eluting sample compound and subsequently signals as a peak on the chromatogram. It is positioned immediately posterior to the

stationary phase in order to detect the compounds as they elute from the column. There are many types of detectors that can be used with HPLC [27].

Some of the common detectors include: Refractive Index (RI), Ultra-Violet (UV), Fluorescent, Radiochemical, Electrochemical, Mass Spectroscopy (MS), Nuclear Magnetic Resonance (NMR), and Light Scattering (LS).

Ultra-Violet (UV) detectors measure the ability of a sample to absorb light. It is the most commonly used because of its high sensitivity, reproducibility and its ability to operate at fixed, multiple or variable wavelengths. Diode Array detectors are the most powerful detectors and can measure a spectrum of wavelengths simultaneously. UV detectors have sensitivity to approximately 10^{-8} or 10^{-9} gm/ml [10, 27].

Refractive Index (RI) detectors measure the difference between the refractive index of the mobile phase containing the chromatographic compound passing through the column and that of the mobile phase alone. Though least sensitive, IR detectors responds to any solute and have detection limit of 10^{-7} g/ml and is especially valuable for compounds that do not show any UV absorption [1, 16].

Fluorescent detectors measure the ability of a compound to absorb then re-emit light at given wavelengths. It is highly sensitive and selective for fluorescent compounds and its limit of detection is found to be 10-^{11}g/ml [1, 16]. Radiochemical detection involves the use of radio-labeled material, usually tritium (3H) or carbon-14 (^{14}C). It operates by detection of fluorescence associated with beta-particle

ionization, and it is most popular in metabolite research. Has sensitivity limit up to 10^{-9} to 10^{-10} g/ml [1, 16].

Electrochemical detectors measure compounds that undergo oxidation or reduction reactions. Usually, the reactions are accomplished by measuring gain or loss of electrons from migrating samples as they pass between electrodes at a given difference in electrical potential. They are not gradient elution compatible and have sensitivity of 10^{-12} to 10^{-13} g/ml [1, 16].

Mass Spectroscopy (MS) Detectors- The sample compound or molecule is ionized, it is passed through a mass analyzer, and the ion current is detected. Has detection limit of 10^{-8} to 10^{-10} g/ml [1, 16].

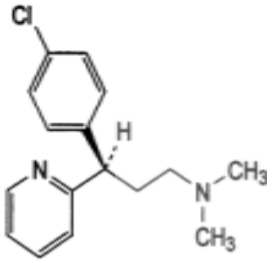
Nuclear Magnetic Resonance (NMR) Detectors- Certain nuclei with odd- numbered masses, including H and ^{13}C, spin about an axis in a random fashion. Each H or C will produce different spectra depending on their location and adjacent molecules, or elements in the compound, because all nuclei in molecules are surrounded by electron clouds which change the encompassing magnetic field and thereby alter the absorption frequency. [1, 16].

Light-Scattering (LS) Detectors- When a source emits a parallel beam of light which strikes particles in solution, some light is reflected, absorbed, transmitted, or scattered. [1, 16].

2.0.11 Profile of pure samples of analyte and surrogates

The pure samples of analyte and surrogates are;

2.0.11.1 Chlorpheniramine Maleate



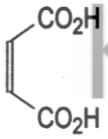


Fig. 2.1 The Chemical structure of Chlorpheniramine Maleate [11].

Chlorpheniramine Maleate, also known as (3RS)-3-(4-Chlorophenyl)-N,N-dimethyl-3-(pyridin-2-yl)propan-1-amine hydrogen (Z)-butenedioate, has an empirical formular of $C_{16}H_{19}ClN_2,C_4H_4O_4$ and molecular mass of 390.9 g/mol. Its solubility in water is 0.55 g/100 ml [11, 29].

Chlorpheniramine Maleate is an antihistamine used to relieve symptoms of allergy, hay fever, and common cold. These symptoms include rash, watery eyes, itchy eyes/nose/throat/skin, cough, runny nose, and sneezing. This medication works by blocking a certain natural substance (histamine) that the body makes during an allergic reaction. By blocking another natural substance made by the body (acetylcholine), it helps dry up some body fluids to relieve symptoms such as watery eyes and runny nose [29].

Some common side effects of Chlorpheniramine include drowsiness, dizziness, headache, constipation, stomach upset, blurred vision, decreased coordination, or dry mouth, nose, throat [29].

2.0.11.1.1 Assay of Chlorpheniramine Maleate

A non aqueous titration is involved in the assay of Chlorpheniramine Maleate because of the weak acid, Maleic acid, attached to it as an enantiomer. Titrations involving very weak acids and very weak bases do not give sharp endpoints in aqueous solutions. These titrations are therefore carried out in non aqueous solvents.

This is done by dissolving 0.150 g in 25 ml of anhydrous acetic acid and titrated with 0.1M Perchloric Acid and then determining the end-point potentiometrically. 1ml of 0.1M Perchloric Acid is equivalent to 19.54 mg of Chlorpheniramine Maleate. [4, 11]

2.0.11.2 Metformin Hydrochloride

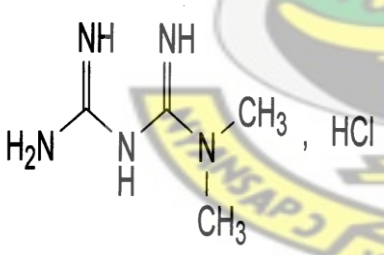


Fig.2.2 The Chemical structure of Metformin Hydrochloride [11].

Metformin Hydrochloride, also known as 1,1-Dimethylbiguanide hydrochloride, has an empirical formula of $C_4H_{12}ClN_5$. Its molecular formular is 165.6g/mol and is freely

soluble in water, slightly soluble in alcohol, practically insoluble in acetone and in Methylene Chloride [11].

Metformin is an oral anti-diabetic drug in the biguanide class. Metformin belongs to the class of medications called oral hypoglycemics, which are medications that lower blood sugar. It is used to control blood glucose (blood sugar) for people with type 2 (non-insulin-dependent) diabetes. [30, 31].

Metformin lowers the levels of glucose (sugar) in blood in three different ways. First, it reduces the amount of glucose produced by your liver; second, it reduces the amount of glucose absorbed from food through your stomach; and third, it improves the effectiveness of insulin in the body in reducing glucose already in the blood [27].

2.0.11.2.1 Assay of Metformin Hydrochloride

A non aqueous titration is involved in the assay of Metformin Hydrochloride by dissolving 0.1g in 4ml of anhydrous Formic acid. 80 ml of Acetonitrile is added and the titration is carried out immediately with 0.1M Perchloric acid, determining the end-point potentiometrically. 1 ml of 0.1M Perchloric acid is equivalent to 16.56 mg of Metformin Hydrochloride [11].

2.0.11.3 Caffeine

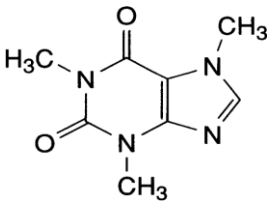


Fig. 2.3 Chemical structure of Caffeine [11]

The IUPAC name of Caffeine is 1,3,7-trimethyl-3,7-dihydro-1*H*-purine-2,6-dione with a molecular formula of $C_8H_{10}N_4O_2$. Its molar mass is 194.19 g/mol and has a density of 1.23 g/cm^3. It is an odorless, white needles or powder with a melting point of 227–228 °C, (anhydrous) boiling point of 178 °C. Its solubility in water is 2.17 g/100 mL (25 °C) 18.0 g/100 mL (80 °C) and 67.0 g/100 mL (100 °C) [11, 32].

Caffeine is a bitter, white crystalline xanthine alkaloid that is a psychoactive stimulant. In humans, caffeine acts as a central nervous system (CNS) stimulant, temporarily warding off drowsiness and restoring alertness [32].

2.0.11.4 Ascorbic acid

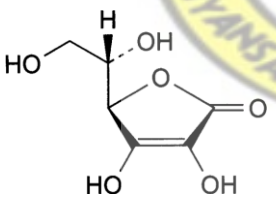


Fig. 2.4 The Chemical structure of Ascorbic acid [11].

Ascorbic acid has the molecular formula $C_6H_8O_6$ and the molecular weight of 176.1g/mol. It melts at about 190 °C, with decomposition. Ascorbic acid is a naturally

occurring organic compound with antioxidant properties. It is a white solid and it dissolves well in water to give mildly acidic solutions. Most importantly, ascorbic acid is a mild reducing agent and therefore degrades upon exposure to oxygen, especially in the presence of metal ions and light. Ascorbic acid is used in the treatment of vitamin C deficiency, thus preventing scurvy, the disease caused by a deficiency of vitamin C. Being derived from glucose, many animals are able to produce it, but humans require it as a nutritional supplement [11, 33].

2.0.11.5 Piroxicam

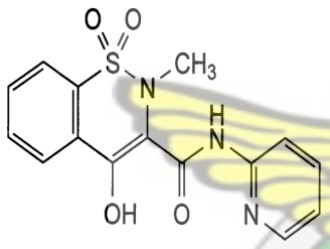


Fig. 2.5 The Chemical structure of Piroxicam [11].

The IUPAC name of Piroxicam is 4-hydroxy-2-methyl-*N*-(pyridin-2-yl)-2*H*-1,2-benzothiazine-3-carboxamide 1,1-dioxide. It has the molecular formula $C_{15}H_{13}N_3O_4S$ and its molecular weight is 331.4g/mol. It appears as a white or slightly yellow, crystalline powder, practically insoluble in water, soluble in methylene chloride, slightly soluble in ethanol. It shows polymorphism [11].

Piroxicam is a non-steroidal anti-inflammatory drug of the oxicam class used to relieve the symptoms of rheumatoid and osteoarthritis, primary dysmenorrhoea, postoperative

pain; and act as an analgesic, especially where there is an inflammatory component. As a side effect, Piroxicam use can result in gastrointestinal toxicity, tinnitus, dizziness, headache, rash and pruritus [34].

2.0.11.6 Metronidazole

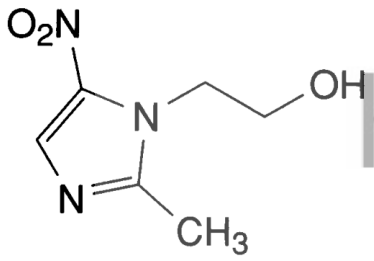


Fig.2.6 The Chemical structure of Metronidazole [11]

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, alcohol and methylene chloride [11, 30].

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 [35].

2.0.11.7 Paracetamol

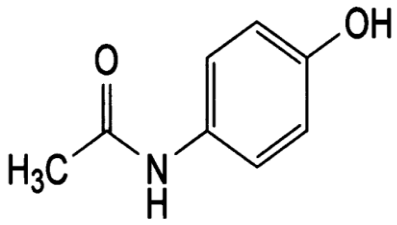


Fig.2.7 The Chemical structure of Paracetamol [11].

Paracetamol is also known as *N*-(4-Hydroxyphenyl) acetamide and have an empirical formular of $C_8H_9NO_2$. Its molecular mass is 151.2g/mol. It appears as white, crystalline powder and is sparingly soluble in water, freely soluble in alcohol, slightly soluble in methylene chloride [11].

Paracetamol, known as acetaminophen is a widely used over-the-counter analgesic (pain reliever) and antipyretic (fever reducer). It is commonly used for the relief of headaches, other minor aches and pains, and is a major ingredient in numerous cold and flu remedies. In combination with opioid analgesics, Paracetamol can also be used in the management of more severe pain such as post surgical pain and providing palliative care in advanced cancer patients [36].

Table 2.0 pKa and effective pH working range for analytes [6, 7].

Analyte	pKa (25ºC)	Effective pH working range
Paracetamol	9.5	8.0 – 11.0
Ascorbic Acid	4.2, 11.6	2.7 – 5.7
Metronidazole	2.5	1.0 – 4.0
Metformin Hydrochloride	2.8, 11.5	1.3 – 4.3
Caffeine	14.0, 10.4	8.9 – 11.9
Chlorpheniramine Maleate	9.13	7.63 – 10.63
Piroxicam	6.3	4.8 – 7.9

CHAPTER THREE

3.0 Experimental Methods

3.1 Materials/ Reagents

Anhydrous Formic Acid (BDH Analar grade), Acetonitrile, Glacial Acetic Acid (BDH Analar grade), Butanol 99.99%, Methanol (HPLC grade, 99.99%), Methanol (BDH Analar grade), Sodium Acetate 99.5%, Hydrochloric Acid 36% BDH, Anhydrous Acetic Acid BDH, Acetic Anhydride BDH, Toluene, Perchloric Acid BDH, Sulphuric Acid BDH, Iodine, Starch solution, Dragendorff's TS, Cerium Sulphate, Ether, Sodium Hydroxide pellets, 99% (BDH), Sulphamic acid (BDH Analar grade), Ethyl Acetate, Ammonium Hydroxide, Iodine crystals, Potassium Hydrogen Phthalate, Potassium Dihydrogen Phosphate 100% (BDH) were provided by the Department of Pharmaceutical Chemistry, KNUST.

3.2 Acquisition of pure samples of drugs and surrogates

The following pure samples were obtained from Pharmanova Ghana Limited, Physicochemical Laboratory of the Food and Drugs Board, Amponsah Effah Pharmaceuticals Limited and Regional Medical Stores of the Ghana Health Service, Ashanti Region, Kumasi.

Table 3.0 Profile of pure samples

Sample	Batch number	Manufacturing Date	Expiry Date	Assay (%)
Chlorpheniramine Maleate	BL/SC/C/0608012	05/08	04/13	98.34
Ascorbic acid	0011847	20/06/2009	19/06/2012	99.27
Caffeine , anhydrous	0912007-P1019	12/09	12/13	98.71
Piroxicam	K8-10	04/10	07/11	100.08
Metformin Hydrochloride	Q137	28/09/2010	31/02/2012	99.50
Metronidazole	09011801	09/11/09	12/09/2012	99.80
Paracetamol	J8-299	18/2/2009	23/12/2011	99.54

Chlorpheniramine Maleate Tablets used in this project were manufactured in Ghana by Pharmanova Limited, Kinapharma Limited, Letap Pharmaceuticals Limited and Amponsah Effah Pharmaceuticals Limited; while the Metformin Hydrochloride Tablets were manufactured by Hovid BHD (Malaysia), Denk Pharma (Germany), Pharma DOR (China) and Ernest Chemist Limited (Ghana). Both tablets were bought from pharmacy shops in Ayeduasi and Tech Junction, both are suburbs of Kumasi.

Table 3.1 Profile of drug samples

Tablet	Strength (mg)	Manufacturer	Batch number	Manufacturing Date	Expiry Date
Chlorpheniramine Maleate, BP	4	Pharmanova Ghana Ltd	B9006	12/10	06/13
Chlorpheniramine Maleate, BP	4	Kinapharma Ltd	10119	05/10	09/13
Chlorpheniramine Maleate, BP	4	Letap Pharmaceuticals Ltd	110084	07/10	12/12
Chlorpheniramine Maleate, BP	4	Amponsah Effah Pharmaceuticals Ltd	50.001	06/10	11/12
Metformin Hydrochloride, BP	500	Hovid	BA05407	05/10	05/13
Metformin Hydrochloride	500	Denk	680	02/10	01/15
Metformin Hydrochloride	500	Pharma DOR	091001	10/09	10/12
Metformin Hydrochloride	500	Ernest Chemist	4112K	12/10	12/14

3.3 Instrumentation / Apparatus

- EUTECH Instruments Cyberscan pH Meter
- Stuart Melting Point SMP 10 Apparatus
- Whatman Filter paper 11.0 cm
- Phenomenex Hypersil 5µC18 BDS, 250x4.6mm
- Phenomenex Lichrosorb 10 RP-18, 250x4.6mm
- Pump, Kontron Instruments, Applied Biosystems
- Cecil Ce 2041 2000Series-UV Spectrophotometer
- T_{90} + UV/VIS Spectrometer; PG Instruments Limited
- Melting point Capillary tubes

- Buchi Rotary Evaporator
- Adam Analytical Weighing Balance
- Applied Biosystems 783 programmable Absorbance Detector
- Chromato-Vue C-70 UV View System (UVP inc) 254nm Short wave; 365nm Long wave;
- Clifton Sonicator, Nickel – Electro Limited.

3.4 Identification Tests

The following identification tests were carried out.

3.4.1 Colour test

Colour test was performed on the following samples

3.4.1.1 Chlorpheniramine Maleate

1.0 mg of Chlorpheniramine Maleate was dissolved in 5ml of distilled water and 2 drops of Dragendorff's TS were added.

3.4.1.2 Caffeine

To Caffeine (0.0156g), Hydrogen Peroxide solution (0.1ml) and 0.3ml of dilute Hydrochloric Acid (0.1M) (17ml HCl diluted in 100ml distilled water) were added. The resulting solution was heated to dryness in a water-bath until a yellowish-red residue was obtained. Ammonia (0.1ml) was added.

3.4.1.3 Ascorbic acid

1.001 g was dissolved in carbon dioxide-free water and diluted to 20 ml with the same solvent. To 1ml of this solution, 0.2ml of dilute nitric acid and 0.2ml of silver nitrate solution was added. The carbon dioxide free water was obtained by vigorously boiling distilled water in a 200ml beaker for 20 minutes and protected from the atmosphere by covering with a plastic foil and kept in a dark cupboard and cooled.

3.4.1.4 Paracetamol

1ml of 1M HCl was added to 0.1020g Paracetamol, The mixture was heated to boil for 3 minutes and distilled water (1ml) was added. It was then cooled in an ice bath. 0.049g of Potassium dichromate was dissolved in 10ml of distilled water and 0.05ml of this solution was added to the Paracetamol solution.

3.4.2 Ultra-Violet Spectroscopy test

3.4.2.1 Metronidazole

0.04g of the pure Metronidazole was dissolved in 0.1M HCl and diluted to 100.0 ml with the same acid. 5.0 ml of the solution was diluted to 100.0 ml with the same 0.1M HCl and the resulting solution was examined between 230 nm and 350 nm.

3.4.3 Thin layer chromatography

3.4.3.1 Chlorpheniramine Maleate

A quantity of the powdered tablets of the four brands containing 4mg of Chlorpheniramine Maleate was weighed into a 10ml volumetric flask and dissolved in 5ml methanol and sonicated for 10minutes. It was then diluted to the mark with distilled water and filtered. 1.2mg/ml of the pure Chlorpheniramine Maleate powder was prepared in a mixture of methanol and water (1:1). 10µL of both solutions were applied separately to a thin layer chromatographic plate of about 15cm x 5cm coated with a 0.25mm layer of thin layer chromatographic silica gel mixture. The spots were allowed to dry and the chromatogram was developed in a solvent system consisting of a mixture of ethyl acetate, methanol and ammonium hydroxide (100:5:5), placed in an air-tight chromatographic chamber until the solvent front has moved about three-fourth of the length of the plate. The plate was subsequently removed and air-dried. The spots were located by visual inspection and examined under short wavelength ultraviolet light (254nm).

3.4.3.2 Metformin Hydrochloride

Twenty (20) tablets of the four brands of Metformin Hydrochloride were powdered and 0.02g of the powdered Metformin Hydrochloride tablets was dissolved in 5ml of distilled water. 0.02g of the pure Metformin Hydrochloride was also dissolved in 5ml of distilled water. 5µL of both solutions were applied separately to a thin layer chromatographic plate of about 15cm x 5cm coated with a 0.25mm layer of thin layer chromatographic silica gel mixture. The spots were allowed to dry and the chromatogram was developed in a solvent system consisting of a mixture of glacial Acetic Acid, Butanol and water in a

ratio of 1:4:5, placed in an air-tight chromatographic chamber until the solvent front has moved over a path of about 15cm along the plate. The plate was subsequently removed and air-dried. The spots were located by visual inspection and examined under short wavelength ultraviolet light (254nm).

3.4.4 Melting Point Determination

The dry pure powder of each of the reference standards and analytes were introduced into separate capillary tubes sealed at one end. The solid was shaken down the tube by tapping the sealed end on a hard surface so as to form a tightly packed column from 3 to 5 mm in height. These were then placed in a melting point determination apparatus and their various melting points determined.

3.4.5 Determination of pH of pure samples

0.05g each of the pure form of the analytes were weighed and dissolved in 50ml of distilled water and their respective pH was determined.

3.4.6 Determination of wavelength of maximum absorption

0.05g each of the pure form of the analytes were weighed and dissolved in 100ml of distilled water and 5ml of this solution was diluted to 100ml with the same solvent. The resulting solution was scanned between 200nm and 350nm using a Cecil CE 2041 2000 Series-UV Spectrophotometer.

3.5 Assay of pure samples

3.5.1 Chlorpheniramine Maleate

3.5.1.1 Standardization of 0.1M Perchloric acid ($HClO_4$) using Potassium Hydrogen Phthalate ($C_8H_5KO_4$)

Approximately 0.5007g of Potassium Hydrogen Phthalate was weighed and dissolved in 50.00ml of Glacial Acetic acid. The cooled solution was then titrated against 0.1M Perchloric acid using Oracet Blue as the indicator and the endpoint determined.

3.5.1.2 Method of assay

Chlorpheniramine Maleate (0.150 g) powder was dissolved in 25 ml of anhydrous Acetic acid and titrated with 0.1M of the standardized Perchloric acid and the end-point was determined potentiometrically. 1 ml of 0.1 M perchloric acid is equivalent to 19.54 mg of Chlorpheniramine Maleate.

3.5.2 Caffeine

3.5.2.1 Method of assay

Caffeine (0.170g) was dissolved with heating in 5ml of anhydrous Acetic acid. It was allowed to cool and 10ml of Acetic Anhydride and 20 ml of Toluene were added. It was then titrated with 0.1M Perchloric acid and the end-point was determined potentiometrically.1ml of 0.1M Perchloric acid is equivalent to 19.42mg of Caffeine.

3.5.3 Piroxicam

3.5.3.1 Method of assay

Piroxicam (0.250g) was dissolved in 60ml of a mixture of equal volumes of Acetic Anhydride and Anhydrous Acetic acid and titrated with 0.1M Perchloric acid and the end-point was determined potentiometrically. 1ml of 0.1M Perchloric acid is equivalent to 33.14mg of Piroxicam.

3.5.4 Ascorbic Acid

3.5.4.1 Standardization of 0.05M Iodine solution with Sodium Thiosulphate

Exactly 20ml of 0.05M Iodine solution was pipetted into a conical flask and titrated with 0.1M Sodium Thiosulphate from the burette until the Iodine is decolourized at the endpoint. The procedure was repeated twice.

3.5.4.2 Method of assay

Ascorbic acid (0.150 g) was dissolved in a mixture of 10ml of dilute Sulphuric acid and 80ml of carbon dioxide-free water. 1ml of starch solution was added and titrated with 0.05M Iodine until a persistent violet-blue colour is obtained. 1ml of 0.05M Iodine is equivalent to 8.81mg of Ascorbic acid. The carbon dioxide free water was obtained by vigorously boiling distilled water in a 200ml beaker for 20 minutes and protected from the atmosphere by covering with a plastic foil and kept in a dark cupboard and cooled.

3.5.5 Metformin Hydrochloride

3.5.5.1 Method of assay

Metformin (0.100g) was dissolved in 4 ml of anhydrous formic acid and 80 ml of acetonitrile was added. The titration was carried out immediately with 0.1M Perchloric

acid, determining the end-point potentiometrically. 1 ml of 0.1 M Perchloric acid is equivalent to 16.56mg of Metformin Hydrochloride.

3.5.6 Metronidazole

3.5.6.1 Method of assay

Metronidazole (0.150g) was dissolved in 50ml of Anhydrous Acetic Acid and it was titrated with 0.1M Perchloric acid and the end-point was determined potentiometrically. 1 ml of 0.1M Perchloric acid is equivalent to 17.12 mg of Metronidazole.

3.5.7 Paracetamol

3.5.7.1 Method of assay

Paracetamol (0.3011g) was dissolved in a mixture of 10 ml of purified water and 30 ml of dilute Sulphuric acid .The mixture was boiled under a reflux condenser for 1 hour, cooled and diluted to 100.0 ml with water. To 20.0ml of the solution, 40ml of water, 40g of ice, 15ml of dilute Hydrochloric acid and 0.1ml of Ferroin were added. It was then titrated with 0.1M Cerium Sulphate until a greenish-yellow colour was obtained. A blank titration was also carried out. 1 ml of 0.1 M Cerium Sulphate is equivalent to 7.56mg of Paracetamol.

3.6 Uniformity of weight

Twenty tablets each of all the samples were weighed. The tablets were also weighed individually and the average weight was determined. Deviation of each tablet for the average weight was calculated and the number of tablets deviating from the average weight according to a pharmacopoeial specification determined.

3.7 Determination of Percentage content using Standard Method from the British Pharmacopoeia, 2007.

3.7.1 Chlorpheniramine Maleate Tablets (4mg)

Twenty (20) tablets were weighed and powdered. A quantity of the powder containing 3mg of Chlorpheniramine Maleate was shaken with 20ml of 0.05M Sulphuric acid for 5 minutes and 20ml of Ether was added and shaken carefully. The acid layer was filtered into a second separating funnel. The ether layer was then extracted with two 10ml quantities of 0.05M Sulphuric acid and each acid layer was filtered into the second separating funnel and washed with 0.05M Sulphuric acid. The combined acid extracts and washings were made just alkaline to litmus paper with 1M Sodium Hydroxide and 2ml was added in excess and extracted with two 50 ml quantities of Ether. Each Ether extract was washed with the same 20ml of water and extracted with successive quantities of 20, 20 and 5ml of 0.25M Sulphuric acid. The combined acid extracts was diluted to 50ml with 0.25M Sulphuric acid and 10ml was diluted to 25ml with 0.25M Sulphuric acid and the absorbance of the resulting solution was measured at the maximum at 265nm. The content of Chlorpheniramine Maleate was calculated taking 212 as the value of A (1%, 1cm) at the maximum at 265nm.

3.7.2 Metformin Hydrochloride Tablets (500mg)

Twenty (20) tablets were weighed and powdered. A quantity of the powder containing 0.1g of Metformin Hydrochloride was shaken with 70ml of water for 15 minutes. It was then diluted to 100ml with distilled water and filtered, discarding the first 20ml. 10 ml of the filtrate was then diluted to 100ml with distilled water and 10ml of the resulting

solution was again diluted to 100ml with water. The absorbance of the resulting solution was measured at the maximum at 232nm. The content of the Metformin Hydrochloride was measured taking 798 as the value of A (1%, 1 cm) at the maximum at 232nm.

3.8 HPLC Analyses

Preliminary information about the analytes and the surrogates were gathered. These included the structure, molecular weight, stability, pH and UV-Visible absorption pattern.

3.8.1 Chromatographic mode and Column selection

The reversed-phase chromatographic mode was selected because both the analytes and the surrogate reference standards were polar. This also informed the choice of using Octyldecylsilane (ODS) bonded column. Compared to C8, C18 is more hydrophobic and less retentive and has a stable bonded phase [9]. Phenomenex Hypersil 5µC18 BDS, 250x4.6mm column was chosen for the project.

3.8.2 Detector Selection

From the information gathered on the structures of the analytes and the surrogate reference standards that they possess chromophores and therefore absorb UV/Vis light, a UV/Vis detector was chosen because of its high sensitivity, reproducibility and its ability to operate at fixed, multiple or variable wavelengths.

3.8.3 Mobile phase selection

After trial of various mobile phase solutions, Phosphate buffer (Potassium Dihydrogen Phosphate) was selected for the analysis of Chlorpheniramine Maleate Tablets and Acetate buffer (Sodium Acetate) for the analysis of Metformin Hydrochloride Tablets.

This is because of the effective control of the pH of the mobile phase, and hence gave good resolutions of peaks.

Control of the pH of the mobile phase is paramount because all the compounds used in this project have ionizable functional groups which are strongly influenced by the pH of the mobile phase. Phosphate buffer has pKa values of 2.1, 7.2 and 12.3 and Acetate buffer a pKa value of 4.8. The effective working pH ranges for phosphate buffer are 0.6 – 3.60; 5.70 – 8.7 and 10.8 – 13.8 whiles that of Acetate buffer is 3.8 – 5.8 [39]. At pH < 2, the SiO bonds are subjected to acidic hydrolytic cleavage, causing the loss of the bonded phase. At pH > 8, the silica structure is prone to dissolution [8]. In order to avoid these mishaps, pH below 2 and above 8 was avoided. Therefore the pH range chosen for the Phosphate buffer was 6.35 – 6.39 and that of the mobile phase combination was 7.44 – 7.48 whiles that chosen for Acetate buffer was 5.12 – 5.16 and the mobile phase combination was 5.44 – 5.48. The mobile phase combination used was Methanol and Phosphate buffer in a ratio of 50:50 for the Chlorpheniramine Maleate Tablets and Methanol and Acetate Buffer in a ratio of 30:70 for the Metformin Hydrochloride Tablets.

3.8.4 Operating parameters

In order to reduce the retention time, analyses time as well as the back pressure, a flow rate of 1ml/min was chosen.

3.8.5 Wavelength selection

A single wavelength value of 266nm was chosen for Chlorpheniramine Maleate tablet and its surrogate reference standards (Ascorbic Acid, Caffeine and Piroxicam) and a

different single wavelength value of 245nm for Metformin Hydrochloride tablet and its surrogate reference standards (Paracetamol and Metronidazole). This is a compromise that was stricken after careful examination of the wavelength of maximum absorption of both the analytes and their surrogates so that at these wavelengths, all the compounds will exhibit reasonable UV absorption and thus be detected by the UV-Visible detector.

3.8.6 Preparation of Mobile phase

For Chlorpheniramine Maleate tablet with its surrogate reference standards, phosphate buffer (0.025M, pH ± 0.02) and methanol with ratios of 50 and 50 respectively was found to give good resolutions of peaks with a temperature reading of 29.7^{o}C. The Phosphate buffer (0.025M) was prepared by weighing 2.722g of Potassium Dihydrogen Orthophosphate into a 100ml volumetric flask and dissolved with distilled water. The pH of the solution was adjusted to 6.37 ± 0.02 with 0.2M Sodium Hydroxide before it was topped up to the mark. 50ml of both the buffer and Methanol were measured and they were mixed thoroughly.

For Metformin Hydrochloride with its surrogate reference standards, Acetate buffer (0.025M, pH 5.12 ± 0.02) and Methanol with a ratio of 70:30 respectively was found to be appropriate with a temperature reading of 30.8^{o}C. The Acetate buffer was prepared by weighing and dissolving 1.36g of Sodium Acetate with distilled water in a 100ml volumetric flask, adjusting the pH to 5.12 ± 0.02 with 0.6ml of Acetic acid and topping it up to the mark.

3.8.7 Summary of chromatographic conditions

3.8.7.1 Chlorpheniramine Maleate and its surrogate reference standards

Stationary phase: ODS C 18 Phenomenex 250 x 4.6mm column

Mobile phase: Methanol : Phosphate buffer in a ratio of 1:1

Flow rate: 1ml/min

Detector : UV-visible detector

Wavelength: 266nm

pH of buffer: 6.37 ± 0.02

pH of mobile phase: 7.46 ± 0.02

Sensitivity: 0.050

Injector Volume: 20µl

3.8.7.2 Metformin Hydrochloride and its surrogate reference standards

Stationary phase: ODS C 18 Phenomenex 250 x 4.6mm column

Mobile phase: Methanol : Acetate buffer in a ratio of 3:7

Flow rate: 1ml/min

Detector : UV-visible detector

Wavelength: 245nm

pH of buffer: 5.14 ± 0.02

pH of mobile phase: 5.46 ± 0.02

Sensitivity: 0.050

Injector Volume: 20µl

3.9 Analytical Performance Parameters

3.9.1 Limit of Detection (LOD) and Limit of Quantification (LOQ) of the surrogate reference standards; Piroxicam, Ascorbic Acid, Caffeine, Metronidazole and Paracetamol

A stock solution of 0.02% w/v of all the surrogate reference standards were prepared and serially diluted to 5 different concentrations. Twenty micro-litres (20µl) of the resultant solutions were injected into the column. The peak areas were then read. The Limit of Detection (LOD) and the Limit of Quantification (LOQ) were determined using the following formulae;

$$LOD = \frac{3.3 \times \sigma}{S};$$

$$LOQ = \frac{10 \times \sigma}{S}$$

Where;

σ = residual standard deviation and;

S = Slope of the calibration curve drawn

3.10 Validation Parameters

3.10.1 Linearity

Linearity should be evaluated by visual inspection of a plot of signals as a function of the analyte concentration or content. If there is a linear relationship, test should be evaluated by appropriate statistical methods for example by calculation of a regression line [37].

A stock solution of 0.02% w/v of all the pure samples were prepared and serially diluted to four different concentrations. Twenty micro-litres (20µl) of the resultant solutions were injected into the column. The peak areas were read and plotted against their respective concentrations.

3.10.2 Specificity and Selectivity

The Specificity and Selectivity describe the capacity of the analytical method to measure the drug in the presence of impurities or excipients [2, 37]. A quantity of 0.02%w/v of the pure sample of Chlorpheniramine Maleate and Metformin Hydrochloride was prepared and 20µl is injected three times and their retention time noted. Chlorpheniramine Maleate tablets and Metformin Hydrochloride tablets powder of 0.02%w/v is also prepared and 20µl is injected and its retention time was noted. The resolutions of the chromatograms of the pure Chlorpheniramine Maleate and the Chlorpheniramine Maleate tablet were compared and that of the pure Metformin Hydrochloride and the Metformin Hydrochloride tablet were also compared.

3.10.3 Repeatability (Precision)

3.10.3.1 Intra-day Variation

The process involved in the new method was repeated three times on three different occasions in a day. Fresh mobile phases and diluents were prepared, and the analytes as well as the surrogate reference standards were reweighed in accordance with earlier measurements, with the chromatographic conditions maintained throughout. The results were subjected to T-test to evaluate the precision.

3.10.3.2 Inter-day Variation

The processes involved in the new method were repeated every two days on three occasions with five concentrations. As a result, fresh mobile phases and diluents were prepared, and the analytes as well as the surrogate reference standards were reweighed in accordance with earlier measurements, with the chromatographic conditions maintained throughout. The results were subjected to T-test to evaluate the precision.

3.10.4 Sensitivity

This is a measurement of the lowest concentration of analyte that the system can measure. [2]. Successive low concentrations of the surrogate reference standards as well as the analytes were prepared and injected. Calibration curves of all the surrogate reference standards as well as the analytes were plotted and their respective slopes were determined.

3.10.5 Robustness

In the determination of the robustness of the method developed, a different column with the same length was used, while all the other chromatographic conditions were maintained [37]. This was done to investigate the performance of the method when small, deliberate changes were made to the already-established chromatographic conditions. The results obtained for this second column was compared to that obtained for the first column that was originally used, by subjecting the results obtained to t-Test.

3.11 Determination of the constant K

The constant K relates the concentration and the area of the analyte to the concentration and area of the standard. In this determination, initial concentrations of 0.016%w/v of the analyte Chlorpheniramine Maleate and 0.0098%w/v of Caffeine (surrogate), 0.015%w/v of Chlorpheniramine Maleate and 0.02%w/v of Piroxicam (surrogate) and 0.015%w/v of Chlorpheniramine Maleate and 0.00225%w/v of Ascorbic Acid (surrogate) were prepared. Also 0.024%w/v of the analyte Metformin Hydrochloride and 0.024%w/v of Metronidazole (surrogate) and 0.024%w/v of Metformin Hydrochloride and 0.024%w/v of Paracetamol (surrogate) were prepared. Three milliliters each of these initial concentrations of Chlorpheniramine Maleate and its surrogate reference standards were then mixed together individually and the same was done for Metformin Hydrochloride and its surrogate reference standards. Four serial dilutions of this initial 6ml mixture of the analytes and their respective surrogate reference standards were prepared and 20µl was injected. The chromatograms were recorded and the peak areas were read accordingly. The constant K for each analyte with its surrogate reference standard was then determined.

3.12 Analysis of Commercial Samples using the Surrogate Reference Standards

3.12.1 Chlorpheniramine Maleate

Twenty tablets of each of the four different brands of Chlorpheniramine Maleate tablet were powdered. A quantity of the powdered Chlorpheniramine Maleate tablets equivalent to 4.0mg of pure Chlorpheniramine Maleate was dissolved in 25ml of distilled water, to give a concentration of 0.016%w/v. The resulting solution was filtered using a micro filter. Subsequently, 0.0098%w/v of Caffeine, 0.02%w/v of Piroxicam and 0.00225%w/v of Ascorbic Acid were also prepared. These are the surrogate reference standards of Chlorpheniramine Maleate. Three milliliters each of these initial concentrations of Chlorpheniramine Maleate and its surrogate reference standards were then mixed together individually. 20µl of the resulting solution was injected and the corresponding peak area was read. Four serial dilutions of this initial 6ml mixture of Chlorpheniramine Maleate and its surrogate reference standards were prepared and 20µl was injected and the corresponding peak area was read.

Table 3.2 Weight taken, equivalent weight of Chlorpheniramine Maleate and final concentration

Manufacturer	**Weight of Powdered tablet (g)**	**Equivalent weight of Chlorpheniramine Maleate (mg)**	**Final Concentration (%w/v)**
Kinapharma Ltd	0.1646 (0.1646)	4.0	0.016
Pharmanova Ltd	0.1133 (0.1133)	4.0	0.016
Letap Pharmaceuticals	0.1269 (0.1269)	4.0	0.016
Amponsah Effah Pharmaceuticals	0.1009 (0.1009)	4.0	0.016

Average weight of each tablet in bracket

3.12.2 Metformin Hydrochloride

Twenty tablets of each of the four different brands of Metformin Hydrochloride tablet were powdered. A quantity of the powdered Metformin Hydrochloride tablets equivalent to 0.0960g pure Metformin Hydrochloride was dissolved in 25ml of distilled water to give a concentration of 0.024%w/v. The resulting solution was filtered using a micro filter. Subsequently, 0.024%w/v of Metronidazole and 0.024%w/v of Paracetamol were also prepared. These are the surrogate reference standards of Metformin Hydrochloride. Three milliliters each of these initial concentrations of Metformin Hydrochloride and its surrogate reference standards were then mixed together individually. 20µl of the resulting solution was injected and the corresponding peak area was read. Four serial dilutions of this initial 6ml mixture of Metformin Hydrochloride and its surrogate reference standards were prepared and 20µl was injected and the corresponding peak area was read.

Table 3.3 Weight taken, equivalent weight of Metformin Hydrochloride and final concentration

Manufacturer	**Weight of Powdered tablet (g)**	**Equivalent weight of Metformin Hydrochloride (g)**	**Final Concentration (%w/v)**
Hovid	0.1079 (0.5619)	0.096	0.024
Denk	0.1267 (0.6599)	0.096	0.024
Pharma DOR	0.1130 (0.5888)	0.096	0.024
Ernest Chemist	0.1204 (0.6271)	0.096	0.024

Average weight of each tablet in bracket

3.13 Validation Measures

3.13.1 Accuracy and Precision

The results obtained from the chromatograms were subjected to the following statistical tools to investigate their accuracy and precision: F-test and t-Test.

3.14 General procedure for the use of surrogate reference standard in quantitative High Performance Liquid Chromatography

- Performance of preliminary tests of the pure forms of analytes and surrogate reference standards. These include identification tests, ultra-violet spectroscopy test to determine the wavelength of maximum absorbance, thin layer chromatography, melting point determination, pH determination and assay of the commercial samples to ascertain the level of purity.
- Performance of weight uniformity test and determination of the percentage content of the various brands of the analyte using the standard method in any of the pharmacopoeias.
- Determination of suitable conditions for the HPLC analysis based on the results of the preliminary tests performed. These include chromatographic mode, column selection, detector selection, suitable mobile phase, flow rate and wavelength selection.
- Limit of detection and limit of quantification is carried out on all the surrogate reference standards. These are termed as the analytical performance parameters.

- Validation parameters such as linearity, specificity and selectivity, precision, sensitivity and robustness are then carried out.
- In the determination of the constant K, appropriate concentrations but equal volumes of the analyte and the surrogate are mixed together and injected. Serial dilutions of the initial mixture based on the result obtained from the limit of detection test carried out previously are done and also injected and the areas of the peaks are recorded. The results obtained are used to calculate the constant K by the formular;

$$K = \frac{A_{analyte} \times C_{standard}}{C_{analyte} \times A_{standard}}$$

Where;

$A_{analyte}$ = peak area of the analyte

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

$C_{analyte}$ = concentration of the analyte

$C_{standard}$ = concentration of the standard

- On the analysis of the commercial samples, twenty tablets or capsules each (depending on the type of formulation) of the various brands of the analytes are powdered. A quantity of the powdered formulation equivalent to an amount which when dissolved will give a concentration equal to that of the previous concentration of the pure analyte is taken and prepared.

- This preparation of the analyte is then mixed with its appropriate surrogate reference standard and injected and the peak area recorded.

  Once K, $A_{analyte}$ and $C_{standard}$ are known for a particular system, $C_{analyte}$ can be calculated.

  Hence percentage content = $\frac{\text{Actual concentration}}{\text{Nominal concentration}} \times 100\ \%$

- Statistical parameters such as T-test and F-test are applied to the results obtained from the percentage contents to evaluate the accuracy of the new method as compared to the standard method.

CHAPTER FOUR

4.0 Results and Calculations

4.1 Identification Tests

4.1.1 Colour Test

Table 4.0 Colour Test Results

Sample	Result	Inference
Chlorpheniramine Maleate	A red-orange precipitate is formed.	Positive
Caffeine	The yellowish-red residue obtained after heating to dryness in a water bath changed to violet-red upon addition of 0.1ml of ammonia.	Positive
Ascorbic acid	A grey precipitate is formed	Positive
Paracetamol	A violet color develops which does not change to red.	Positive

4.1.2 Ultra-Violet Spectroscopy test

4.1.2.1 Metronidazole

Examined between 230 nm and 350 nm, the solution shows an absorption maximum at 277 nm and a minimum at 240 nm. The specific absorbance at the maximum is 365 to 395. This implies that the substance is Metronidazole.

4.1.3 Thin layer chromatography

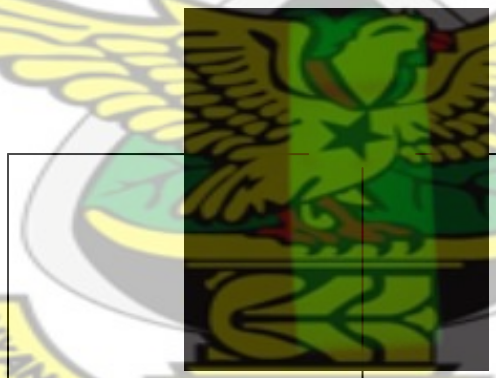
Rf value = (Distance the substance travels from the origin)
(Distance the solvent travels from the origin)

The Rf value for the pure sample was 0.78 when paired with brands from Amponsah Effah, Pharmanova Ltd, Letap Pharmaceuticals and Kinapharma Ltd.

Table 4.1 Rf values for the brands of Chlorpheniramine Maleate

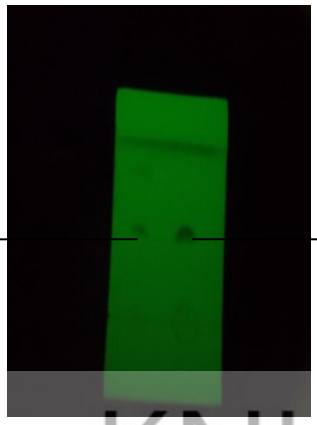
Brand	Distance of substance from origin (cm)	Distance of solvent from origin (cm)	Rf value
Amponsah Effah	7.8	10.0	0.78
Pharmanova	7.8	10.0	0.78
Letap	7.8	10.0	0.78
Kinapharma	7.8	10.0	0.78

4.1.3.1 Chlorpheniramine Maleate



Amponsah Effah Pharmanova Pure Chlorpheniramine Maleate

Fig. 4.0 Thin Layer Chromatogram of pure Chlorpheniramine Maleate and Chlorpheniramine Maleate tablets manufactured by Amponsah Effah Pharmaceuticals and Pharmanova Limited.



Kinapharma Pure Chlorpheniramine Maleate

Fig. 4.1 Thin Layer Chromatogram of pure Chlorpheniramine Maleate and Chlorpheniramine Maleate tablets manufactured by Kinapharma Limited.

Letap Pharmaceuticals pure Chlorpheniramine Maleate

Fig. 4.2 Thin Layer Chromatogram of pure Chlorpheniramine Maleate and Chlorpheniramine Maleate tablets manufactured by Letap Pharmaceuticals.

4.1.3.2 Metformin Hydrochloride

The Rf value for the pure sample was 0.68 when paired with brand from Hovid, Denk, Pharma DOR and Ernest Chemist.

Table 4.2 Rf values for the brands of Metformin Hydrochloride

Brand	Distance of substance from origin (cm)	Distance of solvent from origin (cm)	Rf value
Hovid	6.8	10.0	0.68
Denk	6.8	10.0	0.68
Pharma DOR	6.8	10.0	0.68
Ernest Chemist	6.8	10.0	0.68

Fig. 4.3 Thin Layer Chromatogram of pure Metformin Hydrochloride and Metformin Hydrochloride tablets manufactured by Hovid.

Fig. 4.4 Thin Layer Chromatogram of pure Metformin Hydrochloride and Metformin Hydrochloride tablets manufactured by Denk.

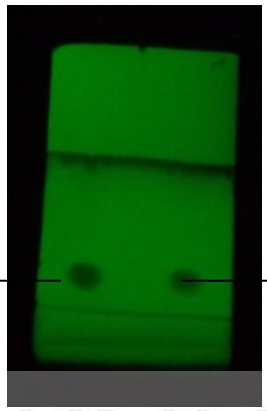


Fig. 4.5 Thin Layer Chromatogram of pure Metformin Hydrochloride and Metformin Hydrochloride tablets manufactured by Pharma DOR.

Fig. 4.6 Thin Layer Chromatogram of pure Metformin Hydrochloride and Metformin Hydrochloride tablets produced by Ernest Chemist.

4.1.4 Melting point determination

Table 4.3 British Pharmacopoeia and experimental melting range of pure samples.

Drug	British Pharmacopoeia value (ºC)	Experimental Values (ºC)
Chlorpheniramine Maleate	130 - 135	132 - 135
Caffeine	234 - 239	236 - 239
Piroxicam	240 - 245	241 - 244
Ascorbic acid	190 - 192	190 - 191
Metformin Hydrochloride	222 - 226	223 - 225
Metronidazole	159 - 163	160 - 163
Paracetamol	168 - 172	168 - 170

4.1.5 Determination of pH of pure samples

Table 4.4 British Pharmacopoeia and experimental pH range of samples.

Analyte	British Pharmacopoeia pH range	Mean experimental pH
Chlorpheniramine Maleate	4.0 – 5.5	4.10 ± 0.02
Caffeine	6.0 – 7.5	6.11 ± 0.02
Ascorbic acid	2.0 – 3.0	2.13 ± 0.03
Piroxicam	4.5 – 7.0	4.96 ± 0.20
Metformin Hydrochloride	6.7 – 7.0	6.80 ± 0.10
Metronidazole	6.5 – 6.9	6.64 ± 0.02
Paracetamol	5.3 – 6.5	5.92 ± 0.10

4.1.6 Determination of wavelength of maximum absorption

Table 4.5 Wavelength of maximum absorption of pure samples

Analyte	Wavelength of maximum absorption (nm)
Chlorpheniramine Maleate	261.00
Caffeine	273.00
Ascorbic acid	264.00
Piroxicam	245.00
Metformin Hydrochloride	236.00
Metronidazole	310.00
Paracetamol	245.00

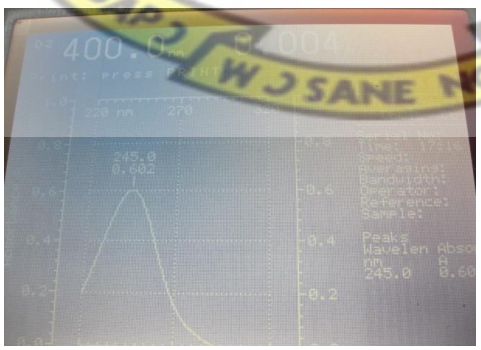


Fig. 4.7 UV Spectrum of Paracetamol

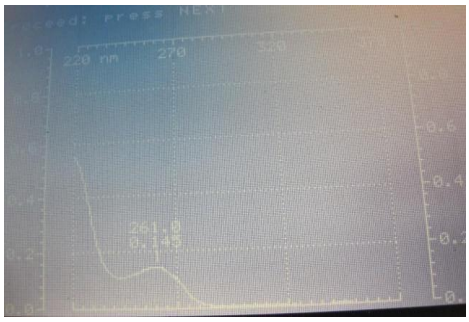


Fig. 4.8 UV Spectrum of Chlorpheniramine Maleate

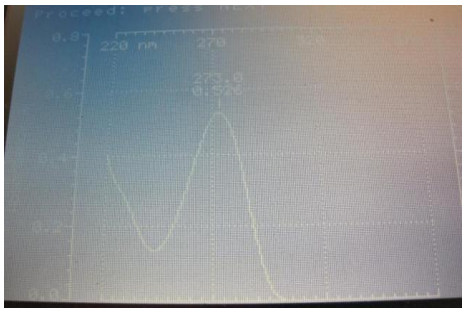


Fig. 4.9 UV Spectrum of Caffeine

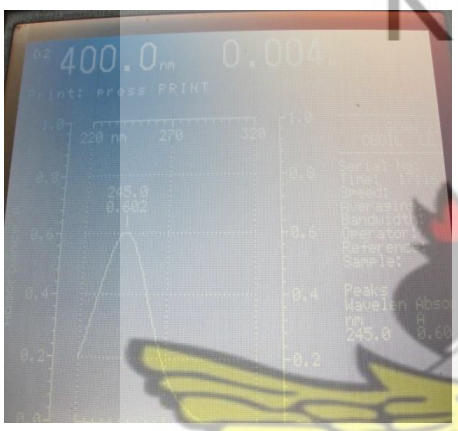


Fig. 4.10 UV Spectrum of Piroxicam

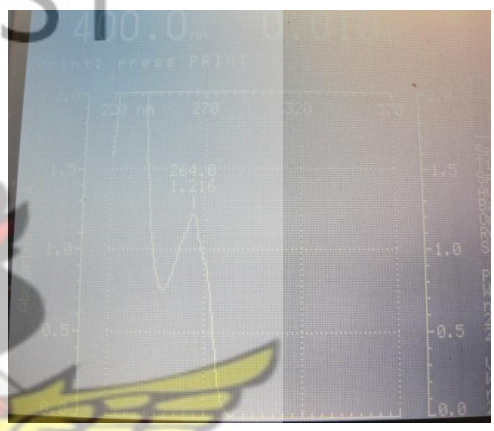


Fig. 4.11 UV Spectrum of Ascorbic acid

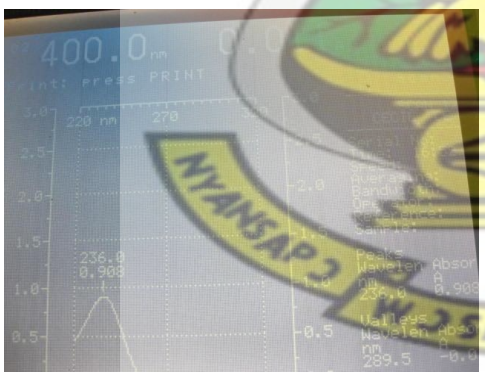


Fig. 4.12 UV Spectrum of Metformin Hydrochloride

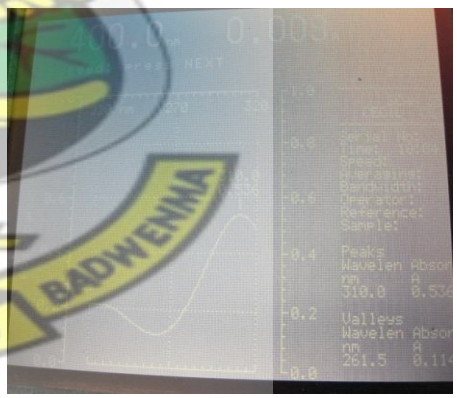


Fig. 4.13 UV Spectrum of Metronidazole

4.2 Assay of pure samples

Refer to appendix II A.P1-A.P7 for sample calculations.

Table 4.6 Average percentage purity of analytes and surrogates (n = 2)

Sample	Average percentage purity (%)
Chlorpheniramine Maleate	98.4 ± 0.02
Metformin Hydrochloride	99.3 ± 0.01
Caffeine	98.5 ± 0.01
Piroxicam	100.1 ± 0.02
Ascorbic acid	99.9 ± 0.05
Metronidazole	99.8 ± 0.03
Paracetamol	99.7 ± 0.03

4.3 Uniformity of weight

Refer to appendix UCK.1-UME.8 for table of uniformity of weight

4.4 Percentage content of analytes using the standard method in the British Pharmacopoeia, 2007.

Refer to appendix IV for sample calculations and UV spectra.

Table 4.7 Table of average percentage content of Chlorpheniramine Maleate in Chlorpheniramine Maleate tablets. (n = 5)

Sample Number	Kinapharma Ghana Limited	Pharmanova Ghana Limited	Letap Pharmaceuticals Ltd	Amponsah Effah Pharmaceuticals Ltd
1	104.5	94.6	100.6	98.6
2	104.5	94.4	100.5	98.4
3	104.4	94.5	100.6	98.5
4	104.6	94.6	100.9	98.6
5	104.3	94.4	100.4	98.1
Average Percentage Content	104.46 ± 0.05	94.50 ± 0.04	100.60 ± 0.08	98.44 ± 0.09

Table 4.8 Table of average percentage content of Metformin Hydrochloride tablet (n = 5)

Sample Number	Hovid	Pharma DOR	Denk	Ernest Chemist
1	104.1	95.9	100.1	99.7
2	105.0	95.8	99.1	101.1
3	104.9	96.8	99.8	99.8
4	104.0	97.0	99.6	98.0
5	104.3	96.7	99.9	100.8
Average Percentage Content	104.5 ± 0.2	96.4 ± 0.2	99.7 ± 0.2	99.7 ± 0.5

4.5 Chromatographic conditions

4.5.1 Chlorpheniramine Maleate

Stationary phase: ODS C 18 Phenomenex 250 x 4.6mm column

Mobile phase: methanol : phosphate buffer in a ratio of 1:1

Flow rate: 1ml/min

Detector : UV-visible detector

Wavelength: 266nm

pH of buffer: 6.35

pH of mobile phase: 7.44

Sensitivity: 0.050

Injector Volume: 20µl

4.5.2 Metformin Hydrochloride

Stationary phase: ODS C18 Phenomenex 250 x 4.6mm column

Mobile phase: Methanol : Acetate buffer in a ratio of 30:70

Flow rate: 1ml/min

Detector : UV-visible detector

Wavelength: 245nm

pH of buffer: 5.12

pH of mobile phase: 5.46

Sensitivity: 0.050

Injector Volume: 20µl

4.6 Chromatograms

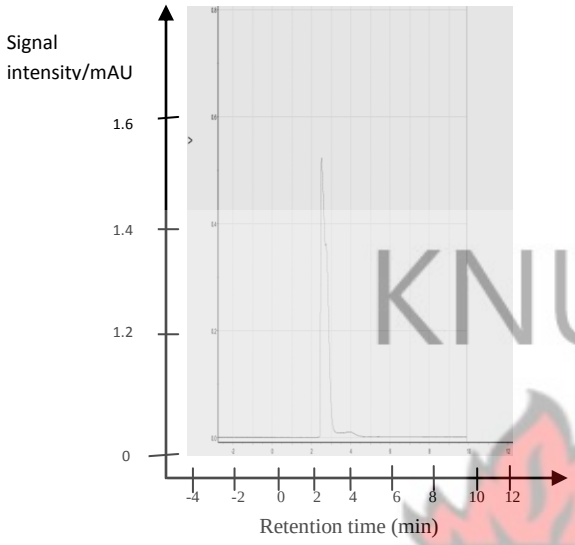


Fig. 4.22 Chromatogram of pure Chlorpheniramine Maleate

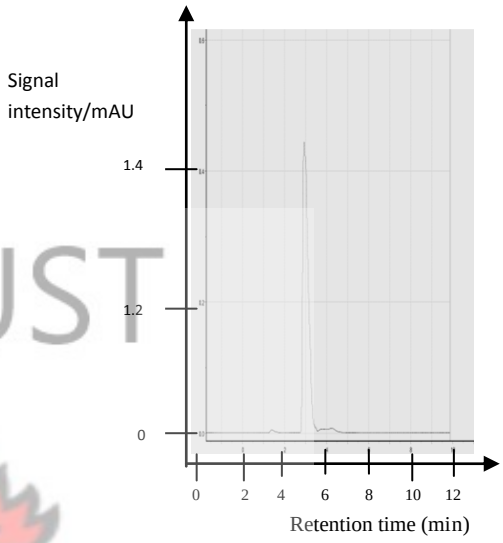


Fig.4.23 Chromatogram of pure Ascorbic acid

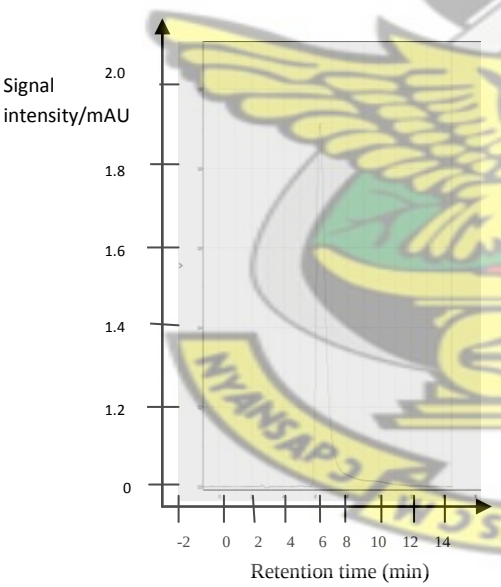


Fig. 4.24 Chromatogram of pure Piroxicam

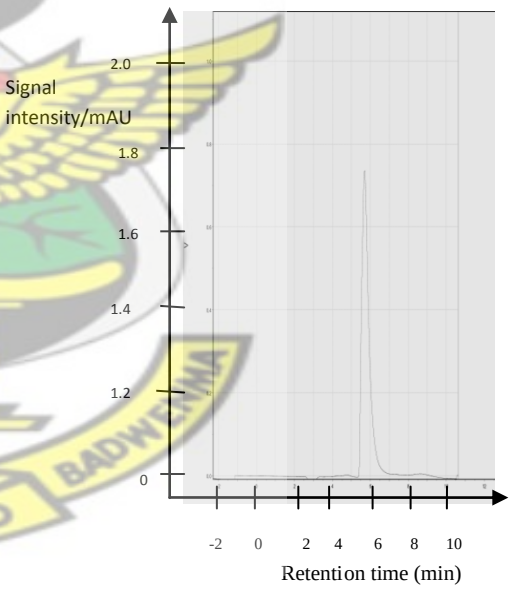


Fig. 4.25 Chromatogram of pure Caffeine

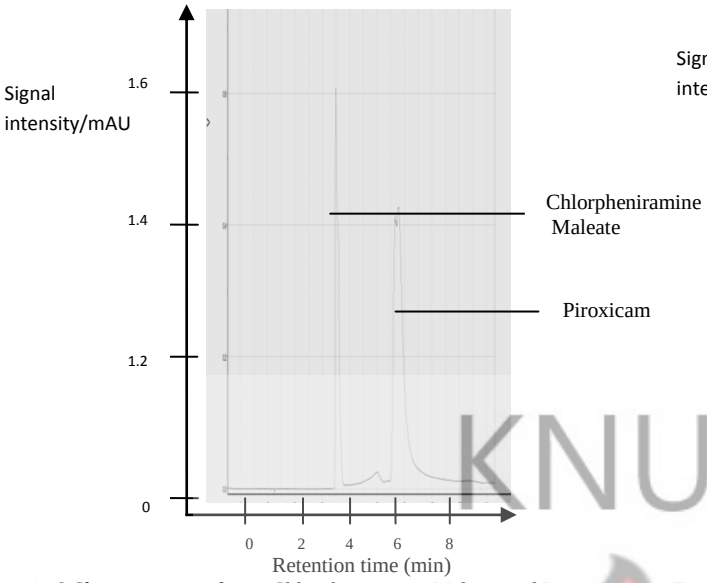


Fig. 4.26 Chromatogram of pure Chlorpheniramine Maleate and Piroxicam

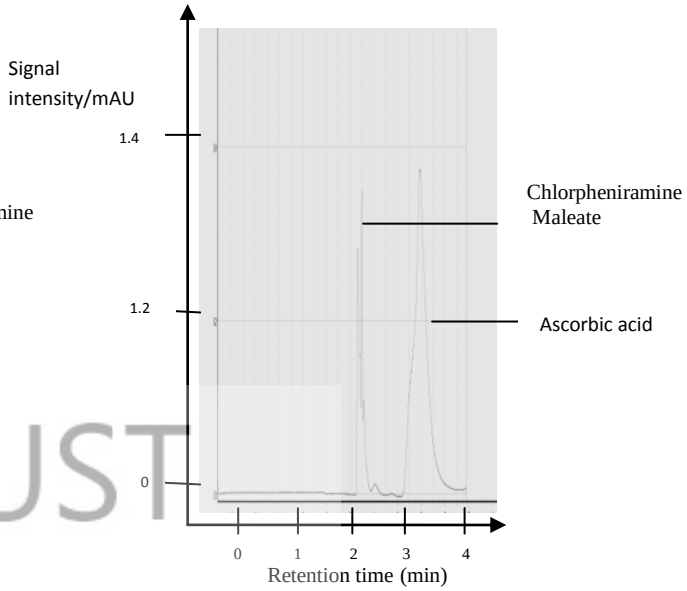


Fig. 4.27 Chromatogram of pure Chlorpheniramine Maleate and Ascorbic acid

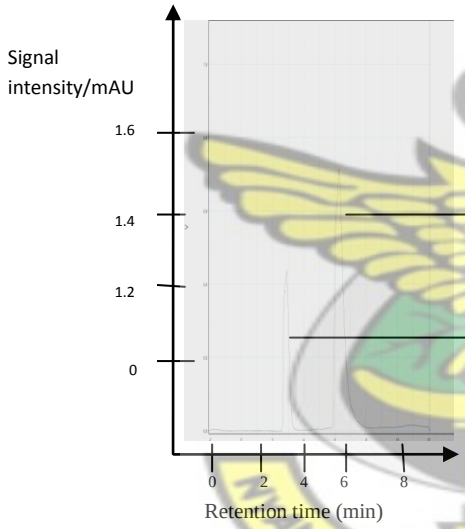


Fig. 4.28 Chromatogram of pure Chlorpheniramine Maleate and Caffeine

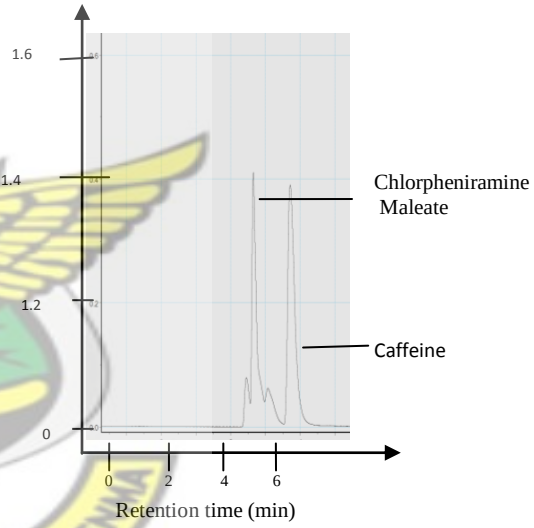


Fig.4.29 Chromatogram of Chlorpheniramine Maleate produced by Letap and Caffeine

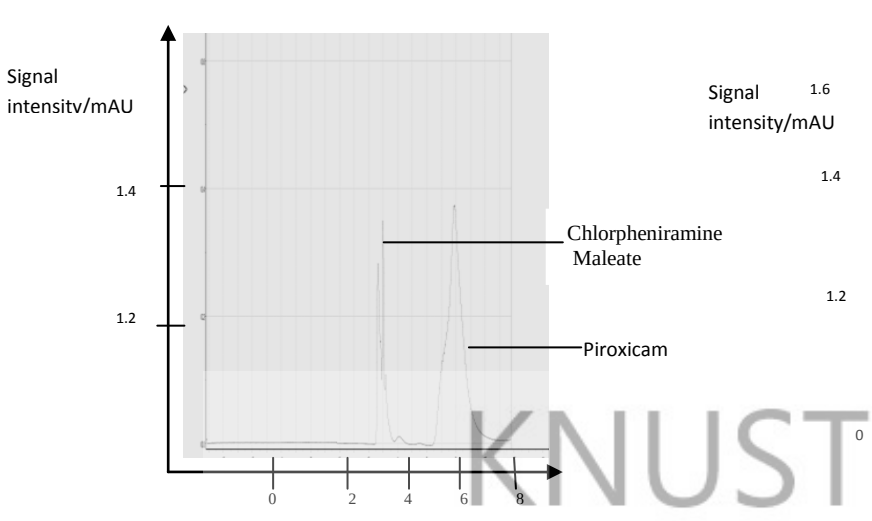


Fig. 4.30 Chromatogram of Chlorpheniramine Maleate produced by Amponsah Effah Pharmaceuticals and Piroxicam

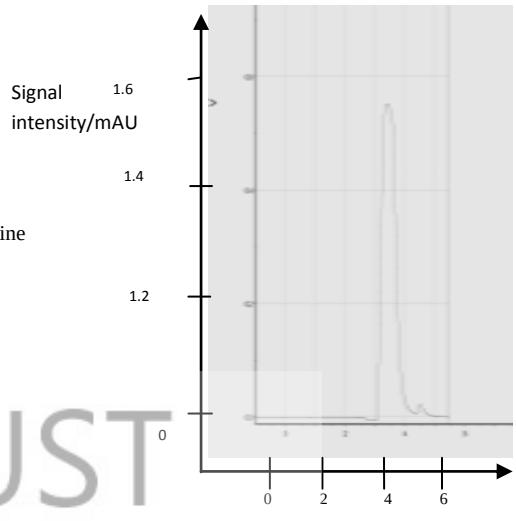


Fig. 4.31 Chromatogram of pure Metformin Hydrochloride

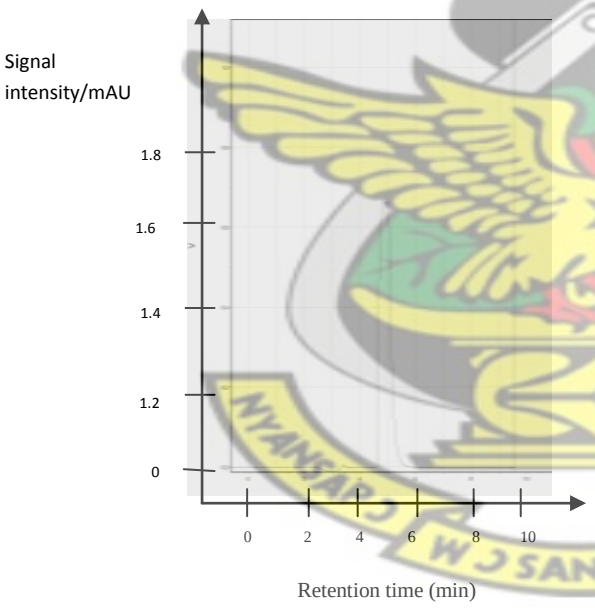


Fig. 4.32 Chromatogram of pure Metronidazole

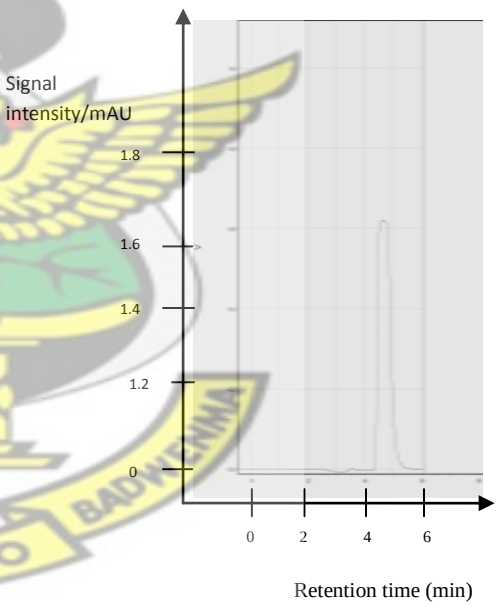


Fig. 4.33 Chromatogram of pure Paracetamol

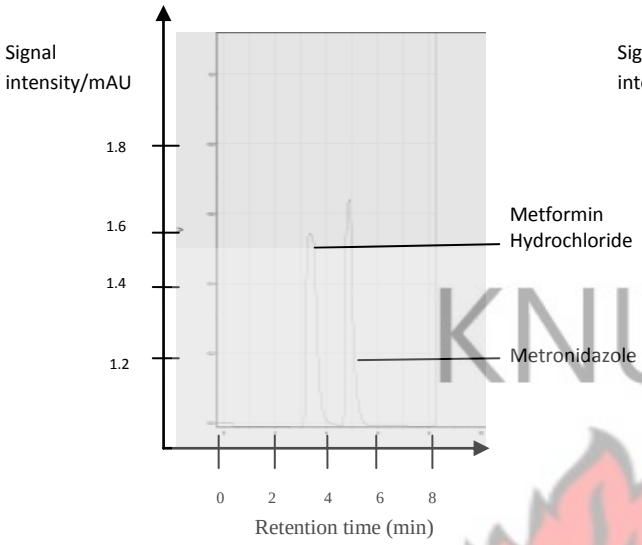


Fig. 4.34 Chromatogram of pure Metformin Hydrochloride and Metronidazole

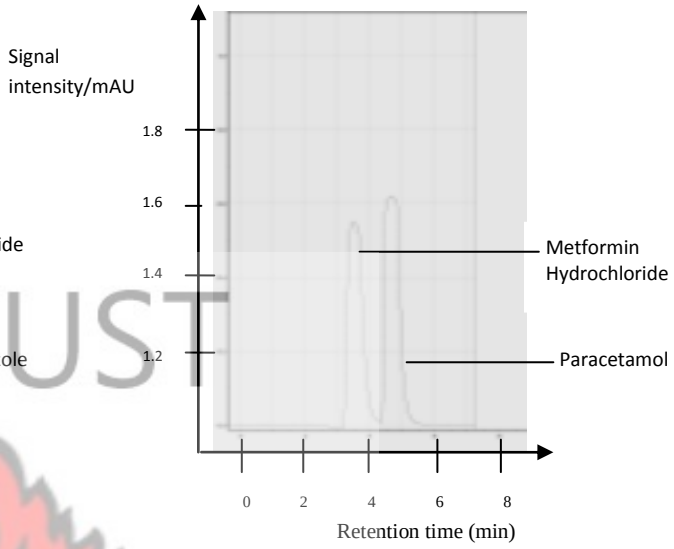


Fig. 4.35 Chromatogram of pure Metformin Hydrochloride and Paracetamol

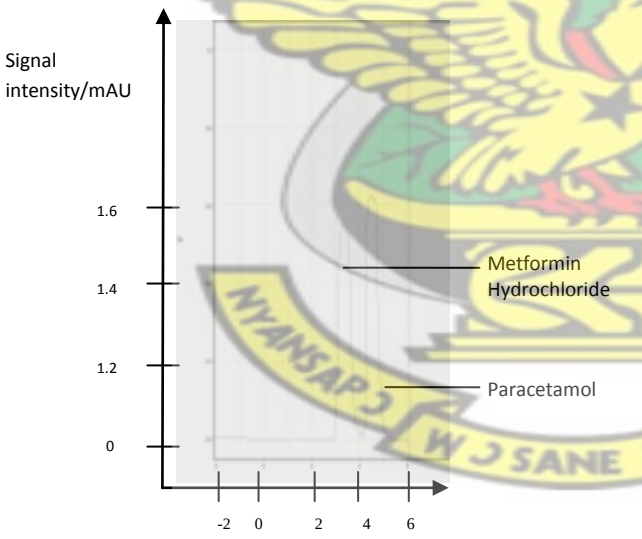


Fig. 4.36 Chromatogram of Metformin Hydrochloride produced by Hovid and Paracetamol

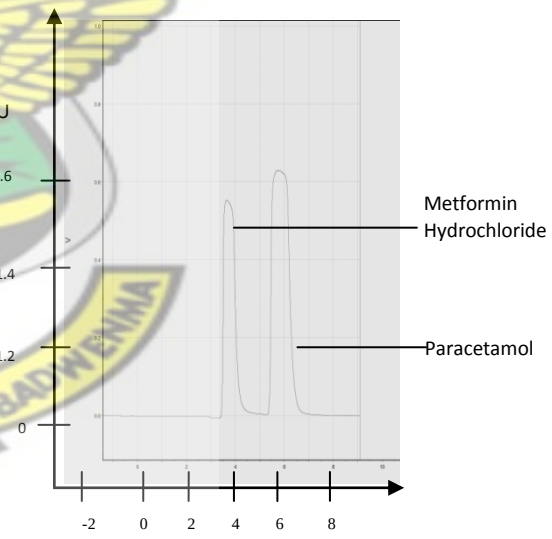


Fig. 4.37 Chromatogram of Metformin Hydrochloride produced by Ernest Chemist and Paracetamol

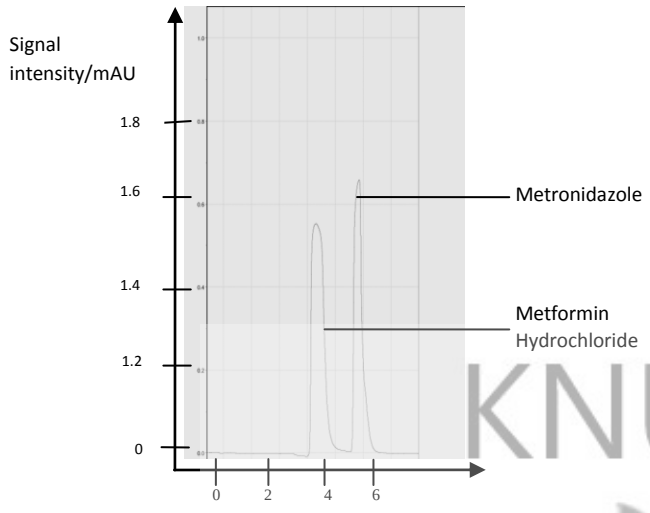


Fig. 4.38 Chromatogram of Metformin Hydrochloride produced by Denk and Metronidazole

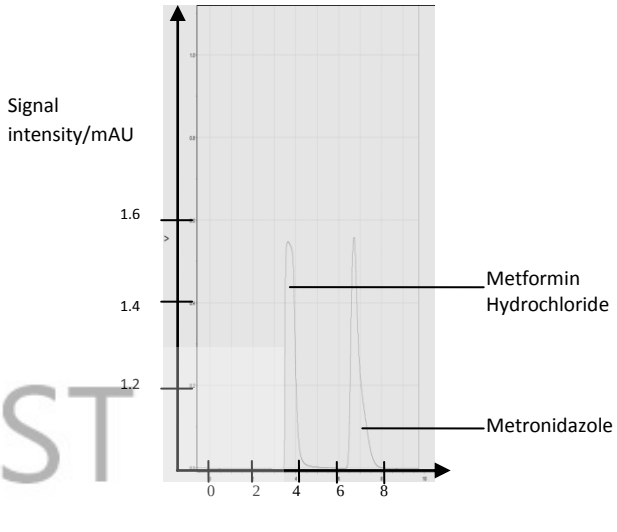


Fig. 4.39 Chromatogram of Metformin Hydrochloride produced by Pharma DOR and Metronidazole

4.7 Retention times

Table 4.9 Mean retention times for pure form of both analytes and surrogates (n = 5)

Samples	Mean retention time (min)
Chlorpheniramine Maleate	2.6 ± 0.09
Metformin Hydrochloride	3.4 ± 0.03
Ascorbic acid	3.2 ± 0.02
Piroxicam	6.5 ± 0.02
Caffeine	5.9 ± 0.02
Metronidazole	5.3 ± 0.20
Paracetamol	4.6 ± 0.02

4.8 Analytical Performance Parameters

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

LOD = $3.3\sigma / S$

LOQ = $10\sigma / S$

Where; σ = residual standard deviation i.e. $\sigma_{res} = \{\Sigma(Y - Y_{est}) / n\text{-}1\}^2$,

Y = y values (Peak Area) from a calibration curve

Y_{est} = y values calculated using the equation of line; $y = mx + c$

n = number of determinations

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

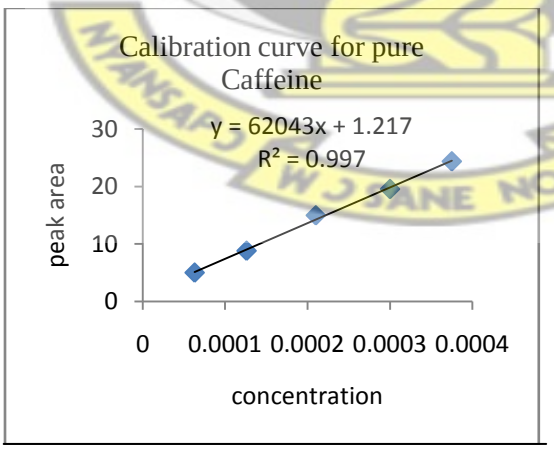


Fig.4.14 Calibration graph for Caffeine

4.8.1.1 Caffeine

Table 4.10 Table of concentration and peak area of Caffeine

Concentration	Peak area (Y)	Y_{est}	$Y - Y_{est}$
0.000375	24.39	24.48333	-0.09333
0.000300	19.56	19.83010	-0.27010
0.000210	14.98	14.24623	0.73377
0.000126	8.80	9.034618	-0.23462
0.000063	4.99	5.125909	-0.13591
			$\Sigma[(Y - Y_{est})] = 1.46773$

The equation of the line, y = 62043x + 1.2172

$$\sigma_{res} = \{\Sigma(Y - Y_{est}) / n-1\}^2$$

from the equation above;

$$\sigma_{res} = \{ 1.46773 / 4\}^2$$

$$= 0.1346$$

Therefore LOD = (3.3 x 0.1346) / 62043

$$= 7.16 \times 10^{-6}$$

LOQ = (10 x 0.1346) / 62043

$$= 2.17 \times 10^{-5}$$

Table 4.11 Results for LOD and LOQ

Sample	Limit of Detection (% w/v)	Limit of Quantification (% w/v)
Chlorpheniramine Maleate	8.77×10^{-5}	2.66×10^{-4}
Ascorbic acid	9.55×10^{-6}	2.90×10^{-5}
Caffeine	7.16×10^{-6}	2.17×10^{-5}
Piroxicam	4.36×10^{-4}	1.32×10^{-3}
Metformin Hydrochloride	3.62×10^{-3}	1.09×10^{-2}
Metronidazole	8.85×10^{-3}	2.68×10^{-2}
Paracetamol	4.02×10^{-3}	1.22×10^{-2}

4.8.2 Linearity

Refer to appendix V and VI for calibration curves and their corresponding correlation coefficients.

4.8.3 Specificity and Selectivity

Below are the representative chromatograms of the pure analytes and the formulations of the various brands.

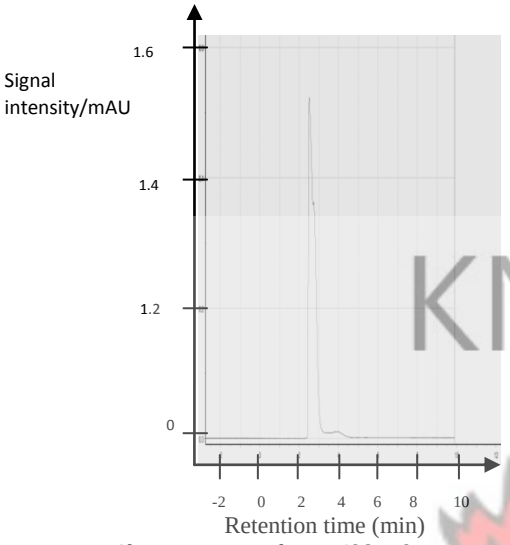


Fig.4.15 Chromatogram of pure Chlorpheniramine Maleate

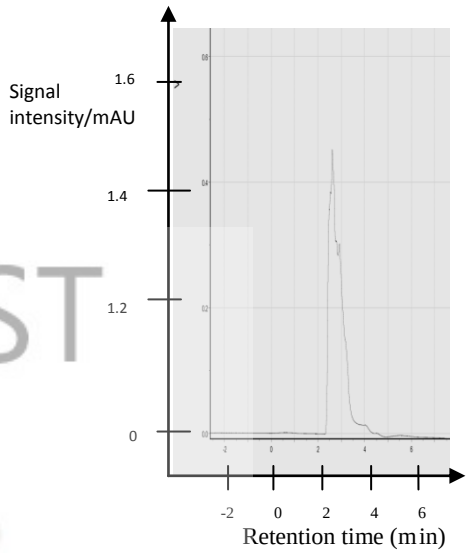


Fig 4.16 Chromatogram of Chlorpheniramine Maleate tablet Produced by Amponsah Effah

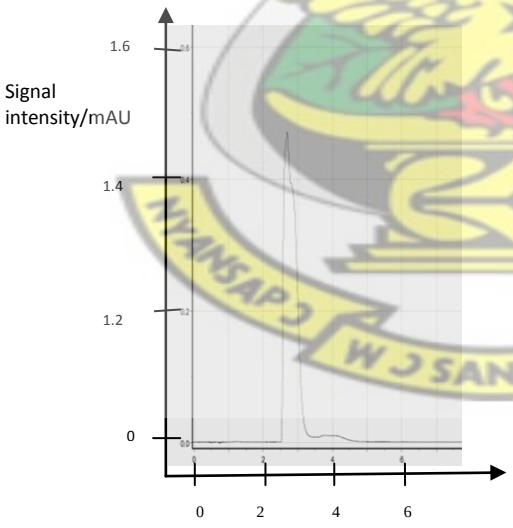


Fig.4.17 Chromatogram of Chlorpheniramine Maleate tablet produced by Kinapharma Ltd

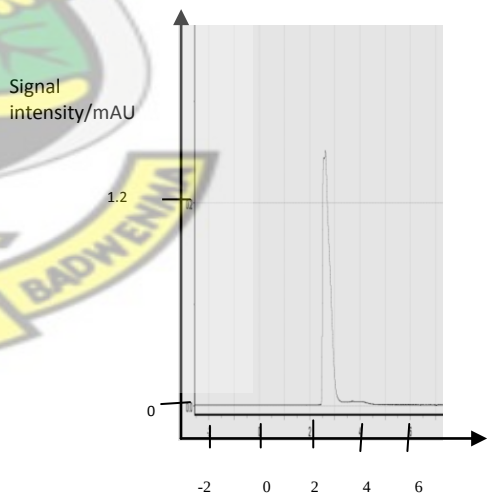
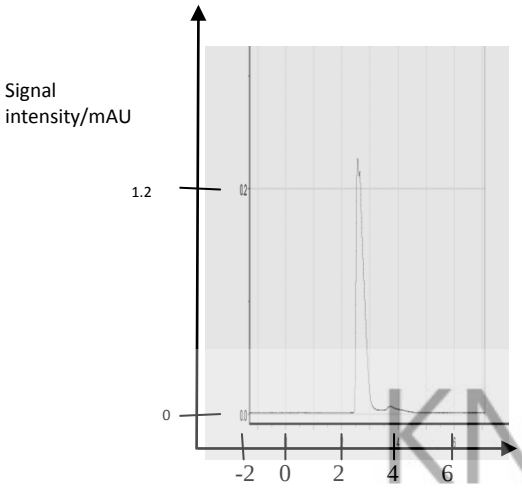
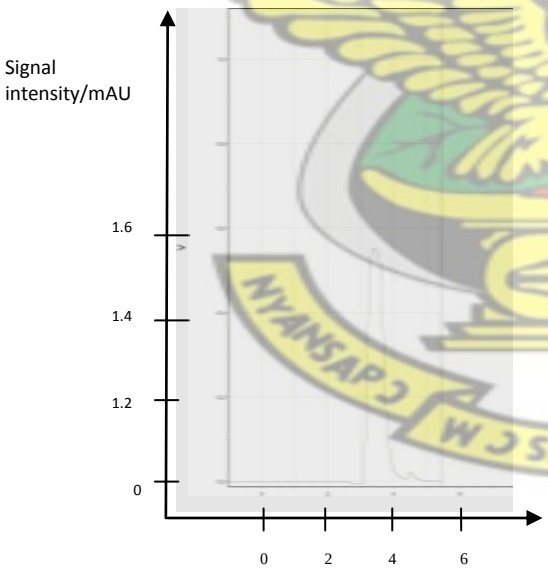


Fig.4.18 Chromatogram of Chlorpheniramine Maleate tablet produced by Pharmanova Ltd



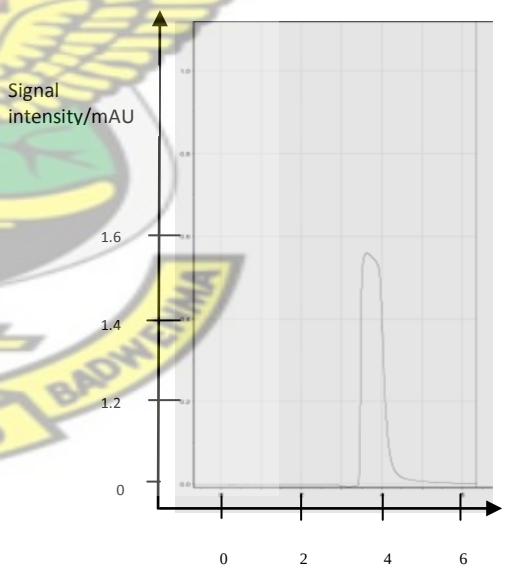
Retention time (min)

Fig.4.19 Chromatogram of Chlorpheniramine Maleate tablet produced by Letap



Retention time (min)

Fig.4.20 Chromatogram of pure Metformin Hydrochloride



Retention time (min)

Fig.4.21Chromatogram of Metformin Hydrochloride tablet produced by Denk Pharma

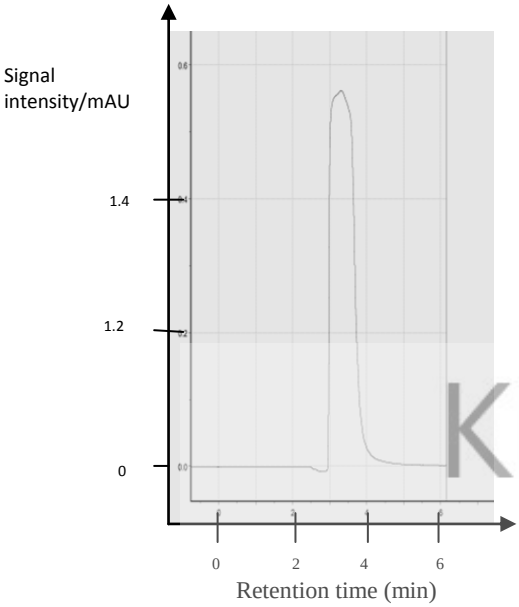


Fig.4.22 Chromatogram of Metformin Hydrochloride tablet produced by Hovid

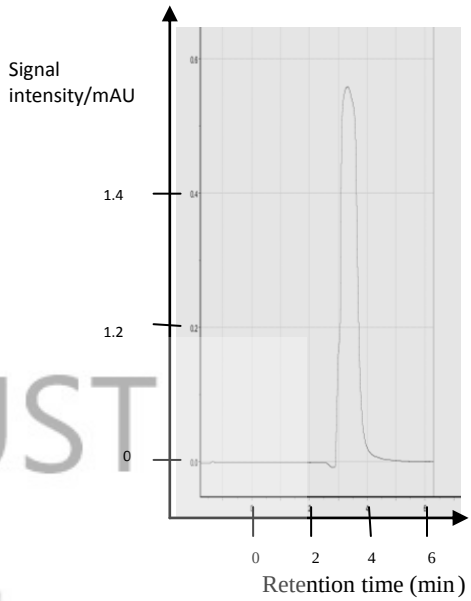


Fig.4.23 Chromatogram of Metformin Hydrochloride tablet produced by Pharma DOR

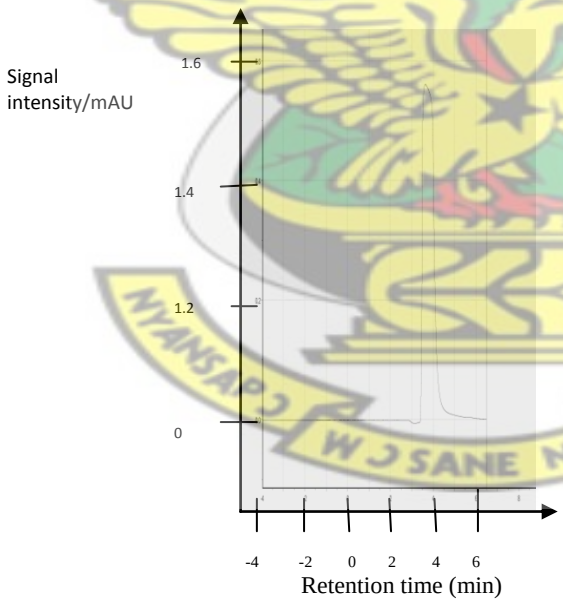


Fig.4.24 Chromatogram of Metformin Hydrochloride tablet produced by Ernest Chemist

4.9 Determination of K values

4.9.1 Determination of the constant K for Chlorpheniramine Maleate

The constant K was calculated using the formular

$$K = \frac{A_{analyte} \times C_{standard}}{C_{analyte} \times A_{standard}}$$

Where;

$A_{analyte}$ = peak area of the analyte

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

$C_{analyte}$ = concentration of the analyte

$C_{standard}$ = concentration of the standard

Table 4.12 Determination of K values for pure Chlorpheniramine Maleate using Caffeine as the surrogate reference standard

Peak Area of Caffeine, As	Concentration of Caffeine, Cs	Peak Area of Chlorpheniramine Maleate, Aa	Concentration of Chlorpheniramine Maleate, Ca	K
26.67	0.009800	9.39	0.01600	0.2225
18.82	0.006860	6.62	0.01120	0.2200
9.54	0.003430	3.30	0.00560	0.2119
5.73	0.002058	2.33	0.00392	0.2135
2.54	0.001029	1.18	0.00196	0.2439
			Average K = 0.2224 ± 0.006	

Table 4.13 Determination of K values for pure Chlorpheniramine Maleate using Piroxicam as the surrogate reference standard.

Peak Area of Piroxicam, As	Concentration of Piroxicam, Cs	Peak Area of Chlorpheniramine Maleate, Aa	Concentration of Chlorpheniramine Maleate, Ca	K
15.11	0.02000	9.19	0.015000	0.8109
10.68	0.01400	6.45	0.010500	0.8075
5.41	0.00700	3.32	0.005250	0.8182
2.02	0.00250	2.09	0.003150	0.8013
0.98	0.00125	1.00	0.001575	0.8098
			Average K = 0.8095 ± 0.003	

Table 4.14 Determination of K values for pure Chlorpheniramine Maleate using Ascorbic acid as the surrogate reference standard.

Peak Area of Ascorbic acid, As	Concentration of Ascorbic acid, Cs	Peak Area of Chlorpheniramine Maleate, Aa	Concentration of Chlorpheniramine Maleate, Ca	K
16.07	0.0022500	16.14	0.01500	0.1507
11.35	0.0015750	11.40	0.010500	0.1506
7.72	0.0011000	5.90	0.005250	0.1601
3.96	0.0005510	4.18	0.003675	0.1583
2.89	0.0003859	3.09	0.002570	0.1605
			Average K = 0.1560 ± 0.002	

4.9.2 Determination of the constant K for Metformin Hydrochloride

Table 4.15 Determination of K values for pure Metformin Hydrochloride using Metronidazole as the surrogate reference standard.

Peak Area of Metronidazole, As	Concentration of Metronidazole, Cs	Peak Area of Metformin Hydrochloride, Aa	Concentration of Metformin Hydrochloride, Ca	K
12.73	0.24000	16.64	0.24000	1.3071
9.10	0.12000	12.78	0.12000	1.4033
4.88	0.04800	6.39	0.04800	1.3094
2.19	0.01920	2.86	0.01920	1.3059
1.02	0.00576	1.33	0.00576	1.3040
			Average K = 1.3262 ± 0.02	

Table 4.16 Determination of K values for pure Metformin Hydrochloride using Paracetamol as the surrogate reference standard.

Peak Area of Paracetamol, As	Concentration of Paracetamol, Cs	Peak Area of Metformin Hydrochloride, Aa	Concentration of Metformin Hydrochloride, Ca	K
22.53	0.24000	19.82	0.24000	0.8797
20.81	0.14000	18.31	0.14000	0.8798
17.64	0.12000	13.81	0.12000	0.7829
12.76	0.04800	10.96	0.04800	0.8589
2.90	0.01920	2.64	0.01920	0.9103
			Average K = 0.8623 ± 0.02	

Table 4.17 K values for Chlorpheniramine Maleate

Determination	Piroxicam	Caffeine	Ascorbic Acid
1	0.8109	0.2225	0.1507
2	0.8075	0.2200	0.1506
3	0.8182	0.2119	0.1601
4	0.8013	0.2135	0.1583
5	0.8098	0.2439	0.1605
Average K	0.8095 ± 0.003	0.2224 ± 0.006	0.1560 ± 0.002

Table 4.18 K values for Metformin Hydrochloride

Determination	Metronidazole	Paracetamol
1	1.3071	0.8797
2	1.4033	0.8798
3	1.3094	0.7829
4	1.3059	0.8589
5	1.3040	0.9103
Average K	1.3262 ± 0.02	0.8623 ± 0.02

4.9.3 Variation of K values with changes in Concentration of analyte

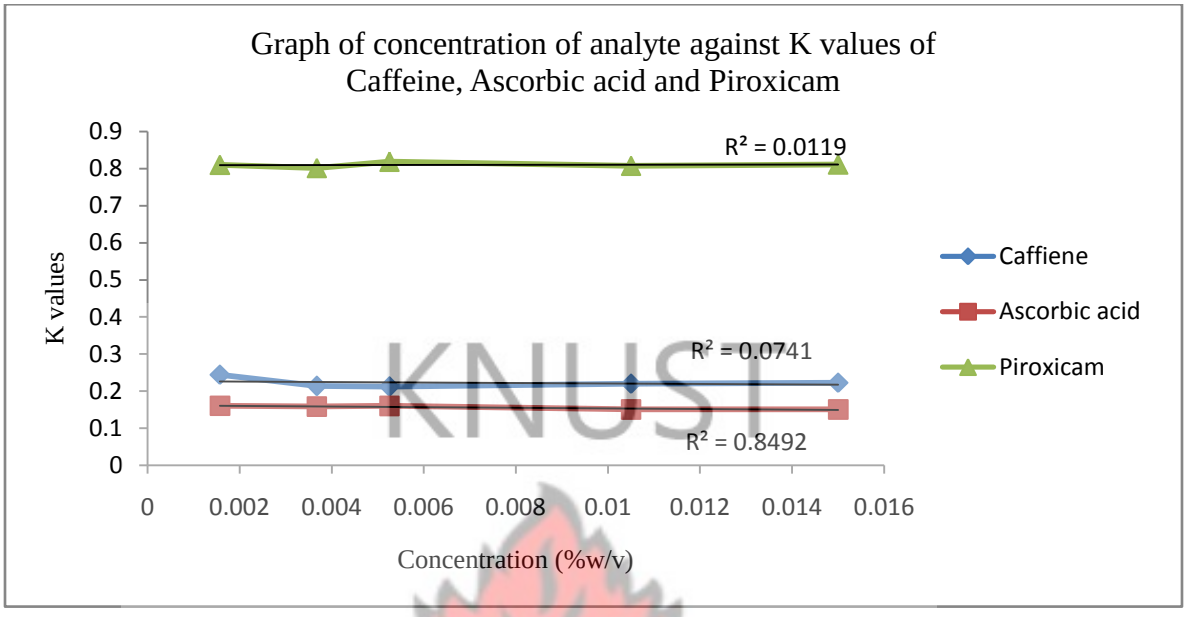


Fig.4.25 Graph of concentration of Chlorpheniramine Maleate (analyte) against K values of Caffeine, Ascorbic acid and Piroxicam

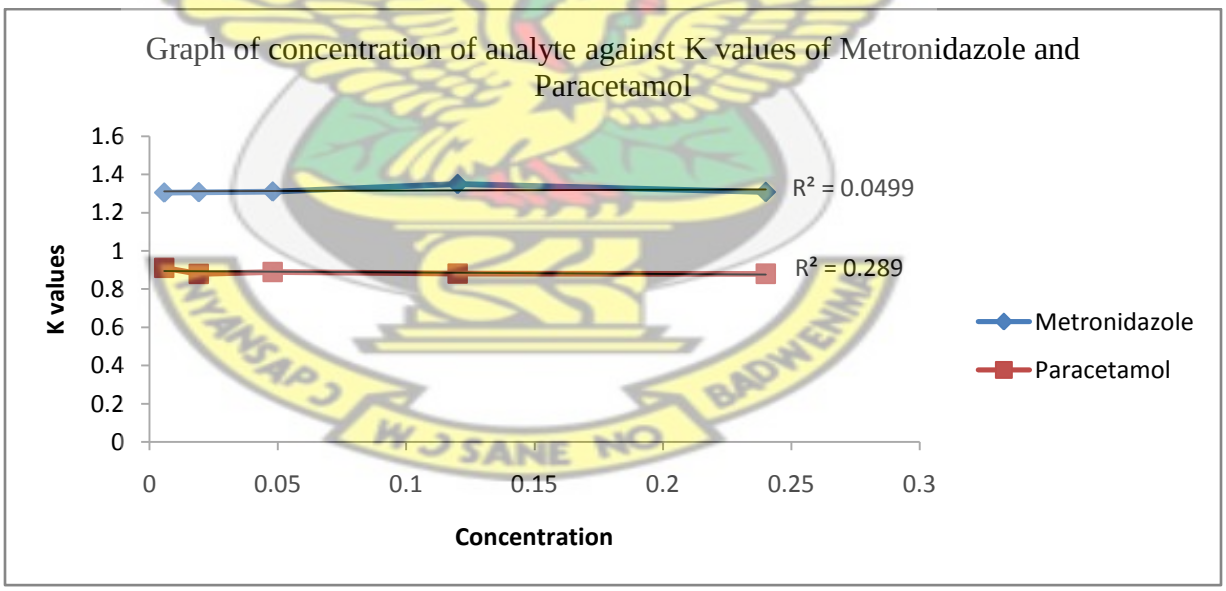


Fig.26 Graph of concentration of Metformin Hydrochloride (analyte) against K values of Metronidazole and Paracetamol

4.10 Repeatability

4.10.1 Intra-day Variation

Table 4.19 Intra-day variation of the percentage content of Chlorpheniramine Maleate in Chlorpheniramine Maleate tablet manufactured by Amponsah Effah Pharmaceuticals using Piroxicam as surrogate reference standard

Percentage content of Chlorpheniramine Maleate Tablet (%)		
Test 1	Test 2	Difference = (Day 1 - Day 2)
97.9	97.0	0.9
98.2	97.9	0.3
98.3	98.0	0.3
98.4	98.3	0.1
97.6	97.5	0.1
		Average X_d = 0.34

Batch number: 50.001

$t_{exp} = (X_d / S_d) \times \sqrt{N}$

where;

X_d = the mean difference between paired values,

S_d = the estimated standard deviation of the differences and

N = number of values within the sets. [38]

X_d = 0.34

S_d = 0.33

N = 5

$t_{exp} = (X_d / S_d) \times \sqrt{N}$

$= (0.34 / 0.33) \times \sqrt{5}$

$= 2.30$

Critical value of t (t_{stat}) at P = 0.05 (95%) level = 2.78 [38]

Table 4.20 Intra-day variation of the percentage content of Metformin Hydrochloride in Metformin Hydrochloride tablets manufactured by Hovid using Paracetamol as surrogate reference standard

Percentage content of Metformin Hydrochloride (%)		
Day 1	Day 2	Difference = (Day 1 - Day 2)
104.0	103.5	0.5
104.8	104.0	0.8
104.3	104.9	-0.6
103.3	103.3	0
102.3	101.7	0.6
		Average X_d = 0.26

Batch number: BA050407

$t_{exp} = (X_d / S_d) \times \sqrt{N}$

$X_d = 0.26$

$S_d = 0.56$

$N = 5$

$t_{exp} = (X_d / S_d) \times \sqrt{N}$

$= (0.26 / 0.56) \times \sqrt{5}$

$= 1.04$

4.10.2 Inter-day Variation

Table 4.21 Inter-day variation of the percentage content of Chlorpheniramine Maleate in Chlorpheniramine Maleate tablet manufactured by Amponsah Effah Pharmaceuticals using Piroxicam as surrogate reference standard

Percentage content of Chlorpheniramine Maleate Tablet (%)		
Day 1	Day 2	Difference = (Day 1 - Day 2)
97.9	97.5	0.4
98.2	98.0	0.2
98.3	98.2	0.1
98.4	98.7	-0.3
97.6	97.1	0.5
		Average X_d = 0.18

Batch number: 50.001

$t_{exp} = (X_d / S_d) \times \sqrt{N}$

where;

X_d = the mean difference between paired values,

S_d = the estimated standard deviation of the differences and

N = number of values within the sets. [38]

$X_d = 0.18$

$S_d = 0.31$

N = 5

$t_{exp} = (X_d / S_d) \times \sqrt{N}$

$= (0.18 / 0.31) \times \sqrt{5}$

$= 1.29$

Table 4.22 Inter-day variation of the percentage content of Metformin Hydrochloride in Metformin Hydrochloride tablets manufactured by Hovid using Paracetamol as surrogate reference standard

Percentage content of Metformin Hydrochloride (%)		
Day 1	Day 2	Difference = (Day 1 - Day 2)
104.0	104.1	-0.1
104.8	104.3	0.5
104.3	103.2	1.1
103.3	102.3	1
102.3	104.2	-1.9
		Average $X_d = 0.12$

Batch number: BA050407

$t_{exp} = (X_d / S_d) \times \sqrt{N}$

$X_d = 0.12$

$S_d = 1.22$

N = 5

$t_{exp} = (X_d / S_d) \times \sqrt{N}$

$= (0.12 / 1.22) \times \sqrt{5}$

$= 0.22$

4.11 Sensitivity

Refer to appendix V for calibration curves

Table 4.23 Results for LOD and LOQ

Sample	Limit of Detection (% w/v)	Limit of Quantification (% w/v)
Chlorpheniramine Maleate	8.77×10^{-5}	2.66×10^{-4}
Ascorbic acid	9.55×10^{-6}	2.90×10^{-5}
Caffeine	7.16×10^{-6}	2.17×10^{-5}
Piroxicam	4.36×10^{-4}	1.32×10^{-3}
Metformin Hydrochloride	3.62×10^{-3}	1.09×10^{-2}
Metronidazole	8.85×10^{-3}	2.68×10^{-2}
Paracetamol	4.02×10^{-3}	1.22×10^{-2}

4.12 Robustness

Table 4.24 Variation of the percentage content of Chlorpheniramine Maleate in Chlorpheniramine Maleate tablet manufactured by Amponsah Effah Pharmaceuticals using Piroxicam as surrogate reference standard

Percentage content of Chlorpheniramine Maleate Tablet (%)		
Condition 1	Condition 2	Difference = (Condition 1 – Condition 2)
97.5	97.6	-0.1
98.0	97.2	0.8
98.2	97.9	0.3
98.7	98.1	0.6
98.1	97.7	0.4
		Average X_d = 0.40

$t_{exp} = (X_d / S_d) \times \sqrt{N}$

$X_d = 0.40$

$S_d = 0.34$

$N = 5$

$t_{exp} = (X_d / S_d) \times \sqrt{N}$

$= (0.40/0.34) \times \sqrt{5}$

$= 2.63$

Critical value of t (t_{stat}) at P = 0.05 (95%) level = 2.78 [38]

Table 4.25 Variation of the percentage content of Metformin Hydrochloride in Metformin Hydrochloride tablets manufactured by Hovid using Paracetamol as surrogate reference standard

Percentage content of Metformin Hydrochloride (%)		
Day 1	Day 2	Difference = (Day 1 - Day 2)
104.1	103.9	0.2
104.3	103.8	0.5
104.0	104.1	-0.1
104.0	103.7	0.3
104.2	104.8	-0.6
		Average X_d = 0.06

$t_{exp} = (X_d / S_d) \times \sqrt{N}$

$X_d = 0.06$

$S_d = 0.43$

$N = 5$

$t_{exp} = (X_d / S_d) \times \sqrt{N}$

$= (0.06/0.43) \times \sqrt{5}$

$= 0.31$

4.13 Determination of Percentage content using the K values

4.13.1 Sample calculation of Percentage content of Chlorpheniramine Maleate in Chlorpheniramine Maleate tablets using the new method

Manufacturer: Letap Pharmaceuticals

Average weight of tablet = 0.1269g

Weight of powder taken = 0.1269g

Therefore, 0.1269g of crashed Chlorpheniramine Maleate tablet = 4.0mg of Chlorpheniramine Maleate was dissolved and injected. This gives a concentration of 0.016%w/v with a peak area of 6.65.

Surrogate standard = Piroxicam

The average K value for Piroxicam is 0.8095

Using the hypothetical formular;

$$K = \frac{A_{analyte} \times C_{standard}}{C_{analyte} \times A_{standard}}$$

Where;

$A_{analyte}$ = peak area of the analyte

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

$C_{analyte}$ = concentration of the analyte

$C_{standard}$ = concentration of the standard

$$C_{analyte} = \frac{A_{analyte} \times C_{standard}}{K \times A_{standard}}$$

$\Rightarrow (6.65 \times 0.020) / (0.8095 \times 10.30)$

$= 0.015951$ % w/v

Percentage content = (Actual concentration / Nominal concentration) x 100

$= (0.015951/0.016) \times 100$

$= 99.6\%$

4.13.2 Sample calculation of Percentage content of Metformin Hydrochloride in Metformin Hydrochloride tablets using the new method

Manufacturer: Hovid

Average weight of tablet = 0.5619g

Weight of powder taken = 0.0674g

Therefore, 0.5619g of crashed Metformin Hydrochloride tablet = 500.0mg of Metformin Hydrochloride; hence, 0.0674g will contain 60mg of pure Metformin Hydrochloride. Therefore 60.0mg of Metformin Hydrochloride was actually dissolved and injected. This gives a concentration of 0.24%w/v with a peak area of 15.82.

Surrogate standard = Metronidazole

The average K value for Metronidazole is 1.3262

Using the hypothetical formular;

$$K = \frac{A_{analyte} \times C_{standard}}{C_{analyte} \times A_{standard}}$$

Where;

$A_{analyte}$ = peak area of the analyte

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

$C_{analyte}$ = concentration of the analyte

$C_{standard}$ = concentration of the standard

$$C_{analyte} = \frac{A_{analyte} \times C_{standard}}{K \times A_{standard}}$$

$\Rightarrow$ (15.82 x 0.24) / (1.3262 x 11.45)

= 0.25004 % w/v

Percentage content = (Actual concentration / Nominal concentration) x 100

= (0.25004/0.24) x 100

= 104.2%

4.13.3 Percentage contents for the brands of Chlorpheniramine Maleate

Table 4.26 Table of percentage contents of Chlorpheniramine Maleate produced by Letap Pharmaceuticals using the surrogate reference standards

Sample	Surrogate reference standard	Average percentage content (%)
Chlorpheniramine Maleate Tablet	Piroxicam	100.2 ± 0.2
Chlorpheniramine Maleate Tablet	Ascorbic acid	96.82 ± 0.6
Chlorpheniramine Maleate Tablet	Caffeine	100.5 ± 0.3

Table 4.27 Table of percentage contents of Chlorpheniramine Maleate produced by Amponsah Effah Pharmaceuticals using the surrogate reference standards

Sample	Surrogate reference standard	Average percentage content (%)
Chlorpheniramine Maleate Tablet	Piroxicam	98.1 ± 0.1
Chlorpheniramine Maleate Tablet	Ascorbic acid	98.4 ± 0.5
Chlorpheniramine Maleate Tablet	Caffeine	98.2 ± 0.2

Table 4.28 Table of percentage contents of Chlorpheniramine Maleate produced by Pharmanova Limited using the surrogate reference standards

Sample	Surrogate reference standard	Average percentage content (%)
Chlorpheniramine Maleate Tablet	Piroxicam	94.0 ± 0.2
Chlorpheniramine Maleate Tablet	Ascorbic acid	94.2 ± 0.3
Chlorpheniramine Maleate Tablet	Caffeine	94.3 ± 0.4

Table 4.29 Table of percentage contents of Chlorpheniramine Maleate produced by Kinapharma Limited using the surrogate reference standards

Sample	Surrogate reference standard	Average percentage content (%)
Chlorpheniramine Maleate Tablet	Piroxicam	105.1 ± 0.1
Chlorpheniramine Maleate Tablet	Ascorbic acid	104.1 ± 0.2
Chlorpheniramine Maleate Tablet	Caffeine	104.2 ± 0.2

4.13.4 Percentage contents for the brands of Metformin Hydrochloride

Table 4.30 Table of percentage contents of Metformin Hydrochloride produced by Hovid Bhd using the surrogate reference standards

Sample	Surrogate reference standard	Average percentage content (%)
Metformin Hydrochloride Tablet	Metronidazole	104.4 ± 0.2
Metformin Hydrochloride Tablet	Paracetamol	103.7± 0.4

Table 4.31 Table of percentage contents of Metformin Hydrochloride produced by Pharma DOR using the surrogate reference standards

Sample	Surrogate reference standard	Average percentage content (%)
Metformin Hydrochloride Tablet	Metronidazole	96.0 ± 0.1
Metformin Hydrochloride Tablet	Paracetamol	101.3 ± 0.2

Table 4.32 Table of percentage contents of Metformin Hydrochloride produced by Denk using the surrogate reference standards

Sample	Surrogate reference standard	Average percentage content (%)
Metformin Hydrochloride Tablet	Metronidazole	99.4 ± 0.2
Metformin Hydrochloride Tablet	Paracetamol	99.9 ± 0.3

Table 4.33 Table of percentage contents of Metformin Hydrochloride produced by Ernest Chemist using the surrogate reference standards

Sample	Surrogate reference standard	Average percentage content (%)
Metformin Hydrochloride Tablet	Metronidazole	104.5 ± 0.2
Metformin Hydrochloride Tablet	Paracetamol	98.4± 0.2

4.14 Percentage content of analytes using the standard method in the British Pharmacopoeia, 2007.

Refer to appendix IV for sample calculations and UV spectra

Table 4.34 Table of average percentage content of Chlorpheniramine Maleate in Chlorpheniramine Maleate tablets. (n = 5)

Sample Number	Kinapharma Ghana Limited	Pharmanova Ghana Limited	Letap Pharmaceuticals Ltd	Amponsah Effah Pharmaceuticals Ltd
1	104.5	94.6	100.6	98.6
2	104.5	94.4	100.5	98.4
3	104.4	94.5	100.6	98.5
4	104.6	94.6	100.9	98.6
5	104.3	94.4	100.4	98.1
Average Percentage Content	104.46 ± 0.05	94.50 ± 0.04	100.60 ± 0.08	98.44 ± 0.09

Table 4.35 Table of average percentage content of Metformin Hydrochloride tablet (n = 5)

Sample Number	Hovid	Pharma DOR	Denk	Ernest Chemist
1	104.1	95.9	100.1	99.7
2	105.0	95.8	99.1	101.1
3	104.9	96.8	99.8	99.8
4	104.0	97.0	99.6	98.0
5	104.3	96.7	99.9	100.8
Average Percentage Content	104.5 ± 0.2	96.4 ± 0.2	99.7 ± 0.2	99.7 ± 0.5

4.15 Comparison of the Method Developed with Standard Method (BP 2007) using t-Test

$t_{exp} = (X_d / S_d) \times \sqrt{N}$

where;

X_d = the mean difference between paired values,

S_d = the estimated standard deviation of the differences and

N = number of values within the sets. [38]

4.15.1 Sample calculation for t_{exp}

The percentage content for pure Chlorpheniramine Maleate in Chlorpheniramine Maleate tablet from Letap Pharmaceuticals Ltd., using the standard method in the BP 2007 and the developed method with Piroxicam as the surrogate reference standard is indicated in the table below:

Table 4.36. Table of difference in percentage content of Chlorpheniramine Maleate Tablet using standard method and the new method

Percentage content of Chlorpheniramine Maleate Tablet (%)		
Standard method	New method	Difference = (Standard method – New method)
100.6	99.6	1
100.5	100.7	-0.2
100.6	100.2	0.4
100.9	99.9	1
100.4	100.6	-0.2
		Average X_d = 0.4

$X_d = 0.4$

$S_d = 0.6$

$N = 5$

$t_{exp} = (X_d / S_d) \times \sqrt{N}$

$= (0.4 / 0.6) \times \sqrt{5}$

$= 1.49$

Sample size, n of the standard method = 5; number of degrees of freedom = 4

Sample size, n of the new method = 5; number of degrees of freedom = 4

Critical value of t (t_{stat}) at P = 0.05 (95%) level = 2.78 [38]

Table 4.37 Table for t-Test for Chlorpheniramine Maleate tablets manufactured by Letap Pharmaceuticals

Standard Method (B.P., 2007)	**New Method (Piroxicam)**	**New Method (Ascorbic acid)**	**New Method (Caffeine)**
100.6	99.6	95.6	101.2
100.5	100.7	96.4	100.2
100.6	100.2	95.9	100.1
100.9	99.9	98.6	101.2
100.4	100.6	97.6	99.8
Average = 100.60 ± 0.08	t_{exp} = 1.49	t_{exp} = 8.11	t_{exp} = 0.43

Table 4.38 Table for t-Test for Chlorpheniramine Maleate tablets manufactured by Pharmanova Ltd

Standard Method (B.P., 2007)	**New Method (Piroxicam)**	**New Method (Ascorbic acid)**	**New Method (Caffeine)**
94.6	93.3	93.5	95.6
94.4	93.9	94.3	93.9
94.5	94.2	93.8	94.0
94.6	94.0	94.1	95.1
94.4	94.7	95.2	93.1
Average = 94.5 ± 0.04	t_{exp} = 1.88	t_{exp} = 0.99	t_{exp} = 0.39

Table 4.39 Table for t-Test for Chlorpheniramine Maleate tablets manufactured by Amponsah Effah Pharmaceuticals

Standard Method (B.P., 2007)	**New Method (Piroxicam)**	**New Method (Ascorbic acid)**	**New Method (Caffeine)**
98.6	97.9	99.1	98.0
98.4	98.2	98.0	98.9
98.5	98.3	99.8	98.2
98.6	98.4	98.1	97.7
98.1	97.6	97.1	98.3
Average = 98.44 ± 0.20	t_{exp} = 3.50	t_{exp} = 0.05	t_{exp} = 0.86

Table 4.40 Table for t-Test for Chlorpheniramine Maleate tablets manufactured by Kinapharma Ltd

Standard Method (B.P., 2007)	**New Method (Piroxicam)**	**New Method (Ascorbic acid)**	**New Method (Caffeine)**
104.5	105.0	103.9	104.3
104.5	104.9	104.1	104.2
104.4	105.2	104.8	104.0
104.6	104.8	103.9	103.9
104.3	105.5	103.8	104.8
Average = 104.46 ± 0.10	t_{exp} = 3.93	t_{exp} = 1.35	t_{exp} = 0.54

Table 4.41 Table for t-Test for Metformin Hydrochloride tablets manufactured by Hovid

Standard Method (B.P., 2007)	**New Method (Metronidazole)**	**New Method (Paracetamol)**
104.1	104.2	104.0
105.0	104.9	104.8
104.9	104.8	104.3
104.0	103.9	103.3
104.3	104.1	102.3
Average = 104.5 ± 0.2	t_{exp} = 1.63	t_{exp} = 2.12

Table 4.42 Table for t-Test for Metformin Hydrochloride tablets manufactured by Denk

Standard Method (B.P., 2007)	**New Method (Metronidazole)**	**New Method (Paracetamol)**
100.1	99.2	100.2
99.1	99.3	100.5
99.8	100.1	99.7
99.6	98.8	99.0
99.9	99.7	100.2
Average = 99.7 ± 0.2	t_{exp} = 1.14	t_{exp} = 0.66

Table 4.43 Table for t-Test for Metformin Hydrochloride tablets manufactured by Pharma DOR

Standard Method (B.P., 2007)	**New Method (Metronidazole)**	**New Method (Paracetamol)**
95.9	95.5	101.2
95.8	96.0	101.3
96.8	96.2	101.1
97.0	96.2	102
96.7	96.3	101.1
Average = 96.4 ± 0.2	t_{exp} = 2.42	t_{exp} = 20.67

Table 4.44 Table for t-Test for Metformin Hydrochloride tablets manufactured by Ernest Chemist

Standard Method (B.P., 2007)	**New Method (Metronidazole)**	**New Method (Paracetamol)**
99.7	104.6	98.4
100.1	104.4	99.0
99.8	103.9	98.3
98.0	104.7	98.2
100.8	105.0	98.0
Average = 99.68 ± 0.4	t_{exp} = 9.97	t_{exp} = 2.72

4.16 Relative Precision of the New Method to the Standard Method

4.16.1 Assay of Chlorpheniramine Maleate tablets

Null Hypothesis: There are no significant differences between the precisions of the two methods at the 95% probability level.

The standard method and the developed method were subjected to the F-test to determine whether their sets of data differ in precision; a two-sided test.

$F = S_1^2 / S_2^2$

Where:

S_1^2 and S_2^2 are the variances of either the standard method or the new method, depending on which value is larger, with the largest variance value being the numerator so that

$F > 1$.

Sample size, n of the standard method = 5; number of degrees of freedom = 4

Sample size, n of the new method = 5; number of degrees of freedom = 4

Critical value of F at P = 0.05 (95%) level = 9.605 [38]

Manufacturer: Letap Pharmaceuticals

Mean of Standard method, B.P. 2007 = 100.6

Standard deviation, S of Standard method, B.P. 2007 = 0.1871

Therefore variance, S^2 of Standard method, B.P. 2007 = 0.0350

Table 4.45 Relative Precision of the New Method to the Standard method with respect to the assay of Chlorpheniramine Maleate tablets from Letap Pharmaceutical

Surrogate reference standard	Mean	Standard deviation	Variance	F_{exp}
Piroxicam	100.2	0.4637	0.22	6.286
Ascorbic Acid	96.82	1.2538	1.57	44.857
Caffeine	100.5	0.6557	0.43	12.286

Manufacturer: Pharmanova Limited

Mean of Standard method, B.P. 2007 = 94.5

Standard deviation, S of Standard method, B.P. 2007 = 0.1

Therefore variance, S^2 of Standard method, B.P. 2007 = 0.01

Table 4.46 Relative Precision of the New Method to the Standard method with respect to the assay of Chlorpheniramine Maleate tablets from Pharmanova

Surrogate reference standard	Mean	Standard deviation	Variance	F_{exp}
Piroxicam	94.02	0.5069	0.26	26.000
Ascorbic Acid	94.18	0.6458	0.42	42.000
Caffeine	94.34	1.001	1.00	100.000

Manufacturer: Amponsah Effah Pharmaceuticals Ltd

Mean of Standard method, B.P. 2007 = 98.44

Standard deviation, S of Standard method, B.P. 2007 = 0.2073

Therefore variance, S^2 of Standard method, B.P. 2007 = 0.043

Table 4.47 Relative Precision of the New Method to the Standard method with respect to the assay of Chlorpheniramine Maleate tablets from Amponsah Effah Pharmaceutical

Surrogate reference standard	Mean	Standard deviation	Variance	F_{exp}
Piroxicam	98.08	0.3271	0.107	2.488
Ascorbic Acid	98.42	1.0474	1.097	25.512
Caffeine	98.22	0.4438	0.197	4.581

Manufacturer: Kinapharma Limited

Mean of Standard method, B.P. 2007 = 104.5

Standard deviation, S of Standard method, B.P. 2007 = 0.1140

Therefore variance, S^2 of Standard method, B.P. 2007 = 0.0129

Table 4.48 Relative Precision of the New Method to the Standard method with respect to the assay of Chlorpheniramine Maleate tablets from Kinapharma Limited

Surrogate reference standard	Mean	Standard deviation	Variance	F_{exp}
Piroxicam	105.8	0.2774	0.077	5.969
Ascorbic Acid	104.1	0.4062	0.165	12.791
Caffeine	104.2	0.3507	0.123	9.535

4.16.2 Assay of Metformin Hydrochloride tablets

Manufacturer: Hovid

Mean of Standard method, B.P. 2007 = 104.5

Standard deviation, S of Standard method, B.P. 2007 = 0.4615

Therefore variance, S^2 of Standard method, B.P. 2007 = 0.2130

Table 4.49 Relative Precision of the New Method to the Standard method with respect to the assay of Metformin Hydrochloride tablets from Hovid

Surrogate reference standard	Mean	Standard deviation	Variance	F_{exp}
Metronidazole	104.4	0.4438	0.1969	1.082
Paracetamol	103.7	0.9711	0.9430	4.427

Manufacturer: Denk

Mean of Standard method, B.P. 2007 = 99.7

Standard deviation, S of Standard method, B.P. 2007 = 0.0.3808

Therefore variance, S^2 of Standard method, B.P. 2007 = 0.1450

Table 4.50 Relative Precision of the New Method to the Standard method with respect to the assay of Metformin Hydrochloride tablets from Denk

Surrogate reference standard	Mean	Standard deviation	Variance	F_{exp}
Metronidazole	99.42	0.4970	0.2470	1.703
Paracetamol	99.9	0.5891	0.3470	2.393

Manufacturer: Pharma DOR

Mean of Standard method, B.P. 2007 = 96.44

Standard deviation, S of Standard method, B.P. 2007 = 0.5504

Therefore variance, S^2 of Standard method, B.P. 2007 = 0.3029

Table 4.51 Relative Precision of the New Method to the Standard method with respect to the assay of Metformin Hydrochloride tablets from Pharma DOR

Surrogate reference standard	Mean	Standard deviation	Variance	F_{exp}
Metronidazole	96.0	0.3209	0.1030	2.941
Paracetamol	101.3	0.3782	0.143	2.118

Manufacturer: Ernest Chemist

Mean of Standard method, B.P. 2007 = 99.7

Standard deviation, S of Standard method, B.P. 2007 = 1.0329

Therefore variance, S^2 of Standard method, B.P. 2007 = 1.067

Table 4.52 Relative Precision of the New Method to the Standard method with respect to the assay of Metformin Hydrochloride tablets from Ernest Chemist

Surrogate reference standard	Mean	Standard deviation	Variance	F_{exp}
Metronidazole	104.5	0.4087	0.1670	6.389
Paracetamol	98.4	0.3768	0.1419	7.520

CHAPTER FIVE

5.0 Discussion, Conclusion and Recommendations

5.1 Discussion

5.1.0 Identification Tests

The various identification tests were performed to verify the identity of the pure samples before being used in the analysis [11].

5.1.0.1 Colour Test

Colour test was performed on the pure forms of Chlorpheniramine Maleate, Caffeine, Ascorbic acid and Paracetamol. The results obtained conform to that stated in the British Pharmacopoeia, 2007.

5.1.0.2 Ultra-Violet Spectroscopy

The maximum and minimum absorption at 277nm and 240nm respectively for Metronidazole and the specific absorbance at the maximum being 365 to 395 conforms to that stated in the British Pharmacopoeia and therefore identifies the substance as Metronidazole.

5.1.0.3 Thin Layer Chromatography

The Rf value of 0.78 obtained from the pure form of Chlorpheniramine Maleate was the same as the Rf value obtained for the brands from Amponsah Effah Pharmaceuticals Limited, Pharmanova Limited, Letap Pharmaceuticals Ltd. and Kinapharma Limited as shown in Table 4.1. Both the pure form and the brands were spotted on the same thin

layer chromatographic plate and this identifies the brands of the tablets as containing Chlorpheniramine Maleate.

Rf value of 0.68 as shown in Table 4.2 were obtained for brands of Metformin Hydrochloride tablets from Hovid, Denk, Pharma DOR and Ernest Chemist and they were the same as the Rf value obtained for the pure Metformin Hydrochloride spotted on the same thin layer chromatographic plate. This therefore identifies the brands of the tablets as containing Metformin Hydrochloride.

5.1.0.4 Melting Point determination

The experimental melting point ranges obtained for all the pure samples used, as shown in Table 4.3 were within the range of the literature melting point range as stated in the British Pharmacopoeia, 2007 and hence the pure samples were not contaminated.

5.1.0.5 pH determination

The experimental pH range of all the samples were within the range of the pH stated in the British Pharmacopoeia, 2007. Refer to Table 4.4.

5.1.0.6 Wavelength of maximum absorption

The wavelength of maximum absorption of all the pure samples was determined to be able to select a suitable wavelength for detection by the Detector of the High Performance Liquid Chromatography. As shown in Table 4.5, it is observed that the wavelength of maximum absorption for Chlorpheniramine Maleate tablet and its surrogate reference standards (Ascorbic Acid, Caffeine and Piroxicam) were within the range of 245mn and 273nm and that of Metformin Hydrochloride tablet and its surrogate

reference standards (Paracetamol and Metronidazole was 236nm and 310nm. A single wavelength value of 266nm was therefore chosen for Chlorpheniramine Maleate tablet and its surrogate reference standards (Ascorbic Acid, Caffeine and Piroxicam) and a different single wavelength value of 245nm for Metformin Hydrochloride tablet and its surrogate reference standards (Paracetamol and Metronidazole). This is a compromise that was stricken so that at these wavelengths, all the compounds will exhibit reasonable UV absorption and thus be detected by the UV-Visible detector.

5.1.1 Assay of pure samples

5.1.1.0 Chlorpheniramine Maleate

The percentage purity of pure Chlorpheniramine Maleate as stated in the British Pharmacopoeia should be within the range of 98.0% – 101.0%. The percentage purity of pure Chlorpheniramine Maleate performed using the procedure in the British Pharmacopoeia gave a percentage purity of 98.3%. This establishes the level of purity of the sample as within the accepted range. The sample is therefore pure and hence suitable for the analysis.

5.1.1.1 Caffeine

The British Pharmacopoeia gives the percentage purity of Caffeine as between 98.5% - 101.5%. Using the procedure stated in the British Pharmacopoeia for the assay of Caffeine, the percentage purity of Caffeine was found to be 98.7%. This establishes the level of purity of the sample as within the accepted range. The sample is therefore pure and hence suitable for the analysis.

5.1.1.2 Piroxicam

The percentage purity of Piroxicam was determined using the procedure in the British Pharmacopoeia and was found to be 100.1%. This falls within the range stated in the British Pharmacopoeia as 98.5% - 101.0%. This establishes the level of purity of the sample as within the accepted range. The sample is therefore pure and hence suitable for the analysis.

5.1.1.3 Ascorbic Acid

Ascorbic acid should have a percentage purity between the range of 99.0% - 100.5 according to the British Pharmacopoeia. It was found to have a percentage purity of 99.3% when the procedure in the British Pharmacopoeia was used for the analyses. This establishes the level of purity of the sample as within the accepted range. The sample is therefore pure and hence suitable for the analysis.

5.1.1.4 Metformin Hydrochloride

The British Pharmacopoeia gives the percentage purity of Metformin Hydrochloride as between 98.5% - 101.0%. Using the procedure stated in the British Pharmacopoeia for the assay of Metformin Hydrochloride, the percentage purity of Metformin Hydrochloride was found to be 99.5%. This establishes the level of purity of the sample as within the accepted range. The sample is therefore pure and hence suitable for the analysis.

5.1.1.5 Metronidazole

The percentage purity of pure Metronidazole as stated in the British Pharmacopoeia should be within the range of 99.0% – 101.0%. The percentage purity of pure Metronidazole performed using the procedure in the British Pharmacopoeia gave a percentage purity of 99.8%. This establishes the level of purity of the sample as within the accepted range. The sample is therefore pure and hence suitable for the analysis.

5.1.1.6 Paracetamol

The percentage purity of Paracetamol was determined using the procedure in the British Pharmacopoeia and was found to be 99.5%. This falls within the range stated in the British Pharmacopoeia as 99.0% - 101.0%. This establishes the level of purity of the sample as within the accepted range. The sample is therefore pure and hence suitable for the analysis.

5.1.2 Uniformity of weight test

Table 5.1 Uniformity of weight of tablets (uncoated and film-coated)

Average weight of tablet	Percentage deviation permissible
80 mg or less	± 10
More than 80 mg and less than 250mg	± 7.5
250mg or more	± 5

Source: [11].

In the determination of the uniformity of weight, 20 individual tablets were taken at random and the average weight was determined. According to the British Pharmacopoeia, not more than 2 of the individual masses should deviate from the average mass by more

than the percentage deviation shown in Table 5.1 and none should deviate by more than twice that percentage.

The average weight of Chlorpheniramine Maleate tablets manufactured by Kinapharma Limited, Amponsah Effah Pharmaceuticals, Pharmanova Limited and Letap Pharmaceuticals was 164.600mg, 100.860mg, 113.305mg and 126.985mg respectively. A look at Appendix III (UCK.1, UCP.3 and UCL.4) show that none of the tablets deviates by 7.5% as the percentage deviation for tablets with an average mass more than 80mg and less than 250mg, according to the British Pharmacopoeia. Hence, the batch of Chlorpheniramine Maleate tablets from Kinapharma Limited, Pharmanova Limited and Letap Pharmaceuticals which were taken through the uniformity of weight test were therefore within the control limits of pharmacopoeial standards. The tablets from Amponsah Effah Pharmaceuticals were not within the control limits of pharmacopoeial standards because a look at Appendix III UCA.2 shows that 3 tablets (more than 2) have percentage deviations of 8.0706%, 8.6655% and 10.2578% which is greater than the 7.5% percentage deviation limit for tablets with an average mass more than 80mg and less than 250mg, according to the British Pharmacopoeia, 2007.

The average weight of Metformin Hydrochloride tablets manufactured by Pharma DOR, Hovid, Denk and Ernest Chemist Limited was 587.645mg, 561.96mg, 659.9mg and 627.06mg respectively. A look at Appendix III (UMH.5, UMD.6, UMP.7 and UME.8) shows that none of the tablets deviates by 5%, with the exception of the brand from Pharma DOR in which only one tablet deviated by more than 5% i.e. 5.012% as the percentage deviation permissible for tablets with an average mass of 250mg or more, according to the British Pharmacopoeia. Hence, the batch of Metformin Hydrochloride

tablets manufactured by Pharma DOR, Hovid, Denk and Ernest Chemist Limited which were taken through the uniformity of weight test were therefore within the control limits of pharmacopoeial standards.

5.1.3 Determination of Percentage content of analyte

5.1.3.1 Chlorpheniramine Maleate in Chlorpheniramine Maleate tablets using Standard Method in the British Pharmacopoeia, 2007.

Chlorpheniramine Maleate tablets manufactured by Kinapharma Limited, Amponsah Effah Pharmaceuticals Limited, Pharmanova Limited and Letap Pharmaceutical Limited gave an average percentage content of 104.5 ± 0.05%, 98.4 ± 0.09%, 94.5 ± 0.04 and 100.6 ± 0.08% respectively.

The British pharmacopoeia gives the percentage range of the content of Chlorpheniramine Maleate in Chlorpheniramine Maleate tablets as between 92.5% to 107.5%. This implies that, the Chlorpheniramine Maleate tablets were within the range and hence passed the test.

5.1.3.2 Metformin Hydrochloride in Metformin Hydrochloride tablets using the Standard Method in the British Pharmacopoeia, 2007

Metformin Hydrochloride tablets manufactured by Hovid, Denk, Pharma DOR and Ernest Chemist Limited gave an average percentage content of 104.5 ± 0.2%, 99.7 ± 0.2%, 96.4 ± 0.2 and 99.7 ± 0.5% respectively.

The British Pharmacopoeia gives the percentage range of the content of Metformin Hydrochloride in Metformin Hydrochloride tablets as between 95.0% to 105.0%. This implies that, the Metformin Hydrochloride tablets were within the range and hence passed the test.

5.1.4 HPLC Method Development

Amines are more basic and more polar than amides [39]. This accounts for the relatively low retention time recorded for Metformin Hydrochloride and high retention time for Paracetamol. This is because Metformin Hydrochloride, (an amine), being more polar, has less affinity for the non-polar stationary phase i.e. the ODS Column and hence is not retained on the stationary phase. Paracetamol on the other hand, is an amide and is less basic and less polar (more non-polar), and hence has a high affinity for the non-polar stationary phase used and thus is much more retained on it.

Carboxylic acids are generally polar substances and therefore form strong intermolecular hydrogen bonds in two places i.e. the H at the ends of its kind and that of water [40]. The carboxylic acid group, Maleic Acid attached to Chlorpheniramine as an isomer contributes to the relatively low retention time recorded for Chlorpheniramine Maleate and also due to the strong hydrogen bond formed with the mobile phase which consists of Phosphate buffer and Methanol.

The polarity of Ascorbic acid due to the strong H – O bond contributed to the low retention time observed. This is because of the low affinity of the polar Ascorbic acid to the non-polar stationary phase and hence there is less time spent on the column.

The strength of an acid or base is determined by the value of its Ka or Kb and consequently its pKa or pKb respectively. The stronger the acid or base is, the larger the value of its Ka or Kb and the lower the pKa or pKb respectively [41].

From Table 4.2, it is noted that Paracetamol, Caffeine, Metformin Hydrochloride, Metronidazole, Chlorpheniramine Maleate and Piroxicam were within the useful pH working range of 6.2 – 8.2 and 3.8 – 5.8 for Phosphate buffer and Acetate buffer respectively and are therefore expected to give good peaks within these ranges. At pH < 2, the SiO bonds are subjected to acidic hydrolytic cleavage, causing the loss of the bonded phase. At pH > 8, the silica structure is prone to dissolution [8]. In order to avoid these mishaps, pH below 2 and above 8 was avoided and therefore the pH range chosen for the Phosphate buffer was 6.35 – 6.39 and that of the Acetate buffer was 5.44 – 5.48. It was observed after a number of individual injections of the analytes and their combinations that analytes with their pH range closer to the pH of the mobile phase gave good, well resolved peaks and therefore the chosen pH for both mobile phase solutions were maintained throughout the analyses.

The mobile phase system chosen for the analyses of Chlorpheniramine Maleate is thus Phosphate buffer and Methanol (50:50) at a pH of 6.37 ± 0.02 and that of Metformin Hydrochloride in Acetate buffer and Methanol (70:30) at a pH of 5.46 ± 0.02.

5.1.5 Analytical Performance Parameters

5.1.5.1 Linearity

The linearity of the method was tested by using the calibration curves of the concentration of the analytes and the surrogate reference standards plotted against their peak areas. As shown in Appendix V and Appendix VI, it is observed that all the samples analyzed were linear over a concentration range (0.01500%w/v – 0.00125%w/v) for Chlorpheniramine Maleate and (0.2%w/v – 0.062%w/v) for Metformin Hydrochloride, with the correlation coefficient, r in the range of 0.9910 – 0.9989. This is within the accepted range of $-1 \leq r \leq +1$. [38].

5.1.5.2 Specificity and Selectivity

The specificity and selectivity describe the capacity of the analytical method to measure the drug in the presence of impurities or excipients [2, 37]. Comparing the chromatogram of the pure Chlorpheniramine Maleate in Fig.15 and that for Chlorpheniramine Maleate in all the brands of Chlorpheniramine Maleate tablet in Fig.4.16 to Fig.4.19, there was similarity in the resolutions and the shape of the peaks. The same can be said of the peak of pure Metformin Hydrochloride in Fig.4.20 and that of all the brands of Metformin Hydrochloride tablets in Fig.4.21 to Fig.4.24. This implies that excipients and impurities in the tablets did not interfere with the analyses of these drugs using the method developed, since all the samples were reasonably resolved with no overlapping bands. It is clear from the chromatograms that the analytical conditions could separate and resolve reasonably the study samples.

5.1.5.3 Repeatability (Precision)

5.1.5.3.1 Intra-day Variation

The precision refers to the variability of the results in repeated analyses of the sample under identical experimental conditions. [38]. This was done by repeating the process involved in the new method for two times on two different occasions in a day. The results obtained were used to find the percentage content of the analytes. The percentage contents obtained from the two tests were subjected to t-Test to verify if there was any significant difference between the two test results. As shown in Table 4.19 and Table 4.20, the t_{exp} obtained was 2.30 when Chlorpheniramine Maleate in Chlorpheniramine Maleate tablet manufactured by Amponsah Effah Pharmaceuticals using Piroxicam as surrogate reference standard was analyzed and that for the percentage content of Metformin Hydrochloride in Metformin Hydrochloride tablets manufactured by Hovid using Paracetamol as surrogate reference standard was 1.04. Since both t_{exp} values were lower than the t_{stat} which is 2.78, it implies that there was no significant difference between the intra-day analyses. The analyses can therefore be repeated under the stated experimental conditions regardless of the time of the day of the analyses.

5.1.5.3.2 Inter-day Variation

The precision refers to the variability of the results in repeated analyses of the sample under identical experimental conditions. [38]. This was done by repeating the process involved in the new method on two different days. The results obtained were used to find the percentage content of the analytes. The percentage contents obtained from the two

days were subjected to t-Test to verify if there was any significant difference between the results for the two days. As shown in Table 4.21 and Table 4.22, the t_{exp} obtained was 1.29 when Chlorpheniramine Maleate in Chlorpheniramine Maleate tablet manufactured by Amponsah Effah Pharmaceuticals using Piroxicam as surrogate reference standard was analyzed and that for the percentage content of Metformin Hydrochloride in Metformin Hydrochloride tablets manufactured by Hovid using Paracetamol as surrogate reference standard was 0.22. Since both t_{exp} values were lower than the t_{stat} which is 2.78, it implies that there was no significant difference between the intra-day analyses. The analyses can therefore be repeated under the stated experimental conditions regardless of the day of the analyses.

5.1.5.4 Sensitivity

This is a measurement of the lowest concentration of analyte that the system can measure. [38]. The LOD and the LOQ results obtained for all the samples used in the analyses gave the lowest concentration of all the samples that can be determined and quantified. (Refer to Table 4.23). This gave the appropriate working concentration for the samples and hence the quality of the analytical results can be assessed because the indices of analytical performance are known.

5.1.5.5 Robustness

The robustness of the new method is done to investigate the performance of the method when small, deliberate changes were made to the already-established chromatographic conditions. As shown in Section 4.12, t-Test was applied to the results obtained from

condition 1 and condition 2 and the t_{exp} was 2.63 for Chlorpheniramine Maleate and 0.31 for Metformin Hydrochloride. These values are lower than the t_{stat} of 2.78 [38] indicating that there is no significant difference between the results of condition 1 and condition 2. The new method is therefore robust under the stated experimental parameters.

5.1.5.6 Accuracy

Accuracy may be inferred once precision, linearity and specificity have been established [37]. From the results obtained, all the samples analyzed were linear over a certain concentration range with the correlation coefficient, **r** within the accepted range which is $-1 \leq \mathbf{r} \leq +1$. [38].

The percentage contents obtained from the standard method and the new method were subjected to statistical tests to investigate their accuracy and precision. These statistical tests are F-test and T-test.

As shown from Table 4.37 to Table 4.44 for the t-Test and from Table 4.45 to Table 4.52 for the F-Test, no significant difference was observed between results of the standard method and the new method, in spite of few exceptions during the analyses of some of the brands. The new method can be said to be accurate

5.1.6 Determination of the constant K

The values of the peak areas and the concentrations of both analytes and surrogate reference standards were put into the equation for the determination of the constant K. From Fig. 4.25 and Fig. 4.26, it was observed that changes in concentration of both analytes and surrogates had no significant effect on the K values obtained. This observation is made from the almost straight line graph obtained from the graphs.

It was also observed from the profile of the samples, that the chromophores in both analyte and surrogates which are the unsaturated groups responsible for absorption in the UV-Visible Region as well as auxochromes were much more in Metformin Hydrochloride than in Paracetamol, but Paracetamol gave a higher peak area than Metformin Hydrochloride. This could be attributed to the fact that the wavelength selected for the HPLC Detector, 245nm is the wavelength of maximum absorption for Paracetamol at a pH of 5.92 ± 0.10, since the wavelength of maximum absorption for Paracetamol depends on the pH of the solution. There was therefore a corresponding low K value of 0.8623 obtained when Paracetamol was used as the surrogate reference standard for Metformin Hydrochloride since peak area of surrogate reference standard is inversely proportional to K as indicated in Equation 1. The same observation is made when Metronidazole with less number of chromophores as compared to that of Metformin Hydrochloride, gave a low peak area compared to Metformin Hydrochloride which gave a relatively higher peak area, is used as the surrogate reference standard for Metformin Hydrochloride and hence gave a high K value of 1.3262.

With Chlorpheniramine Maleate, Caffeine had a high peak area of 26.67Vs as compared to Chlorpheniramine Maleate, 9.39Vs, Piroxicam, 15.11Vs and Ascorbic acid, 16.07Vs

though Piroxicam has more chromophores and auxochromes, as shown in the profile of the samples. This could be due to the wavelength of 266nm chosen for the HPLC Detector. This wavelength is closer to the wavelength of maximum absorption for Caffeine which is 273nm than for Piroxicam which is 245nm. A corresponding higher K value of 0.8095 was therefore obtained when Piroxicam was used as the surrogate reference standard as compared to that obtained for Caffeine which is 0.2224. The same is observed for Ascorbic acid. With the number of chromophores for Ascorbic Acid being smaller than that of Piroxicam, it was expected that Ascorbic Acid should give a lower peak area than Piroxicam, but the reverse rather occurred. This could also be attributed to the closeness of the wavelength of maximum absorption for Ascorbic Acid which is 264nm being very close to the wavelength chosen for the detector which is 266, as compared to that of Piroxicam with a wavelength of maximum absorption of 245nm. A corresponding low K value of 0.1560 was therefore obtained for Ascorbic Acid and a relatively higher K value of 0.8095 was obtained for Piroxicam.

The surrogate constant K defined mathematically by Equation 1 for Piroxicam, Caffeine, and Ascorbic acid were 0.8095, 0.2224 and 0.1560 respectively. These are the surrogate reference standards for Chlorpheniramine Maleate. The surrogate reference standards for Metformin Hydrochloride; Paracetamol and Metronidazole gave K values of 0.8623 and 1.3262 respectively. It is observed that the molecular weight ratio of the analyte to surrogate reference standard have an effect on the K values. The molecular weight in g/mol of Piroxicam, Caffeine, Ascorbic acid and Chlorpheniramine Maleate are 331.4, 194.2, 176.1 and 390.9 respectively and the corresponding molecular weight ratios for Piroxicam, Caffeine and Ascorbic acid were 1.17, 2.01 and 2.22 respectively. This shows

that the lower the molecular weight ratio of analyte to surrogate reference standard, the higher the K value with the opposite also being true. The same trend is observed for Metformin Hydrochloride and its surrogate reference standards. The molecular weight in g/mol of Metformin Hydrochloride, Paracetamol and Metronidazole is 165.6, 151.2 and 171.2 respectively with their corresponding molecular weight ratio of analyte to surrogate being 1.09 and 0.96 respectively.

The values of the surrogate constant K showed a variation that could be linked to the structural and inherent physico-chemical differences among the surrogate standards. The constant is characteristic for each surrogate reference under a set of experimental conditions.

5.1.7 Determination of Percentage Content using the constant K

The K values obtained from the new method were used to determine the percentage content of Chlorpheniramine Maleate in the brands of Chlorpheniramine Maleate Tablet and Metformin Hydrochloride in the brands of Metformin Hydrochloride Tablet using their respective surrogate reference standards. This is recorded in Table 4.26 to Table 4.33. According to the British Pharmacopoeia, the percentage content of Chlorpheniramine Maleate in Chlorpheniramine Maleate tablet and Metformin Hydrochloride in Metformin Hydrochloride tablet should be between 92.5 % - 107.5% and 95.0% - 105.0%. The percentage contents obtained for the two analytes using the new method were therefore within the accepted range. Although the percentage content obtained when the standard method was used also fell within the accepted range, the results were subjected to t-Test to ascertain whether there is any significant difference

between the two results obtained from using the standard method and the developed method. The null hypothesis states that the means of the two methods do not differ significantly at the 95% probability level. The acceptance or rejection of the null hypothesis is discussed specifically for each surrogate reference standard.

5.1.8 Comparison of the Method Developed with Standard Method (BP 2007) using t-Test

5.1.8.1 Chlorpheniramine Maleate Tablets

Null Hypothesis: The means of the two methods do not differ significantly at the 95% probability level.

The experimental values of t (t_{exp}) for Chlorpheniramine Maleate tablets manufactured by Pharmanova Limited were 1.88, 0.99 and 0.39 for Piroxicam, Ascorbic Acid and Caffeine reference standards respectively. The calculated t-values are smaller than the critical value of 2.78. Hence, there is no significant difference between the two methods in the analyses of Chlorpheniramine Maleate tablets manufactured by Pharmanova Limited. The null hypothesis is accepted at the 95% probability level. Therefore Piroxicam, Ascorbic Acid and Caffeine are appropriate surrogate reference standards for the analyses of Chlorpheniramine Maleate tablet. However, this assertion was not supported by the analyses of Chlorpheniramine Maleate tablet of the other brands.

The experimental values of t (t_{exp}) for Chlorpheniramine Maleate tablets manufactured by Amponsah Effah Pharmaceuticals were 3.50, 0.05 and 0.86 for Piroxicam, Ascorbic Acid and Caffeine reference standards respectively. The calculated t-values of Ascorbic acid and Caffeine are smaller than the critical value of 2.78, whereas that from Piroxicam is greater than the critical value of 2.78. Hence, there is no significant difference between the two methods when Ascorbic acid and Caffeine were used as the surrogate reference standards, whereas there is a significant difference between the two methods when Piroxicam was used as the surrogate reference standard to analyze Chlorpheniramine Maleate tablets manufactured by Amponsah Effah Pharmaceuticals. The null hypothesis is accepted at the 95% probability level for Ascorbic acid and Caffeine and rejected for Piroxicam. This could be attributed to the solubility of the samples used in water. Whiles Caffeine, Ascorbic acid and Chlorpheniramine Maleate are soluble in water which was used for the dissolution before running on the aqueous mobile phase, Piroxicam is practically insoluble in water and hence alcohol was used for its dissolution.

The experimental values of t (t_{exp}) for Chlorpheniramine Maleate tablets manufactured by Kinapharma Limited were 3.93, 1.35 and 0.54 for Piroxicam, Ascorbic Acid and Caffeine reference standards respectively. The calculated t values of Ascorbic acid and Caffeine are smaller than the critical value of 2.78, whereas that from Piroxicam is greater than the critical value of 2.78. Hence, there is no significant difference between the two methods when Ascorbic acid and Caffeine were used as the surrogate reference standards, whereas there is a significant difference between the two methods when Piroxicam was used as the surrogate reference standard to analyze Chlorpheniramine Maleate tablets

manufactured by Kinapharma Limited. The null hypothesis is accepted at the 95% probability level for Ascorbic acid and Caffeine and rejected for Piroxicam.

The experimental values of t (t_{exp}) for Chlorpheniramine Maleate tablets manufactured by Letap Pharmaceuticals were 1.49, 8.11 and 0.43 for Piroxicam, Ascorbic Acid and Caffeine reference standards respectively. The calculated t-values of Piroxicam and Caffeine are smaller than the critical value of 2.78, whereas that from Ascorbic acid is greater than the critical value of 2.78. Hence, there is no significant difference between the two methods when Piroxicam and Caffeine were used as the surrogate reference standards, whereas there is a significant difference between the two methods when Ascorbic acid was used as the surrogate reference standard to analyze Chlorpheniramine Maleate tablet manufactured by Letap Pharmaceuticals. The null hypothesis is accepted at the 95% probability level for Piroxicam and Caffeine and rejected for Ascorbic acid.

5.1.8.2 Metformin Hydrochloride Tablets

The experimental values of t (t_{exp}) for Metformin Hydrochloride tablets manufactured by Hovid were 1.63 and 2.12 for Metronidazole and Paracetamol surrogate reference standards respectively. The experimental t-values (t_{exp}) are smaller than the critical value of 2.78. Hence, there is no significant difference between the two methods in the analyses of Metformin Hydrochloride tablet manufactured by Hovid. The null hypothesis is accepted at the 95% probability level.

The experimental values of t (t_{exp}) for Metformin Hydrochloride tablets manufactured by Denk were 1.14 and 0.66 for Metronidazole and Paracetamol surrogate reference

standards respectively. The calculated t-values are smaller than the critical value of 2.78. Hence, there is no significant difference between the two methods in the analyses of Metformin Hydrochloride tablet manufactured by Denk. The null hypothesis is accepted at the 95% probability level.

The experimental values of t (t_{exp}) for Metformin Hydrochloride tablets manufactured by Pharma DOR were 2.42 and 20.67 for Metronidazole and Paracetamol surrogate reference standards respectively. The (t_{exp}) of Metronidazole which is 2.42 is smaller than the critical value of 2.78, whereas that from Paracetamol which is 20.67 is greater than the critical value of 2.78. Hence, there is no significant difference between the two methods when Metronidazole was used as the surrogate reference standard, whereas there is a significant difference between the two methods when Paracetamol was used as the surrogate reference standard to analyze Metformin Hydrochloride tablets manufactured by Pharma DOR.

The experimental values of t (t_{exp}) for Metformin Hydrochloride tablets manufactured by Ernest Chemist were 9.97 and 2.72 for Metronidazole and Paracetamol surrogate reference standards respectively. The (t_{exp}) of Paracetamol is smaller than the critical value of 2.78, whereas that from Metronidazole is greater than the critical value of 2.78. Hence, there is no significant difference between the two methods when Paracetamol was used as the surrogate reference standard, whereas there is a significant difference between the two methods when Metronidazole was used as the surrogate reference standard to analyze Metformin Hydrochloride tablets manufactured by Ernest Chemist.

It is observed that there is a level of inconsistency with respect to the performance of the surrogate reference standards in the analysis of Metformin Hydrochloride tablets manufactured by Pharma DOR and Ernest Chemist. This could be attributed to formulation factors of the tablets from these brands.

It is worth noting that, in spite of the significant differences in some of the assay methods, the proposed method produced assay results that were within monograph specifications of the British Pharmacopoeia. Nonetheless, the significant difference realized in some cases made it difficult to establish the general relative accuracy of the new method to those of the pharmacopoeia.

5.1.9 Relative Precision of the New Method to the Standard Method

5.1.9.1 Relative Precision of the New Method to the Standard Method with respect to the Assay of Chlorpheniramine Maleate tablets

Null Hypothesis: The Standard Method and the New Method do not differ in their precision.

The Standard Method and the New Method were subjected to the F-test to determine whether their sets of data differ in precision; a two-sided test. The critical value of F (F_{stat}) at the probability level of 95% level is 9.605. [38]. The calculated F-test value, F_{exp} of Chlorpheniramine Maleate tablets from Letap Pharmaceutical Limited using Piroxicam, Ascorbic Acid and Caffeine as the surrogate reference standards against the Standard Method (B.P. 2007) were 6.286, 44.857 and 12.286 respectively. The F_{exp} obtained when Piroxicam was used as the surrogate reference standard is less than the

critical value of 9.605. Hence, the two methods do not differ significantly in their precision when Piroxicam is used as the surrogate reference standard. However, the F_{exp} obtained when Ascorbic Acid and Caffeine were used as the surrogate reference standard were greater than the critical value of 9.605. Hence, the two methods differ significantly in their precision when Ascorbic Acid and Caffeine were used as the surrogate reference standards. The null hypothesis is therefore accepted for Piroxicam as a surrogate reference standard and is rejected when Ascorbic Acid and Caffeine were used as the surrogate reference standard at the 95% probability level.

Chlorpheniramine Maleate tablets manufactured by Pharmanova Limited using Piroxicam, Ascorbic Acid and Caffeine as the surrogate reference standards against the Standard Method (B.P. 2007) were 26.0, 42.0 and 100.0 respectively. The F_{exp} are greater than the critical value of 9.605. Hence, the two methods differ significantly in their precision. The null hypothesis is rejected at the 95% probability level.

The F_{exp} of Chlorpheniramine Maleate tablets manufactured by Amponsah Effah Pharmaceutical Limited using Piroxicam, Ascorbic Acid and Caffeine as the surrogate reference standards against the Standard Method (B.P. 2007) were 2.488, 25.512 and 4.581 respectively. The calculated F-value obtained when Piroxicam and Caffeine were used as the surrogate reference standard is less than the critical value of 9.605. Hence, the two methods do not differ significantly in their precision when Piroxicam and Caffeine were used as the surrogate reference standard. However, the calculated F-value obtained when Ascorbic Acid was used as the surrogate reference standard is greater than the critical value of 9.605. Hence, the two methods differ significantly in their precision

when Ascorbic Acid was used as the surrogate reference standard. The null hypothesis is therefore accepted for Piroxicam and Caffeine as surrogate reference standard and is rejected when Ascorbic Acid was used as the surrogate reference standard at the 95% probability level.

The calculated F-test value of Chlorpheniramine Maleate tablets manufactured by Kinapharma Limited using Piroxicam, Ascorbic Acid and Caffeine as the surrogate reference standards against the Standard Method (B.P. 2007) were 5.969, 12.791 and 9.535 respectively. The calculated F-value obtained when Piroxicam and Caffeine were used as the surrogate reference standard is less than the critical value of 9.605. Hence, the two methods do not differ significantly in their precision when Piroxicam and Caffeine were used as the surrogate reference standard. However, the calculated F-value obtained when Ascorbic Acid was used as the surrogate reference standard is greater than the critical value of 9.605. Hence, the two methods differ significantly in their precision when Ascorbic Acid was used as the surrogate reference standard. The null hypothesis is therefore accepted for Piroxicam and Caffeine as surrogate reference standard and is rejected when Ascorbic Acid was used as the surrogate reference standard at the 95% probability level.

5.1.9.2 Relative Precision of the New Method to the Standard Method with respect to the Assay of Metformin Hydrochloride Tablets

Null Hypothesis: The Standard Method and the New Method do not differ in their precision.

Metformin Hydrochloride tablets manufactured by Hovid using Metronidazole and Paracetamol as the surrogate reference standards against the Standard Method (B.P. 2007) were 1.082 and 4.427 respectively. The calculated F-values are less than the critical value of 9.605. Hence, the two methods do not differ significantly in their precision. The null hypothesis is accepted at the 95% probability level.

Metformin Hydrochloride tablets manufactured by Denk using Metronidazole and Paracetamol as the surrogate reference standards against the Standard Method (B.P. 2007) were 1.073 and 2.393 respectively. The calculated F-values are less than the critical value of 9.605. Hence, the two methods do not differ significantly in their precision. The null hypothesis is accepted at the 95% probability level.

Metformin Hydrochloride tablets manufactured by Pharma DOR using Metronidazole and Paracetamol as the surrogate reference standards against the Standard Method (B.P. 2007) were 2.941 and 2.118 respectively. The calculated F-values are less than the critical value of 9.605. Hence, the two methods do not differ significantly in their precision. The null hypothesis is accepted at the 95% probability level.

Metformin Hydrochloride tablets manufactured by Ernest Chemist using Metronidazole and Paracetamol as the surrogate reference standards against the Standard Method (B.P. 2007) were 6.389 and 7.520 respectively. The calculated F-values are less than the

critical value of 9.605. Hence, the two methods do not differ significantly in their precision. The null hypothesis is accepted at the 95% probability level.

It is observed that of all the brands of Metformin Hydrochloride analyzed, the two methods did not differ significantly in their precision when Paracetamol and Metronidazole are used as surrogate reference standards. This could be attributed to the similarity in their physico-chemical parameter in terms of solubility.

5.2 Conclusion

This study sought to investigate the use of compounds that were physico-chemically related to assay Chlorpheniramine Maleate tablets and Metformin Hydrochloride tablets. This was made possible by the use of Caffeine, Ascorbic acid and Piroxicam as surrogate reference standards for the assay of Chlorpheniramine Maleate tablet and Paracetamol and Metronidazole for the assay of Metformin Hydrochloride tablets.

A mobile phase system of Phosphate buffer and Methanol (50:50) at a pH of 6.37 ± 0.02 was found to be appropriate for the assay of pure Chlorpheniramine Maleate and for that matter the percentage content of Chlorpheniramine Maleate in Chlorpheniramine Maleate tablet using Piroxicam, Ascorbic acid and Caffeine as surrogate reference standards. Acetate buffer and Methanol (70:30) at a pH of 5.46 ± 0.02 was also found to be appropriate for the assay of pure Metformin Hydrochloride and the percentage content of Metformin Hydrochloride in Metformin Hydrochloride tablet, using Metronidazole and Paracetamol as surrogate reference standards.

The percentage contents of both Chlorpheniramine Maleate and Metformin Hydrochloride in their tablets (formulations) were found when the various K values obtained from the analyses of their respective pure samples, using their corresponding surrogate reference standards were inserted into the hypothetical formular. The surrogate reference standards for Chlorpheniramine Maleate were Caffeine, Piroxicam and Ascorbic Acid and their average K values are 0.2224 ± 0.006, 0.8095 ± 0.003 and 0.1560 ± 0.002 respectively. The surrogate reference standards for Metformin Hydrochloride

were Metronidazole and Paracetamol and their respective average K values are 1.3262 ± 0.02 and 0.8623 ± 0.02.

Analytical Performance Parameters such as Limit of Detection (LOD), Limit of Quantitation (LOQ), Specificity and Selectivity, Repeatability and Robustness were carried out and appreciable results were obtained and hence the method developed was inferred to be accurate.

t-Test and F-Test were used as statistical tools to compare the two methods and also to test whether there was a significant difference between their precisions. The results obtained show that there was no significant difference between the two methods and in their precisions though some brands of both analytes show significant differences between the two methods.

Similarity in physico-chemical parameters between analyte and surrogate is favourable as observed in all the brands of Metformin Hydrochloride analyzed when Paracetamol and Metronidazole are used as surrogate reference standards, the two methods did not differ significantly in their precision because of the similarity in their solubility. Similar trend was observed in the analyses of Chlorpheniramine Maleate where the two methods did not differ significantly in their precision for all the brands when Piroxicam was used as the surrogate reference standard due to closeness of the wavelength of maximum absorption.

The percentage contents of both Chlorpheniramine Maleate and Metformin Hydrochloride in their respective tablets obtained from the assay procedures of both the

standard method and the new method were all within the control limits in the British Pharmacopoeia, which is the specification used.

By using the chromatographic conditions stated and the surrogates for both analytes, the new method is therefore valid and can be used to assay both pure and formulated Chlorpheniramine Maleate and Metformin Hydrochloride.

5.3 Recommendation

Though pure reference standards are required for the analysis of most pharmaceutical preparations, they are expensive. From the results of this study, it is possible to use surrogate reference standards for the same analysis of these pharmaceutical preparations and achieve comparable results. It is therefore recommended that more surrogate reference standards are found to carry out these analysis, especially by local institutions and regulatory bodies, in place of the pure reference standards.

The value of K was observed to be affected by the structure similarities and differences between the analyte and the surrogate reference standards with regard to the number of chromophores and auxochromes they both contain. Further research should be carried out to substantiate this observation.

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APPENDICES

Appendix I: Preparation of solutions

PS.1 Preparation of 1M Hydrochloric acid

36.5g/mol in 1000ml ≡ 1M HCl

9.125g/mol in 250ml ≡ 1M HCl

Percentage purity = 36%

36% = 9.125g/mol

So 100% = 25.35g/mol

Specific gravity of HCl = 1.18g/ml

Therefore volume = 25.35g / 1.18g/ml

= 21.5ml

Distilled water (100ml) was measured and poured into a 250ml volumetric flask. Hydrochloric acid (25.5ml) was pipetted into the flask and swirled for thorough mixing. It was then filled to the mark with distilled water and stoppered.

PS.2 Preparation of 0.1MPerchloric acid

8.5ml of Perchloric acid was slowly added to 900ml of glacial acetic acid with continuous and efficient mixing and 30ml of acetic anhydride was then added and the volume was made up to 1000ml with Acetic acid. This was done to prevent the formation of the explosive acetyl Perchlorate. The solution was left for 24 hours before using. This allows for the complete rendering of the mixture virtually anhydrous.

PS.3 Preparation of 0.2M Sodium Hydroxide

40g/mol NaOH in 1000ml ≡ 0.2M NaOH
1.6g/mol NaOH in 200ml ≡ 0.2M NaOH
Percentage purity of NaOH = 99.0%
Hence 100% = 1.6g
Therefore 1.6g of NaOH was weighed and dissolved in about 60ml of distilled water in a 200ml volumetric flask and made up to the mark. This is equal to 0.2M NaOH solution.

PS.4 Preparation of 0.1M Cerium (IV) Sulphate

632.55g/mol of Cerium in 1000ml = 1M Ce
63.255g of Cerium in 1000ml = 0.1M Ce

6.3255g of Cerium in 100ml = 0.1M Ce
Cerium (IV) Sulphate (6.3255g) was weighed and dissolved in distilled water (40ml) topping it up to 100ml in a 100ml volumetric flask. It was then stoppered and labeled.

PS.5 Preparation of 0.1M H_2SO_4

Specific gravity of H_2SO_4 = 1.835g/ml
98.05g H_2SO_4 in 1000ml ≡ 1M H_2SO_4
0.4904g/mol in 100ml ≡ 0.05MH_2SO_4
Percentage purity of H_2SO_4 = 98%
98% = 0.4904g/mol
100% ≡ 0.5g/mol
But volume, V = mass/specific gravity
Therefore V = 0.5g/1.835g/ml = 0.3ml
Hence 0.3ml of the stock solution of H_2SO_4 was taken and diluted to the 100ml mark with distilled water.

PS.6 Preparation of 0.05M Iodine
253.8g/mol of Iodine in 1000ml ≡ 0.05M I
1.27g/mol of Iodine in 100ml ≡ 0.05M I
Percentage purity of I = 99.0%
Hence 100% = 1.28g/mol
Therefore 1.28g of I was weighed and dissolved in about 60ml of distilled water in a 100ml and swirled vigorously and made up to the mark. This is equal to 0.05M I solution.

PS.7 Preparation of 0.1M $Na_2S_2O_3$

248g/mol of $Na_2S_2O_3$ in 1000ml ≡ 1M $Na_2S_2O_3$
2.48g/mol of $Na_2S_2O_3$ in 100ml ≡ 0.1M $Na_2S_2O_3$
Percentage purity of $Na_2S_2O_3$ = 98.0%
Hence 100% = 2.5g/mol
Therefore 2.5g of $Na_2S_2O_3$ was weighed and dissolved in about 60ml of distilled water in a 100ml and swirled vigorously and made up to the mark. This is equal to 0.1M $Na_2S_2O_3$ solution.

Appendix II: Assay of pure samples

AP.1 Chlorpheniramine Maleate

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

Amount of $C_8H_5KO_4$ weighed = 0.5007g

Nominal weight of $C_8H_5KO_4$ = 0.5g

Factor of $C_8H_5KO_4$ = actual weight / nominal weight

= 0.5007g / 0.5g

= 1.0014

TP.1 Titration table for the standardization of 0.1M Perchloric acid ($HClO_4$)

Burette readings (ml)	1st	2nd	Blank
Final reading	25.70	25.70	0.50
Initial reading	0.00	0.00	0.00
Titre	25.70	25.70	0.50

Titre value = (25.70 – 0.5) ml
= 25.20ml

Volume of $HClO_4$ = 25.20ml

Volume of $C_8H_5KO_4$ = 25.00ml

$F(HClO_4) = F(C_8H_5KO_4) \times V(C_8H_5KO_4) / V(HClO_4)$

= 1.0014 x 25.0ml / 25.20ml

= 0.9934

Assay

TC.2 Titration table for pure Chlorpheniramine Maleate

Burette readings (ml)	1st	2nd	Blank
Final reading	7.70(0.1501g)	7.71(0.1503)	0.10
Initial reading	0.00	0.00	0.00
Titre	7.70	7.71	0.10

Titre 1= 7.70ml
Titre value = 7.70ml – Blank (0.1ml)
= 7.60ml

Factor of perchloric acid = 0.9934

Actual titre = 7.6 x 0.9934
= 7.5498ml

1 ml of 0.1 M perchloric acid is equivalent to 0.01954g of $C_{20}H_{23}ClN_2O_4$.

Actual amount of Chlorpheniramine Maleate = 7.5498 x 0.01954g

= 0.1475g

Thus the percentage purity (titre 1) = (0.1475/0.15) x 100

= 98.34%

Titre 2= 7.71ml
Titre value = 7.71ml – Blank (0.1ml) = 7.61ml
Factor of perchloric acid = 0.9934

Actual titre = 7.61 x 0.9934
= 7.5597ml

1 ml of 0.1 M perchloric acid is equivalent to 0.01954g of $C_{20}H_{23}ClN_2O_4$.

Actual amount of Chlorpheniramine Maleate = 7.5498 x 0.01954g

= 0.1477g

Thus the percentage purity (titre 2) = (0.1477/0.15) x 100

= 98.46%
Therefore average percentage purity = (98.34 + 98.46)%/2
= 98.4%

AP.2 Caffeine

Assay

Table 4.6 Titration table for pure Caffeine

Burette readings (ml)	1st	2nd	Blank
Final reading	8.90(0.1702)	8.80(0.1700)	0.20
Initial reading	0.00	0.00	0.00
Titre	8.90	8.80	0.20

Titre 1= 8.90ml

Titre value = 8.90ml – Blank (0.2ml) = 8.70ml
Factor of perchloric Acid = 0.9934

Actual titre = 8.70 x 0.9934

= 8.6426ml

1 ml of 0.1 M Perchloric acid is equivalent to 0.01942g of $C_8H_{10}N_4O_2$.

Actual amount of Caffeine = 8.6426 x 0.01942g

= 0.1678g

Thus the percentage purity (titre 1) = (0.1678/0.17) x 100%

= 98.7%

The percentage purity (titre 2) = (0.16711/0.17) x 100

= 98.3%

Therefore average percentage purity = (98.7 + 98.3)%/2

= 98.5

AP.3 Piroxicam

Assay

Table 4.7 Titration table for pure Piroxicam

Burette readings (ml)	1st	2nd	Blank
Final reading	7.70(0.2501)	7.70(0.2501)	0.10
Initial reading	0.00	0.00	0.00
Titre	7.70	7.70	0.10

Titre (1)= 7.70

Titre value = 7.70ml – Blank (0.1ml)

= 7.60ml

Factor of perchloric Acid = 0.9934

Actual titre = 7.60 x 0.9934

= 7.5498ml

1 ml of 0.1 M perchloric acid is equivalent to 0.03314g of $C_{15}H_{13}N_3O_4S$.

Actual amount of Piroxicam = 7.5498 x 0.03314g

= 0.2502g

The percentage purity (titre 1) = (0.2502/0.25) x 100

= 100.08%

The percentage purity (titre 2) = (0.2502/0.25) x 100

= 100.08%

Therefore average percentage purity = (100.08 + 100.08)%/2

= 100.08

AP.4 Ascorbic acid

Assay

Table 4.8 Titration table for pure Ascorbic acid

Burette readings (ml)	1st	2nd	Blank
Final reading	17.00(0.1500)	17.20(0.01502)	0.10
Initial reading	0.00	0.00	0.00
Titre	17.00	17.20	0.10

Titre (1) = 17.00

Titre value = 17.00ml – Blank (0.1ml) = 16.90ml

If 1 ml of 0.05 M Iodine is equivalent to 0.00881g of $C_6H_8O_6$,
Then 16.90ml = 16.90 x 0.00881g
= 0.1489g

Therefore the percentage purity (titre 1) = (0.1489/0.15) x 100
= 99.27%
The percentage purity (titre 2) = (0.1507/0.15) x 100

= 100.5%
Therefore average percentage purity = (99.27 + 100.5)%/2
= 99.9%

AP.5 Metformin Hydrochloride

Assay

Table 4.9 Titration table for pure Metformin Hydrochloride

Burette readings (ml)	1st	2nd	Blank
Final reading	6.10(0.1001)	6.20(0.1003)	0.10
Initial reading	0.00	0.00	0.00
Titre	6.10	6.20	0.10

Titre = 6.10

Titre value = 6.10ml – Blank (0.1ml)
= 6.00ml
Factor of Perchloric Acid = 0.9934

Actual titre = 6.0 x 0.9934

= 5.9604ml

1 ml of 0.1 M Perchloric acid is equivalent to 0.01656g of Metformin Hydrochloride.

Actual amount of Metformin Hydrochloride = 5.9604 x 0.01656g

= 0.09834g

The percentage purity (Titre 1) = (0.09834/0.10) x 100

= 98.34%

The percentage purity (titre 2) = (0.1003/0.10) x 100

= 100.08%

Therefore average percentage purity = (98.34 + 100.3)%/2

= 99.3

AP.6 Metronidazole

Assay

Table 4.10 Titration table for pure Metronidazole

Burette readings (ml)	1st	2nd	Blank
Final reading	9.00(0.1500)	9.00(0.1500)	0.20
Initial reading	0.00	0.00	0.00
Titre	9.00	9.00	0.20

Titre (1) = 9.00

Titre value = 9.0ml – Blank (0.2ml)

= 8.80ml

Factor of Perchloric Acid = 0.9934

Actual titre = 8.80 x 0.9934

= 8.7419ml

1 ml of 0.1 M Perchloric acid is equivalent to 0.01712g of Metronidazole.

Actual amount of Metronidazole = 8.7419 x 0.01712g

= 0.1497g

The percentage purity (titre 1) = (0.1497/0.15) x 100

= 99.80%

The percentage purity (titre 2) = (0.1497/0.15) x 100

= 99.80%

Therefore average percentage purity = (99.80 + 99.80)%/2

= 99.80%

AP.7 Paracetamol

Assay

Table 4.11 Titration table for pure Paracetamol

Burette readings (ml)	1st	2nd	Blank
Final titre	8.00(0.3011)	8.10(0.3020)	0.10
Initial titre	0.00	0.00	0.00
Titre	7.90	8.10	0.10

Titre (ml) = 8.00

Actual titre (ml) = 8.00 – 0.10(Blank)

= 7.90ml

Mass of pure sample taken = 0.3011g

It was dissolved in 100ml of purified water and 20ml of the resulting solution was pipetted out and used for the titration.

If 100ml ≡ 0.3011g of pure Paracetamol powder,

20ml ≡ (20/100) x 0.3011g

≡ 0.06022g

From the British Pharmacopoeia,

1ml of 0.1M Ce ≡ 7.56mg of Paracetamol

Therefore 7.90ml of Ce ≡ $\frac{7.90\text{ml} \times 7.56\text{mg}}{1\text{ml}}$

≡ 59.724mg

= 0.059724g

Thus the percentage purity (titre 1) = $\frac{0.059724\text{g} \times 100}{0.06022\text{g}}$

= 99.2%

Thus the percentage purity (titre 2) = (0.06048/0.0604) x 100

= 100.1%

Therefore average percentage purity = (99.2 + 100.1)%/2

= 99.7%

Appendix III Uniformity of weight

UCK.1 Uniformity of weight of Chlorpheniramine Maleate tablets produced by Kinapharma Limited

Tablet	Weight (g)	Deviation	% Deviation
1	0.1693	0.0047	2.8554
2	0.1644	-0.0002	-0.1215
3	0.1652	0.0006	0.3645
4	0.1623	-0.0023	-1.3973
5	0.1658	0.0012	0.7290
6	0.1651	0.0005	0.3037
7	0.1654	0.0008	0.4860
8	0.1646	0	0.0000
9	0.1642	-0.0004	-0.2430
10	0.1664	0.0018	1.0935
11	0.1609	-0.0037	-2.2478
12	0.1627	-0.0019	-1.1543
13	0.1639	-0.0007	-0.4252
14	0.1657	0.0011	0.6682
15	0.1662	0.0016	0.9720
16	0.1663	0.0017	1.0328
17	0.1609	-0.0037	-2.2478
18	0.1662	0.0016	0.9720
19	0.1652	0.0006	0.3645
20	0.1613	-0.0033	-2.0048
Average weight = 0.1646			

UCA.2 Uniformity of weight of Chlorpheniramine Maleate tablets produced by Amponsah Effah Pharmaceuticals

Tablet	Weight (g)	Deviation	% Deviation
1	0.0977	-0.00316	-3.1330
2	0.1090	0.00814	8.0705
3	0.1000	-0.00086	-0.8526
4	0.0988	-0.00206	-2.0424
5	0.1074	0.00654	6.4842
6	0.1096	0.00874	8.6654
7	0.0992	-0.00166	-1.6458
8	0.1036	0.00274	2.7166
9	0.0990	-0.00186	-1.8441
10	0.0989	-0.00196	-1.9432
11	0.0994	-0.00146	-1.4475
12	0.1006	-0.00026	-0.2577
13	0.0961	-0.00476	-4.7194
14	0.1112	0.01034	10.2518
15	0.0989	-0.00196	-1.9432
16	0.1017	0.00084	0.8328
17	0.0942	-0.00666	-6.6032
18	0.0986	-0.00226	-2.2407
19	0.0970	-0.00386	-3.8270
20	0.0963	-0.00456	-4.5211
Average weight =0.1009			

UCP.3 Uniformity of weight of Chlorpheniramine Maleate tablets produced by Pharmanova Limited

Tablet	Weight (g)	Deviation	% Deviation
1	0.1141	0.000795	0.7016
2	0.1163	0.002995	2.6433
3	0.1119	-0.001405	-1.2400
4	0.1060	-0.007305	-6.4472
5	0.1121	-0.001205	-1.0635
6	0.1129	-0.000405	-0.3574
7	0.1103	-0.003005	-2.6521
8	0.1069	-0.006405	-5.6528
9	0.1151	0.001795	1.5842
10	0.1158	0.002495	2.2020
11	0.1194	0.006095	5.3792
12	0.1150	0.001695	1.4959
13	0.1160	0.002695	2.3785
14	0.1126	-0.000705	-0.6222
15	0.1179	0.004595	4.0554
16	0.1093	-0.004005	-3.5347
17	0.1143	0.000995	0.8781
18	0.1142	0.000895	0.7899
19	0.1143	0.000995	0.8781
20	0.1117	-0.001605	-1.4165
Average weight = 0.113305			

UCL.4 Uniformity of weight of Chlorpheniramine Maleate Tablets produced by Letap Pharmaceuticals

Tablet	Weight (g)	Deviation	% Deviation
1	0.1294	0.002415	1.9017
2	0.1275	0.000515	0.4055
3	0.1287	0.001715	1.3505
4	0.1258	-0.001185	-0.9331
5	0.1278	0.000815	0.6418
6	0.1220	-0.004985	-3.9256
7	0.1272	0.000215	0.1693
8	0.1262	-0.000785	-0.6181
9	0.1267	-0.000285	-0.2244
10	0.1255	-0.001485	-1.1694
11	0.1260	-0.000985	-0.7756
12	0.1262	-0.000785	-0.6181
13	0.1286	0.001615	1.2718
14	0.1254	-0.001585	-1.2481
15	0.1283	0.001315	1.0355
16	0.1256	-0.001385	-1.0906
17	0.1265	-0.000485	-0.3819
18	0.1264	-0.000585	-0.4606
19	0.1291	0.002115	1.6655
20	0.1308	0.003815	3.0042
Average weight = 0.126985			

UMH.5 Uniformity of weight of Metformin Hydrochloride tablets produced by Hovid

Tablet	Weight (g)	Deviation	% Deviation
1	0.6699	0.01	1.5153
2	0.6591	-0.0008	-0.1212
3	0.6484	-0.0115	-1.7426
4	0.6666	0.0067	1.0153
5	0.6649	0.005	0.7576
6	0.6627	0.0028	0.4243
7	0.6663	0.0064	0.9698
8	0.6657	0.0058	0.8789
9	0.6587	-0.0012	-0.1818
10	0.6544	-0.0055	-0.8334
11	0.6634	0.0035	0.5303
12	0.6548	-0.0051	-0.7728
13	0.6618	0.0019	0.2879
14	0.6585	-0.0014	-0.2121
15	0.659	-0.0009	-0.1363
16	0.6574	-0.0025	-0.3788
17	0.6537	-0.0062	-0.9395
18	0.6626	0.0027	0.4091
19	0.6552	-0.0047	-0.7122
20	0.6549	-0.005	-0.7576
		Average weight = 0.6599	

UMD.6 Uniformity of weight of Metformin Hydrochloride tablets produced by Denk

Tablet	Weight (g)	Deviation	% Deviation
1	0.5637	0.00174	0.3096
2	0.5726	0.01064	1.8933
3	0.5556	-0.00636	-1.1317
4	0.5554	-0.00656	-1.1673
5	0.5707	0.00874	1.5552
6	0.5559	-0.00606	-1.0783
7	0.5639	0.00194	0.3452
8	0.5657	0.00374	0.6655
9	0.5641	0.00214	0.3808
10	0.554	-0.00796	-1.4164
11	0.5604	-0.00156	-0.2775
12	0.5569	-0.00506	-0.9004
13	0.565	0.00304	0.5409
14	0.5589	-0.00306	-0.5445
15	0.5545	-0.00746	-1.3274
16	0.5665	0.00454	0.8078
17	0.5635	0.00154	0.2740
18	0.5622	0.00024	0.0427
19	0.5627	0.00074	0.1316
20	0.567	0.00504	0.8968
Average weight = 0.56196			

UMP.7 Uniformity of weight of Metformin Hydrochloride tablets produced by Pharma DOR

Tablet	Weight (g)	Deviation	% Deviation
1	0.5914	0.003755	0.6389
2	0.5968	0.009155	1.5579
3	0.597	0.009355	1.5919
4	0.5879	0.000255	0.0433
5	0.5796	-0.008045	-1.3690
6	0.5789	-0.008745	-1.4881
7	0.573	-0.014645	-2.4921
8	0.5651	-0.022545	-3.8364
9	0.5992	0.011555	1.9663
10	0.5714	-0.016245	-2.7644
11	0.6003	0.012655	2.1535
12	0.6171	0.029455	5.0123
13	0.5996	0.011955	2.0343
14	0.5929	0.005255	0.8942
15	0.6108	0.023155	3.9403
16	0.5827	-0.004945	-0.8414
17	0.5626	-0.025045	-4.2619
18	0.6073	0.019655	3.3447
19	0.5694	-0.018245	-3.1047
20	0.5699	-0.017745	-3.0196
Average weight = 0.587645			

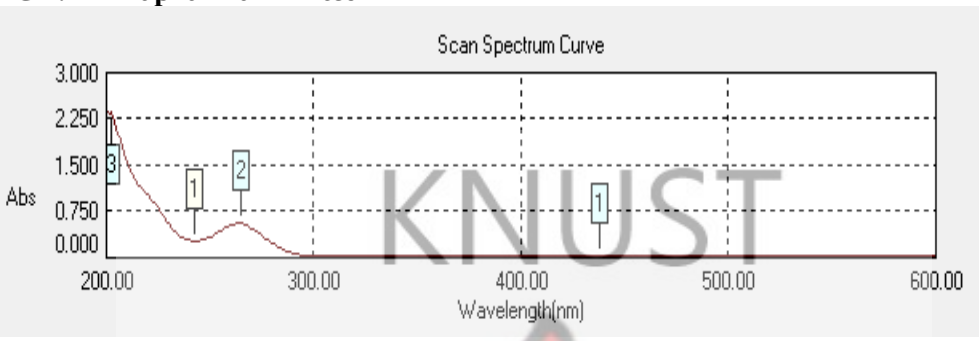
UME.8 Uniformity of weight of Metformin Hydrochloride tablets produced by Ernest Chemist

Tablet	Weight (g)	Deviation	% Deviation
1	0.6279	0.00084	0.1339
2	0.6321	0.00504	0.8037
3	0.6313	0.00424	0.6761
4	0.6299	0.00284	0.4529
5	0.6303	0.00324	0.5166
6	0.6341	0.00704	1.1226
7	0.6488	0.02174	3.4669
8	0.632	0.00494	0.7878
9	0.5953	-0.03176	-5.0649
10	0.624	-0.00306	-0.4879
11	0.6061	-0.02096	-3.3425
12	0.6265	-0.00056	-0.0893
13	0.6288	0.00174	0.2774
14	0.644	0.01694	2.7014
15	0.6179	-0.00916	-1.4607
16	0.6202	-0.00686	-1.0939
17	0.6255	-0.00156	-0.2487
18	0.6159	-0.01116	-1.7797
19	0.6346	0.00754	1.2024
20	0.636	0.00894	1.4257
	Average weight = 0.62706		

Appendix IV Percentage content of analytes using Standard Method from the British Pharmacopoeia, 2007

PCC.1 Percentage content of Chlorpheniramine Maleate in Chlorpheniramine Maleate tablet using standard method

PCK.1 Kinapharma Limited



USK.1 UV Spectrum at 265nm of Chlorpheniramine Maleate tablets produced by Kinapharma Limited

TAK.1Table of absorbance and wavelength of Chlorpheniramine Maleate tablets produced by Kinapharma Limited

No.	P/V	Wavelength(nm)	Absorbance
1	Peak	438.00	0.024
2	Peak	265.00	0.532
3	Peak	203.00	2.361
1	Valley	243.00	0.26

Absorbance = 0.532 at a wavelength of 265.00nm

A quantity of the powder containing 3mg of Chlorpheniramine Maleate was diluted to 50ml with 0.25M Sulphuric acid. This contains 0.006%w/v of Chlorpheniramine Maleate. 10ml was again diluted to 25ml with 0.25M Sulphuric acid making the final concentration of the Chlorpheniramine Maleate to be 0.0024% w/v.

Absorbance = abc , where: a = A(1%, 1cm) = 212

b = path length

c = concentration

therefore concentration c = 0.532/212x1cm

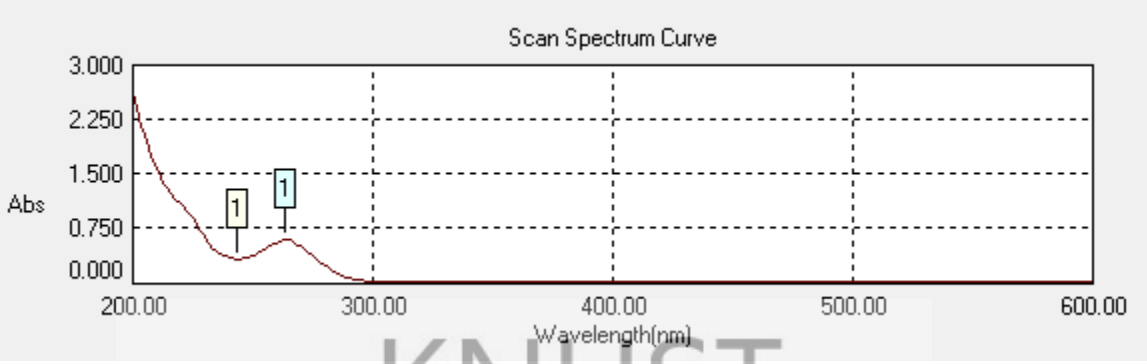
= 0.002509% w/v

Hence the percentage content of Chlorpheniramine Maleate in the tablet

= (0.002509/0.0024)% x 100

= 104.5%

PCP.2 Pharmanova Limited



USP.2 UV Spectrum at 265nm of Chlorpheniramine Maleate tablets produced by Pharmanova Limited

USP.2 Table of absorbance and wavelength of Chlorpheniramine Maleate tablets produced by Pharmanova Limited

No.	P/V	Wavelength(nm)	Absorbance
1	Peak	264.00	0.482
1	Valley	244.00	0.331

Absorbance = 0.482 at a wavelength of 264.00nm

A quantity of the powder containing 3mg of Chlorpheniramine Maleate was diluted to 50ml with 0.25M Sulphuric acid. This contains 0.006%w/v of Chlorpheniramine Maleate. 10ml was again diluted to 25ml with 0.25M Sulphuric acid making the final concentration of the Chlorpheniramine Maleate to be 0.0024% w/v.

Absorbance = abc , where: a = A(1%, 1cm) = 212

b = path length

c = concentration

therefore concentration c = 0.482/212x1cm

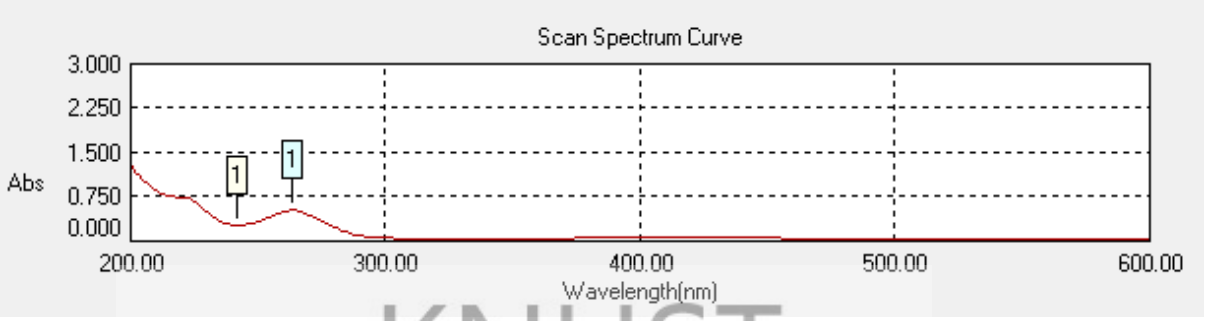
= 0.00227% w/v

Hence the percentage content of Chlorpheniramine Maleate in the tablet

= (0.00227/0.0024)% x 100

= 94.6%

PCL.3 Letap Pharmaceuticals



USL.3 UV Spectrum at 265nm of Chlorpheniramine Maleate tablets produced by Letap Pharmaceuticals

TAL.3 Table of absorbance and wavelength of Chlorpheniramine Maleate tablets produced by Letap Pharmaceuticals

No.	P/V	Wavelength(nm)	Absorbance
1	Peak	264.00	0.512
1	Valley	242.00	0.257

Absorbance = 0.512 at a wavelength of 264.00nm

A quantity of the powder containing 3mg of Chlorpheniramine Maleate was diluted to 50ml with 0.25M Sulphuric acid. This contains 0.006%w/v of Chlorpheniramine Maleate. 10ml was again diluted to 25ml with 0.25M Sulphuric acid making the final concentration of the Chlorpheniramine Maleate to be 0.0024% w/v.

Absorbance = abc , where: a = A(1%, 1cm) = 212

b = path length

c = concentration

therefore concentration c = 0.512/212x1cm

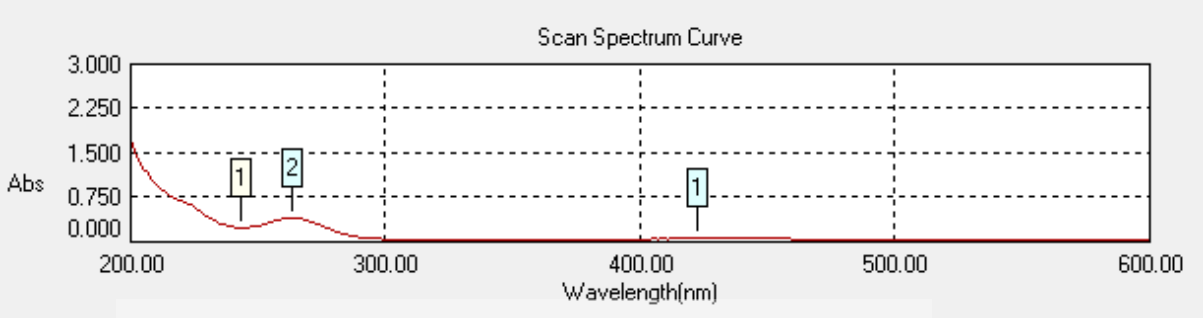
= 0.002415% w/v

Hence the percentage content of Chlorpheniramine Maleate in the tablet

= (0.002415/0.0024)% x 100

= 100.6%

PCA.4 Amponsah Effah Pharmaceuticals



USA.4 UV Spectrum at 265nm of Chlorpheniramine Maleate tablets produced by Amponsah Effah Pharmaceuticals

TAA.4 Table of absorbance and wavelength of Chlorpheniramine Maleate tablets produced by Amponsah Effah Pharmaceuticals

No.	P/V	Wavelength(nm)	Absorbance
1	Peak	423.00	0.039
2	Peak	264.00	0.502
1	Valley	244.00	0.226

Absorbance = 0.502 at a wavelength of 264.00nm

A quantity of the powder containing 3mg of Chlorpheniramine Maleate was diluted to 50ml with 0.25M Sulphuric acid. This contains 0.006%w/v of Chlorpheniramine Maleate. 10ml was again diluted to 25ml with 0.25M Sulphuric acid making the final concentration of the Chlorpheniramine Maleate to be 0.0024% w/v.

Absorbance = abc , where: a = A(1%, 1cm) = 212

b = path length

c = concentration

therefore concentration c = 0.502/212x1cm

= 0.002367% w/v

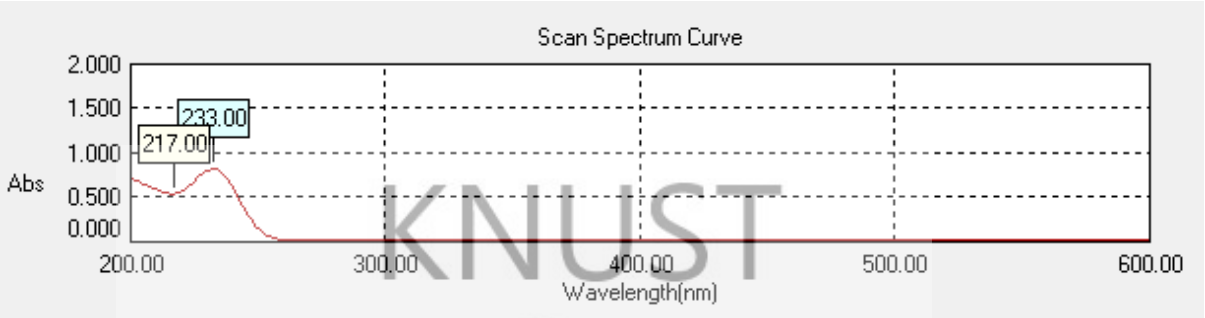
Hence the percentage content of Chlorpheniramine Maleate in the tablet

= (0.002367/0.0024)% x 100

= 98.6%

PCM.2 Percentage content of Metformin Hydrochloride in Metformin Hydrochloride tablets using standard method

PCH.5 Hovid Bdh.



USH.5 UV Spectrum at 233nm of Metformin Hydrochloride tablets manufactured by Hovid

TAH.5 Table of absorbance and wavelength of Metformin Hydrochloride tablets manufactured by Hovid

No.	P/V	Wavelength(nm)	Abs
1	Peak	233.00	0.831
1	Valley	217.00	0.537

Absorbance = 0.831 at a wavelength of 233.00nm

A quantity of the powder containing 0.1g of Metformin Hydrochloride was diluted to 100ml with distilled water. This contains 0.1%w/v of Metformin Hydrochloride. 10ml was again diluted to 100ml with distilled water and 10ml was again diluted to 100ml with distilled water making the final concentration of the Metformin Hydrochloride to be 0.001% w/v.

Absorbance = abc, where: a = A(1%, 1cm) = 798

b = path length

c = concentration

therefore concentration c = 0.831/798x1cm

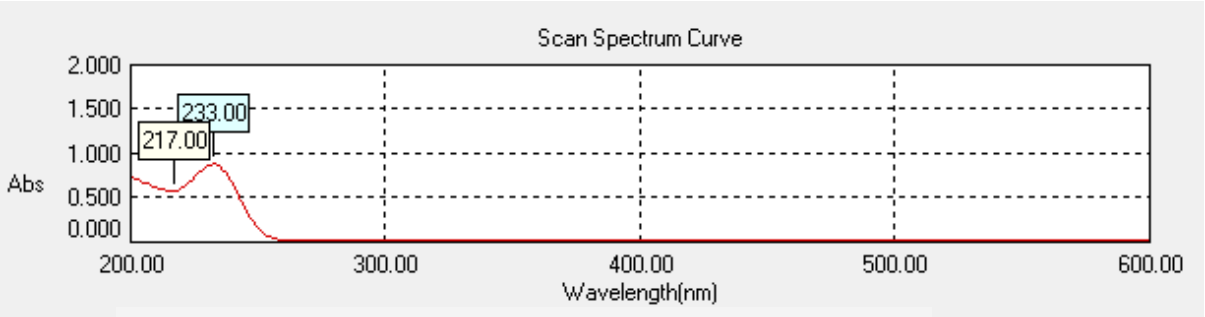
= 0.0010413% w/v

Hence the percentage content of Metformin Hydrochloride in the tablet

= (0.0010413/0.001)% x 100

= 104.1%

PCPD.6 Pharma DOR



USPD.5 UV Spectrum at 233nm of Metformin Hydrochloride tablets manufactured by Pharma DOR

TAPD.5 Table of absorbance and wavelength of Metformin Hydrochloride tablets manufactured by Pharma DOR

No.	P/V	Wavelength(nm)	Abs
1	Peak	233.00	0.765
1	Valley	217.00	0.569

Absorbance = 0.765 at a wavelength of 233.00nm

A quantity of the powder containing 0.1g of Metformin Hydrochloride was diluted to 100ml with distilled water. This contains 0.1%w/v of Metformin Hydrochloride. 10ml was again diluted to 100ml with distilled water and 10ml was again diluted to 100ml with distilled water making the final concentration of the Metformin Hydrochloride to be 0.001% w/v.

Absorbance = abc , where: a = A(1%, 1cm) = 798

b = path length

c = concentration

therefore concentration c = 0.765/798x1cm

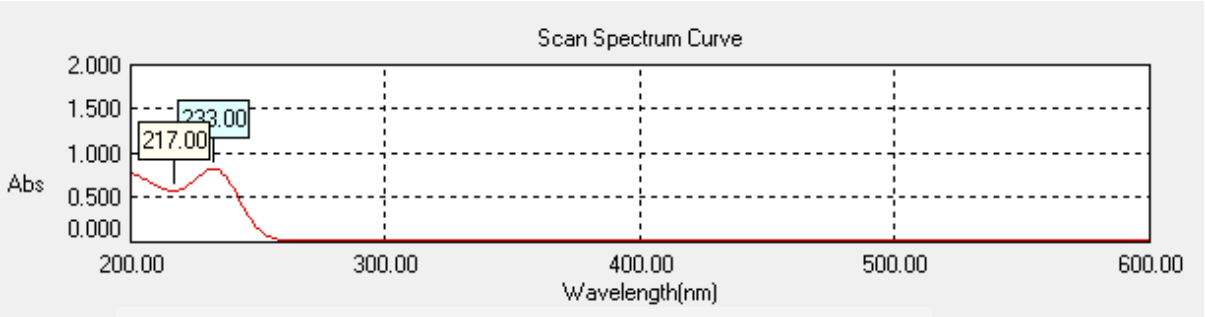
= 0.000959% w/v

Hence the percentage content of Metformin Hydrochloride in the tablet

= (0.000959/0.001)% x 100

= 95.9%

PCD.7 Denk



USD.7 UV Spectrum at 233nm of Metformin Hydrochloride tablets manufactured by Denk

TAD.7 Table of absorbance and wavelength of Metformin Hydrochloride tablets manufactured by Denk

No.	P/V	Wavelength(nm)	Abs
1	Peak	233.00	0.799
1	Valley	217.00	0.572

Absorbance = 0.799 at a wavelength of 233.00nm

A quantity of the powder containing 0.1g of Metformin Hydrochloride was diluted to 100ml with distilled water. This contains 0.1%w/v of Metformin Hydrochloride. 10ml was again diluted to 100ml with distilled water and 10ml was again diluted to 100ml with distilled water making the final concentration of the Metformin Hydrochloride to be 0.001% w/v.

Absorbance = abc , where: a = A(1%, 1cm) = 798

b = path length

c = concentration

therefore concentration c = 0.799/798x1cm

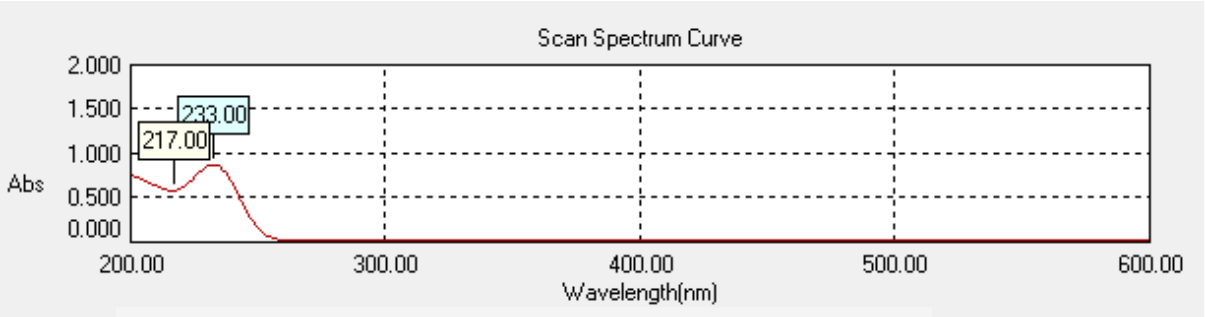
= 0.001001% w/v

Hence the percentage content of Metformin Hydrochloride in the tablet

= (0.001001/0.001)% x 100

= 100.1%

PCE.8 Ernest Chemist



USE.8 UV Spectrum at 233nm of Metformin Hydrochloride tablets manufactured by Ernest Chemist

TAE.8 Table of absorbance and wavelength of Metformin Hydrochloride tablets manufactured by Ernest Chemist

No.	P/V	Wavelength(nm)	Abs
1	Peak	233.00	0.796
1	Valley	217.00	0.576

Absorbance = 0.796 at a wavelength of 233.00nm

A quantity of the powder containing 0.1g of Metformin Hydrochloride was diluted to 100ml with distilled water. This contains 0.1%w/v of Metformin Hydrochloride. 10ml was again diluted to 100ml with distilled water and 10ml was again diluted to 100ml with distilled water making the final concentration of the Metformin Hydrochloride to be 0.001% w/v.

Absorbance = abc , where: a = A(1%, 1cm) = 798

b = path length

c = concentration

therefore concentration c = 0.796/798x1cm

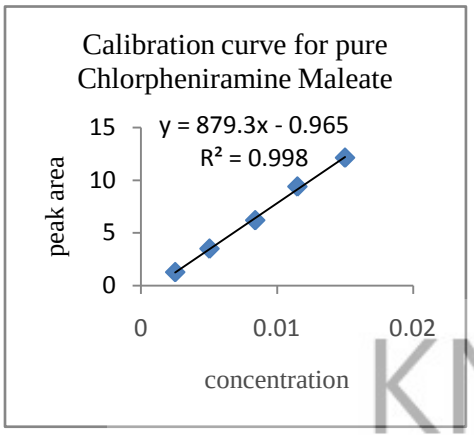
= 0.000997% w/v

Hence the percentage content of Metformin Hydrochloride in the tablet

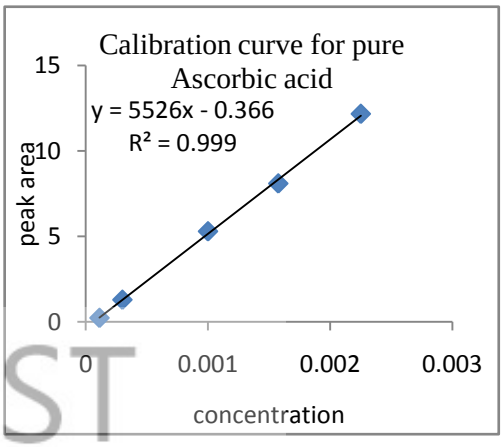
= (0.000997/0.001)% x 100

= 99.7%

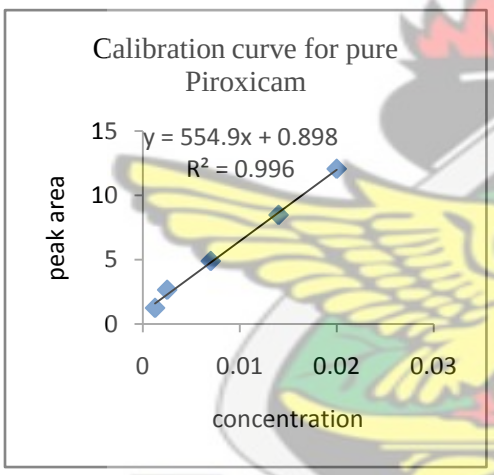
Appendix V Calibration curves of pure samples



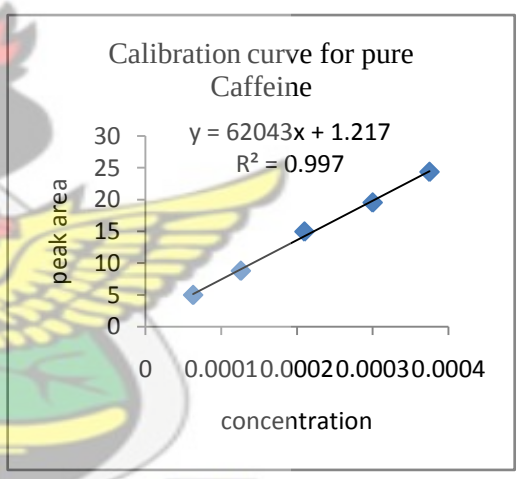
CCC.1 Calibration curve for Chlorpheniramine Maleate



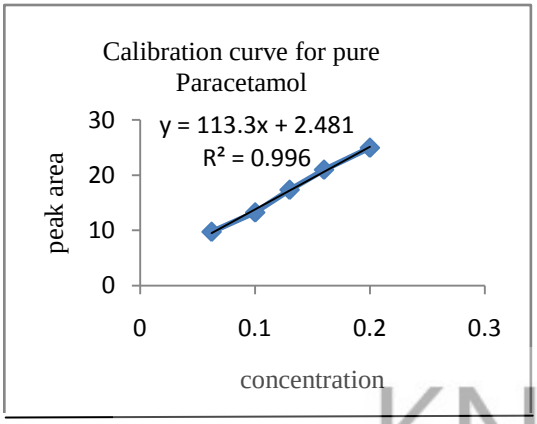
CCA.2 Calibration curve for Ascorbic acid



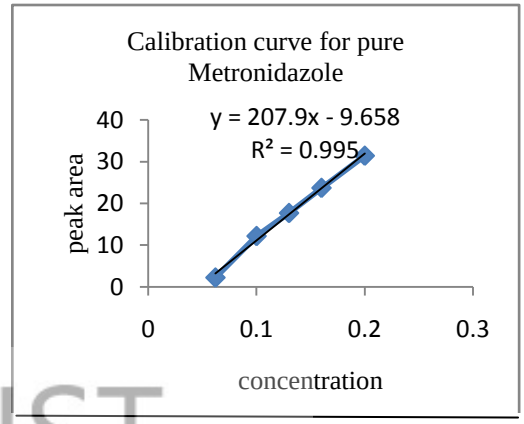
CCP.3 Calibration curve for Piroxicam



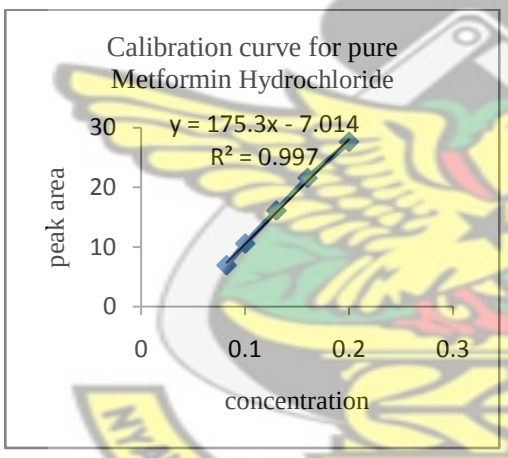
CCC.4 Calibration curve for Caffeine



CCP.5 Calibration curve for Paracetamol



CCM.6 Calibration curve for Metronidazole



CCC.7 Calibration curve for Metformin Hydrochloride

Appendix VI Linearity

LC.1 Table of linearity of Chlorpheniramine Maleate

Linearity of Chlorpheniramine Maleate; Concentration (%w/v) range = 0.015-0.00252	
Equation of Line	**Correlation coefficient, R^2**
y = 879.32x - 0.9652	0.9984
y = 879.32x - 0.9649	0.9982
y = 879.32x - 0.9640	0.9979

LA.2 Table of linearity of Ascorbic acid

Linearity of Ascorbic acid; Concentration (%w/v) range = 0.00225-0.000113	
Equation of Line	**Correlation coefficient, R^2**
y = 5526x - 0.3664	0.9991
y = 5526x - 0.3669	0.9989
y = 5526x - 0.3672	0.9981

LP.3 Table of linearity of Piroxicam

Linearity of Piroxicam; Concentration (%w/v) range = 0.02-0.00125	
Equation of Line	**Correlation coefficient, R^2**
y = 554.98x + 0.8989	0.9960
y = 554.98x + 0.8992	0.9910
y = 554.98x + 0.8996	0.9820

LC.4 Table of linearity of Caffeine

Linearity of Caffeine; Concentration (%w/v) range = 0.000357-0.000063	
Equation of Line	**Correlation coefficient, R^2**
y = 62043x + 1.2171	0.9952
y = 62043x + 1.2172	0.9972
y = 62043x + 1.2170	0.9982

LM.5 Table of linearity of Metformin Hydrochloride

Linearity of Metformin Hydrochloride; Concentration (%w/v) range = 0.2 – 0.082	
Equation of Line	**Correlation coefficient, R^2**
y = 175.37x - 7.0142	0.9973
y = 175.37x - 7.0141	0.9972
y = 175.37x - 7.0143	0.9970

LM.6 Table of linearity of Metronidazole

Linearity of Metronidazole; Concentration (%w/v) range = 0.2 – 0.062	
Equation of Line	**Correlation coefficient, R^2**
y = 207.98x – 9.6588	0.9950
y = 207.98x – 9.6589	0.9960
y = 207.98x – 9.6579	0.9930

LP.7 Table of linearity of Paracetamol

Linearity of Paracetamol; Concentration (%w/v) range = 0.2 – 0.062	
Equation of Line	**Correlation coefficient, R^2**
y = 113.33x + 2.4817	0.9961
y = 113.33x + 2.4817	0.9961
y = 113.33x + 2.4817	0.9961

Appendix VII Percentage content of Chlorpheniramine Maleate and Metformin Hydrochloride using K values

DPM.1 Determination of Metformin Hydrochloride in Metformin Hydrochloride tablets

PMHP.1 Percentage content of Metformin Hydrochloride in Metformin Hydrochloride tablets manufactured by Hovid Bdh using Paracetamol as the surrogate reference standard.

K value = 0.8623

Peak area of Metformin Hydrochloride, Aa	Concentration (%w/v) of Paracetamol, Cs	Area of Paracetamol, As	(Aa x Cs) / (K x As)	Concentration (%w/v) of Metformin Hydrochloride	Percentage content
18.50	0.24000	20.64	0.2495	0.2400	104.0
13.99	0.09600	15.48	0.0998	0.0996	104.8
8.46	0.02880	9.40	0.0299	0.0288	104.3
1.87	0.01008	2.10	0.0104	0.0101	103.3
0.82	0.00252	0.93	0.0026	0.0252	102.3

PMHM.2 Percentage content of Metformin Hydrochloride in Metformin Hydrochloride tablets manufactured by Hovid Bdh using Metronidazole as the surrogate reference standard.

K value = 1.3262

Peak area of Metformin Hydrochloride, Aa	Concentration (%w/v) of Metronidazole, Cs	Area of Metronidazole, As	(Aa x Cs) / (K x As)	Concentration (%w/v) of Metformin Hydrochloride, Ca	Percentage content
15.82	0.240000	11.45	0.250040	0.24000	104.2
11.45	0.096000	8.23	0.100700	0.09600	104.9
3.49	0.028800	2.51	0.030000	0.02880	104.8
1.31	0.008640	0.95	0.008980	0.00864	103.9
0.58	0.001728	0.42	0.001798	0.00173	104.1

PMPM.3 Percentage content of Metformin Hydrochloride in Metformin Hydrochloride tablets manufactured by Pharma DOR using Metronidazole as the surrogate reference standard

K value = 1.3262

Peak area of Metformin Hydrochloride, Aa	Concentration (%w/v) of Metronidazole, Cs	Area of Metronidazole, As	(Aa x Cs) / (K x As)	Concentration (%w/v) of Metformin Hydrochloride, Ca	Percentage content
17.60	0.24000	13.89	0.24000	0.24000	95.5
8.98	0.08400	7.05	0.08400	0.08400	96.0
3.00	0.02520	2.35	0.02520	0.02520	96.2
1.39	0.00630	1.09	0.00630	0.00630	96.2
0.46	0.00189	0.36	0.00189	0.00189	96.3

PMPP.4 Percentage content of Metformin Hydrochloride in Metformin Hydrochloride tablets manufactured by Pharma DOR using Paracetamol as the surrogate reference standard.

K value = 0.8623

Peak area of Metformin Hydrochloride, Aa	Concentration (%w/v) of Paracetamol, Cs	Area of Paracetamol, As	(Aa x Cs) / (K x As)	Concentration (%w/v) of Metformin Hydrochloride, Ca	Percentage content
17.59	0.24000	21.20	0.23090	0.2400	101.2
11.25	0.07200	13.68	0.06867	0.0720	101.3
5.03	0.02520	5.77	0.02548	0.0252	101.1
1.63	0.00504	1.91	0.005141	0.00504	102.0
0.68	0.00126	0.78	0.001274	0.00126	101.1

PMEM.5 Percentage content of Metformin Hydrochloride in Metformin Hydrochloride tablets manufactured by Ernest Chemist using Metronidazole as the surrogate reference standard. K value = 1.3262

Peak area of Metformin Hydrochloride, Aa	Concentration (%w/v) of Metronidazole, Cs	Area of Metronidazole, As	(Aa x Cs) / (K x As)	Concentration (%w/v) of Metformin Hydrochloride, Ca	Percentage content
19.47	0.24000	14.04	0.25104	0.240	104.6
8.37	0.084000	7.05	0.07511	0.072	104.4
2.45	0.029400	2.42	0.02244	0.0216	103.9
0.93	0.007350	0.76	0.006782	0.0648	104.7
0.44	0.002205	0.43	0.001701	0.00162	105.0

PMEP.6 Percentage content of Metformin Hydrochloride in Metformin Hydrochloride tablets manufactured by Ernest Chemist using Paracetamol as the surrogate reference standard.

K value = 0.8623

Peak area of Metformin Hydrochloride, Aa	Concentration (%w/v) of Paracetamol, Cs	Area of Paracetamol, As	(Aa x Cs) / (K x As)	Concentration (%w/v) of Metformin Hydrochloride, Ca	Percentage content
18.41	0.2400	21.19	0.23620	0.24000	98.4
12.90	0.0840	17.63	0.07130	0.07200	99.0
6.91	0.0294	11.09	0.02120	0.02160	98.3
3.01	0.00735	4.03	0.00636	0.00648	98.2
2.45	0.002205	3.95	0.001587	0.00162	98.0

PMDM.7 Percentage content of Metformin Hydrochloride in Metformin Hydrochloride tablets manufactured by Denk using Metronidazole as the surrogate reference standard.

K value = 1.3262

Peak area of Metformin Hydrochloride, Aa	Concentration (%w/v) of Metronidazole, Cs	Area of Metronidazole, As	(Aa x Cs) / (K x As)	Concentration (%w/v) of Metformin Hydrochloride, Ca	Percentage content
21.82	0.24000	16.59	0.2380	0.24000	99.2
11.11	0.07200	8.44	0.0716	0.07200	99.3
7.49	0.02160	5.64	0.0216	0.02160	100.1
3.68	0.00649	2.81	0.00639	0.00649	98.8
2.01	0.00259	1.52	0.00259	0.00259	99.7

PMDP.8 Percentage content of Metformin Hydrochloride in Metformin Hydrochloride tablets manufactured by Denk using Paracetamol as the surrogate reference standard. K value = 0.8623

Peak area of Metformin Hydrochloride, Aa	Concentration (%w/v) of Paracetamol, Cs	Area of Paracetamol, As	(Aa x Cs) / (K x As)	Concentration (%w/v) of Metformin Hydrochloride, Ca	Percentage content
17.74	0.24000	20.53	0.24050	0.24000	100.2
7.15	0.07200	8.25	0.07330	0.07200	100.5
5.05	0.02160	5.87	0.02155	0.02160	99.7
3.03	0.00649	3.55	0.00641	0.00649	99.0
2.31	0.00259	2.67	0.00260	0.00259	100.2

DPC.2 Determination of Chlorpheniramine Maleate in Chlorpheniramine Maleate tablets

PCLP.9 Percentage content of Chlorpheniramine Maleate in Chlorpheniramine Maleate tablets manufactured by Letap Pharmaceuticals using Piroxicam as the surrogate reference standard

K value = 0.8095

Peak area of Chlorpheniramine Maleate, Aa	Concentration (%w/v) of Piroxicam, Cs	Area of Piroxicam, As	(Aa x Cs) / (K x As)	Concentration (%w/v) of Chlorpheniramine Maleate, Ca	Percentage content
6.65	0.02000	10.30	0.015951	0.016000	99.6
4.31	0.01300	7.21	0.009798	0.009730	100.7
2.22	0.00680	3.82	0.004875	0.004865	100.2
1.31	0.00300	1.71	0.002916	0.002919	99.9
0.67	0.00135	0.77	0.001468	0.001459	100.6

PCLC.10 Percentage content of Chlorpheniramine Maleate in Chlorpheniramine Maleate tablets manufactured by Letap Pharmaceuticals using Caffeine as the surrogate reference standard.

K value = 0.2224

Peak area of Chlorpheniramine Maleate, Aa	Concentration (%w/v) of Caffeine, Cs	Area of Caffeine, As	(Aa x Cs) / (K x As)	Concentration (%w/v) of Chlorpheniramine Maleate, Ca	Percentage content
15.87	0.009800	17.14	0.04080	0.04030	101.2
14.11	0.006860	13.47	0.03230	0.03220	100.2
12.67	0.003430	8.64	0.02262	0.02260	100.1
9.14	0.002058	7.39	0.01144	0.01130	101.2
5.66	0.001029	6.82	0.00384	0.00385	99.8

PCLA.11 Percentage content of Chlorpheniramine Maleate in Chlorpheniramine Maleate tablets manufactured by Letap Pharmaceuticals using Ascorbic acid, as the surrogate reference standard. K value = 0.156

Peak area of Chlorpheniramine Maleate, Aa	Concentration (%w/v) of Ascorbic acid, Cs	Area of Ascorbic acid, As	(Aa x Cs) / (K x As)	Concentration (%w/v) of Chlorpheniramine Maleate, Ca	Percentage content
11.77	0.002300	13.44	0.012910	0.0135	95.6
7.72	0.001840	11.66	0.007810	0.0081	96.4
5.91	0.000920	8.86	0.003932	0.0041	95.9
4.81	0.000552	7.19	0.002380	0.0024	98.2
2.01	0.000441	2.39	0.007124	0.0073	97.6

PCPC.12 Percentage content of Chlorpheniramine Maleate in Chlorpheniramine Maleate tablets manufactured by Pharmanova Limited using Caffeine as the surrogate reference standard. K value = 0.2224

Peak area of Chlorpheniramine Maleate, Aa	Concentration (%w/v) of Caffeine, Cs	Area of Caffeine, As	(Aa x Cs) / (K x As)	Concentration (%w/v) of Chlorpheniramine Maleate, Ca	Percentage content
12.49	0.009800	32.37	0.01700	0.01800	95.6
10.87	0.007840	28.34	0.01350	0.01440	93.9
9.03	0.003920	16.78	0.00948	0.01008	94.0
7.66	0.0027440	19.73	0.00479	0.00504	95.1
4.28	0.0008232	11.24	0.00141	0.00151	93.1

PCPA.13 Percentage content of Chlorpheniramine Maleate in Chlorpheniramine Maleate tablets manufactured by Pharmanova Limited using Ascorbic acid as the surrogate reference standard. K value = 0.156

Peak area of Chlorpheniramine Maleate, Aa	Concentration (%w/v) of Ascorbic acid, Cs	Area of Ascorbic acid, As	(Aa x Cs) / (K x As)	Concentration (%w/v) of Chlorpheniramine Maleate, Ca	Percentage content
10.38	0.002300	9.09	0.01683	0.01800	93.5
9.01	0.001840	7.81	0.01360	0.01440	94.3
8.11	0.000920	5.06	0.00946	0.01008	93.8
6.25	0.000552	4.67	0.00474	0.00504	94.1
2.76	0.000442	5.42	0.00144	0.00151	95.2

PCPP.14 Percentage content of Chlorpheniramine Maleate in Chlorpheniramine Maleate tablets manufactured by Pharmanova Limited using Piroxicam as the surrogate reference standard. K value = 0.8095

Peak area of Chlorpheniramine Maleate, Aa	Concentration (%w/v) of Piroxicam, Cs	Area of Piroxicam, As	(Aa x Cs) / (K x As)	Concentration (%w/v) of Chlorpheniramine Maleate, Ca	Percentage content
6.77	0.02000	10.54	0.01586	0.01700	93.3
5.28	0.01400	7.15	0.00127	0.01360	93.9
4.01	0.00700	4.51	0.00769	0.00816	94.2
2.63	0.00250	2.12	0.00384	0.00408	94.0
0.91	0.00125	0.61	0.00232	0.002448	94.7

PCKA.15 Percentage content of Chlorpheniramine Maleate in Chlorpheniramine Maleate tablets manufactured by Kinapharma Limited using Ascorbic acid as the surrogate reference standard. K value = 0.156

Peak area of Chlorpheniramine Maleate, Aa	Concentration (%w/v) of Ascorbic acid, Cs	Area of Ascorbic acid, As	(Aa x Cs) / (K x As)	Concentration (%w/v) of Chlorpheniramine Maleate, Ca	Percentage content
14.76	0.002250	11.38	0.018700	0.01800	103.9
11.89	0.001578	8.01	0.014990	0.01440	104.1
9.47	0.000788	4.53	0.010560	0.01000	104.8
8.61	0.000315	3.32	0.005240	0.00504	103.9
3.22	0.000945	1.24	0.001569	0.00151	103.8

PCKC.16 Percentage content of Chlorpheniramine Maleate in Chlorpheniramine Maleate tablets manufactured by Kinapharma Limited using Caffeine as the surrogate reference standard.

K value = 0.2224

Peak area of Chlorpheniramine Maleate, Aa	Concentration (%w/v) of Caffeine, Cs	Area of Caffeine, As	(Aa x Cs) / (K x As)	Concentration (%w/v) of Chlorpheniramine Maleate, Ca	Percentage content
15.09	0.00980	31.88	0.02086	0.0200	104.3
14.10	0.006860	29.79	0.01460	0.0140	104.2
12.76	0.003430	22.53	0.00874	0.0084	104.0
10.89	0.007150	24.06	0.00349	0.0034	103.9
7.66	0.000686	22.37	0.00106	0.00101	104.8

PCKP.17 Percentage content of Chlorpheniramine Maleate in Chlorpheniramine Maleate tablets manufactured by Kinapharma Limited using Piroxicam as the surrogate reference standard. K value = 0.8095

Peak area of Chlorpheniramine Maleate, Aa	Concentration (%w/v) of Piroxicam, Cs	Area of Piroxicam, As	(Aa x Cs) / (K x As)	Concentration (%w/v) of Chlorpheniramine Maleate prepared	Percentage content
14.89	0.01250	12.17	0.01890	0.01800	105.0
13.02	0.01000	10.65	0.01510	0.01440	104.9
12.61	0.00700	10.29	0.01060	0.01008	105.2
11.11	0.00350	9.09	0.00528	0.00504	104.8
10.54	0.00105	8.59	0.00159	0.00151	105.5

PCAC.18 Percentage content of Chlorpheniramine Maleate in Chlorpheniramine Maleate tablets manufactured by Amponsah Effah Pharmaceuticals using Caffeine as the surrogate reference standard. K value = 0.2224

Peak area of Chlorpheniramine Maleate, Aa	Concentration (%w/v) of Caffeine, Cs	Area of Caffeine, As	(Aa x Cs) / (K x As)	Concentration (%w/v) of Chlorpheniramine Maleate, Ca	Percentage content
6.53	0.009800	16.31	0.01764	0.01800	98.0
5.10	0.006860	11.08	0.01420	0.01440	98.9
4.22	0.003430	6.58	0.00989	0.01008	98.2
2.15	0.002058	4.04	0.00492	0.00504	97.7
0.67	0.001029	2.08	0.00149	0.00151	98.3

PCAA.19 Percentage content of Chlorpheniramine Maleate in Chlorpheniramine Maleate tablets manufactured by Amponsah Effah Pharmaceuticals using Ascorbic acid as the surrogate reference standard. K value = 0.156

Peak area of Chlorpheniramine Maleate, Aa	Concentration (%w/v) of Ascorbic acid, Cs	Area of Ascorbic acid, As	(Aa x Cs) / (K x As)	Concentration (%w/v) of Chlorpheniramine Maleate, Ca	Percentage content
8.26	0.002300	6.84	0.017800	0.01800	99.1
6.72	0.001840	5.62	0.014112	0.01440	98.0
4.17	0.000920	2.44	0.010060	0.01010	99.8
2.91	0.000552	2.09	0.004940	0.00504	98.1
0.63	0.000441	1.21	0.001468	0.00151	97.1

PCAP.20 Percentage content of Chlorpheniramine Maleate in Chlorpheniramine Maleate tablets manufactured by Amponsah Effah Pharmaceuticals using Piroxicam as the surrogate reference standard. K value = 0.8095

Peak area of Chlorpheniramine Maleate, Aa	Concentration (%w/v) of Piroxicam, Cs	Area of Piroxicam, As	(Aa x Cs) / (K x As)	Concentration (%w/v) of Chlorpheniramine Maleate, Ca	Percentage content
17.64	0.02000	24.73	0.01800	0.01762	97.9
14.39	0.01600	20.17	0.01440	0.01410	98.2
9.20	0.00960	11.01	0.01008	0.00991	98.3
6.08	0.00480	7.27	0.00504	0.00496	98.4
1.35	0.00144	1.64	0.001512	0.00146	97.6