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

QUALITY IN GHANAIAN PHARMACEUTICAL PRODUCTION: A CASE STUDY OF A COMBINATION ANALGESIC

BY
ANDREW GORDON

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI

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BY

ANDREW GORDON

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DECLARATION

It is hereby declared that this thesis is the outcome of research work undertaken by the author. Any assistance obtained has been duly acknowledged. It is neither in part nor whole been presented for another degree elsewhere.

HEAD OF DEPARTMENT

……
Dr. R. K. Adosraku

SUPERVISOR

……..
Dr. R. K. Adosraku

CANDIDATE

……
ANDREW GORDON

Abstract

In the study of a combined analgesic produced in Ghana, the following quality parameters were used in evaluating the oral solid dosage form:

1. Visual appearance,
2. Friability
3. Test of identity
4. Uniformity of weight
5. Test for purity (limit test)
6. Disintegration
7. Dissolution
8. Content of active ingredient.

Thirty seven (37) batches of the products selected within four months were used for the study.

HPLC was used for the assay of the Aspirin, paracetamol and caffeine combination. In all assay benzoic acid was used as internal standard. Water, methanol and glacial acetic acid in the ratio of (75:25:0.2) by volume at pH 2.98 was used as the mobile phase. The compounds were eluted isocratically using a spherisorb S5ODS1 phase (25cm x 4.6mm) column. The flow rate was 1.3 ml per minute. The detector was set at 298nm with an Absorbance Unit Fraction (AUF) of 0.2. The Limit of Detection (LOD) and Limit of Quantification (LOQ) were established for paracetamol, aspirin, caffeine and salicylic acid to be (15.178 ug /ml and 75.89 ug / ml), (7.0 ug /ml and 35.0 ug / ml), (2.1054 ug /ml and 10.5271 ug / ml) and (0.154 ug /ml and 0.7698 ug / ml) respectively.

The compounds were eluted in the following order: paracetamol, salicylic acid, Aspirin, and caffeine. The average retention times were (3.640 ± 0.011) min, (7.668 ± 0.028) min, (9.258 ± 0.030) min and (18.848 ± 0.100) min.

UV spectrophotometric method was used as an alternative method for the assay of the dissolution product. The product was assayed without extraction. Simultaneous equation was used to resolve the spectral overlap caused by the constituent active ingredients in the tablet. The λ maximum and specific absorbance for paracetamol, aspirin and caffeine in the medium 0.01M

hydrochloric acidic were 244 nm (A^1_1 678.5), 229 nm (A^1_1 461.5) and 274 nm (A^1_1 497) respectively.

The dissolution profile showed a rapid dissolution rate with 91.87% of the batches having 70% of all their active ingredients going into the dissolution medium within 20 minutes. The rapid dissolution makes quick bioavailability of the active ingredients, enhancing rapid absorption for action.

The weight uniformity test and friability test performed had only few batches failing. Ninety seven (97%) of all the batches used passed the weight uniformity test. Ninety seven point three (97.3%) of the batches passed the friability test with only one batch failing.

The paracetamol content in the tablets had the least standard deviation of 6.4 indicating the least variance within the batches. Eighty six point five (86.5%) of the total batches used had their paracetamol content passing the assay test.

Aspirin had the highest standard deviation (14.36) and therefore was the Component with the largest variation in the batches assayed compared with paracetamol and caffeine with standard deviation of 6.4 and 11.4 respectively.

The caffeine content was found to be higher than the stipulated amount (30mg) in all the batches except one. Ninety seven point three (97.3%) of all the batches used therefore failed the assay test.

Seven (7) out of the 37 batches assayed failed the salicylic acid limit test ,that is they had a percentage salicylic acid content higher than 3.0%.

All other parameters except the following: the excessive caffeine content and high variation in aspirin content were not satisfactory

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1 CHAPTER ONE: INTRODUCTION

In Ghana as a result of trade liberalization and the boost in the local pharmaceutical manufacturing sector, people perceive the pharmaceutical market as a commodity market and an easy means of making profits. The general disregard to lay down rules 'Quality assurance' and the desire to reap huge financial profits and the motivating factors for quackery and faking makes it necessary for independent assessment of the quality of pharmaceutical product.

Quality assurance is a wide-ranging concept covering all matters that individually or collectively influence the quality of a product. Quality assurance incorporates good manufacturing practice (GMP), Quality control as well as other factors, including product design and development. [1]

Good manufacturing practice is that part of quality assurance which ensures that products can be consistently produced and controlled to maintain the quality standards appropriate for their intended use and as required by the marketing authorization. GMP is aimed primarily at diminishing the risks inherent in any pharmaceutical production. Such risks are essentially of two types: Cross-contamination (in particular of unexpected contaminants) and mix-ups (confusion). [1, 2]

Quality control is concerned with sampling, specifications and testing, organization, documentation and release procedures. It also ensures that the necessary and relevant tests are actually carried out without the materials are not released for use, nor products released for sale or supply, until their quality has been judged to be satisfactory. Quality control is not confined to laboratory operations but must be involved in all decisions concerning the quality of the product and the entire manufacturing process.

The concept of quality assurance has evolved from simple checks of the final products, such as test for appearance, colour, odour, identity, hardness, average weight or volume per unit and has expanded into complex quality assurance systems, which span through the whole manufacturing process.

The purpose of a quality assurance system is to ensure an absolute quality product such that: Each product (tablet) will contain the amount of active drug claimed on the label within the stated limit, as well as other essentials parameters such as bioavailability of the product.

It is necessary that the drug in the dosage form administered is dissolved upon administration. It should have the required bioavailability profile, that the drug is stable in the presence of an adjuvant and in normal or adverse weather conditions. It also indicates that the tablet has been developed with pure ingredients and in an environment which would not lead to the contamination of the product. It also indicates that the dosage form is therapeutically effective in the patient. [3]

An expert committee set up by WHO has produced reports on the quality assurance of pharmaceutical preparations. In these reports recommended practices for the manufacture and quality control of drugs are outlined. The following outlines are the required tests in the evaluation of oral solid dosage forms:

1. Visual appearance

2. Odour,

3. Taste,

4. Hardness,

5. Friability,

6. Moisture content,

7. Standard and test of identity,

8. Standard and test of homogeneity (uniformity of weight)

9. Standard and test for purity

10. Standards and assay of active ingredients

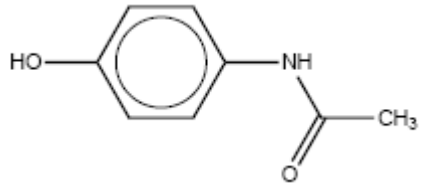
11. Standard and test of performance (dissolution and disintegration)

11. Stability test and storage conditions. [3]

1.1 Profile of active ingredients of sample under study

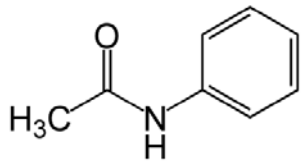
1.1.1 Paracetamol

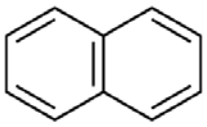
The structure of paracetamol:

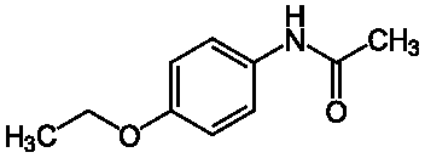


In the 1880s, Professor Kussmaul at the University of Strassburg, Department of Internal Medicine, asked two assistants to administer naphthalene as a treatment for intestinal worms. The medicine had little effect on worms, but one patient had a great reduction in fever temperature. It was found that this patient had infact been given acetanilide instead of naphthalene due to a mistake at the pharmacy. The young assistants quickly published the discovery of this new antipyretic (fever- reducing drug). However it had serious side effect: it deactivates some of the haemoglobin in the red blood cell. Many medicines were tried to improve on it and marketed derivatives like phenacitin. In 1893 Joseph von Mering made paracetamol a phenol amide. Paracetamol has since been used as fever reducing agent. (4)

The structure of acetanilide: The structure of naphthalene: Structure of Phenacitin:







Properties

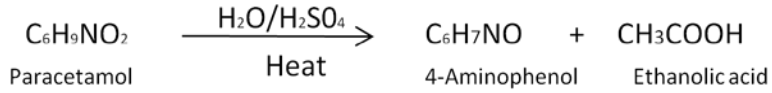
Paracetamol is a white crystalline powder with a melting range of 168 -172^{o}C. Paracetamol has a solubility of 100g / dm^{3} of ethanol, 76.9g / dm^{3} of acetone and 14.3g / dm^{3} of water. It is very soluble in chloroform and practically insoluble in ether. In acidic aqueous medium, it dissipates a minimum uv absorption at 245nm. However, in alkaline aqueous medium there is a maximum uv absorption. Dissociation constant is Ka 9.5 at 25^{o}C**.** (5)

Assay

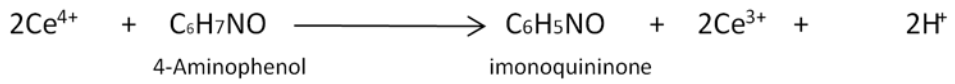
Paracetamol can be analyzed by titration with ceric ammonium sulphate. Ferroin is used as indicator in the titration. It can also be assayed by a uv spectrophotometric method or the use of HPLC.

Reaction equation for the Titration

1. Heating the sample (conversion of paracetamol to 4- aminophenol)



2. Titration of sample (Oxidation of to 4- aminophenol to iminoquinone)



3. Only after all 4- aminophenol have been converted to iminoquinone that ferroin indicator is than reduced to ferriin blue

Uses

Paracetamol is used as antipyretic and analgesic agents. It is also effective in **arthritic** and **rheumatic** as well as **dysmenorrhoea, myalgias** and **neuralgias**. It is used for patients woh are sensitive to aspirin and experience other reactions.

Mode of action (pharmacological effect):

Recent research has shown that the *cyclo-oxygenase* enzyme COX-3, found in the brain and spinal cord is selectively inhibited by paracetamol, and it is distinct from the two already known *cyclo-oxygenase* enzymes COX-1 and COX-2 .[6]

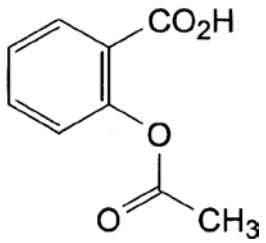
Early work had suggested that the fever reducing action of paracetamol was due to activity in the brain, while its lack of any clinically useful anti-inflammatory action was consistent with a lack of prostaglandin inhibition peripherally in the body. [7]

Like aspirin, the effect of paracetamol is due to inhibition of prostaglandin synthesis. Paracetamol and aspirin inhibit the same enzyme (*cyclo-oxygenase*) responsible for the

biosynthesis of prostaglandins. By reducing the synthesis of prostaglandins, paracetamol produces analgesia by elevating the pain threshold and reduces fever by 'resetting' the hypothalamic heat-regulating centre of the brain. However, in contrast to aspirin, paracetamol is thought to act almost exclusively on the central nervous system, with little peripheral effect. This may explain why paracetamol, even in higher doses, has limited anti-inflammatory effect and is associated with fewer gastro-intestinal side effects. The delay of the action of paracetamol is due to the fact that it is only absorbed in the duodenum once it has left the stomach.

1.1.2 Aspirin

Structure of aspirin



Salicylates, in the form of willow bark, were used as an analgesic during the time of Hippocrates [8], and their antipyretic effects have been recognized for more than 200 years [9]. Acetylsalicylic acid was introduced in the late 1890s [10] and has been used to treat a variety of Inflammatory conditions. [11.12] However, the antiplatelet activity of this agent was not recognized until almost 70 years later. [4]

Properties

Aspirin is a white crystalline powder or colourless crystal, with a melting point at about 142^{o}C [12]. It has a dissociation constant Ka 3.5(25^{o}C). Aspirin has a solubility of 3.3 g / dm^{3} of water, 142.9 g / dm^{3} of acetone, 50.0 g / dm^{3} of ether, and 58.8 g / dm^{3} of chloroform. [5]

Assay

The assay of aspirin involves a simple process (back titration). This process involves the hydrolysis of a known quantity of the aspirin or tablet containing specific quantity of the aspirin with a known quantity of 0.1MNaOH. Phenol red solution is used as an indicator and

the excess sodium hydroxide is titrated with a standardized 0.1MHCl. Titration is also performed on equivalent volume of 0.1MNaOH used to hydrolyze the aspirin.

Reaction equation for the Titration

However, in the titration involving the combination of aspirin with caffeine, the assay is quiet modified. This modified method involves refluxing of a known quantity of the aspirin with sodium citrate. After reflux, the hydrolyzed acids formed are titrated with 0.5MNaOH using phenolphthalein solution as an indicator. [7]

Uses

Aspirin or acetylsalicylic acid (acetosal) is a drug in the family of Salicylates, often used as an **analgesic** (against minor pains and aches), **antipyretic** (against fever), and anti-inflammatory. It also has an **anticoagulant** (blood-thinning) effect. Aspirin's low dosage (75–100 mg) is by now consolidated for secondary prevention of both **ischemic stroke** and **cardiovascular events**. In the acute phase, higher dosages (>300 mg) are employed. [13-15] Some few undesirable effects have been observed in the use of aspirin especially in higher dosage form. Its primary undesirable side effects, especially in higher doses, are gastrointestinal distress (including ulcers and stomach bleeding) and tinnitus "A ringing or booming sensation in one or both ears; a symptom of an ear infection". Another side effect, due to its anticoagulant properties, is increased bleeding in menstruating women. Because there appears to be a connection between aspirin and *Reye's syndrome*, aspirin is no longer used to control flu-like symptoms or the symptoms of chickenpox in minors. Although Aspirin is recognized as anti-inflammatory, analgesic and antipyretic, it is presently applied in several other clinical situations. Besides, a possible role in the incidence reduction of colon, lung and breast cancer and even of *Alzheimer's* disease has been suggested. [16-17]

Mode of action

Aspirin is an extremely potent pyretic and anti-inflammatory [18] agent in several species. [19] Aspirin owes it therapeutic activity to their ability to prevent prostaglandin biosynthesis. Aspirin causes the irreversible inhibition of *cyclooxygenase* activity (COX), defined also as *endoperoxide H synthase-1*, by acetylation of a specific serine at the activesite of the enzyme [20]. At present, there are three different COX isoforms. COX-1 is the constitutive isoform

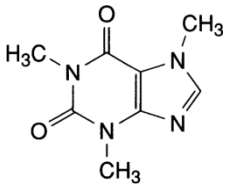
involving physiological processes such as gastric mucosa protection, platelet aggregation and kidney function. COX-2 is induced by various stimuli such as mitogens, growth factors, cancer promoters and lipopolysaccharide, and constitutes the principal cause of PG synthesis during inflammatory reactions. And COX-3 located in the Brain. [21]

Synthesis of aspirin:

Salicylic acid is acetylated using acetic anhydride, yielding aspirin and acetic acid as a by-product. It is a common experiment performed in organic chemistry laboratories, and generally tends to produce low yields due to the relative difficulty of its extraction from an aqueous state. The trick to getting the reaction to work is, to acidify with Phosphoric acid and heat the reagents under reflux with a boiling water bath for between 40 minutes and an hour.

1.1.3 Caffeine

Structure of caffeine



Caffeine is the most widely consumed central-nervous-system stimulant.
Caffeine is an alkaloid, actually a xanthine derivative which is usually extracted from coffee and tea leaves. It can also be synthesized from theophyline and theobromine by methylation. Caffeine is usually extracted and not synthesized. The main reason being that because of the preference for decaffeinated coffee by consumers there is abundant of it as by-product from the decaffeination**.**

Properties

A white, crystalline powder or silky, white crystals, sublimes readily, sparingly soluble in water, freely soluble in boiling water and slightly soluble in ethanol. It dissolves in concentrated solutions of alkali benzoates or Salicylates. [22] At room temperature it is odourless and slightly bitter, and sublimes at 178 ºC. Caffeine has melting point range of 235 ºC -239 ºC. [23]

Assay

The assay of caffeine usually involves non aqueous titration and spectrophotometric methods. For tablets containing Caffeine, like aspirin, the caffeine in an aqueous solution is basified and extracted with chloroform. The caffeine containing chloroform is evaporated to dryness and re-dissolved in distilled water. The concentration is determined through a calibration scanned at 273nm through a UV spectrophotometer. [22]

Mode of Action

Three main mechanisms of action of caffeine on the central nervous system have been described. Mobilization of intracellular calcium and inhibition of specific phosphodiesterases only occur at high non-physiological concentrations of caffeine. The only likely mechanism of action of the methylxanthine is the antagonism at the level of adenosine receptors. Caffeine increases energy metabolism throughout the brain but decreases at the same time cerebral blood flow, inducing a relative brain hypoperfusion. Caffeine activates noradrenaline neurons and seems to affect the local release of dopamine. Many of the alerting effects of caffeine may be related to the action of the methylxanthine on serotonin neurons. The effects of caffeine on learning, memory, performance and coordination are rather related to the methylxanthine action on arousal, vigilance and fatigue. Caffeine exerts obvious effects on anxiety and sleep which vary according to individual sensitivity to the methylxanthine. However, children in general do not appear more sensitive to methylxanthine effects than adults. The central nervous system does not seem to develop a great tolerance to the effects of caffeine although dependence and withdrawal symptoms are reported. [24]

Uses

Caffeine has central nervous system stimulant properties. Its action is mainly on the higher centres and it can produce a condition of wakefulness and increased mental activity. It has bronchi dilation properties and may stimulate the respiratory centre, increasing the rate and depth of respiration. Caffeine facilitates the performance of muscular work and increases the total work which can be performed by the muscle. It is also interesting to note that the diuretic action of caffeine is weaker than that of theophyline. Smaller doses enhance mental alertness and wakefulness, less fatigue and dieresis. Excessive dosage causes **insomnia** and restlessness and tremor in some people. [25]

Extraction of caffeine

For laboratory preparation 100ml of coffee extract and approximately 4g of sodium carbonate is added to the extract in a separating funnel. The mixture is swirled until the sodium carbonate dissolves. 4 of 25ml of chloroform is added and swirled for about 10 minutes. Shaking of the mixture will cause emulsion to form. The immiscible mixture is allowed to separate and the chloroform layer drained off.

The chloroform layer is evaporated slowly to 10ml. At this volume the crystals of caffeine begin to precipitate. This can therefore be filtered off and the crystals washed with 20ml of cool water (5^oC to 8^oC) and after with 20ml chloroform cooled to a temperature of 5^oC to 8^oC.

1.1.4 The combined dosage form

The combined Aspirin, Paracetamol and Caffeine

Combination of aspirin, paracetamol (acetaminophen) and caffeine has been used successfully to treat different kinds of pain including migraine attacks. Even when this formulation has been marketed for a long time, the exact molecular mechanisms underlying its therapeutic effectiveness have not been completely elucidated. Paracetamol and aspirin may act by inhibiting the synthesis of prostaglandins in the CNS and through a peripheral action by blocking pain impulse generation. The peripheral action may also be due to inhibition of the synthesis and actions of other substances that sensitize pain receptors to mechanical or chemical stimulation. Paracetamol may act predominantly on the CNS whilst aspirin may produce analgesic through peripheral actions. Caffeine on the other hand induces the constriction of the cerebral blood vessels. This leads to a decrease in cerebral blood flow and oxygen tension in the brain. This may contribute to the relief of some types of headaches. It has also been suggested that the addition of caffeine to Paracetamol and aspirin may provide a more rapid onset of action and or enhanced pain relief with lower doses of analgesics. However, caffeine which stimulates the CNS inhibits sleep and this may be detrimental to patients with Migraine since sleep contributes to the relief of migraine headaches. [26, 27]

Dissolution

The bioavailability of active drug substances, are monitored basically in vitro. This is an important aspect of quality control in drug manufacturing ensuring the formulation is capable of releasing the active component within a stipulated time frame. [22]

1.2 Tablets and Formulation

1.2.1 Tablet

Tablets may be defined as solid pharmaceutical dosage forms containing drug substances with or without diluents and prepared either by compression or moulding method.

Tablets remain popular as a dosage form because of the advantages afforded to the manufacturers that is, simplicity and the economy of preparation, stability and convenience in packaging, shipping, and dispensing. It is also convenient for the consumer because of the accuracy of dosage, its portability, its ease of administration, and its blandness of taste compared to the inconvenience experienced through the use of injection and infusion. [25]

Although tablets are more frequently discoid in shape, they may also be round, oval, oblong, cylindrical, or triangular. Tablets may differ in weight and sizes depending on the weight of the active ingredients and its intended method of administration. :

Three most unique methods are used in the preparation of tablets and these include dry granulation, wet granulation and direct compression. Granulation may be defined as a size enlargement process which converts small particles into physically stronger & larger agglomerates. Granulation method can be broadly classified into two types: Wet granulation and Dry granulation [41]. .With the dry granulation, when tablet ingredients are sensitive to moisture or are unable to withstand elevation during drying, and when the tablet ingredients have sufficient inherent or coherent properties, slugging may be used for granules. The basic steps in dry granulation include weighing, mixing, slugging, dry screening, lubrication, and compression. Powdered materials contain a considerable amount of air; under pressure, this air is expelled and a dense piece of tablet is formed. The more time allowed for this air to escape, the better the tablet or slug [28, 29].

1.2.2 Granulation

1.2.2.1 Dry granulation

The ingredients in the formulation are intimately mixed and pre-compressed on heavy duty tablet machines. The slug which is formed is ground to a uniform size and compressed into the finished tablet. In drugs known to be unstable towards moisture, many attempts are made to exclude water in the manufacture process. For instance by using a dry-granulating method, or by using water-free organic solvents in wet-granulating methods. Dry granulation" refers to a granulation process where no external fluid is added during processing. In such an embodiment, preferably the therapeutic compounds being granulated contain a total of at least about 4 wt % moisture, more preferably from about 5 wt % to about 15 wt % moisture[25], and most preferably from about 6 wt % to about 10 wt % moisture, based on the total weight of the therapeutic compounds. Such moisture is preferably inherently present in the therapeutic compounds as supplied. Preferably, the moisture present is water. It is believed that having some moisture in the therapeutic compounds aids in achieving an intimate admixture during dry granulation. Dry granulation may be accomplished after admixing in a suitable piece of mixing equipment, and/or using compaction equipment, such as a roll press, to compact the dry blend into a desired shape to form an intimate admixture. In a preferred embodiment, dry granulation is carried out using a Chilsonator™ press. [31, 32]

1.2.2.2 Wet granulation

Wet-granulation methods involve weighing out ingredients (including a solvent), mixing the ingredients, granulating them, screening them damp, drying them, dry-screening, lubrication, and compressing the resultant admixture into tablets. Such procedures result in tablets having at least adequate tablet homogeneity. Wet-granulation methods may have a disadvantage when certain solvents, which may not be desired in view of environmental and safety concerns, are used. [28, 32]

1.2.3 Direct Compression

A compressible vehicle is blended with the medicinal agent, and if necessary, with a lubricant and a disintegrant, and then the blend is compressed. Substances that are commonly used as

directly compressible vehicles are: anhydrous lactose, dicalcium phosphate (Emcompress), granulated mannitol, microcrystalline cellulose (Avicel), hydrolyzed starch, and a blend of sugar, invert sugar, starch and magnesium stearate.[31,28]

1.2.4 Diluents

Diluents are added to pharmaceutical products to increase the bulk in order to make the tablets a practical size for compression since the active ingredient may be too small.

Usually steroidal hormone tablets may contain about 10mg; hence, it is important to add excipients to make the tabletting possible. Example of diluents include; Di-calcium phosphate, Calcium sulphate, lactose, sorbitol, sucrose, kaolin, mannitol, dry starch, powdered sugar, and inositol.

Most tablet formulators tend to use consistently only one or two diluents selected from the above group on their tablet formulations. Usually the selection is based on experience and the cost of production. It is therefore necessary to subject any new therapeutic medication to experiment to ascertain which of the diluents would be most appropriate for the formulation. For example, calcium salt used for the broad-spectrum antibiotic tetracycline has been shown to interfere with the drug's absorption from the gastrointestinal tract. When drugs have low water solubility, it is recommended that a more water soluble diluent is used to avoid bioavailability problems. (25, 33, 34, 35)

1.2.5 Binders (Granulators)

These additives are used to impart cohesive quality to the tablet formulation and ensure that the tablet remains intact after the compression. They are also as binders or granulators. Some of the materials used for binders are; starch, gelatine, sucrose, glucose, dextrose, molasses, and lactose. Also included is the use of natural and synthetic gums such as, acacia, sodium alginate, extract of Irish moss, panwar gum, ghitti gum, mucilage of isapol husks, carboxymethylcellulose, methylcellulose, polyvinylpyrrolidon, veegum, and starch. Others such as waxes, water and alcohol as well as polyethylene glycol may also be considered as binding agents. Alcohol and water are not binders in the true sense, however, their solvent

action on some ingredients such as lactose and starch, changes the powdered material to granules and the residual moisture retained enables the material to adhere together when compressed.[25,34,35]

The quantity of binder used has considerable influence on the characteristics of the compressed tablets. The use of too much binder or too strong a binder will make a hard tablet which will not disintegrate easily and which will cause excessive wear of the punches and dies. Active ingredients which has no cohesive or less cohesive characteristics will need a more and stronger binder than those with these qualities.

Starch paste; the most widely used starch is that of corn starch. The concentration usually used ranges from 10% to 20%. Binder is prepared by dispersing corn starch in sufficient cold purified water to make a 10% w/w solution and warming in a water bath with continuous stirring until a translucent paste forms.

Gelatin solution; this binder is used as a concentration ranging from 10% to 20%. This is used whilst warm or it may solidify. The Gelatin is prepared by adding to cold purified water and allowed to stand until it is hydrated. The preparation is dissolved by warming in a water bath.

Ethyl cellulose; this is insoluble in water. It is used effectively as a binder when dissolved in alcohol or as a dry binder in a granulation which is then wet with alcohol. As a binder in solution it is used as 5% solution. It is used as a binder for moisture sensitive materials. The binder used for the tablet can usually affect the disintegration time. [25,34]

1.2.6 Lubricants

This additive to tablet formulation improves the rate of flow of the tablet granulation, prevents adhesion of the tablet material to the surface of the dies and punches, reduces inter particle friction, and facilitates the ejection of the tablets from the die cavity. Examples of commonly used lubricants include; talc, magnesium stearate, calcium stearate, stearic acid, and hydrogenated vegetable oil. Poor selection or excessive amounts can result in producing water proofing tablets which results in poor dissolution and poor disintegration. In the selection of lubricant, proper attention must be given to its compatibility with the drug agent. Using aspirin as an example, the use of talc with high calcium content would increase aspirin

decomposition. However in the manufacture of aspirin tablet, hydrogenated vegetable oil is preferred since its stability with aspirin is pronounced. Another problem is the use of magnesium stearate which has water proofing properties and can retard disintegration and dissolution. To overcome these waterproofing characteristics, sodium lauryl sulphate is sometimes included. [25, 34, 35]

1.2.7 Disintegrator

These are substances, or mixture of substances, added to a tablet to facilitate its break-up or disintegration after administration. After drug administration the active ingredient must effectively be released from the tablet to be absorbed by the body. Examples of disintegration agents include; starches “corn and potato starches”, cellulose align gums, and clay. The most popular disintegrators are corn and potato starch which have been well-dried and powdered. Starch present as disintegrators absorbs water, swells and disintegrates there by increasing the surface area and hence making the drug available for dissolution. The swelling ability of starch makes it the preferred choice because it enhances dissolution. The percentage of starch usually used ranges from 5% to 15%, and this depends on the extent of disintegration that is required. For the desired effect of disintegration to be felt, starch is added to the powder blend in the dry state. The disintegration agent is usually mixed with the active ingredients and diluents prior to granulation. [25, 33, 34]

1.2.8 Uniformity of weight

Standard of uniformity of weight is applied to tablets and capsules which are supplied in unit-dose forms and uniformity of volume to single dose pro-injections because they are subject to more variation than comparable preparations supplied in multi-dose forms.

As stated in the pharmacopoeia when twenty tablets are selected at random and a uniformity test performed, not more than two tablets should deviate from the average weight by a greater percentage as illustrated below and not even one should deviate by twice that value.

An average tablet weighing 80mg or less should have a permissible deviation of ±10%. On the other hand a tablet having an average mass of 80mg to 250mg should have a percentage

deviation of ±7.5%. And finally, an average mass of tablets containing over 250mg of active ingredient should have a percentage deviation of ±5.0%. [36]

1.2.9 Hardness & Friability

The resistance of tablets to abrasion, shipping, or breakage under storage conditions, transportation and handling before usage depends on its hardness. Quantitatively, hardness of tablet is determined either in N (Newton) or kg. The minimum satisfactory for tablet hardness is 4kg or 40N. Hardness determination is made throughout the tablets' manufacturing to determine the need for pressure adjustment in the tabletting machine. The degree of hardness affects the dissolution of the tablet; hence, the hardness needs to be controlled. Another indicator for hardness is the friability. In here rather than measuring the force required to crush the tablet, the instrument is used to evaluate the ability of the tablet to withstand abrasion in packaging, handling and shipping. A number of tablets are weighed and placed in the friabilator and the machine, allowed to operate for 4 minutes at a total of 100 revolutions. For tablets with a unit mass equal to or less than 650 mg, a sample of whole tablets corresponding as near as possible to 6.5 g is used. For those higher than 650 mg, 10 whole tablets are used. A maximum loss of mass obtained from a single test not greater than 1.0 percent is considered acceptable for most products, [23]

1.2.10 Disintegration and Dissolution

Dissolution of tablet is used to study the rate of dissolution of a tablet following compendia parameters. Disintegration time determination is a useful tool in product control. However, disintegration of tablet does not imply the availability for absorption. A drug can have a rapid disintegration time but does not mean the drug is biologically available. The dissolution rate of the drug form the primary particles of the tablet is the important factor in drug absorption and for many formulations is the rate limiting step.

Dissolution usually involves a dissolution tank containing dissolution bowls and paddles with dissolution medium volume usually 900ml .The number of unit test include the use of 6 unite bowls. The standardized BP criteria for published tests using either the basket or the paddle are that, for each unit tested, not less than 70% of the active ingredient(s) dissolve

within 45 minutes. If one unit fails to meet this requirement, a re-test may be carried out using the same number of units; all units in the re-test must comply. [22]

1.2.11 Thin Layer Chromatography (TLC)

Thin-layer chromatography (TLC) is a separation technique in which a stationary phase consisting of an appropriate material is spread in a uniform thin layer on a support (plate) of glass, metal or plastic. The separation is based on adsorption, partition, ion-exchange or the combinations of these mechanisms. The separation is carried out by migration of solutes in a solvent or a suitable mixture of solvents (mobile phase) through the thin-layer (stationary phase). When a mixture of analytes is spotted on a thin layer plate, dried and placed in a chromatographic chamber, the analytes move across the plate at different rates depending on the extent of adsorption or partitioning on the plates and its solubility in the mobile phases. [22]

Some of the stationary phases used for TLC include silica gel, cellulose, alumina (aluminium oxide), magnesium silicate, ion exchange resins and reversed phases like paraffin and ODS.

TLC is one of the most widely used techniques for the separation of pharmaceutical products and their identification. This method of characterization has gained popularity and favour as an analytical method because of its simplicity, reliability as well as its simple method location procedures.

Location of spots

Most organic compounds are colourless, to locate these compounds, they must be made visible, preferably by a non destructive technique. Most compounds can be located by examining the plate containing a chromophore under a 254nm wavelength. In this process the absorbing compounds are seen as dark spots. These spots can be ringed up with a pencil. Compounds that naturally fluoresce can be located at a wavelength of 350nm as coloured spots. Another very important but destructive test is to spray ethanolic sulphuric acid on the plate and gently warm in an oven. Organic material when treated in such manner, char-up and are seen as dark spots.

Retention Factor (R_f)

The basic chromatographic measurement of a substance in TLC is the Retention factor (**R_f**). The distance travelled by the substance is measured from the centre of the spot, which is easily determined when the spot is round to the origin on the plate spotted. However if the spot is tailing, it is measured from the middle of the dense area of that spot. **R_f** values needed for the identification of samples are usually advised to be run at the same time and on the same plate with both known and unknown side by side. The R_f value if quoted as a fraction ranges between 0 to 1 and if quoted as a percentage 0 to 100.[37]

$$R_f = \left(\frac{Distance\ travelled\ by\ solute\ from\ origin}{Distance\ travelled\ by\ solvent\ from\ origin}\right) X\ 100$$

1.3 Statistics in pharmaceutical analysis

Sampling

Sampling is one of the fundamental methods essential during chemical analysis of bulk products. An analyst has to deal with portions of the sample since in most cases it is impractical or impossible to analyze the entire sample under consideration. In such a situation, random sampling is done and this represents the whole population. Samples are taken in such a way that all the members of the population have an equal chance of being included. [38]

Comparing means

If several means are compared, the ANOVA is used. ANOVA means analysis of variance. There are several programs that can perform this task. Microsoft excel and SPSS are but a few. ANOVA can be used either as one way ANOVA or two way ANOVA. One way ANOVA is used if there is only one independent variable to be compared while two way ANOVA is used if two independent variables are compared. ANOVA tests the null hypothesis that is, the means of all the groups being compared are equal and produces a statistical value called F which is equivalent to the t-statistic from a t-test.

If the F value generated is lower than the tabulated value then the null hypothesis is accepted. However, if the F value generated is higher than the tabulate, then the null hypothesis is rejected. [37]

Statistic in quality control

Even though one could arbitrarily determine when to declare a process out of control, it is a common practice to apply statistical principles to do so. The method for constructing the upper and lower control limits is a straightforward application of the principles in statistics. In all production processes, there is a need to monitor the extent to which products meet specifications. In the most general terms, there are two "enemies" of product quality:

(1) Deviations from target specifications, and

(2) Excessive variability around target specifications.

The general approach to on-line quality control is straightforward: a number of sample of certain size are extracted from the ongoing production process. The values obtained are then used to produce line charts of the variability in those samples, and their closeness to target specifications considered. If a trend emerges in those lines, or if samples fall outside pre-specified limits, the process is then declared to be out of control and action is taken to find the cause of the problem. These types of charts are sometimes also referred to as Shewhart control charts (named after W. A. Shewhart who is generally credited as being the first to introduce these methods). In the case of pharmaceutical quality control, the limit set by the pharmacopoeia could be used as the upper and lower control limit, Unless otherwise stated.[39]

1.4 Analytical Parameters

The validity of analysis involves the basic principles of validation of the analytical process. Validation of an analytical method is the process by which laboratory studies establish that the performance characteristics of the method meet the requirements for the intended analytical application. Validation is required for any new or amended method to ensure that it is capable of giving reproducible and reliable results, when used by different operators employing the same equipment in the same or different laboratories. [40]

Typical analytical parameters used in assay validation include:

Precision

Accuracy

Ruggedness

Limit of detection

Limit of quantification

Selectivity

Specificity

Precision

The precision of an analytical method is the degree of agreement among individual test results obtained when the method is applied to multiple sampling of a homogenous sample. Precision is a measure of the reproducibility of the whole analytical method. [23] Precision is determined by using the method to assay a sample for a sufficient number of times to obtain statistically valid results (that is between 6 - 1 0). The precision is then expressed as the relative standard deviation. %RSD = (Standard deviation / Mean) x 100%. The relative standard deviation should be less than 2.0 %.[40]

Accuracy

Accuracy is a measure of the closeness of test results obtained by a method to the true value. [23] Accuracy indicates the deviation between the mean value found and the true value. It is determined by applying the method to samples to which known amounts of analyte have been added. These should be analysed against standard and blank solutions to ensure that no interference exists. The accuracy is then calculated from the test results as a percentage of the analyte recovered by the assay. [40]

Ruggedness

Ruggedness is the degree of reproducibility of results obtained by the analysis of the same sample under a variety of normal test conditions that is, different analysts, laboratories, instruments, reagents, assay temperatures, small variations in mobile phase, different days etc. (ie from laboratory to laboratory, from analyst to analyst). [23]

1.5 Limit of Detection

This is the lowest concentration in a sample that can be detected, but not necessarily quantified, under the stated experimental conditions. The limit of detection is important for impurity tests and the assays of dosages containing low drug levels and placebos.

The limit of detection is generally quoted as the concentration yielding a signal-to-noise ratio of 2:1 and is confirmed by analyzing a number of samples near this value (6) using the following equation. The signal-to-noise ratio is determined by [27]: s = H/h

Where H = height of the peak corresponding to the component, s= signal and h = absolute value of the largest noise fluctuation from the baseline of the chromatogram of a blank solution. Another method for the determination of the LOD is based on standard deviation of the response and slope of the calibration curve, LOD = 3 x STD/ slope. [40]

1.6 Limit of Quantification

This is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy. It is quoted as the concentration yielding a signal-to-noise ratio of 10: 1 and is confirmed by analyzing a number of samples near this value. Another method for the determination of the LOD is based on standard deviation of the response and slope of the calibration curve, LOQ = 10 x STD/ slope. [26, 40]

1.7 Selectivity and specificity

Selectivity is the ability to measure accurately and specifically the analyte in the presence of components that may be expected to be present in the sample matrix.

Specificity for an assay ensures that the signal measured comes from the substance of interest, and that there is no interference from excipient and/or degradation products and /or impurities. Determination of this can be carried out by assessing the peak identity and purity. Diode array detectors can facilitate the development and validation of HPLC assays. [26, 41]

1.8 Theory and instrumentation of analytical methods

1.8.1 High performance liquid chromatography(HPLC)

This is now the most widely used method of assay and separation technique. The simple high performance liquid chromatographic method developed in the late 1960s had evolved into high pressure and high speed chromatography. HPLC has many advantages over the classical column chromatography. With the packed stationary phase made of smaller particle size, there are improved resolution of substances, faster separation with increased precision and accuracy. The separation principles used for effective separation involves adsorption, partition, ion exchange and gel permeation. [42]

Instrumentation

The basic instruments consist of a mobile phase reservoir, a high–pressure pump, and injector, a stationary phase embedded in a stainless steel column, a detector and a chart recorder.

High Pressure Pumps

It is an important part needed to deliver a constant flow of the mobile phase with a decisive pressure. Most pumps are able to deliver a constant pressure rage of -600bar. A dual – Piston reciprocating pump is performed due to its pulse-free flow. In this system as one shaft phase is filling the valve another phase is pumping the mobile phase. Unlike a single piston pump a damping device is required to smoothen the flow. This is necessary so as to avoid excessive noise at high level of sensitivity causing high base line noise preventing small quantities of substances to be detected. [22]

Injector system

The sample solution is introduced into the flowing mobile phase at or near the head of the column using an injection system which can operate at high pressure. They contain Fixed-loop and variable volume devices which are operated manually or by an auto-sampler. Manual partial filling of loops may lead to poorer injection volume precision. The sample is introduced into the loop when the valve is in the load position. At this stage the eluent flows from the pump to the column through another passage. When the valve is switched to inject, the loop is redirected to flow into the column conveying the sample into its destination. [22]

Column

The columns are made of highly polished stainless steel usually having a column length of 10cm to 30cm and an internal diameter of 4.5mm to 5mm. however, longer and larger pore sided columns are available and are used usually for commercial purposes.

The most widely used stationary phase is silica (SiO_2, XH_2O), usually modified for the manufacturing of the stationary phase. The stationary phase consists of a network of siloxane

linkages (Si-O-Si) in a rigid three dimensional structure containing interconnecting pores. The pore size and the amount of silanol groups are controlled in the manufacturing process.

In a straight stationary phase column the silanol groups are vital as they are involved in adsorption chromatography. Usually used in the separation of polar compounds

Silica can be modified to the reversed stationary phase. This is done by a controlled reaction of organochlorosilanes with the silanol groups or the use of organoalkoxysilanes which modifies the surface of the silica. The linkage of these hydrocarbons to the surface impacts a non polarity to the surface and enhances partitioning, thus the separation of lipophilic compounds.

The most popular stationary phase material used is the (ODS) Octadexyl-silica C18.Others include, octyl (C_8), Phenyl (C_6H_5), Cyanopropyl ($(CH_2)_3$-CN) and aminopropyl ($(CH_2)_3$-NH_2).[22,23]

Pharmaceutical products contain both lipophilic and polar groups. These groups are usually exploited during separation on columns. [41]

Detectors

Four main types of detectors are frequently used in High performance liquid chromatography. These are the electrochemical detectors, Fluorescent detector, Refractive index detector, Mass spectrometers, Radioactivity detectors and the Ultra-Violet visible detectors. Among these, the most widely used is the Ultra- Violet Visible detectors. [22, 23, 41]

1.8.2 UV visible Spectrophotometric analysis

The technique of Ultraviolet- visible spectrophotometry is one of the most frequently employed technology employed in pharmaceutical analysis. The wavelength used ranges from 190 nm – 380 nm for ultraviolet radiation and 380 nm–800 nm for visible radiation. [42]

Different lights are needed for the generation of the radiation needed. Hydrogen discharge lamps and xenon arc lamps are needed for ultraviolet radiation generation, and tungsten filament lamps and deuterium discharge lamps generate the radiation in the visible region. Since these light sources generate large range of wavelengths, there are monochromator filters incorporated in the machine that is able to filter and produce light of only one

wavelength needed and specified by the user. Examples of monochromator include prisms and diffraction gratings. [22,41]

Light from the monochomator passes through the cuvette containing the sample to the light detector system. The signals generated are compared with the incident light and the amount of light absorbed displayed by the machine. However, depending on the machine used, other parameters can be displayed.

The Beer-Lambert law is the basis for all analytical absorption spectrophotometry.

The law states that, “The amount of monochromatic light absorbed by a solution is related to the path length of a solution through which the light passes and the concentration of the solution”.

Mathematically: $a = A \times b \times c$

a = Absorbance

A= specific absorbance if concentration is in %w/v

b= Path-length in cm

c= concentration in %w/v

Or

$a = \varepsilon bc$

a = Absorbance

ε = molar extinction coefficient

b = Path-length in cm

c = concentration in g/L

The law holds when monochromatic light is used and the solution used is diluted and stray light is excluded. Therefore, the plot of absorbance against varying concentration for a cell of unit thickness, usually 1cm should give a straight line passing through the origin. This is termed the calibration curve. The calibration curve can be used for the determination of concentration of an unknown when the absorbance has being determined.

However, for a solution containing a mixture of compounds with each having a different maximum absorbance with spectral overlaps, the overall absorbance at their maximum wavelength will be equal to the summation of the specific absorbance of their product multiplying the concentration. [22, 42, 41]

1.9 Review of Analytical methods

Separation method for simultaneous determination of paracetamol, caffeine, acetylsalicylic acid, and internal standard benzoic acid was developed based on a novel reversed-phase sequential injection chromatography (SIC) technique with UV detection. The mobile phase used was acetonitrile-(0.01 M) phosphate buffer (10: 90, *v/v*) pH 4.05, flow rate 0.6 ml min^{-1}. UV detection was at 210 and 230 nm. The validation parameters showed good results: linearity (r >0.999) for all compounds, detection limits in the range 0.3-0.8 μg mL^{-1}, repeatability (RSD) of peak heights between runs in the range 1.10-4.30% at three concentration levels and intra-day repeatability of the retention times in the range 0.28-0.43%. The analysis time was < 6 mins. The method was found to be applicable for the routine analysis of the active compounds paracetamol, caffeine, and acetylsalicylic acid in pharmaceutical tablets. [43]

Acetylsalicylic acid, paracetamol, caffeine and Phenobarbital in tablets can be detected and resolved using HPLC. Separation can be achieved using Bio SiL HL C18, 5 microm, 250 x 4.6 mm column. Mixture of acetonitrile-water (25:75 v/v) at pH 2.5 adjusted with phosphoric acid using a flow rate of 2.0ml min-1.UV detection should be at 207 nm range 0.01 AUFS. Under the same conditions it is possible to determine the level of salicylic acid. The reproducibility (recovery values: 98.74-102.08% for acetylsalicylic acid, 99.93-102.11% for paracetamol, 98.25-102.12% for caffeine and 98.15-102.3% for Phenobarbital). The proposed HPLC method has been applied for the determination of acetylsalicylic acid. The developed method is rapid and sensitive and therefore suitable for routine control of these drugs in dosage form. [44]

This method describes a rapid reversed-phase liquid chromatographic method, with UV detection, for the simultaneous determination of acetylsalicylic acid, caffeine, paracetamol in pharmaceutical preparations. A reversed-phase C18 Nucleosil column is used. The mobile

phase consists of 2 successive eluants: water (5min) and acetonitrile-water (75:25, v/v; 9 min), both adjusted to pH 2.1 with phosphoric acid. Before determination acetylsalicylic acid, the aspirin is completely converted to salicylic acid by alkaline hydrolysis (this method would not be appropriate for the determination of aspirin when salicylic acid is to be assayed differently. Salicylic acid, caffeine and paracetamol are all detected at 285 nm. Calibration curves were linear for salicylic acid, caffeine and paracetamol in the range of 50-500 mg/L. Recoveries ranged from 92.6 to 105.5%. [45]

The analytical separation could be accomplished using an ODS-2 reversed-phase column (5 mm particle size, 125 ×4.6 mm) (Scharlab) that was connected to a 30 mm guard operculum of similar characteristics (Scharlab). Using a flow rate of 1.0mlmin-1with the detector set at 274 nm using ambient conditions. The mobile phase for the separation involves 0.05 moll-l SDS: 6% propanol: 0.01 mol-1 NaH_2PO_4 at pH 3. The elution time of Paracetamol, aspirin and caffeine are listed restively, 1.7min, 3.4min and 5.0min respectively. [42]

Analytical separation of paracetamol, aspirin, caffeine and salicylic acid could be analysed on an ODS column using a mobile phase of water, methanol and glacial acetic acid in the ratio (69:28:3) using a flow rate of 2ml per minutes at 275nm. Benzoic acid was used as an internal standard dissolved in methanol and glacial acetic acid in the ratio (95:5). [26]

1.10 Aim of the study

The aims of the study are:

- To assess the production consistency of EFPAC.
- To subject the batches under study to various quality control parameters. These parameters include: disintegration, dissolution, hardness, weight uniformity test, limit of salicylic acid and the assay.
- Developing of a suitable HPLC method to assay the samples.
- To develop a readily and easily accessible Spectrophotometric method for the assay of the tablets.
- To subject the result to a statistical analysis.
- To Perform a Quality control chart on the assayed products to display the trend of production lapses and adjustments needed to be done.
- To help in making decisions to streamline the production process were faults are shown.

2 CHAPTER TWO: EXPERIMENTAL

2.1 Materials

2.1.1 chemicals and reagents

Methanol (Crude, Distilled twice with the rotator Evaporator)

Acetic acid (BDH Analar Grade)

Distilled water

Hydrochloric acid (BDH Analar Grade)

Benzoic acid (BDH GPR Grade)

Salicylic acid (GPR Grade)

Paracetamol (BN 0620720, DM November 2006, DE November 2010)

Aspirin (BN 200609003, DM September 2006, DE September 2010)

Caffeine anhydrous powder (BN 200703002, DM January 2007, DE November 2011,)

Sulphuric acid (BDH analar grade)

Iodine crystals (BDH GPR Grade)

Potassium iodide

Silica gel(with 12%-13% calcium sulphate) Risons laboratory reagent

Sodium hydroxide pellet (BDH analar grade)

2.1.2 Equipments

Melting point capillary tubes

Adam-Analytical balance

Cecil CS 2041 UV/Visible Spectrophotometer 2000 series

Fused silica cuvettes

LC-10AT Shimadzu Liquid chromatograph Pump

Applied Biosystems 783 A Programmable absorbance detector

Shimadzu CR 501 Chromatopac

Erweka TA Friabilator

Erweka Disintegrator

S5ODS 1 spherisorb column

20µl loop size for the injector

0.45 µm PTFE ACRC DISK CR 13 micro filter

2.1.3 Drug samples analyzed

EFPAC Blister Packed tablets

Manufacturer: Amponsah Effah Pharmaceutical Company.

For information on batches used, refer to Table 2.1.3.0.

2.1.4 Product description:

Packaging:

The tablets were packaged in a turquoise blister pack made of aluminium foil which is internally laminated with a rubber film.

The average dimension of the foil was 158 mm by 65 mm.

On one side of the blister pack, the following inscriptions were observed: the manufacturer's name, country of origin, dosage, tablet's active content and contra-indication.

The other side of the blister pack contains the inscription "EFPAC blows your pain away".

Each blister pack had 10 tablets.

Tablet Characteristics:

The tablet was yellowish in colour with dispersed white patches.

The tablet is uncoated and discoid in shape with depression at the circumference on each side. The tablets had an average dimension of 12.2 mm by 3.7 mm.

The word EFPAC was engraved on one side of the tablet.

2.2 Sample characterization

2.2.1 Melting point determination

One gram (1.0 g) each of pure samples of Paracetamol, Caffeine, Aspirin, Benzoic acid and salicylic acid were dried to constant mass over anhydrous silica gel for 24 hours. Five melting point capillary tubes were selected and each filled with only one of the pure substances to about 2mm above the bottom of the tube. The melting points were then determined. For results refer to table 1.

2.2.2 Identification of paracetamol (specific test)

A. A quantity of powder containing 0.1015g of the previously dried paracetamol was dissolved in 50ml methanol and diluted to 100ml with the same solvent. 1.0ml of the prepared solution was taken and 0.5 ml of 0.1M hydrochloric acid was added. The mixture was further diluted to 100ml with methanol. The absorbance was then read at 249nm. Refer to section 3.1.2(A) for results.

B. A quantity of powder containing 0.1 g of Paracetamol was dissolved in 1ml hydrochloric acid and the mixture boiled for 3 minutes. 1 ml of distilled water was added and the sample cooled in an ice bath for 4 minutes. 0.05 ml of 0.0167M of potassium dichromate was added. Refer to section 3.1.2(B).

2.2.3 Identification of aspirin (specific test)

A. A quantity of powder containing 0.2 g of aspirin was dissolved in 4 ml of 2M sodium hydroxide and boiled for 3 minutes. The solution was cooled down and 5 ml of 1M of sulphuric acid was added. Precipitate formed was washed with water and dried to constant weight and melting point determined. Refer to section 3.1.3(A) for results.

B. Twenty three milligram (23 mg) of the precipitate formed in test 'A' was dissolved in 10 ml distilled water by heating. 0.5 ml of ferric chloride solution was added to the cooled dissolved precipitate solution. An aliquot of 0.1 ml acetic acid solution was added after 3minutes. Refer to section 3.1.3(B) for results.

2.2.4 Identification of caffeine (specific test)

A volume of 0.05 ml of iodinated potassium iodide solution (2 g iodine and 4 g potassium iodide in 100 ml water) was added to 2ml saturated caffeine solution. An aliquot of 0.1 ml of 2 M HCl was added. After, 2 minutes 0.1ml of 2M sodium hydroxide was added for neutralization. Refer to section 3.1.4 for results.

2.2.5 Identification of benzoic acid(specific test)

A volume of 1 ml of 5 g of benzoic acid dissolved in 100 ml reagent grade ethanol was diluted with 0.5 ml ferric chloride solution in a test tube. A volume of 10 ml ether was added and the sample shook for 1 minute. Refer to section 3.1.5 for results.

2.2.6 Identification of salicylic acid (specific test)

A quantity of powder containing 30 mg of salicylate was dissolved in 5 ml of 0.05 M sodium hydroxide solution. The dissolved salicylate was diluted to 20 ml with water. 1 ml of the solution was taken and 0.5 ml of ferric chloride solution was added. After 1 minute, 0.1 ml acetic acid was added. Refer to section 3.1.6 for results.

2.2.7 Assay of Standard drug samples

2.2.7.1 Assay of standard aspirin:

A quantity of pure aspirin sample weighing 0.5469 g was placed in to a conical flask and 10 ml alcohol added. A 50ml volume of 0.5M NaOH was also added. The flask was plugged for 1 hour. The solution was then titrated with 0.5Molar HCl. Phenol red was used as an indicator. A blank titration was performed. The process was repeated for two other masses of aspirin (0.5464 g and 0.5468 g). Each ml of 0.5 M Sodium hydroxide is equivalent to 0.045g of aspirin. [22]

Standardization of 0.5 M NaOH with sulphamic acid:

A quantity of sulphamic acid weighting 1.2112 g was placed in to conical flask. Twenty (25) ml of distilled water was added and sample whirled to dissolve. The prepared solution was titrated with 0.5 M NaOH using phenolphthalein as an indicator. The process was repeated for two other masses (1.2112 g and 1.2112 g)

Standardization of 0.5 M HCl with 0.5 M NaOH:

A volume of 25 ml 0.5 M NaOH solution was pipetted in to a conical flask. The solution was titrated with O.5 M HCl using methyl orange as an indicator.

2.2.7.2 Assay of salicylic acid in standard aspirin sample:

The chromatographic apparatus consisted of C-18 column, UV detector set at 302 nm and a mobile phase consisting of water methanol and glacial acetic acid in the ratio 69:28:3. Benzoic acid was used as internal standard.

A quantity of aspirin weighing 3.7931 mg was taken and dissolved to 25 ml using methanol. An aliquot of 4 ml was taken and diluted to 10 ml. The sample was injected for the chromatogram. A concentration of 0.0021mg/ ml of pure salicylic acid was also prepared. The salicylate was injected for the chromatogram. [26]

2.2.7.3 Assay of standard paracetamol sample:

A quantity of paracetamol sample weighing 0.3197 g was dissolve in a mixture of 10 ml of water and 30 ml of 0.1 M sulphuric acid. The sample was boiled under reflux for one hour, cooled and diluted to 100 ml with water. To 20 ml of the solution, 40 ml of water, 40 g of ice, 15 ml of 0.2 M hydrochloric acid, and 0.1 ml of ferroin sulphate solution were added and titrated with 0.1 M ammonium cerium (IV) sulphate Vs until a yellow colour was obtained. Three replicate were performed. The procedure was repeated without the paracetamol. Each ml of 0.1 M ammonium cerium (IV) sulphate is equivalent to 0.007560 g of $C_8H_9NO_2$.

Standardization of sodium thiosulphate with potassium bromate:

Twenty (20) ml of 0.0167M potassium bromate was pipetted into a conical flask. Forty (40) ml of water, 10 ml of potassium iodide solution and 5 ml of 7 M hydrochloric acid were added. The solution was titrated with 0.1 M sodium thiosulphate solution using 1 ml of starch solution as indicator. Each ml of 0.1M sodium thiosulphate is equivalent to 2.784 mg of $KBrO_3$. The process was repeated.

Standardizing Cerium ammonium (IV) sulphate with 0.1 M sodium thiosulphate:

Twenty five (20) ml of Cerium ammonium sulphate was pipetted in to a conical flask. Two (2) g of potassium iodide and 150 ml of water of water was added. This was titrated immediately with 0.1 M sodium thiosulphate using 1 ml of starch solution as indicator. Two other replicates were performed.

2.2.7.4 Assay of Caffeine Standard powder:

A quantity of caffeine weighing 0.6836 g was dissolved and heated in 20 ml anhydrous acetic acid for 5 minutes. This was transferred into a 100 ml volumetric flask. Forty (40) ml of acetic acid anhydride was added and toped to the mark with toluene solution. Twenty (25) ml of the prepared solution was then titrated with 0.1 M perchloric acid. Each (1) ml of 0.1 M perchloric acid is equivalent to 19.42 mg of caffeine.

Preparation of 0.1 M perchloric acid:

A volume of glacial acetic acid (130 ml) was measured into a 200 ml volumetric flask. Perchloric acid (2.1 ml of 60%) was slowly added with continuous and efficient mixing. Six (6) ml acetic anhydride was added. The addition was made slowly with continuous whirling.

The volume was adjusted to 200 ml with glacial acetic acid. The solution was allowed to stand for 24 hrs before used.

Standardization of (0.1 M) perchloric acid:

Potassium hydrogen phthalate was used in the standardization of the perchloric acid. Potassium hydrogen phthalate (0.2394 g) was weighed into a conical flask. A volume of glacial acetic acid (25 ml) was pipetted into the conical flask. The solution was warm to dissolve the salt. The resulting solution was then titrated potentiometrically with the 0.1M perchloric acid. Each ml of 0.1M perchloric acid is equivalent to 0.020414g of perchloric acid. This was repeated for two other masses of Potassium hydrogen phthalate (0.2394 g and 0.2394 g).

2.2.8 Identification of APIs in the Tablets using thin layer chromatography(TLC)

The TLC plates were prepared by layering a silica gel of 0.5mm on a glass plate and activated for 20 minutes at 120^{o}C in an oven.

A combination of chloroform and methanol in the ratio 9:1 was used as mobile phase for the caffeine. Developing the thin layer chromatogram for aspirin and paracetamol, chloroform and acetone in the ratio 4:1 was used. Pure samples of aspirin, paracetamol, caffeine and Ground EFPAC tablets were dissolved separately in methanol and used for the spotting.

Two TLC plates were used. For the first plate, the dissolved caffeine and the dissolved tablet were spotted, dried and placed in the chromatographic tank containing the chloroform and methanol mixture.

On the second TLC plate, aspirin, paracetamol and the dissolved tablet solutions were spotted and placed in the chromatographic tank containing chloroform and acetone. The spots after separation were located under 254nm UV light. Refer to section 3.1.6

2.2.9 Friability test

Thirteen (13) tablets from a particular batch were removed from the blister pack foil and weighed collectively before placing in the friabilator. The tablets were allowed to revolve for 4 minutes approximating to 100 revolutions. The lose tables were removed and reweighed. The method was repeated for the rest of the batches under study. Refer to table 11.

2.2.10 Uniformity of weight test

Twenty (20) tablets from a particular batch were removed from the blister pack foil and weighed individually and collectively. The differences between the masses of the individual tablets and the mean from the 20 tablets weighed together were calculated. The percentage deviations were also calculated. This method was repeated for the other batches. Refer to section 6.3 for the various tables of weight uniformity test.

2.2.11 Disintegration test

Six (6) tablets were removed from a blister pack from each batch and each placed in the cylindrical tubes in the disintegration basket. The perforated disk was placed on top of each tablet. The bottom of the dissolution basket was placed at least 15mm below the surface of the water and the machine was made to operate, whilst the time taken for each tablet to disintegrate was recorded. The same procedure was repeated for the other batches. Refer to table 49

2.3 HPLC Analysis process

2.3.1 HPLC method of analysis

The mobile phase used for the assay of the Aspirin, paracetamol, caffeine and salicylic acid using benzoic acid as internal standard was Water: Methanol: glacial acetic acid in the ratio (75:25:0.2) pH 2.98. The compounds were eluted isocratically using a spherisorb S5ODS1 (25cm x 4.6mm) column with a flow of 1.3 ml per minute. The detector was set at 298nm with an AUF of 0.2. The compounds were eluted in the following order: Paracetamol, salicylic acid, Aspirin, Benzoic acid and caffeine.

The mean retention time and standard deviation were determined for each compound by injecting 5 replicates of a standard solution. For paracetamol, 0.8ml of 0.8004mg/ml was diluted to 10 ml, 20 ul was injected for the chromatogram. The concentrations used as stock solution for salicylic acid, aspirin, and caffeine were 0.6376mg/ml, 0.6388mg/ml and 0.142mg/ml respectively. 0.8ml each was taken and diluted to 10ml for the chromatogram. The precision was determined by using the chromatogram obtained from the above injections. Refer to table 4.

The accuracy of recovery was determined using the same data. Refer to table 5.

2.3.1.1 Calibration curves

Mobile phase preparation:

One litre of a mobile phase was prepared by admixing of water, methanol and Glacial acetic acid in the ratio (75:25:0.2). This solution was used as the mobile phase for elution.

Preparation of dissolution medium:

One litre of a solution was prepared by admixing of water and methanol in the ratio 50:50. This mixture was used as the dissolution medium for the sample preparation.

Internal standard preparation:

A quantity of powder containing 0.2676g of Benzoic acid was dissolved in the dissolution medium to 100ml and used as the stock solution. 2ml of this stock solution prepared was added to each final prepared solution before injection.

2.3.1.1.a Calibration curve for Aspirin

A quantity of powder containing 25.1mg of standard aspirin powder was dissolved to 25 ml with the dissolution medium and used as stock solution. Aliquots of 0.60 ml, 0.80 ml, 1.00 ml, 1.20 ml, 1.40 ml and finally 1.60 ml of the stock solution were taken. Aliquots of 2.0ml of the internal standard were added to each and these were diluted to 10ml with the dissolution medium. Each sample was then injected for the chromatogram. Refer to section 6.7.1.

2.3.1.1.b Calibration curve for Paracetamol

A quantity of powder containing 64.9 mg of standard paracetamol powder was dissolved to 25 ml with the dissolution medium and used as stock solution. Volumes of 0.15 ml, 0.20 ml, 0.25 ml, 0.30 ml, 0.35 ml and finally 0.40 ml of the stock solution were taken. Aliquots of 2.0 ml of the internal standard were added to each and these were diluted to 10ml with the dissolution medium. Each sample was then injected for the chromatogram. Refer to section 6.7.2.

2.3.1.1.c Calibration curve for Caffeine

A quantity of powder containing 66.9 mg of standard caffeine powder was dissolved to 50ml with the dissolution medium and used as stock solution. Aliquots of 0.15 ml, 0.20 ml, 0.25 ml, 0.30 ml, 0.35 ml and finally 0.40 ml of the stock solution were taken. Aliquots of 2.0ml

of the internal standard were added to each and these were diluted to 10ml with the dissolution medium. Each sample was then injected for the chromatogram. Refer to section 6.7.3.

2.3.1.1.d Calibration curve for salicylic acid

A quantity of powder containing 25.5 mg of standard salicylic acid powder was dissolved to 50 ml with the dissolution medium and used as stock solution. Aliquots of 0.10 ml, 0.15 ml, 0.20 ml, 0.25 ml, 0.30 ml and finally 0.35 ml the stock solution were taken. Aliquots of 2.0ml of the internal standard were added to each and these were diluted to 10ml with the dissolution medium. Each sample was then injected for the chromatogram. Refer to section 6.7.4.

2.3.1.1.e Limit of detection (*LOD*) and limit of quantification (*LOQ)* determination

Standard solutions of paracetamol, aspirin, caffeine and salicylic acid having the concentrations 778.8ug/ml, 168 ug/ml, 52.6355 ug/ml and 4.1569 ug/ml respectively were injected five times and the average peak heights measured. The average heights of the base line noise were measured at the base of each peak area of the injected pure samples. Refer to section 3.2.3

2.3.1.2 Analysis of samples

All the 20 tablets used for the uniformity of weight test were crushed and grounded in to powder. This was repeated for all the other batches. A grounded tablet powder of mass 0.0752 g belonging to batch 137371 was weighed. The mass weighed was dissolved to 50ml using the dissolution medium. An aliquot of 1.6 ml was taken. Another aliquot of 2 ml of the internal standard was added to the 1.6 ml and finally diluted to 10ml. The final solution prepared was filtered with a micro filter and 20 µl injected for the chromatogram. This was repeated twice for the same sample. The other batches were analyzed following the same format. Refer to table 54 and table 55

2.3.1.3 Preparing a quality control chart

This involves the estimation of Upper action line and the Lower action line. These control limits have already been determined and stated in the compendia. The USP stated clearly that

for the combined product of Aspirin, Paracetamol and caffeine 110% and 90% is the range stipulated. Refer to figure 12,13 and 14

2.3.2 UV/ Visible Spectrophotometric analysis

Two litres 0.01M hydrochloric acid was prepared and used as the dissolution medium.

2.3.2.1 Maximum wavelength determination

A quantity of powder containing 59.9 mg of standard aspirin powder was weighed and dissolved to 250 ml with the dissolution medium. An Aliquot of 1.2 ml of the prepared solution was pipetted and diluted to 25 ml giving a concentration of (0.0011%w/v). The maximum wave length was determined using the UV/ Visible spectrophotometer. Standard Paracetamol powder weighing 89.4 mg was dissolved to 250 ml with the dissolution medium. Aliquot of 0.6 ml of the prepared solution was pipette and diluted to 25 ml giving a concentration, 0.0008%w/v. The maximum wave length was determined using the UV/ Visible spectrophotometer.

A quantity of powder containing 54.6 mg of standard caffeine powder was weighed and dissolved to 250 ml with the dissolution medium. 1.2 ml of the prepared solution was pipette and diluted to 25 ml giving a concentration (0.0011%w/v). The maximum wave length was determined using the UV/ Visible spectrophotometer.

2.3.2.2 Calibration curves

Calibration curves were prepared using the maximum wave lengths obtained from the scan. For each pure API, calibration curves were prepared at the three maximum wavelengths.

2.3.2.2.1 Calibration curve for Aspirin

A quantity of powder containing 59.9 mg of standard aspirin powder was weighed and dissolved to 250 ml with the dissolution medium giving a concentration 0.0240%w/v. The 250 ml volume prepared was used as the stock solution from which various volumes were taken and diluted to 25 ml for the calibration curve. The absorbances were read at 229nm, 244nm and 274nm. Refer to section 6.8.1

2.3.2.2.2 Calibration curve for Paracetamol

A quantity of powder containing 89.4 mg of standard paracetamol powder was weighed and dissolved to 250 ml with the dissolution medium giving a concentration 0.0358%w/v. The 250 ml volume prepared was used as the stock solution from which various volumes were taken and diluted to 25 ml for the calibration curve. The absorbances were read at 229nm, 244nm and 274nm. Refer to section 6.8.2

2.3.2.2.3 Calibration curve for caffeine

A quantity of powder containing 54.6 mg of standard caffeine powder was weighed and dissolved to 250 ml with the dissolution medium giving a concentration 0.0218%w/v. The 250 ml volume prepared was used as the stock solution from which various volumes were taken and diluted to 25 ml for the calibration curve. The absorbances were read at 229nm, 244nm and 274nm. Refer to section 6.8.3

2.3.2.3 Dissolution test

Six replicates of dissolution were performed on each batch using ordinary distilled water as the dissolution medium. The dissolution for each tablet took 60 minutes with a paddle revolution rate of 100rpm at 36.5^{o}C. During the course of the process, 5 ml was taken at10 minute intervals up to the 60th minute for some of the batches. For other batches, 5ml was taken only at the 60th minute. Each 5 ml taken was diluted to 50 ml. An aliquot of 3 ml from the 50 ml prepared was taken and re-diluted to 10ml. All the dilutions were made with 0.01M Hydrochloric acid. The absorbances for each sample were taken at 229nm 244nm and 274nm. The Actual amount of Aspirin, Paracetamol and caffeine were calculated using the simultaneous equation. Refer to section 3.2.4.2, table 68, table 69 and table 70.

2.3.3 Comparison of UV. spectrophotometric and HPLC methods of analysis using standard pure samples:

The HPLC method and the uv. spectrophotometric methods were compared and the various means observed analyzed with ANOVA. Pure samples containing 0.0355 mg of caffeine, 0.2001 mg of paracetamol and 0.1597 mg of aspirin were weighed. Each pure sample was dissolved and diluted with distilled water to 250 ml. Aliquots of the stock were taken and diluted with the various diluents for both HPLC and spectrophotometric method. Six (6)

replicates of each were performed and the results analyzed statistically. Refer to section 3.2.3.1

2.3.4 Assay of tablets using standard and developed methods and their comparison.

For aspirin:

A quantity of the tablet containing 0.7 g of aspirin, 20 ml of water and 2 g of sodium citrate was added and sample boiled under a reflux condenser for 30 minutes. The product was allowed to cool. The condenser was washed with 30 ml of warm water and titrated with 0.5M sodium hydroxide using phenolphthalein solution as indicator. Each ml of 0.5M sodium hydroxide is equivalent to 45.04 mg of Aspirin. Seven batches were used for the test.

For caffeine:

A quantity of the powder containing 30 mg of Caffeine was weighed into a 250 ml volumetric flask, 200 ml of water was added and sample shook for 30 minutes. Sufficient water was added to the 250 ml mark. The prepared solution was filtered. Ten (10) ml of the filtrate was taken and 10 ml of 1M sodium hydroxide was added. The caffeine was extract with five 30 ml quantities of chloroform, washing each extract with the same 10 ml of water. The extract was filtered through absorbent cotton previously moistened with chloroform. The chloroform was evaporated and the residue was dissolved in 50 ml warm water in a 100 ml volumetric flask. Sufficient water was added producing 100 ml. The absorbance of the resultant solution was measured at 273 nm. The content of caffeine was calculated taking 504 as the value of A_1^1 (1%, 1 cm) at the maximum at 273 nm. Seven batches were used for the test.

Using the same batches, their contents were assayed using the developed HPLC and the UV. spectrophotometric methods. ANOVA was used to compare the mean of content recovered. Refer to table 71, 72.73 and 74.

3 CHAPTER THREE: RESU LTS AND CALCULATIONS

3.1 Sample characterization

3.1.1 Melting point determination

Table 1 Melting point determination of the various pure compounds

Pure drug substances	**Melting point**
Paracetamol	170 ℃ to 172 ℃
Aspirin	142 ℃ to 144 ℃
Caffeine	234 ℃ to 238 ℃
Benzoic acid	120 ℃ to 122 ℃
Salicylic acid	156 ℃ to 158 ℃

3.1.2 Identification test for paracetamol

A.) Upon scanning, maximum absorbance was 0.894 at 248.5nm.

Mass of paracetamol taken = 0.1015g dissolved to 100 ml (0.1015%w/v)

1 ml of (0.1015%w/v) was taken and diluted to 100 ml,

Concentration = (0.1015%w/v)/100 = 0.001015%w/v

The specific absorbance can be calculated from the Beer-Lambert's law (A = abc)

A = Absorbance of the prepared solution

A = Specific absorbance (A (1%, 1cm)

B = Path length (1cm)

c = Concentration in g/100 ml (%w/v)

Calculation of the specific absorbance (A (1%, 1cm)) = A / b×c

A (1%, 1cm) = 0.894 / (1 × 0.001015)

A (1%, 1cm) = 880.8

Since the calculated specific absorbance falls within 860 and 980, the substance under study is paracetamol.

B.) After adding 1ml water to the heated paracetamol solution, the sample was cooled on an ice bath. No precipitate was formed. Upon adding the potassium dichromate solution, a violet colouration developed. This violet colouration does not change to red. Paracetamol is therefore present.

3.1.3 Identification of Aspirin

A.) Precipitate was formed after addition of the sulphuric acid. The precipitate formed had a melting point range of 158 ℃ to 160 ℃. This confirms the presence of aspirin.

B.) A Violet colouration evolved after adding the ferric chloride solution. No de-colouration was observed when the acetic acid was added. This implies a positive test for salicylate and therefore the presence of aspirin.

3.1.4 Identification of caffeine

A brown precipitate was formed when the hydrochloric acid was added to the iodinated potassium iodide saturated caffeine solution. The precipitate re-dissolved after neutralization with sodium hydroxide.

3.1.5 Assay of Standard drug powder:

3.1.5.1 Assay of Aspirin

Preparation for 0.5Molar HCl solution:

Molar mass: 36.5

Percentage purity: 35.4% $^{w}/v$.

Specific gravity: 1.18g/ml.

36.5gHCl ≡1000ml of 1MHCl

18.25gHCl ≡1000ml of .5MHCl

0.01825gHCl≡1ml of 0.5MHCl

4.5625gHCl≡250 of 0.5MHCl

If 35.4% of impure HCl≡ 4.5625g

Then 100% of appropriate mass ≡ (100%×4.5625g) / 35.4% ≡ 12.8884g

Actual volume to be taken ≡ appropriate mass / specific gravity

≡ (12.8884g) / (1.18g/ml)

≡ 10.92ml (volume of stock solution to be taken)

Preparation of 0.5Molar NaOH:

Percentage purity is 98%^{w}/v.

40g NaOH≡1000ml of 1MNaOH

5g NaOH≡250ml of 0.5NaOH

But purity is only 98%. Therefore finding appropriate mass is =

(100/ 98)× 5.00g = 5.102g

Preparation of the 100ml standard solution sulphamic acid;

97.09gH_2NSO_3H≡1000ml of 1MNaOH

48.545gH_2NSO_3H≡1000ml of 0.5MNaOH

4.8545gH_2NSO_3H≡100ml of 0.5MNaOH

0.4855gH_2NSO_3H≡10ml of 0.5MNaOH

0.0485gH_2NSO_3H≡1ml of 0.5MNaOH

Standardizing 0.5 M sodium hydroxide:

Average mass of sulphamic acid weighed in to the conical flask = 1.2112g

From the Milliequivalent the expected titer volume is = 24.97ml

Actual average titer volume = 24.85 ml

Factor of 0.5 M sodium hydroxide (F_1) = 24.97 / 24.85

F_1= 1.005

Standardizing 0.5 M HCl:

$F_1 \times V_1 = F_2 \times V_2$

F_1=Factor for the NaOH (1.005)

F_2=Factor for the HCl.

V_1= Pipette volume of the NaOH (50.0ml).

V_2=Titer volume of the HCl (49.0ml).

There for F_2=1.0255

Assay calculation for aspirin:

Volume of the sodium hydroxide that reacted with the aspirin:

$= \{V_b - Va\} \times F3$

V_b = Titer volume of the blank containing no Aspirin (49.0ml).

V_a = Titer volume of the sample containing Aspirin (37.15ml).

$= (49.0\text{-}37.15) \times 1.0255$

= 12.15 ml

Total salicylate (Aspirin + salicylic acid) from standard aspirin

= (12.15 ml× 0.045g)/ 1ml= 0.5468 g

The assay of Aspirin using titer metric method gives the total salicylate (Aspirin and salicylic acid)

Total salicylate (Aspirin + salicylic acid) from standard aspirin = 0.5468g

HPLC method for the assay of salicylic acid in aspirin power:

(Cs/Cu) = (Ps/Pu)

Cs =Concentration of standard salicylic acid (0.0021mg/ ml)

Cu =Concentration of Test salicylic acid

Ps = Peak area ratio of standard salicylic acid (0.3542)

Pu = Peak area ratio of Test salicylic acid (0.1330)

Cu = ((0.0021mg/ ml) × (0.1330)) / (0.3542) = 0.000789 mg/ ml

The actual mass of salicylic acid in the aspirin taken = (25×10/ 4) × 0.000789 mg/ ml

= 0.0493 mg

Percentage content of salicylic acid in standard aspirin = (0.0493 mg / 3.7931 mg)× 100

=1.3 %

Percentage content of aspirin in the pure sample:

{(Total salicylate from standard aspirin – salicylic acid in standard aspirin**) / (**mass of aspirin)}× 100

= {(0.5468 g - 1.3% (0.5468 g)) / 0.5469 g} × 100

= 98.67 %

The process of assay and calculation were repeated for the other masses.

The percentage contents were 98.65% and 98.68 %

The average percentage content = 98.67% + 98.65% +98.68 %

Assay of Standard drug powder = 98.67 %

3.1.5.2 Assay of standard paracetamol powder:

Preparation of 0.1 M ammonium cerium (IV) sulphate:

632.6g $2(NH_4)_2SO_4,Ce(SO_4)_2,2H_2O$ = 1000 ml of 1M $2(NH_4)_2SO_4,Ce(SO_4)_2,2H_2O$

6.326g $2(NH_4)_2SO_4,Ce(SO_4)_2,2H_2O$ = 100ml of 0.1M $2(NH_4)_2SO_4,Ce(SO_4)_2,2H_2O$

Mass of ammonium cerium (IV) sulphate need to prepare 100 ml of 0.1 M = (100/ 97.5) × 6.326g

= 6.4882 g

Preparation of 0.0167 M potassium Bromate:

167.0 g KBrO3 = 1000ml of 1 M KBrO3

0.2788 g KBrO3 = 100 ml of 0.0167 M KBrO3.

Standardizing 0.1 M sodium thiosulphate using potassium bromate:

Factor (F1) of KBrO3 = Actual / Nominal = (0.2771 / 0.2788) = 0.9938

Average titer volume of sodium thiosulphate = $\frac{20.2 \text{ ml} + 20.3 \text{ ml} + 20.0 \text{ ml}}{3}$ = 20.167 ml

Factor of 0.1 M sodium thiosulphate = (Factor of KBrO3× Volume of KBrO3) / Titer volume

= (0.9938 × 20) / 20.167

Factor of 0.1 M sodium thiosulphate = 0.9856

Factor of 0.1 M Cerium ammonium (IV) sulphate:

Titer volume = $\frac{20.2 \text{ ml} + 20.3 \text{ ml} + 20.2 \text{ ml}}{3}$ = 20.23 ml

Factor of 0.1 M Cerium ammonium (IV) sulphate = (Factor of sodium thiosulphate × Volume of sodium thiosulphate) / volume of Cerium ammonium sulphate

= 0.9856 × 20.23/ 20

= 0.9969

Titration of paracetamol with 0.1 M Cerium ammonium (IV) sulphate:

The average titer volume of paracetamol = $\frac{8.8 \text{ ml} + 8.9 \text{ ml} + 8.9 \text{ ml}}{3}$ = 8.87 ml

Average titer volume of the blank = 0.3 ml

Volume of ammonium cerium (IV) sulphate that reacted with paracetamol

= (8.87 ml – 0.3 ml) × 0.9969

= 8.54 ml

Actual amount of paracetamol = (0.007560 g × 8.54 ml)/ 1 ml

= 64.5624 mg

Percentage content of paracetamol standard = Actual mount / nominal amount

= 64.5624 mg / (319.7 mg × (20/ 100))

Percentage purity of Standard Paracetamol = 100.97 %

3.1.5.3 Assay of Caffeine Standard powder:

Standardization of perchloric acid with potassium hydrogen phthalate:

Expected titer volume = (0.2394 g / 0.020414g) × 1ml

=11.73 ml

Average titer volume = $\frac{11.7 \text{ ml} + 11.7 \text{ ml} + 11.6 \text{ ml}}{3}$ = 11.67 ml

Factor of perchloric acid = Expected titer volume / Actual titer volume

=11.73 ml / 11.67ml

= 1.005

Actual mass of caffeine in tablet:

Each 20 ml of the prepared caffeine stock contains = 0.6836 g × (25 ml /100)

= 0.1709 g

Average titer volume = $\frac{8.9 \text{ ml} + 8.7 \text{ ml} + 8.9 \text{ ml}}{3}$ = 8.83 ml

Actual amount of Caffeine = (8.83 ml × 0.01942 mg) × 1.005 / 1 ml

=0.1723g

Percentage content = Actual amount of Caffeine / Expected amount present

= (0.1723 mg /0.1709 mg) × 100

= 100.84%

3.1.6 Identification of benzoic acid

A light yellow coloured precipitate was formed which was soluble in ether. This indicates the presence of benzoic acid.

3.1.7 Identification of APIs in the tablets using thin layer chromatography (TLC)

The chromatograms of pure aspirin, caffeine, paracetamol and grounded EFPAC are shown in Figure 1 and Figure 2. Refer to table 1 for Retention Factor (R_f).

Thin layer chromatogram for identification.

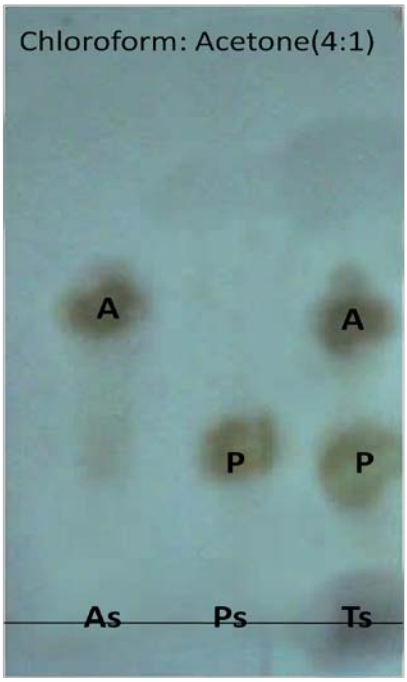


Figure 1

Thin layer chromatogram for aspirin, Paracetamol and EFPAC

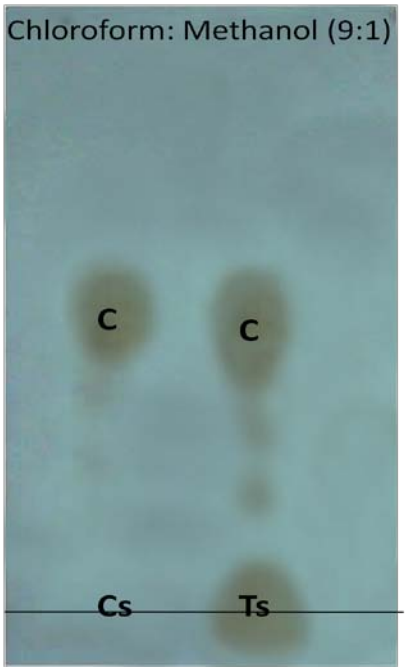


Figure 2

Thin layer chromatogram for caffeine and EFPAC

Key : A= Aspirin
C = Caffeine
P = Paracetamol
As= Standard Aspirin initial sport.
Cs= Standard Caffeine initial spot.
Ps= Standard Paracetamol initial spot
Ts= Tablet initial spot.

Table 2 R_f values calculated from the TLC distribution.

APIs	Caffeine	ASA	Paracetamol
Pure Mean R_f	60.0	44.7	16 .0
Tablet Mean R_f	60.7	44.7	16.7

3.1.8 Identification of salicylic acid

A violet colour was produced after addition of the ferric chloride solution. The violet colour persisted after adding the acetic acid. This confirms the presence of salicylate.

3.2 HPLC Analysis

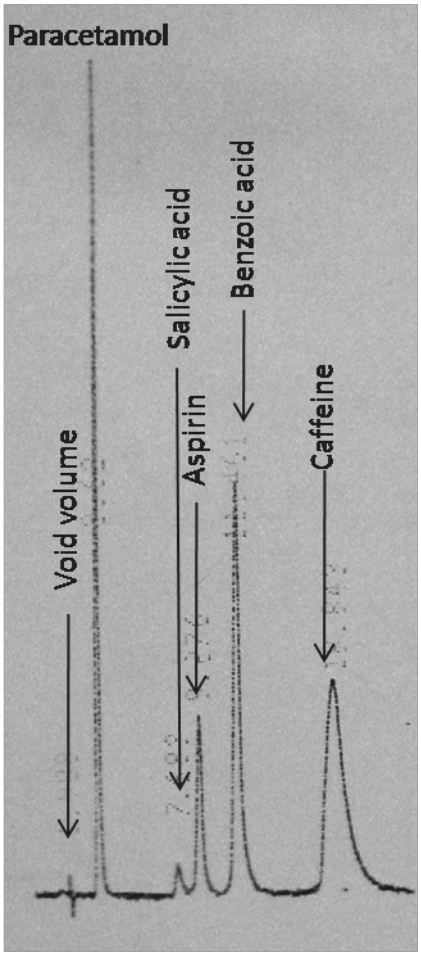


Figure 3a
A typical HPLC Chromatogram of the pure Samples

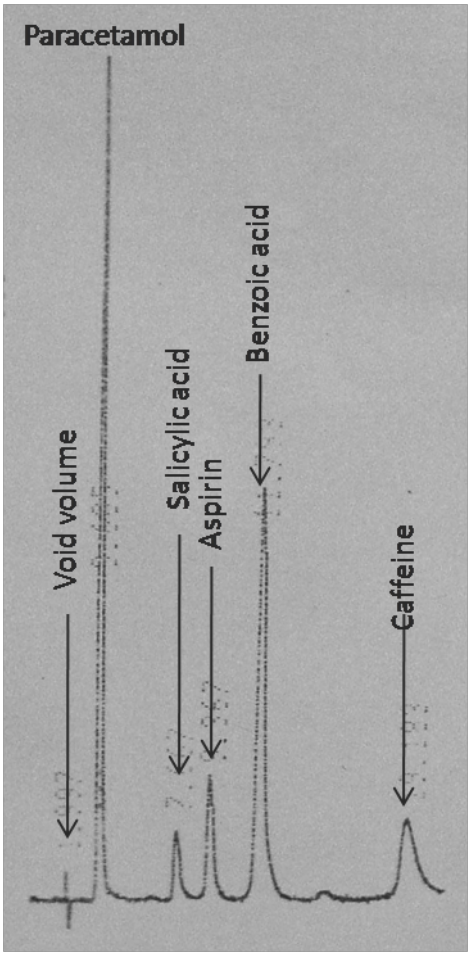


Figure 3b
A typical HPLC Chromatogram of the tablet

3.2.1 Retention time of the APIs

The actual retention mean time lies within the range of mean ± standard deviation

Table 3 Mean retention time of pure samples.

Pure samples	**Mean time(min)**	**SD**	**Actual mean retention time (min)**
paracetamol	3.640	0.011	3.640 ± 0.011
Salicylic acid	7.668	0.028	7.668 ± 0.028
Aspirin	9.258	0.030	9.258 ± 0.030
Caffeine	18.848	0.100	18.848± 0.100

3.2.2 Precision and accuracy determination when using HPLC

Table 4 Precision of method

Pure samples	**Mean mass (g)**	**SD**	**RSD%**
Paracetamol	0.2004	0.0008	0.3992
Salicylic acid	0.1595	0.0003	0.1881
Aspirin	0.1584	0.0011	0.6944
Caffeine	0.0356	0.0006	1.6854

Table 5 Accuracy of method

Pure samples	**Percentage recovered**	**SD**	**RSD%**
paracetamol	100.1	0.4130	0.4124
Salicylic acid	100.1	0.1718	0.1717
Aspirin	99.3	0.7065	0.7112
Caffeine	100.3	1.5810	1.5765

3.2.3 The limit of detection (*LOD*) and limit of quantification (*LOQ)*

LOD = Concentration yielding a signal-to-noise ratio of 2:1

LOQ = Concentration yielding a signal-to-noise ratio of 10:1

LOD

The average base line noise = 1mm

Therefore a concentration yielding a signal to noise ratio of 2:1, should have a peak height of 2x1mm (2mm).

LOQ

The average base line noise = 1mm

Therefore a concentration yielding a signal to noise ratio of 10:1 should have a peak height of 10x1mm (10mm).

Paracetamol:

A known concentration of paracetamol (778.8 ug/ml) yielded an average peak height of 82mm.

LOD = 2mm x {(622.298 ug/ml)/82 mm}

= 15.178 ug /ml

LOQ = 10mm x {(622.298 ug/ml) / 82 mm}

= 75.89 ug / ml

Aspirin:

A known concentration of aspirin (168 ug/ml) yielded an average peak height of 82mm.

LOD = 2mm x {(168 ug/ml)/ 48 mm}

= 7 ug /ml

LOQ = 10mm x {(168 ug/ml) / 48 mm}

= 35.0 ug / ml

Caffeine:

A known concentration of caffeine (52.6355 ug/ml) yielded an average peak height of 50mm.

LOD = 2mm x {(52.6355 ug/ml)/ 50 mm}

= 2.1054 ug /ml

LOQ = 10mm x {(52.6355 ug/ml) / 50 mm}

= 10.5271 ug / ml

Salicylic acid:

A known concentration of salicylic acid (4.1569 ug/ml) yielded an average peak height of 54mm.

LOD = 2mm x {(4.1569 ug/ml)/ 54 mm}

= 0.154 ug /ml

LOQ = 10mm x {(4.1569 ug/ml) / 54 mm}

= 0.7698 ug / ml

3.2.4 UV. Spectrophotometric quantitative analysis

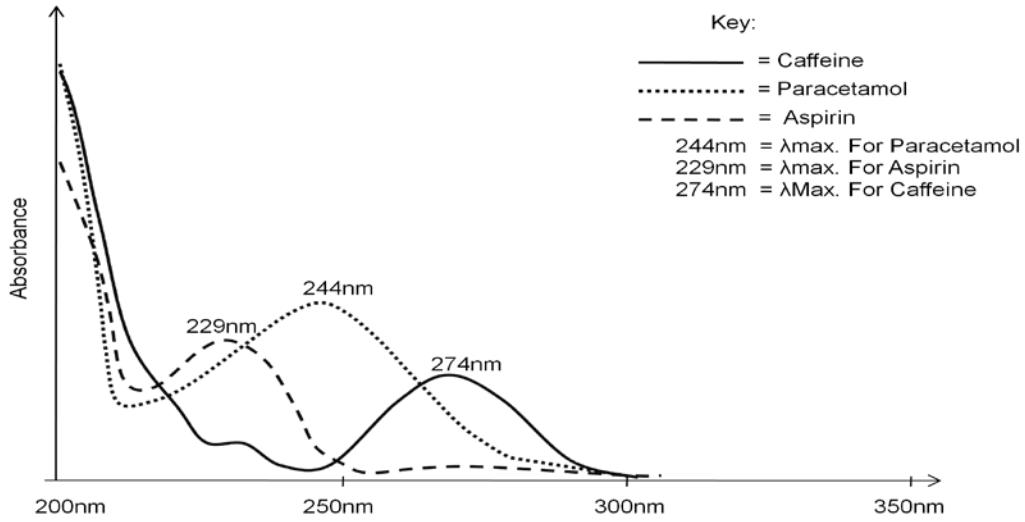


Figure 4a Spectrum of Aspirin, paracetamol and caffeine in 0.01MHCl.

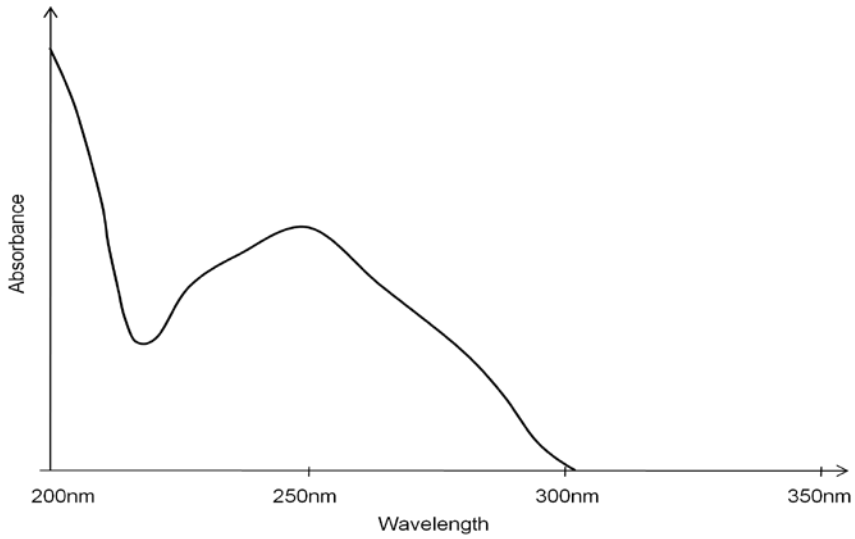


Figure 4b Spectrum of solution containing aspirin, paracetamol and caffeine in 0.01MHCl

From the calibration curve prepared, the various equations of the lines can be combined into a simultaneous equation involving three variables. The three variables therefore represent the various compounds under study.

Table 6 Equations of lines of the calibration curves using UV spectrophotometric analysis.

Wavelength used	Aspirin	Paracetamol	caffeine
229nm	$y = 461.5x_a + 0.011$	$y = 492.3x_p + 0.021$	$y = 278.4x_c - 0.022$
244nm	$y = 142.1x_a + 0.012$	$y = 678.8x_p - 0.005$	$y = 146.3x_c - 0.012$
274nm	$y = 60.72x_a - 0.003$	$y = 151.0x_p + 0.001$	$y = 497.0x_c + 0.000$

Key: y = Absorbance at the various wavelengths

xa = Concentration of aspirin (%w/v)

xp = Concentration of paracetamol (%w/v)

xc = Concentration of caffeine (%w/v)

3.2.4.1 Simultaneous equation for the UV/ visible Spectrophotometric analysis.

Since there was no extraction done, it means each absorbance taken has a contribution from all the three compounds present in the tablets. As such, the absorbance at each wavelength from the assay can be equated to the summation of the specific absorbance of each contributing compound multiplied by the concentration of the compounds contributing to that absorbance.

A_1^1(229nm)×p + A_1^1 (229nm)×a + A_1^1 (229nm)×c = A(229nm)----------Equation one

A_1^1 (244nm)×p + A_1^1 (244nm)×a + A_1^1 (244nm)×c = A(244nm)----------Equation two

A_1^1 (274nm)×p + A_1^1 (274nm)×a + A_1^1 (274nm)×c = A(274nm)----------Equation three

Key:

A_1^1(229nm)×p = Specific absorbance of Paracetamol at 229nm ×Paracetamol concentration

A_1^1(244nm)×p = Specific absorbance of Paracetamol at 244nm×Paracetamol concentration

A_1^1(274nm)×p = Specific absorbance of Paracetamol at 274nm×Paracetamol concentration

A_1^1(229nm)×a = Specific absorbance of Aspirin at 229nm ×Aspirin concentration

A_1^1(244nm)×a = Specific absorbance of Aspirin at 244nm ×Aspirin concentration

A_1^1(274nm)×a = Specific absorbance of Aspirin at 274nm ×Aspirin concentration

A_1^1(229nm)×c = Specific absorbance of caffeine at 229nm ×Caffeine concentration

A_1^1(244nm)×c = Specific absorbance of caffeine at 244nm ×Caffeine concentration

A_1^1(274nm)×c = Specific absorbance of caffeine at 274nm ×Caffeine concentration

A(229nm) = cumulative absorbance of sample at 229nm

A(244nm) = cumulative absorbance of sample at 244nm

A(274nm) = cumulative absorbance of sample at 274nm

Therefore:

$461.5x_a + 492.3x_p + 278.4x_c = y_a + y_p + y_c - 0.01$ (Absorbance at 229nm)……..……eq1

$142.1x_a + 678.8x_p + 146.3x_c = y_a + y_p + y_c + 0.005$ (Absorbance at 244nm)…….……eq2

$60.72x_a + 151.0x_p + 497.0x_c = y_a + y_p + y_c - 0.002$ (Absorbance at 274nm)……..…eq3

3.2.4.2 Sample calculation using UV/ visible Spectrophotometric method

Using batch 137367 as example calculation:

After the dissolution time of 60 minutes, 5 ml was taken and diluted 50 ml with 0.01MHCl. 3 ml was then taken and diluted to 10 ml. The absorbance read at 229nm, 244nm and 274nm were 0.649, 0,604 and 0.201 respectively.

From the equations,

$y_a + y_p + y_c = 0.649$ (Absorbance at 229nm)

$y_a + y_p + y_c = 0.604$ (Absorbance at 244nm)

$y_a + y_p + y_c = 0.201$ (Absorbance at 274nm)

By substitution,

$461.5x_a + 492.3x_p + 278.4x_c = 0.649 - 0.01$ (Absorbance at 229nm)…….……eq1

$142.1x_a + 678.8x_p + 146.3x_c = 0.604 + 0.005$ (Absorbance at 244nm)…….……..….eq2

$60.72x_a + 151.0x_p + 497.0x_c = 0.201 - 0.002$ (Absorbance at 274nm)……eq3

Therefore:

$461.5x_a + 492.3x_p + 278.4x_c = 0.639$ ……eq1

$142.1x_a + 678.8x_p + 146.3x_c = 0.609$ …….eq2

$60.72x_a + 151.0x_p + 497.0x_c = 0.203$ ……..…eq3

Eliminating x_a

-eq2→eq1

$-69955.83\ x_p - 39560.64x_c = -90.8019$ ……..……..3

Eq1→eq2

$313266.2\ x_p + 67517.45x_c = 281.0535$ ……..4

Summation of 3 and 4 =

$243310.37\ x_p + 27956.81\ x_c = 190.251$…….…….5

Eq1 →eq3

$69686.5\ x_p + 229365.5\ x_c = 93.6845$…….……..……6

-eq3 →eq1

-29892.456 x_p -16904.448 x_c = -38.80008……..……7

Summation of 6 and 7 =

39794.044 x_p - 212461.052 x_c = 54.88442……..8

Combining equations 5 and 8 and eliminating x_p

243310.37 x_p + 27956.81 x_c = 190.251……..……..…..…….5

39794.044 x_p - 212461.052 x_c = 54.88442……..……..8

-1112514527 x_c = -7570880.541…….……..9

51693977173 x_c = 13353948.54…….……..10

Summation of 9 and 10 to find x_c

50581462645 x_c = 5783067.996

x_c = 0.000114332 (%w/v) = 0.00114332 mg/ml

Finding **x_p** by substituting **x_c** in to equation 8

39794.044 x_p - 212461.052 (0.000132091) = 54.88442

x_p = 0.000768793 (%w/v) = 0.00768793 mg/ml

Substituting x_p and x_c into equation 3

60.72x_a + 151.0 (0.000766752) + 497.0 (0.000132091) = 0.203

x_a = 0.00049554 (%w/v) = 0.0049554 mg/ml

The actual amount released in the 900ml dissolution bowl=

Amount in mg/ml ×900ml ×(50/5) ×(10/3)

Actual amount of aspirin released = 0.0049554 mg/ml × 900ml ×(50/5) ×(10/3)

= 148.7mg

Percentage content = (146.1mg/ 150mg) × 100 = **99%**

Actual amount of caffeine released = 0.00114332 mg/ml × 900ml ×(50/5) ×(10/3)

=34.3 mg

Percentage content = (34.3 mg / 30mg) ×100 = **114%**

Actual amount of paracetamol released = 0.00768792 mg/ml × 900ml ×(50/5) ×(10/3)

= 230.6 mg

Percentage content = (230.6mg /250 mg)×100 = **92.3%**

3.2.4.3 Comparison of uv spectrophotometric method with the HPLC method.

Table 7 Mass recovered for pure samples using both HPLC and uv. Spectrophotometer

	Sample	Actual mass(mg)	Mass recovered(mg)					
UV Spec.	Caffeine	0.0355	0.0367	0.0380	0.0378	0.0350	0.0350	0.0351
	Paracetamol	0.2001	0.2000	0.2009	0.2054	0.2015	0.2032	0.1992
	Aspirin	0.1597	0.1592	0.1600	0.1563	0.1557	0.1603	0.1576
HPLC	Caffeine	0.0355	0.0361	0.0352	0.0362	0.0349	0.0356	0.0360
	Paracetamol	0.2001	0.1991	0.2002	0.2003	0.2010	0.2012	0.2031
	Aspirin	0.1597	0.1590	0.1595	0.1580	0.1570	0.1597	0.1590

Table 8 Mean mass recovered, standard deviation and standard error for mass recovered

	Number of assay	Mean	Std. Deviation	Std. Error
Caffeine. UV	6	0.036267	0.0014222	0.000581
Caffeine. HPLC	6	0.035667	0.0005279	0.000216
	Number of assay	Mean	Std. Deviation	Std. Error
Paracetamol. UV	6	0.201551	0.002447	0.000999
Paracetamol. HPLC	6	0.20105	0.0010784	0.00044

	Number of assay	Mean	Std. Deviation	Std. Error
Aspirin. UV	6	0.158183	0.0019426	0.000793
Aspirin. HPLC	6	0.158867	0.0007257	0.000296

Table 9 Statistical ANOVA table for comparison

Comparing caffeine recovered	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0	2	0	1.272	0.309
Within Groups	0	15	0		
Total	0	17			
There is no significant difference between the actual mean and the means from the assy. F(2,15)=1.272,p>0.05					
Comparing Paracetamol recovered	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0	2	0	1.367	0.285
Within Groups	0	15	0		
Total	0	17			
There is no significant difference between the actual mean and the means from the assay. F(2,15)=1.367,p>0.05					
comparing aspirin recovered	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0	2	0	2.415	0.123
Within Groups	0	15	0		
Total	0	17			
There is no significant difference between the actual mean and the means from the assay. F(2,15)= 2.415,p>0.05					

4 CHAPTER FOUR
DISCUSSION, CONCLUSION AND RECOMMENDATIONS

4.1 DISCUSSION

4.1.1 Sample characterization

4.1.1.1 Melting point determination

The purity of the compound was ascertained by the determination of the melting point. The melting points of the various APIs were sharp and did fall within the British pharmacopoeia standard. This means the compounds used were pure.

4.1.1.2 Identification of pure samples

Paracetamol

Paracetamol was identified based on the British pharmacopoeia standard. As stated in the pharmacopoeia, the specific absorbance of paracetamol having a concentration of 0.001015%w/v should fall within the range of 860 to 980. As confirmed by the calculation in section 3.1.2, the specific absorbance calculated was 880.8. This implies that paracetamol is present.

Another test performed was the addition of potassium dichromate solution to the cooled pre-heated acidified paracetamol. A violet colouration developed which did not change to red. This implies that paracetamol is present.

Aspirin

Boiling the aspirin in sodium hydroxide solution led to its hydrolysis into salicylic acid and acetic acid. Upon adding sulphuric acid, the salicylic acid present precipitated. The melting point of the dried precipitate was 158 ℃ to 160 ℃. This implies the presence of salicylic acid and therefore aspirin.

Again, the addition of ferric chloride to the pre-dissolved precipitate, leads to the formation of a violet colour solution.

The persistent colour, even after the addition of acetic acid confirms the presence of salicylic acid, hence aspirin.

Caffeine

The hydrochloric acid added to the iodinated potassium iodide saturated caffeine solution leads to the formation of periodide. The periodide precipitate formed dissolved after neutralizing the solution with Sodium hydroxide. This indicates the presence of caffeine.

Benzoic acid

A yellowish coloured precipitate was formed after the addition of ferric chloride to the ethanolic benzoic acid. The precipitate formed was soluble in ether and hence confirms the presence of benzoic acid.

Salicylic acid

The ferric chloride solution leads to the formation of a violet colour solution. The persistent colour, even after the addition of acetic acid confirms the presence of salicylic acid,

Identification of APIs in the tablet used

The Rf values of EFPAC clearly correspond with those of the pure Aspirin, caffeine and paracetamol respectively. This therefore confirms the presence of Aspirin, paracetamol and caffeine in the EFPAC tablet.

4.1.2 Friability test

Thirteen (13) individual tablets were used for the test for each batch. It was expected that none of the tablets would lose a weight of more than 1% of the mass used. This test was essential as the handling and transporting could lead to chipping of the tablet. Loss of tablets by the process would mean less active ingredient would be dispensed. From the test

performed, batch number 137439 had the least percentage loss with 0.0842 % and that of batch number 137400 had a percentage loss of 8.1820% being the highest. Referring to table 11, all the batches passed the test except batch number 137400.

4.1.3 Weight uniformity test

Since 20 individual tablets were selected, not more than two tablets should deviate by ±5.0% and not even a single tablet should deviate by ±10.0%. From the assay, batch number 137367 had zero deviation for two tablets with none falling out of the range specified and therefore passed the test. Batch number 137379, 137424 and 137436 had one tablet each deviating by zero. However, none failed the uniformity test. On the other hand, batch number 137390 and 137385 had one tablet each deviating by zero and another tablet each deviating by more than ±5.0%. They however passed the test. Batch number 137383 failed the test since three tablets had their deviation more than the required ±5.0%. In all, 97% of the batches passed the weight uniformity test and therefore shows high efficiency of mass uniformity and weight distribution.

4.1.4 Disintegration time

From the test, the entire tablets disintegrated within 10 minutes. During the course of the determination, the tablets swell, chip off and disintegrate. Batch number 13737 had the least disintegration time of 1 minute 35.2 seconds. A fast disintegration time like this may impede swallowing and cause vomiting since it would irritate the throat. Batch number 137381 had the longest disintegration time of 6 minutes 13.6 seconds. All the batches passed this particular test. Disintegration time is necessary as the drug formulated needs to break up in time to make its content readily available for dissolution and hence absorption for fast release of pain and headaches.

4.1.5 Assay of Product

The use of HPLC was necessary for the assay of the various products because of its high efficiency. A reversed phase chromatography using a column containing ODS was used for the separation. The use of mobile phase containing Water, Methanol and Glacial acetic in the ratio 75:25:0.2 was used for the assay. The compounds were eluted isocratically using a

spherisorb S5ODS1 phase 5microns (25cm x 4.6mm) column with the flow rate 1.3 ml per minute. The detector was set at 298nm with an Absorbance Unit Fraction (AUF) of 0.2. The wave length 298nm gave an appreciable peak height for all the constituents in the tablet needed for the assay. The mobile phase gave good resolution of all the five compounds involved. Paracetamol eluted first followed by salicylic acid, Aspirin, Benzoic acid and finally caffeine. As indicated in table 3, the last compound elutes at (18.848± 0.100) minutes. The correlation coefficient (r^2) for paracetamol, salicylic acid, aspirin and caffeine were 0.998, 0.999, 0.999 and 0.996 respectively.

Quantification was done based on the internal standard peak area ratio and the preparation of calibration curves for all the compounds assayed. The peak area ratio is the peak area of the sample drug divided by the peak area of the internal standard. The internal standard provides protection against fluctuation of results due to injection problems, day to day variation of environmental conditions, power fluctuations and inherent deficiencies in the instruments. With the elimination of these variations, the precision of the method was improved.

The HPLC method had a high precision and accuracy. As indicated in table 4 and table 5, both had RSD values of less than 2.0% and therefore acceptable. Salicylic acid had the highest precision having a lower relative standard deviation of 0.1881%. Caffeine had the least with 1.6854 %. The lower precision displayed by the caffeine may be due to the tailing effect caused by the interaction with uncapped silanol groups present on the stationary phase used. In terms of accuracy the trend was the same as seen in table 5.

From the assay, paracetamol content had the least standard deviation with 6.4 indicating the least variance within the batches as indicated in section 6.7.9 compared with aspirin and caffeine as indicated in section 6.7.8 and 6.7.10 respectively. The mean percentage paracetamol content of all the batches was 96.74%. As indicated in the USP, any batch that falls within the range 90% to 110% had passed the test. As indicated in the frequency table (refer to table 57) and that of the quality control chart (refer to figure 12), only three batches had their paracetamol content less than 90 percentage, and only one batch exceeded the 110% mark. The frequency distribution table shows a good distribution profile with 86.5% of all the batches passing the test.

Aspirin content within the batches had the highest standard deviation and therefore the component with the widest variation in the products. Refer to section 6.7.8 for the frequency distribution chart and figure 13 for the quality control chart. This variation may be due to the fast hydrolytic process through which aspirin undergoes. Also, the absorption of moisture from the atmosphere as well as the heat generated during the compression process may lead to hydrolysis of aspirin. Excessive moisture in excepients if not controlled may also aggravate the situation. As indicated in the frequency distribution curve and that of the quality control chart, only thirteen batches passed the test indicating a percentage of 35%. 19 batches out of the 37 failed the test and had percentage content less than 90%. Five batches had their percentage content higher than 110%.

The percentage content of caffeine claimed to be contained in each tablet was 30 mg. However, only one batch thus batch number 137407 passed the test with a percentage content of 106.56%. Refer to section 6.7.10. All the other batches failed the test since they have higher percentage content higher than 110%. Refer to figure 14. The manufacturer should therefore take critical steps to control this anomaly.

As indicated in the USP, the limit of salicylic acid in any aspirin containing tablet should be 3.0%. Above this limit the sample should be rejected. From the assay, seven batches failed the limit test. This represents 18.9% of the total batches assayed.

UV spectrophotometric quantitative analysis

UV spectrophotometric method was used as an alternative method to assay of the dissolution product. The product was assayed without extraction. The use of simultaneous equation was necessary to resolve the spectral overlap caused by the constituent active ingredients (Aspirin, paracetamol and caffeine). 0.01M hydrochloric acid was used

It was necessary to reduce the spectral overlap, suppress the specific absorbance of the paracetamol and to reduce the rate of hydrolysis of aspirin in the tablet hence the acid medium used. Also, a tinted blue colour developed when the three APIs were dissolved in sodium hydroxide solution. These therefore made it unfavourable for sodium hydroxide to be used. From literature the λ maximum and specific absorbance for paracetamol, aspirin and caffeine in an acidic medium are 245 nm (A_1^1 668), 230 nm (A_1^1 466) and 273 nm (A_1^1 504)

respectively. From the spectral scan, the λ maximum and specific absorbance for paracetamol, aspirin and caffeine in the 0.01M hydrochloric acidic medium were 244 nm (A^1_1 678.5), 229 nm (A^1_1 461.5) and 274 nm (A^1_1 497) respectively. Refer to figure 4a. As stated in the BP, the permitted deviation of λ maximum in the UV region is ±1. The λ maximum obtained was acceptable. The specific absorbances were also close to that stated in literature.

From the calibration curve performed, the correlation coefficient for paracetamol, aspirin and caffeine had a range of 0.997 to 0.999. This indicates a good correlation within the individual concentrations with their respective absorbencies. Refer to section 6.8.

The HPLC method of assay was compared with that of the UV method developed. Six (6) replicates each of 3 different concentrations were used and the results analyzed statistically using ANOVA. Referring to Table 9, there were no significant differences between the results. The HPLC method was therefore comparable with the UV method developed. Table 8, however shows the variation in the results as indicated by the standard deviation. The standard deviation for each sample analysed by the uv. Spectrophotometric method indicated a lesser precision as compared with the HPLC method which is known for its higher precision and accuracy.

From the dissolution product analyzed, both Paracetamol and caffeine from every batch used passed the test with each having more than 70% content dissolving within 60 minutes. Aspirin content for three batches however failed the test. These batches include 137392, 137402 and 137406. Refer to table 69.

The dissolution profile performed for batch number 137428 shows a rapid dissolution rate with more than 70% of the paracetamol and aspirin content dissolving within 10 minutes from the start. Caffeine however at the 10^{th} minute had 64% of its content dissolved. On the 20^{th} minute almost all the active ingredients in the tablet had gone into solution. Refer to figure 14.

4.2 Conclusion

In monitoring the combination analgesic “EFPAC” for 4 months, the quality of the product was assessed. In this quest, various conventional methods were employed.

From the thin layer chromatography Aspirin, paracetamol and caffeine were identified in the sample tablet. The presence of aspirin in the tablets confirms the presence of salicylic acid. The sample analyzed (EFPAC) passed the friability test and therefore the tablets can be handled and transported without losses through chipping hence intact of the dosage form.

There was less variation in the weight distribution among the tablets as confirmed by the weight uniformity test. From the BP specifications when 20 individual tablets are selected, not more than two tablets should deviate by ±5.0% and not even a single tablet should deviate by ±10.0%. Thirty six (36) batches passed this test. Only one batch failed this test.

Disintegration time is necessary as the drug formulated needs to break up in time to make its content readily available for dissolution and hence absorption for fast relief of pain and headaches. The disintegration time for EFPAC was good since all the batches disintegrated within 10 minutes. However it is important for the manufacturer to monitor the amount of disintegrant added since the tablet is not a dispersive tablet but an uncoated tablet.

Both the HPLC method (used for the assay) and the UV Spectrophotometric (used for the dissolution analysis) passed the statistical t-test (ANOVA) when compared to the actual mass. The test with ANOVA produced a result of no significant difference among the three. However, the HPLC method had a higher precision compared to the UV Spectrophotometric method.

From the assay, paracetamol had 86.5% of the batches passing the test (32 batches). The instability of aspirin due to its fast hydrolysis in the presence of moisture was observed since it gave the widest variation of results from the assay. Only thirteen (13) of the batches passed the assay for the aspirin. Of all the 37 batches used 36 of them failed the test for caffeine (having more than 110% of the labelled content).

Seven (7) out of the 37 batches used failed the salicylic acid limit test. Aspirin which hydrolyses into salicylic acid should therefore be protected by monitoring and controlling the moisture content during production.

EFPAC passed the dissolution test. All the paracetamol and caffeine in each batch passed the test. However, only three batches had their aspirin content failing the dissolution test.

Both manufacturing and expiry dates were absent on the blister pack.

The performance of EFPAC in terms of physical attributes was acceptable. However, the assay results were not satisfactory.

4.3 Recommendations

It is empirical that the manufacturer's method for the analysis be revalidated and compared with pharmacopoeia standard. This is necessary if the desired quality of the drug is to be improved.

It is important for the manufacturer to revalidate the steps in the production process. Any critical control point in the production process which leads to the hydrolysis of aspirin should be revalidated or modified. It is necessary since high salicylic acid content exposes the patient to gastrointestinal problems.

The manufacturer should try either the HPLC or the uv. Spectrophotometric methods developed in this project for the analysis of the Active pharmaceutical ingredients.

To avoid excessive variation in the amount of aspirin in the batches the manufacturer needs to assay the aspirin powder before formulation so as to know what quantity to add to each batch. The manufacturer should be consistent in the amount of aspirin added to each batch.

It is important to monitor the amount of caffeine added to the product. Excessive caffeine in the tablet may suppress sleep and this may be detrimental to those suffering from migraines.

There should be consistent checks by regular bodies on this analgesic product to ensure that the drug complies with specification.

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Both the manufacturing date and expiry date should be inscribed on each blister pack.

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4.5 APPENDIXES

Table 10 Batches of tablets used

Observation Number	Batch number	Manufacturing date	Expiry date
1	137367	29th Oct 2007	
2	137371	31st Oct 2007	
3	137376	30th Oct 2007	
4	137379	5th Nov 2007	
5	137381	6th Nov 2007	
6	137382	9th Nov 2007	
7	137383	12th Nov 2007	
8	137385	14th Nov 2007	
9	137387	16th Nov 2007	
10	137388	3rd Nov 2007	
11	137390	9th Nov 2007	
12	137392	19th Nov 2007	
13	137398	21st Nov 2007	
14	137400	23rd Nov 2007	
15	137401		
16	137402	26th Nov 2007	
17	137404	28th Nov 2007	
18	137405	29th Nov 2007	
19	137406	27th Nov 2007	
20	137407	28th Nov 2007	
21	137409	5th Dec 2007	
22	137411	11th Dec 2007	
23	137413	11th Dec 2007	
24	137414	10th Dec 2007	
25	137422	17th Dec 2007	
26	137423	17th Dec 2007	
27	137424	18th Dec 2007	
28	137425	18th Dec 2007	

Observation Number	Batch number	Manufacturing date	Expiry date
29	137428	21st Dec 2007	
30	137429	27th Dec 2007	
31	137430	31st Dec 2007	
32	137433	2nd Jan 2008	
33	137434	4th Jan 2008	
34	137436	8th Jan 2008	
35	137438	8th Jan 2008	
36	137439	9th Jan 2008	
37	137441	16th Jan 2008	

Table 11 Friability results

Observation number	**Batch number**	**Mass before friabilation(g)**	**Mass after friablitation(g)**	**Percentage mass loss.**
1	137367	6.5247	6.5161	0.1318
2	137371	6.6016	6.5913	0.1560
3	137376	6.5515	6.5416	0.1511
4	137379	6.5313	6.5253	0.0919
5	137381	6.4932	6.4876	0.0862
6	137382	6.5290	6.5166	0.1899
7	137383	6.5445	6.5370	0.1146
8	137385	6.6135	6.5992	0.2162
9	137387	6.5583	6.5174	0.6236
10	137388	6.5620	6.5416	0.3109
11	137390	6.5586	6.5517	0.1052
12	137392	6.5483	6.5377	0.1619
13	137398	6.4843	6.4698	0.2236
14	137400	6.4630	5.9342	8.1820
15	137401	6.4044	6.3925	0.1858
16	137402	6.4383	6.4292	0.1413

17	137404	6.3971	6.3858	0.1766
18	137405	6.4927	6.4847	0.1232
19	137406	6.4954	6.4725	0.3526
20	137407	6.4495	6.4395	0.1551
21	137409	6.3942	6.3565	0.5896
22	137411	6.5440	6.5131	0.4722
23	137413	6.5522	6.5451	0.1084
24	137414	6.5142	6.5045	0.1489
25	137422	6.5928	6.5714	0.3246
26	137423	6.3718	6.3599	0.1868
27	137424	6.5803	6.5695	0.1641
28	137425	6.5851	6.5758	0.1412
29	137428	6.5041	6.4931	0.1691
30	137429	6.5499	6.5395	0.1588
31	137430	6.5125	6.4924	0.3086
32	137433	6.4388	6.4194	0.3013
33	137434	6.5372	6.5285	0.1331
34	137436	6.4543	6.4445	0.1518
35	137438	6.4535	6.4423	0.1735
36	137439	6.2917	6.2864	0.0842
37	137441	6.3930	6.3836	0.1470

6.3 series of tables containing the various batches with their weight uniformity test.

Deviation = Tablet weight – Mean weight

% Deviation = (Deviation / Mean weight)× 100

Table 12

Batch number 137367			
Tablet number	Tablet weight	Deviation	%Deviation
1	0.4973	-0.0018	-0.3606
2	0.5002	0.0011	0.2204
3	0.4904	-0.0087	-1.7431
4	0.5004	0.0013	0.2605
5	0.5070	0.0079	1.5828
6	0.4948	-0.0043	-0.8616
7	0.4940	-0.0051	-1.0218
8	0.4922	-0.0069	-1.3825
9	0.5010	0.0019	0.3807
10	0.5002	0.0011	0.2204
11	0.5030	0.0039	0.7814
12	0.5010	0.0019	0.3807
13	0.5016	0.0025	0.5009
14	0.4991	0.0000	0.0000
15	0.5001	0.0010	0.2004
16	0.4998	0.0007	0.1403
17	0.4991	0.0000	0.0000
18	0.5001	0.0010	0.2004
19	0.4997	0.0006	0.1202
20	0.5010	0.0019	0.3807
Mass of 20 Tablets= 9.982			
Average mass= 0.4991			

Table 13

Batch number 137371			
Tablet number	Tablet weight	Deviation	%Deviation
1	0.5068	0.0026	0.5157
2	0.5026	-0.0016	-0.3173
3	0.5059	0.0017	0.3372
4	0.503	-0.0012	-0.2380
5	0.5094	0.0052	1.0313
6	0.4977	-0.0065	-1.2892
7	0.5105	0.0063	1.2495
8	0.5089	0.0047	0.9322
9	0.5106	0.0064	1.2693
10	0.5104	0.0062	1.2297
11	0.5071	0.0029	0.5752
12	0.5047	0.0005	0.0992
13	0.495	-0.0092	-1.8247
14	0.5	-0.0042	-0.8330
15	0.5011	-0.0031	-0.6148
16	0.5	-0.0042	-0.8330
17	0.5031	-0.0011	-0.2182
18	0.5022	-0.002	-0.3967
19	0.511	0.0068	1.3427
20	0.5106	0.0064	1.2693
Mass of 20 Tablets= 10.0842			
Average mass= 0.5042			

Table 14

Batch number 137376			
Tablet number	Tablet weight	Deviation	%Deviation
1	0.4934	-0.0108	-2.1420
2	0.4932	-0.011	-2.1817
3	0.5098	0.0056	1.1107
4	0.5098	0.0056	1.1107
5	0.5085	0.0043	0.8528
6	0.4983	-0.0059	-1.1702
7	0.4987	-0.0055	-1.0908
8	0.5083	0.0041	0.8132
9	0.5014	-0.0028	-0.5553
10	0.4987	-0.0055	-1.0908
11	0.5108	0.0066	1.309
12	0.4984	-0.0058	-1.1503
13	0.5138	0.0096	1.904
14	0.5	-0.0042	-0.833
15	0.5058	0.0016	0.3173
16	0.5027	-0.0015	-0.2975
17	0.5055	0.0013	0.2578
18	0.5147	0.0105	2.0825
19	0.5084	0.0042	0.833
20	0.5033	-0.0009	-0.1785
Mass of 20 Tablets= 10.0834			
Average mass= 0.5042			

Table 15

Batch number 137379			
Tablet number	Tablet weight	Deviation	%Deviation
1	0.5094	0.009	1.7986
2	0.5052	0.0048	0.9592
3	0.4961	-0.0043	-0.8593
4	0.5017	0.0013	0.2598
5	0.4914	-0.009	-1.7986
6	0.4921	-0.0083	-1.6587
7	0.4966	-0.0038	-0.7594
8	0.5051	0.0047	0.9392
9	0.4949	-0.0055	-1.0991
10	0.4932	-0.0072	-1.4388
11	0.5103	0.0099	1.9784
12	0.5071	0.0067	1.3389
13	0.4961	-0.0043	-0.8593
14	0.5004	0	0
15	0.4996	-0.0008	-0.1599
16	0.5048	0.0044	0.8793
17	0.4931	-0.0073	-1.4588
18	0.5044	0.004	0.7994
19	0.5077	0.0073	1.4588
20	0.4986	-0.0018	-0.3597
Mass of 20 Tablets= 10.0075			
Average mass= 0.5004			

Table 16

Batch number 137381			
Tablet number	Tablet weight	Deviation	%Deviation
1	0.501	0.0015	0.3003
2	0.5072	0.0077	1.5415
3	0.4884	-0.0111	-2.2222
4	0.4909	-0.0086	-1.7217
5	0.5176	0.0181	3.6236
6	0.5012	0.0017	0.3403
7	0.4979	-0.0016	-0.3203
8	0.5058	0.0063	1.2613
9	0.4961	-0.0034	-0.6807
10	0.4956	-0.0039	-0.7808
11	0.5003	0.0008	0.1602
12	0.5095	0.01	2.002
13	0.4991	-0.0004	-0.0801
14	0.4931	-0.0064	-1.2813
15	0.497	-0.0025	-0.5005
16	0.5025	0.003	0.6006
17	0.5037	0.0042	0.8408
18	0.5021	0.0026	0.5205
19	0.4896	-0.0099	-1.982
20	0.4906	-0.0089	-1.7818
Mass of 20 Tablets= 9.9896			
Average mass= 0.4995			

Table 17

Batch number 137382			
Tablet number	Tablet weight	Deviation	%Deviation
1	0.4998	0.0003	0.06006
2	0.5001	0.0006	0.12012
3	0.5001	0.0006	0.12012
4	0.5022	0.0027	0.54054
5	0.4985	-0.001	-0.2002
6	0.5	0.0005	0.1001
7	0.4919	-0.0076	-1.5215
8	0.4994	-1E-04	-0.02
9	0.5006	0.0011	0.22022
10	0.5002	0.0007	0.14014
11	0.499	-0.0005	-0.1001
12	0.4987	-0.0008	-0.1602
13	0.5016	0.0021	0.42042
14	0.4975	-0.002	-0.4004
15	0.5002	0.0007	0.14014
16	0.4992	-0.0003	-0.0601
17	0.5006	0.0011	0.22022
18	0.5005	0.001	0.2002
19	0.5001	0.0006	0.12012
20	0.4998	0.0003	0.06006
Mass of 20 Tablets= 9.99			
Average mass= 0.4995			

Table 18

Batch number 137383			
Tablet number	Tablet weight	Deviation	%Deviation
1	0.5001	0.0002	0.036
2	0.5383	0.0384	7.6815
3	0.5001	0.0002	0.04
4	0.4735	-0.0264	-5.2811
5	0.5032	0.0033	0.6601
6	0.4695	-0.0304	-6.0812
7	0.497	-0.0029	-0.5801
8	0.4918	-0.0081	-1.6203
9	0.495	-0.0049	-0.9802
10	0.4985	-0.0014	-0.2801
11	0.5074	0.0075	1.5003
12	0.5194	0.0195	3.9008
13	0.4967	-0.0032	-0.6401
14	0.5057	0.0058	1.1602
15	0.4849	-0.015	-3.0006
16	0.5058	0.0059	1.1802
17	0.4932	-0.0067	-1.3403
18	0.5145	0.0146	2.9206
19	0.5059	0.006	1.2002
20	0.4945	-0.0054	-1.0802
Mass of 20 Tablets= 9.9971			
Average mass= 0.4999			

Table 19

Batch number 137385			
Tablet number	Tablet weight	Deviation	%Deviation
1	0.5039	0.0023	0.4585
2	0.5002	-0.0014	-0.2791
3	0.5016	0	0
4	0.4949	-0.0067	-1.3357
5	0.4975	-0.0041	-0.8174
6	0.4947	-0.0069	-1.3756
7	0.4958	-0.0058	-1.1563
8	0.4973	-0.0043	-0.8573
9	0.5044	0.0028	0.5582
10	0.5048	0.0032	0.638
11	0.503	0.0014	0.2791
12	0.5126	0.011	2.193
13	0.5039	0.0023	0.4585
14	0.509	0.0074	1.4753
15	0.5026	0.001	0.1994
16	0.4997	-0.0019	-0.3788
17	0.5021	0.0005	0.0997
18	0.5021	0.0005	0.0937
19	0.5099	0.0083	1.6547
20	0.5301	0.0285	5.6818
Mass of 20 Tablets= 10.164			
Average mass= 0.5082			

Table 20

Batch number 137387			
Tablet number	Tablet weight	Deviation	%Deviation
1	0.4936	-0.0108	-2.1412
2	0.5002	-0.0042	-0.8327
3	0.5041	-0.0003	-0.0595
4	0.5079	0.0035	0.6939
5	0.5069	0.0025	0.4956
6	0.4977	-0.0067	-1.3283
7	0.5	-0.0044	-0.8723
8	0.5136	0.0092	1.8239
9	0.522	0.0176	3.4893
10	0.5028	-0.0016	-0.3172
11	0.5036	-0.0008	-0.1586
12	0.4975	-0.0069	-1.368
13	0.5117	0.0073	1.4473
14	0.5023	-0.0021	-0.4163
15	0.5091	0.0047	0.9318
16	0.5059	0.0015	0.2974
17	0.4993	-0.0051	-1.0111
18	0.5015	-0.0029	-0.5749
19	0.5026	-0.0018	-0.3569
20	0.5052	0.0008	0.1586
Mass of 20 Tablets= 10.0881			
Average mass= 0.5044			

Table 21

Batch number 137388			
Tablet number	Tablet weight	Deviation	%Deviation
1	0.507	0.0046	0.9156
2	0.5077	0.0053	1.0549
3	0.502	-0.0004	-0.0796
4	0.5132	0.0108	2.1497
5	0.5029	0.0005	0.0995
6	0.5084	0.006	1.1943
7	0.4983	-0.0041	-0.8161
8	0.5072	0.0048	0.9554
9	0.4941	-0.0083	-1.6521
10	0.5182	0.0158	3.1449
11	0.4829	-0.0195	-3.8814
12	0.4968	-0.0056	-1.1146
13	0.5105	0.0081	1.6123
14	0.5004	-0.002	-0.4001
15	0.5017	-0.0007	-0.1393
16	0.4945	-0.0079	-1.5725
17	0.4985	-0.0039	-0.7763
18	0.4921	-0.0103	-2.0502
19	0.5062	0.0038	0.7564
20	0.4974	-0.005	-0.9952
mass o 20 tabets = 10.0470g			
Average mass = 0.5024g			

Table 22

Batch number 137390			
Tablet number	Tablet weight	Deviation	%Deviation
1	0.5059	0.0022	0.4368
2	0.491	-0.0127	-2.5213
3	0.5047	0.001	0.1985
4	0.499	-0.0047	-0.9331
5	0.5083	0.0046	0.9132
6	0.5064	0.0027	0.536
7	0.5059	0.0022	0.4368
8	0.4656	-0.0381	-7.564
9	0.5162	0.0125	2.4816
10	0.5016	-0.0021	-0.4169
11	0.5054	0.0017	0.3375
12	0.5012	-0.0025	-0.4963
13	0.5063	0.0026	0.5162
14	0.5038	0.0001	0.0199
15	0.5037	0	0
16	0.498	-0.0057	-1.1316
17	0.5156	0.0119	2.3625
18	0.5108	0.0071	1.4096
19	0.5111	0.0074	1.4691
20	0.5012	-0.0025	-0.4963
Mass of 20 Tablets= 10.0739			
Average mass= 0.5037			

Table23

Batch number 137392			
Tablet number	Tablet weight	Deviation	%Deviation
1	0.5039	0.0007	0.1391
2	0.5051	0.0019	0.3776
3	0.499	-0.0042	-0.8347
4	0.508	0.0048	0.9539
5	0.4986	-0.0046	-0.9141
6	0.5997	0.0965	19.1773
7	0.4973	-0.0059	-1.1725
8	0.5008	-0.0024	-0.4769
9	0.5095	0.0063	1.252
10	0.5088	0.0056	1.1129
11	0.51	0.0068	1.3514
12	0.4991	-0.0041	-0.8148
13	0.494	-0.0092	-1.8283
14	0.5066	0.0034	0.6757
15	0.5004	-0.0028	-0.5564
16	0.4995	-0.0037	-0.7353
17	0.5021	-0.0011	-0.2186
18	0.5044	0.0012	0.2385
19	0.5033	0.0001	0.0199
20	0.5081	0.0049	0.9738
Mass of 20 Tablets= 10.0631			
Average mass= 0.5032			

Table 24

Batch number 137398			
Tablet number	Tablet weight	Deviation	%Deviation
1	0.4967	-0.002	-0.401
2	0.4955	-0.0032	-0.6417
3	0.5013	0.0026	0.5214
4	0.4998	0.0011	0.2206
5	0.4959	-0.0028	-0.5615
6	0.5039	0.0052	1.0427
7	0.4892	-0.0095	-1.905
8	0.5006	0.0019	0.381
9	0.4971	-0.0016	-0.3208
10	0.499	0.0003	0.0602
11	0.5067	0.008	1.6042
12	0.4851	-0.0136	-2.7271
13	0.5073	0.0086	1.7245
14	0.5011	0.0024	0.4813
15	0.4907	-0.008	-1.6042
16	0.5047	0.006	1.2031
17	0.4907	-0.008	-1.6042
18	0.4985	-0.0002	-0.0401
19	0.4984	-0.0003	-0.0602
20	0.5095	0.0108	2.1656
mass of 20 tablets =9.9736			
Average mass =0.4987			

Table25

Batch number 137400			
Tablet number	Tablet weight	Deviation	%Deviation
1	0.5009	0.0072	1.4584
2	0.502	0.0083	1.6812
3	0.4939	0.0002	0.0405
4	0.495	0.0013	0.2633
5	0.4817	-0.012	-2.4306
6	0.4942	0.0005	0.1013
7	0.4977	0.004	0.8102
8	0.5046	0.0109	2.2078
9	0.4968	0.0031	0.6279
10	0.4925	-0.0012	-0.2431
11	0.4923	-0.0014	-0.2836
12	0.4991	0.0054	1.0938
13	0.487	-0.0067	-1.3571
14	0.4884	-0.0053	-1.0735
15	0.4921	-0.0016	-0.3241
16	0.4875	-0.0062	-1.2558
17	0.4912	-0.0025	-0.5064
18	0.4913	-0.0024	-0.4861
19	0.4988	0.0051	1.033
20	0.4945	0.0008	0.162
Mass of 20 Tablets= 9.8745			
Average mass= 0.4937			

Table 26

Batch number 137401			
Tablet number	Tablet weight(g)	Deviation	%Deviation
1	0.4912	-0.0118	-2.3459
2	0.5023	-0.0007	-0.1392
3	0.4999	-0.0031	-0.6163
4	0.4882	-0.0148	-2.9423
5	0.501	-0.002	-0.3976
6	0.4945	-0.0085	-1.6899
7	0.4752	-0.0278	-5.5268
8	0.4948	-0.0082	-1.6302
9	0.4933	-0.0097	-1.9284
10	0.4988	-0.0042	-0.835
11	0.4903	-0.0127	-2.5249
12	0.4791	-0.0239	-4.7515
13	0.5118	0.0088	1.7495
14	0.4922	-0.0108	-2.1471
15	0.4908	-0.0122	-2.4254
16	0.5039	0.0009	0.1789
17	0.5011	-0.0019	-0.3777
18	0.4938	-0.0092	-1.829
19	0.4974	-0.0056	-1.1133
20	0.4873	-0.0157	-3.1213
Mass o 20 Tablets = 10.06g			
Average mass= 0.5030g			

Table 27

Batch number 137402			
Tablet number	Tablet weight	Deviation	%Deviation
1	0.493	-0.0056	-1.1231
2	0.49	-0.0086	-1.7248
3	0.4967	-0.0019	-0.3811
4	0.4967	-0.0019	-0.3811
5	0.5043	0.0057	1.1432
6	0.4945	-0.0041	-0.8223
7	0.4918	-0.0068	-1.3638
8	0.5036	0.005	1.0028
9	0.4952	-0.0034	-0.6819
10	0.5042	0.0056	1.1231
11	0.5062	0.0076	1.5243
12	0.5103	0.0117	2.3526
13	0.4987	0.0001	0.0201
14	0.4864	-0.0122	-2.4469
15	0.4932	-0.0054	-1.083
16	0.5019	0.0033	0.6619
17	0.4976	-0.001	-0.2006
18	0.4933	-0.0053	-1.063
19	0.4997	0.0011	0.2206
20	0.5039	0.0053	1.063
Mass of 20 Tablets= 9.9725			
Average mass= 0.4986			

Table 28

Batch number 137404			
Tablet number	Tablet weight	Deviation	%Deviation
1	0.5004	0.0026	0.5223
2	0.5044	0.0066	1.3258
3	0.49	-0.0078	-1.5669
4	0.499	0.0012	0.2411
5	0.5	0.0022	0.4419
6	0.4954	-0.0024	-0.4821
7	0.4954	-0.0024	-0.4821
8	0.4933	-0.0045	-0.904
9	0.4975	-0.0003	-0.0603
10	0.4922	-0.0056	-1.1249
11	0.4966	-0.0012	-0.2411
12	0.4967	-0.0011	-0.221
13	0.4998	0.002	0.4018
14	0.4952	-0.0026	-0.5223
15	0.5031	0.0053	1.0647
16	0.5025	0.0047	0.9442
17	0.4824	-0.0154	-3.0936
18	0.521	0.0232	4.6605
19	0.4953	-0.0025	-0.5022
20	0.4886	-0.0092	-1.8481
Mass of 20 Tablets= 9.9564			
Average mass= 0.4978			

Table 29

Batch number 137405			
Tablet number	Tablet weight	Deviation	%Deviation
1	0.5049	0.0057	1.1418
2	0.5036	0.0044	0.8814
3	0.4995	0.0003	0.0601
4	0.5025	0.0033	0.6611
5	0.4885	-0.0107	-2.1434
6	0.4861	-0.0131	-2.6242
7	0.4999	0.0007	0.1402
8	0.5138	0.0146	2.9247
9	0.4987	-0.0005	-0.1002
10	0.5043	0.0051	1.0216
11	0.4961	-0.0031	-0.621
12	0.499	-0.0002	-0.0401
13	0.5045	0.0053	1.0617
14	0.4906	-0.0086	-1.7228
15	0.5025	0.0033	0.6611
16	0.5078	0.0086	1.7228
17	0.4906	-0.0086	-1.7228
18	0.4986	-0.0006	-0.1202
19	0.4935	-0.0057	-1.1418
20	0.4966	-0.0026	-0.5208
Mass of 20 Tablets= 9.983			
Average mass= 0.4992			

Table 30

Batch number 137406			
Tablet number	Tablet weight	Deviation	%Deviation
1	0.4928	-0.0073	-1.4597
2	0.5074	0.0073	1.4597
3	0.5012	0.0011	0.22
4	0.5029	0.0028	0.5599
5	0.488	-0.0121	-2.4195
6	0.4971	-0.003	-0.5999
7	0.4914	-0.0087	-1.7397
8	0.507	0.0069	1.3797
9	0.4973	-0.0028	-0.5599
10	0.5028	0.0027	0.5399
11	0.5075	0.0074	1.4797
12	0.4913	-0.0088	-1.7596
13	0.4975	-0.0026	-0.5199
14	0.5082	0.0081	1.6197
15	0.5075	0.0074	1.4797
16	0.5008	0.0007	0.14
17	0.5027	0.0026	0.5199
18	0.502	0.0019	0.3799
19	0.4971	-0.003	-0.5999
20	0.5026	0.0025	0.4999
Mass of 20 Tablets= 10.0017			
Average mass= 0.5001			

Table 31

Batch number 137407			
Tablet number	Tablet weight	Deviation	%Deviation
1	0.4977	0.0013	0.2619
2	0.491	-0.0054	-1.0878
3	0.5024	0.006	1.2087
4	0.4977	0.0013	0.2619
5	0.5084	0.012	2.4174
6	0.5023	0.0059	1.1886
7	0.4974	0.001	0.2015
8	0.4882	-0.0082	-1.6519
9	0.4874	-0.009	-1.8131
10	0.4952	-0.0012	-0.2417
11	0.4924	-0.004	-0.8058
12	0.4957	-0.0007	-0.141
13	0.5089	0.0125	2.5181
14	0.5016	0.0052	1.0475
15	0.4953	-0.0011	-0.2216
16	0.5004	0.004	0.8058
17	0.502	0.0056	1.1281
18	0.4858	-0.0106	-2.1354
19	0.4825	-0.0139	-2.8002
20	0.4924	-0.004	-0.8058
Mass of 20 tablets = 9.9276			
Average mass =0.4964			

Table 32

Batch number 137409			
Tablet number	Tablet weight	Deviation	%Deviation
1	0.485	-0.0056	-1.1415
2	0.4965	0.0059	1.20261
3	0.4829	-0.0077	-1.5695
4	0.4871	-0.0035	-0.7134
5	0.4826	-0.008	-1.6307
6	0.497	0.0064	1.30453
7	0.4816	-0.009	-1.8345
8	0.484	-0.0066	-1.3453
9	0.491	0.0004	0.08153
10	0.4904	-0.0002	-0.0408
11	0.5018	0.0112	2.28292
12	0.5003	0.0097	1.97717
13	0.4961	0.0055	1.12108
14	0.4745	-0.0161	-3.2817
15	0.4941	0.0035	0.71341
16	0.5006	0.01	2.03832
17	0.4979	0.0073	1.48797
18	0.4827	-0.0079	-1.6103
19	0.4878	-0.0028	-0.5707
20	0.4981	0.0075	1.52874
Mass of 20 Tablets= 9.812			
Average mass= 0.4906			

Table 33

Batch number 137411			
Tablet number	Tablet weight	Deviation	%Deviation
1	0.5001	-0.003	-0.5963
2	0.4979	-0.0052	-1.0336
3	0.4952	-0.0079	-1.5703
4	0.5098	0.0067	1.3317
5	0.5083	0.0052	1.0336
6	0.4929	-0.0102	-2.0274
7	0.5073	0.0042	0.8348
8	0.5066	0.0035	0.6957
9	0.5023	-0.0008	-0.159
10	0.5058	0.0027	0.5367
11	0.5085	0.0054	1.0733
12	0.5093	0.0062	1.2324
13	0.5028	-0.0003	-0.0596
14	0.4844	-0.0187	-3.717
15	0.5055	0.0024	0.477
16	0.5025	-0.0006	-0.1193
17	0.5081	0.005	0.9938
18	0.5055	0.0024	0.477
19	0.5012	-0.0019	-0.3777
20	0.5016	-0.0015	-0.2982
Mass o 20 Tablets = 10.0622			
Average mass = 0.5031g			

Table 34

Batch number 137413			
Tablet number	Tablet weight	Deviation	%Deviation
1	0.5177	0.0135	2.6775
2	0.511	0.0068	1.3487
3	0.5026	-0.0016	-0.3173
4	0.5052	0.001	0.1983
5	0.5056	0.0014	0.2777
6	0.5105	0.0063	1.2495
7	0.5043	0.0001	0.0198
8	0.5081	0.0039	0.7735
9	0.4955	-0.0087	-1.7255
10	0.5003	-0.0039	-0.7735
11	0.4979	-0.0063	-1.2495
12	0.503	-0.0012	-0.238
13	0.5122	0.008	1.5867
14	0.4964	-0.0078	-1.547
15	0.4998	-0.0044	-0.8727
16	0.5084	0.0042	0.833
17	0.4991	-0.0051	-1.0115
18	0.5027	-0.0015	-0.2975
19	0.5034	-0.0008	-0.1587
20	0.5321	0.0279	5.5335
Mass of 20 Tablets= 10.083			
Average mass= 0.5042			

Table 35

Batch number 137414			
Tablet number	Tablet weight	Deviation	%Deviation
1	0.5025	0.0005	0.0996
2	0.5086	0.0066	1.3147
3	0.5037	0.0017	0.3386
4	0.4937	-0.0083	-1.6534
5	0.4967	-0.0053	-1.0558
6	0.5084	0.0064	1.2749
7	0.5013	-0.0007	-0.1394
8	0.5014	-0.0006	-0.1195
9	0.4978	-0.0042	-0.8367
10	0.5053	0.0033	0.6574
11	0.4971	-0.0049	-0.9761
12	0.4975	-0.0045	-0.8964
13	0.501	-0.001	-0.1992
14	0.5068	0.0048	0.9562
15	0.508	0.006	1.1952
16	0.4978	-0.0042	-0.8367
17	0.5078	0.0058	1.1554
18	0.5009	-0.0011	-0.2191
19	0.4971	-0.0049	-0.9761
20	0.503	0.001	0.1992
Mass of 20 Tablets= 10.0409			
Average mass= 0.502			

Table 36

Batch number 137422			
Tablet number	Tablet weight	Deviation	%Deviation
1	0.4906	-0.0157	-3.1009
2	0.5125	0.0062	1.2246
3	0.4963	-0.01	-1.9751
4	0.5201	0.0138	2.7257
5	0.5131	0.0068	1.3431
6	0.512	0.0057	1.1258
7	0.5101	0.0038	0.7505
8	0.5277	0.0214	4.2267
9	0.5075	0.0012	0.237
10	0.5134	0.0071	1.4023
11	0.5011	-0.0052	-1.0271
12	0.5036	-0.0027	-0.5333
13	0.5042	-0.0021	-0.4148
14	0.5023	-0.004	-0.79
15	0.4899	-0.0164	-3.2392
16	0.503	-0.0033	-0.6518
17	0.4933	-0.013	-2.5676
18	0.5254	0.0191	3.7725
19	0.5	-0.0063	-1.2443
20	0.4957	-0.0106	-2.0936
Mass of 20 Tablets= 10.1255			
Average mass= 0.5063			

Table 37

Batch number 137423			
Tablet number	Tablet weight	Deviation	%Deviation
1	0.4844	-0.0062	-1.2638
2	0.4879	-0.0027	-0.5503
3	0.5066	0.016	3.2613
4	0.4899	-0.0007	-0.1427
5	0.4908	0.0002	0.0408
6	0.4837	-0.0069	-1.4064
7	0.4927	0.0021	0.428
8	0.4887	-0.0019	-0.3873
9	0.4858	-0.0048	-0.9784
10	0.4866	-0.004	-0.8153
11	0.4973	0.0067	1.3657
12	0.4868	-0.0038	-0.7746
13	0.4877	-0.0029	-0.5911
14	0.4995	0.0089	1.8141
15	0.4933	0.0027	0.5503
16	0.485	-0.0056	-1.1415
17	0.4851	-0.0055	-1.1211
18	0.483	-0.0076	-1.5491
19	0.496	0.0054	1.1007
20	0.4952	0.0046	0.9376
Mass of 20 Tablets= 9.8125			
Average mass= 0.4906			

Table 38

Batch number 137424			
Tablet number	Tablet weight	Deviation	%Deviation
1	0.509	0.0074	1.4753
2	0.5	-0.0016	-0.319
3	0.4973	-0.0043	-0.8573
4	0.5038	0.0022	0.4386
5	0.4975	-0.0041	-0.8174
6	0.4947	-0.0069	-1.3756
7	0.4958	-0.0058	-1.1563
8	0.4949	-0.0067	-1.3357
9	0.5044	0.0028	0.5582
10	0.5048	0.0032	0.638
11	0.503	0.0014	0.2791
12	0.5116	0.01	1.9936
13	0.5016	0	0
14	0.5099	0.0083	1.6547
15	0.5011	-0.0005	-0.0997
16	0.5039	0.0023	0.4585
17	0.4991	-0.0025	-0.4984
18	0.5007	-0.0009	-0.1794
19	0.4997	-0.0019	-0.3788
20	0.5001	-0.0015	-0.299
Mass of 20 Tablets= 10.0328			
Average mass= 0.5016			

Table 39

Batch number 137425			
Tablet number	Tablet weight	Deviation	%Deviation
1	0.5107	0.0052	1.0287
2	0.512	0.0065	1.2859
3	0.5113	0.0058	1.1474
4	0.5126	0.0071	1.4045
5	0.4992	-0.0063	-1.2463
6	0.5044	-0.0011	-0.2176
7	0.4983	-0.0072	-1.4243
8	0.5056	0.0001	0.0198
9	0.5089	0.0034	0.6726
10	0.5	-0.0055	-1.088
11	0.5024	-0.0031	-0.6133
12	0.5129	0.0074	1.4639
13	0.4834	-0.0221	-4.3719
14	0.5002	-0.0053	-1.0485
15	0.5092	0.0037	0.7319
16	0.507	0.0015	0.2967
17	0.5038	-0.0017	-0.3363
18	0.5087	0.0032	0.633
19	0.5069	0.0014	0.277
20	0.5099	0.0044	0.8704
Mass of 20 Tablets= 10.11			
Average mass= 0.5055			

Table 40

Batch number 137428			
Tablet number	Tablet weight	Deviation	%Deviation
1	0.4961	-0.0054	-1.0768
2	0.4784	-0.0231	-4.6062
3	0.4952	-0.0063	-1.2562
4	0.5042	0.0027	0.5384
5	0.5095	0.008	1.5952
6	0.5017	0.0002	0.0399
7	0.5107	0.0092	1.8345
8	0.4982	-0.0033	-0.658
9	0.496	-0.0055	-1.0967
10	0.5059	0.0044	0.8774
11	0.5095	0.008	1.5952
12	0.5004	-0.0011	-0.2193
13	0.5026	0.0011	0.2193
14	0.5094	0.0079	1.5753
15	0.5049	0.0034	0.678
16	0.5093	0.0078	1.5553
17	0.4927	-0.0088	-1.7547
18	0.4948	-0.0067	-1.336
19	0.4968	-0.0047	-0.9372
20	0.5108	0.0093	1.8544
Mass of 20 Tablets= 10.0298			
Average mass= 0.5015			

Table 41

Batch number 137429			
Tablet number	Tablet weight	Deviation	%Deviation
1	0.4985	-0.002	-0.3996
2	0.5059	0.0054	1.0789
3	0.5007	0.0002	0.04
4	0.5052	0.0047	0.9391
5	0.5057	0.0052	1.039
6	0.5075	0.007	1.3986
7	0.5016	0.0011	0.2198
8	0.4977	-0.0028	-0.5594
9	0.4925	-0.008	-1.5984
10	0.5132	0.0127	2.5375
11	0.4946	-0.0059	-1.1788
12	0.5015	0.001	0.1998
13	0.493	-0.0075	-1.4985
14	0.4964	-0.0041	-0.8192
15	0.4988	-0.0017	-0.3397
16	0.5027	0.0022	0.4396
17	0.4974	-0.0031	-0.6194
18	0.4965	-0.004	-0.7992
19	0.4873	-0.0132	-2.6374
20	0.5119	0.0114	2.2777
Mass of 20 Tablets = 10.0091			
Average mass = 0.5005			

Table 42

Batch number 137430			
Tablet number	Tablet weight	Deviation	%Deviation
1	0.4983	-0.0031	-0.6183
2	0.5192	0.0178	3.5501
3	0.5021	0.0007	0.1396
4	0.4962	-0.0052	-1.0371
5	0.5029	0.0015	0.2992
6	0.4985	-0.0029	-0.5784
7	0.5102	0.0088	1.7551
8	0.4959	-0.0055	-1.0969
9	0.4967	-0.0047	-0.9374
10	0.51	0.0086	1.7152
11	0.504	0.0026	0.5185
12	0.513	0.0116	2.3135
13	0.4961	-0.0053	-1.057
14	0.5	-0.0014	-0.2792
15	0.497	-0.0044	-0.8775
16	0.5033	0.0019	0.3789
17	0.497	-0.0044	-0.8775
18	0.5066	0.0052	1.0371
19	0.4992	-0.0022	-0.4388
20	0.4975	-0.0039	-0.7778
Mass of 20 Tablets= 10.0277			
Average mass= 0.5014			

Table 43

Batch number 137433			
Tablet number	Tablet weight	Deviation	%Deviation
1	0.5118	0.0117	2.3395
2	0.506	0.0059	1.1798
3	0.4952	-0.0049	-0.9798
4	0.4971	-0.003	-0.5999
5	0.4942	-0.0059	-1.1798
6	0.5053	0.0052	1.0398
7	0.4961	-0.004	-0.7998
8	0.4903	-0.0098	-1.9596
9	0.5051	0.005	0.9998
10	0.5033	0.0032	0.6399
11	0.497	-0.0031	-0.6199
12	0.4994	-0.0007	-0.14
13	0.4939	-0.0062	-1.2398
14	0.4952	-0.0049	-0.9798
15	0.4994	-0.0007	-0.14
16	0.4881	-0.012	-2.3995
17	0.4899	-0.0102	-2.0396
18	0.5022	0.0021	0.4199
19	0.4945	-0.0056	-1.1198
20	0.495	-0.0051	-1.0198
Mass of 20 Tablets= 10.001			
Average mass= 0.5001			

Table 44

Batch number 137434			
Tablet number	Tablet weight	Deviation	%Deviation
1	0.4933	-0.009	-1.7918
2	0.5016	-0.0007	-0.1394
3	0.4963	-0.006	-1.1945
4	0.501	-0.0013	-0.2588
5	0.5143	0.012	2.389
6	0.507	0.0047	0.9357
7	0.5036	0.0013	0.2588
8	0.5085	0.0062	1.2343
9	0.4957	-0.0066	-1.314
10	0.5005	-0.0018	-0.3584
11	0.5008	-0.0015	-0.2986
12	0.4997	-0.0026	-0.5176
13	0.4998	-0.0025	-0.4977
14	0.5008	-0.0015	-0.2986
15	0.5126	0.0103	2.0506
16	0.4997	-0.0026	-0.5176
17	0.4981	-0.0042	-0.8362
18	0.5025	0.0002	0.0398
19	0.5117	0.0094	1.8714
20	0.4949	-0.0074	-1.4732
Mass of 20 Tablets= 10.0458			
Average mass= 0.5023			

Table 45

Batch number 137436			
Tablet number	Tablet weight	Deviation	%Deviation
1	0.5051	0.0071	1.4257
2	0.5042	0.0062	1.245
3	0.4957	-0.0023	-0.4618
4	0.5006	0.0026	0.5221
5	0.5019	0.0039	0.7831
6	0.5012	0.0032	0.6426
7	0.4952	-0.0028	-0.5622
8	0.5023	0.0043	0.8635
9	0.4968	-0.0012	-0.241
10	0.4877	-0.0103	-2.0683
11	0.4969	-0.0011	-0.2209
12	0.4899	-0.0081	-1.6265
13	0.4993	0.0013	0.261
14	0.5058	0.0078	1.5663
15	0.4916	-0.0064	-1.2851
16	0.497	-0.001	-0.2008
17	0.5008	0.0028	0.5622
18	0.498	0	0
19	0.502	0.004	0.8032
20	0.4885	-0.0095	-1.9076
Mass of 20 Tablets= 9.9607			
Average mass= 0.498			

Table 46

Batch number 137438			
Tablet number	Tablet weight	Deviation	%Deviation
1	0.497	0.0006	0.1209
2	0.4917	-0.0047	-0.9468
3	0.502	0.0056	1.1281
4	0.4977	0.0013	0.2619
5	0.5088	0.0124	2.498
6	0.5023	0.0059	1.1886
7	0.4974	0.001	0.2015
8	0.4882	-0.0082	-1.6519
9	0.4874	-0.009	-1.8131
10	0.4955	-0.0009	-0.1813
11	0.4921	-0.0043	-0.8662
12	0.4957	-0.0007	-0.141
13	0.5089	0.0125	2.5181
14	0.5016	0.0052	1.0475
15	0.4973	0.0009	0.1813
16	0.5004	0.004	0.8058
17	0.5002	0.0038	0.7655
18	0.4858	-0.0106	-2.1354
19	0.4825	-0.0139	-2.8002
20	0.4924	-0.004	-0.8058
mass of 20 tablets = 9.9276			
Average mass =0.4964			

Table 47

Batch number 137439			
Tablet number	Tablet weight	Deviation	%Deviation
1	0.5134	0.0192	3.8851
2	0.4873	-0.0069	-1.3962
3	0.4842	-0.01	-2.0235
4	0.5199	0.0257	5.2003
5	0.5217	0.0275	5.5645
6	0.5184	0.0242	4.8968
7	0.4882	-0.006	-1.2141
8	0.4792	-0.015	-3.0352
9	0.4835	-0.0107	-2.1651
10	0.4825	-0.0117	-2.3675
11	0.5213	0.0271	5.4836
12	0.4812	-0.013	-2.6305
13	0.5071	0.0129	2.6103
14	0.4765	-0.0177	-3.5815
15	0.4866	-0.0076	-1.5378
16	0.4884	-0.0058	-1.1736
17	0.4789	-0.0153	-3.0959
18	0.4854	-0.0088	-1.7807
19	0.4936	-0.0006	-0.1214
20	0.4779	-0.0163	-3.2983
Mass of 20 Tablets= 9.8839			
Average mass= 0.4942			

Table 48

Batch number 137441			
Tablet number	Tablet weight	Deviation	%Deviation
1	0.5008	0.0007	0.1400
2	0.5039	0.0038	0.7598
3	0.5008	0.0007	0.1400
4	0.5012	0.0011	0.2200
5	0.4999	-0.0002	-0.0400
6	0.5012	0.0011	0.2200
7	0.4818	-0.0183	-3.6593
8	0.4989	-0.0012	-0.2400
9	0.5009	0.0008	0.1600
10	0.4904	-0.0097	-1.9396
11	0.4988	-0.0013	-0.2599
12	0.4993	-0.0008	-0.1600
13	0.5023	0.0022	0.4399
14	0.5090	0.0089	1.7796
15	0.4997	-0.0004	-0.0800
16	0.5006	0.0005	0.1000
17	0.5032	0.0031	0.6199
18	0.5004	0.0003	0.0600
19	0.4989	-0.0012	-0.2400
20	0.5100	0.0099	1.9796
Mass of 20 Tablets= 10.002			
Average mass= 0.5001			

Table 49 Disintegration time for the various batches of tablets used

		Number of tablets with disintegration time mm:ss:ms						
Number	**Batch number**	**1**	**2**	**3**	**4**	**5**	**6**	**Average time**
1	137367	03:41.0	03:44.7	04:06.3	04:10.1	04:14.9	04:24.0	04:03.5
2	137371	04:30.8	04:32.0	06:03.7	06:12.9	06:20.2	06:21.6	05:40.2
3	137376	00:32.9	01:18.0	01:47.5	01:52.5	01:57.5	02:02.5	01:35.2
4	137379	04:07.1	05:02.5	05:04.7	05:07.5	05:34.8	05:40.9	05:06.3
5	137381	04:24.0	05:43.4	06:13.8	06:51.2	06:59.4	07:10.1	06:13.6
6	137382	01:32.1	01:36.2	01:47.3	01:56.5	01:59.6	02:00.7	01:48.7
7	137383	03:09.1	04:29.4	05:07.2	05:16.6	05:19.4	05:22.5	04:47.4
8	137385	02:50.1	06:00.8	06:38.3	06:40.6	06:45.8	06:53.6	05:58.2
9	137387	01:56.3	02:49.9	03:20.9	04:01.9	04:02.6	04:06.8	03:23.1
10	137388	03:02.9	03:06.3	03:10.0	03:12.9	03:18.1	03:26.7	03:12.8
11	137390	01:48.5	02:09.7	02:31.1	02:41.2	02:47.7	02:55.9	02:29.0
12	137392	02:29.4	02:49.3	03:49.3	04:12.7	04:21.1	04:28.9	03:41.8
13	137398	01:56.1	02:22.9	02:34.4	02:53.6	03:12.5	03:15.5	02:42.5
14	137400	04:00.1	04:27.9	04:31.8	04:41.6	04:47.8	04:52.3	04:33.6
15	137401	02:04.9	02:10.1	03:02.6	03:05.9	03:06.8	03:07.9	02:46.4
16	137402	02:04.2	03:37.8	03:44.7	03:45.0	03:45.4	03:51.0	03:28.0
17	137404	03:00.4	03:07.8	03:16.9	03:26.6	03:30.5	03:34.3	03:19.4
18	137405	03:57.3	04:15.4	04:59.8	05:18.7	05:24.2	05:27.8	04:53.9
19	137406	04:02.3	04:23.7	04:50.0	05:20.1	05:50.2	05:52.6	05:03.1
20	137407	03:02.1	03:03.7	03:05.5	03:06.9	03:38.3	03:45.1	03:16.9
21	137409	02:54.5	03:26.6	03:43.5	04:11.8	04:16.1	04:18.2	03:48.4
22	137413	04:21.0	04:21.7	04:24.5	04:27.6	04:33.2	04:54.9	04:30.5
23	147411	03:51.3	04:19.5	04:26.3	04:35.5	04:38.6	04:43.4	04:25.8
24	137414	04:25.0	04:46.2	04:49.6	04:57.5	05:00.7	05:05.5	04:50.7
25	137422	04:21.1	05:06.0	05:44.1	05:56.7	06:29.4	06:38.7	05:42.7
26	137423	03:01.2	03:39.7	04:00.3	04:21.3	04:32.0	04:34.1	04:01.4
27	137424	02:03.4	02:43.4	03:24.6	04:30.0	04:59.1	04:59.8	03:46.7
28	137425	02:29.5	03:24.1	04:09.8	04:15.8	04:34.6	04:42.3	03:56.0

29	137428	02:09.1	03:52.5	04:28.7	04:51.5	05:43.3	06:35.9	04:36.8
30	137429	04:01.1	04:04.5	04:12.3	04:33.6	04:40.0	04:49.8	04:23.5
31	137430	04:35.9	05:09.4	05:31.8	05:59.7	06:22.7	06:56.2	05:45.9
32	137433	04:59.0	04:59.2	05:02.6	05:12.7	05:30.5	05:35.4	05:13.2
33	137434	03:52.0	04:29.1	04:30.0	04:32.5	04:38.8	04:44.2	04:27.8
34	137436	03:08.0	03:43.2	04:05.9	04:49.2	04:58.2	05:07.2	04:18.6
35	137438	04:21.9	04:43.6	04:48.4	05:08.0	05:08.7	05:18.9	04:54.9
36	137439	04:45.5	05:13.3	06:08.9	06:11.7	06:13.0	06:13.9	05:47.7
37	137441	02:41.3	03:24.8	03:40.0	03:46.2	03:49.1	03:56.9	03:33.1

6.7 Analysis performed with the HPLC.

6.7.1 Calibration curve for aspirin

Table 50 Calibration curve for Aspirin using HPLC

Volume of stock diluted to 10ml (ml)	Concentration(mg/ml)	Peak Area of sample	Peak area of internal standard	Peak are ratio
0.6	0.0581	36.25	121	0.29959
0.8	0.0775	50.00	127	0.3937
1.0	0.0969	62.50	126	0.49603
1.2	0.1162	75.25	125	0.602
1.4	0.1356	88.25	126	0.7004
1.6	0.155	103.25	127	0.81299

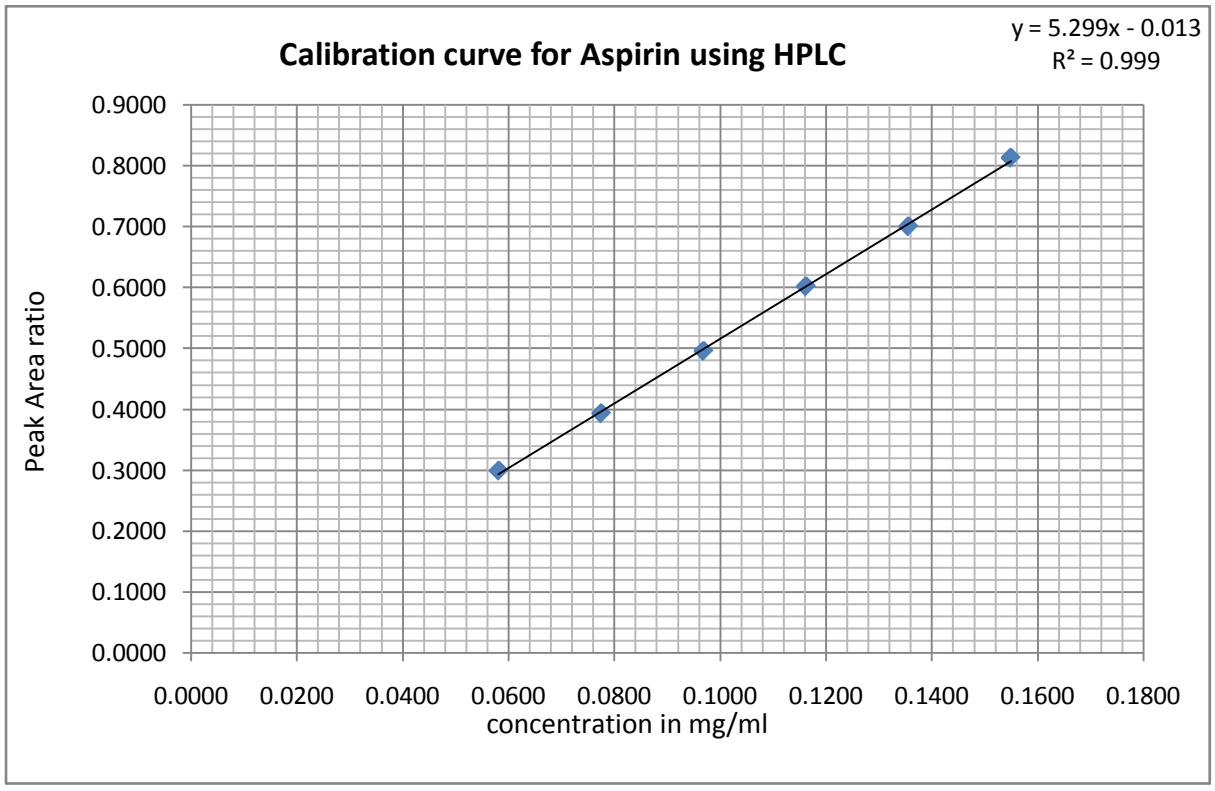


Figure 5 Calibration curve for Aspirin using HPLC

6.7.2 Calibration curve for paracetamol

Table 51 Calibration curve for paracetamol Using HPLC

Volume diluted to 10ml(ml)	Concentration(mg/ml)	Peak Area of sample	Peak area of internal standard	Peak are ratio
0.15	0.038	91	125	0.728
0.2	0.0506	108	112	0.9643
0.25	0.0633	136	117	1.1624
0.3	0.076	164	120	1.3667
0.35	0.0886	192	117	1.641
0.4	0.1013	228	124	1.8387

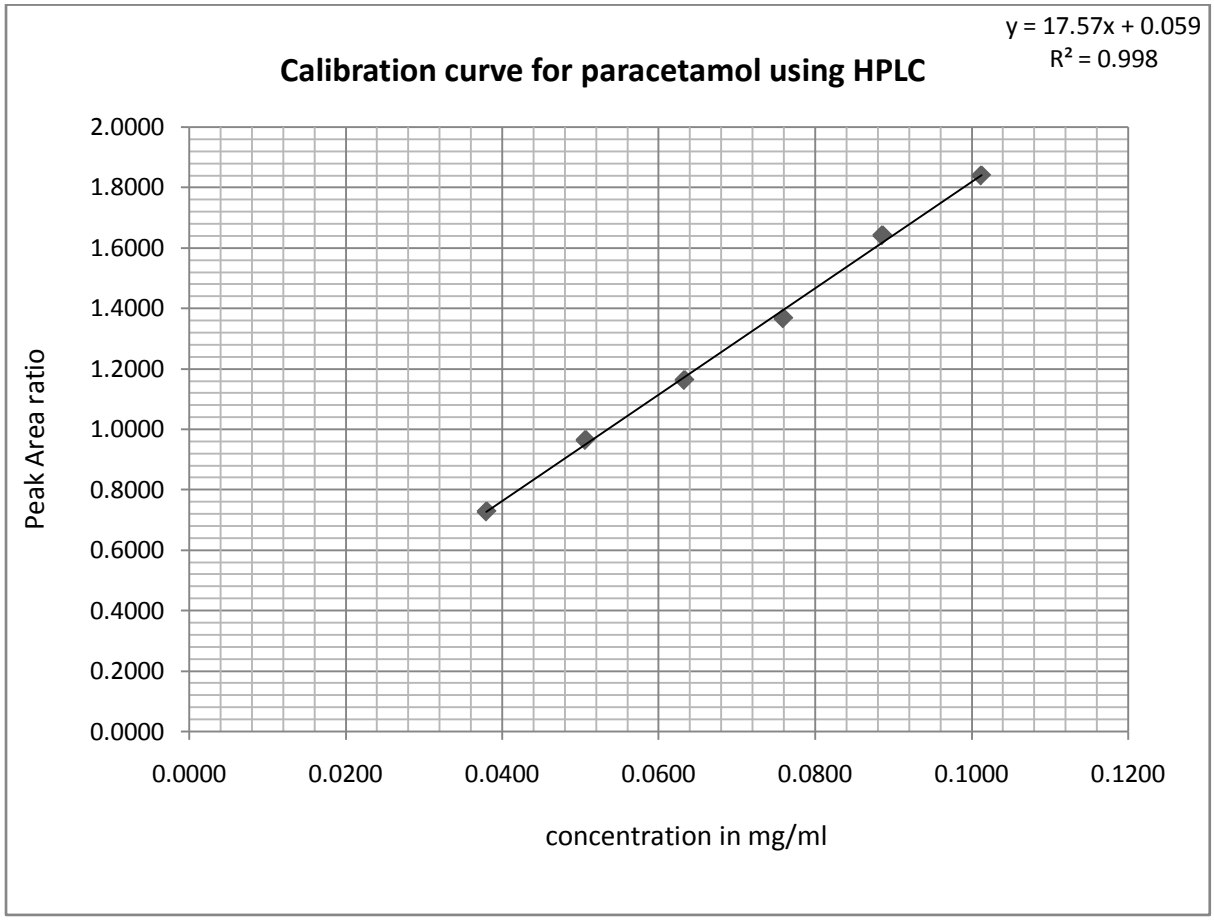


Figure 6 Calibration curve for paracetamol using HPLC

6.7.3 Calibration curve for caffeine

Table 52 Calibration curve for caffeine using HPLC

Volume diluted to 10ml(ml)	Concentration(mg/ml)	Peak Area of sample	Peak area of internal standard	Peak area ratio
0.15	0.0200872	40.7	117	0.347863
0.20	0.0267829	54	124	0.435484
0.25	0.0334786	72	125	0.576000
0.30	0.0401744	82	112	0.732143
0.35	0.0468701	100	117	0.854701
0.40	0.0535658	118	120	0.983333

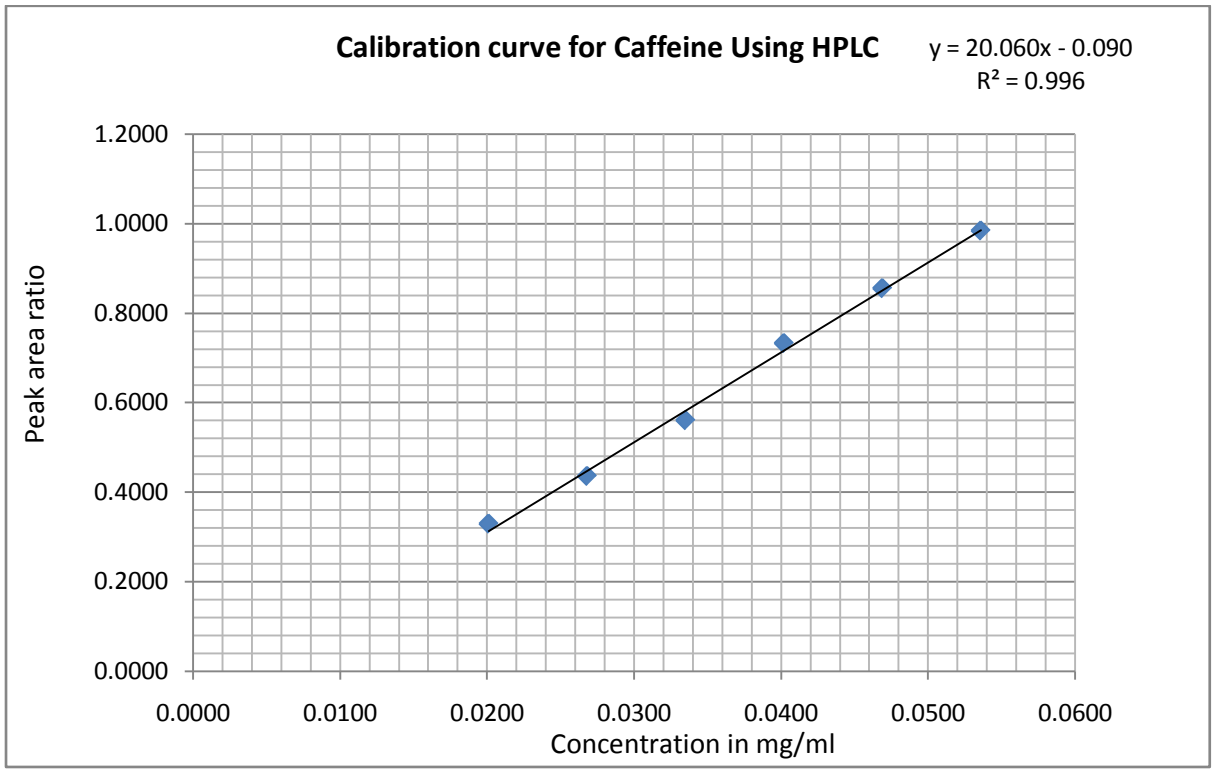


Figure 7 Calibration curve for caffeine using HPLC

6.7.4 Calibration curve for salicylic acid

Table 53 Calibration curve for salicylic acid Using HPLC

Volume of stock diluted to 10ml (ml)	Concentration(mg/ml)	Peak Area of sample	Peak area of internal standard	Peak are ratio
0.1	0.005	36.3	212.3	0.1707
0.15	0.0075	50	214	0.2337
0.2	0.01	62.5	210.6	0.2967
0.25	0.0125	75.3	214	0.3517
0.3	0.015	88.3	212.3	0.4157
0.35	0.0175	103.3	212.3	0.4863

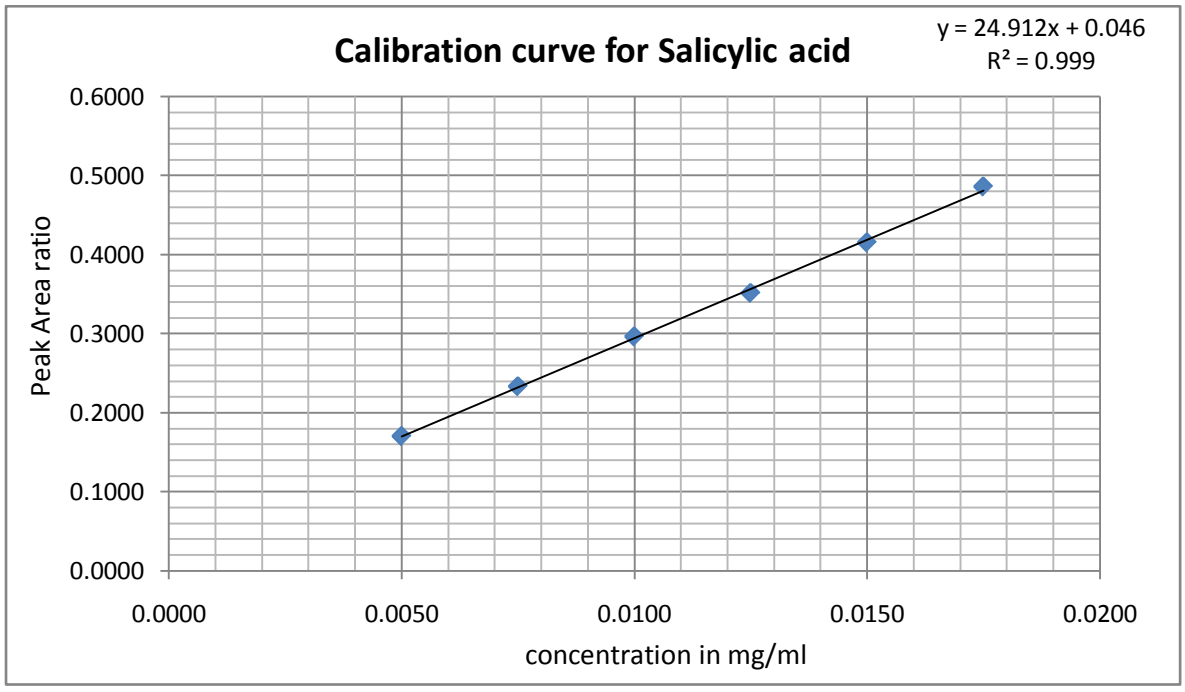


Figure 8 Calibration curve for salicylic acid using HPLC

Table 54 Mean peak area ratios from the chromatogram for each batch of tablets.

		Peak area ratio			
Observation number	**Batch number**	Salicylate	Aspirin	Paracetamol	Caffeine
1	137367	0.1863	0.4098	2.3059	0.3333
2	137371	0.1102	0.3178	2.1695	0.2742
3	137376	0.1731	0.3669	2.2865	0.3512
4	137379	0.1154	0.2788	1.7692	0.2492
5	137381	0.0960	0.2380	1.8080	0.2166
6	137382	0.1353	0.2538	1.5789	0.2113
7	137383	0.1731	0.3846	2.2500	0.3688
8	137385	0.1607	0.5214	2.3277	0.3884
9	137387	0.0870	0.1993	1.5507	0.1971
10	137388	0.0625	0.2539	1.7780	0.2723
11	137390	0.2308	0.5288	2.3558	0.3944
12	137392	0.1434	0.2022	1.6176	0.2154
13	137398	0.2400	0.3000	2.1200	0.2784
14	137400	0.3750	0.4702	2.3077	0.3846
15	137401	0.0894	0.2039	1.5177	0.1443
16	137402	0.2692	0.3365	1.9423	0.2777
17	137404	0.3600	0.4936	2.6800	0.3338
18	137405	0.0781	0.2148	1.5234	0.2450
19	137406	0.0763	0.2119	2.0169	0.2121
20	137407	0.0917	0.2813	1.9833	0.2070
21	137409	0.1071	0.2902	1.8661	0.2027
22	137411	0.1020	0.3061	2.0204	0.2231
23	137413	0.0948	0.2802	1.9828	0.2759
24	137414	0.3077	0.4087	2.1923	0.2923
25	137422	0.1875	0.3646	2.4167	0.3550
26	137423	0.0876	0.2737	1.4891	0.1916
27	137424	0.0656	0.2766	1.7869	0.2241
28	137425	0.0730	0.2281	1.5036	0.1851

29	137428	0.0719	0.2698	1.6691	0.2266
30	137429	0.2200	0.4208	2.3000	0.3286
31	137430	0.1250	0.2604	1.7917	0.1729
32	137433	0.2041	0.3061	2.5816	0.4082
33	137434	0.2449	0.5102	2.2245	0.3182
34	137436	0.2745	0.5751	2.3882	0.3175
35	137438	0.1466	0.3511	2.2328	0.2793
36	137439	0.1455	0.3789	1.9964	0.2791
37	137441	0.2200	0.4600	2.2000	0.2518

Table 55 Mean percentage content for each batch of tablets analysed

		Percentage content			
Observation number	**Batch number**	Salicylate	Aspirin	Paracetamol	Caffeine
1	137367	2.11	98.6	94.78	130.03
2	137371	1.09	87.2	100.67	126.44
3	137376	1.80	84.4	89.53	129.12
4	137379	1.30	84.4	89.45	129.13
5	137381	1.02	78.7	99.22	126.55
6	137382	1.93	89.2	92.02	132.68
7	137383	2.03	98.5	98.22	149.80
8	137385	1.68	123.4	94.77	145.56
9	137387	0.94	75.1	95.55	133.76
10	137388	0.35	82.9	96.67	148.28
11	137390	2.68	123.6	94.77	145.59
12	137392	2.00	69.0	90.45	128.96
13	137398	3.37	84.5	100.72	131.05
14	137400	4.83	109.4	92.11	141.61
15	137401	1.01	77.8	94.70	110.56
16	137402	3.86	94.1	91.76	130.40
17	137404	4.46	111.9	104.74	123.32
18	137405	0.70	75.0	87.28	145.30
19	137406	0.60	68.0	107.15	120.28
20	137407	0.82	80.2	94.92	106.56
21	137409	1.19	89.0	95.98	113.08
22	137411	1.06	93.4	103.92	120.68
23	137413	0.93	86.0	102.16	141.42
24	137414	4.24	107.1	98.07	127.95
25	137422	2.24	94.1	106.30	146.12
26	137423	0.87	89.9	81.16	116.24
27	137424	0.40	87.7	94.72	125.27
28	137425	0.63	85.4	92.61	128.26

29	137428	0.55	90.7	93.41	133.65
30	137429	2.58	100.3	93.74	127.50
31	137430	1.71	91.9	105.38	116.27
32	137433	2.33	73.2	104.67	150.56
33	137434	3.17	110.0	97.79	134.21
34	137436	3.30	108.1	94.45	120.30
35	137438	1.88	104.8	113.21	140.00
36	137439	1.68	101.2	90.55	125.57
37	137441	2.93	116.0	101.74	118.21

6.7.8 Frequency distribution for Aspirin in the assayed batches

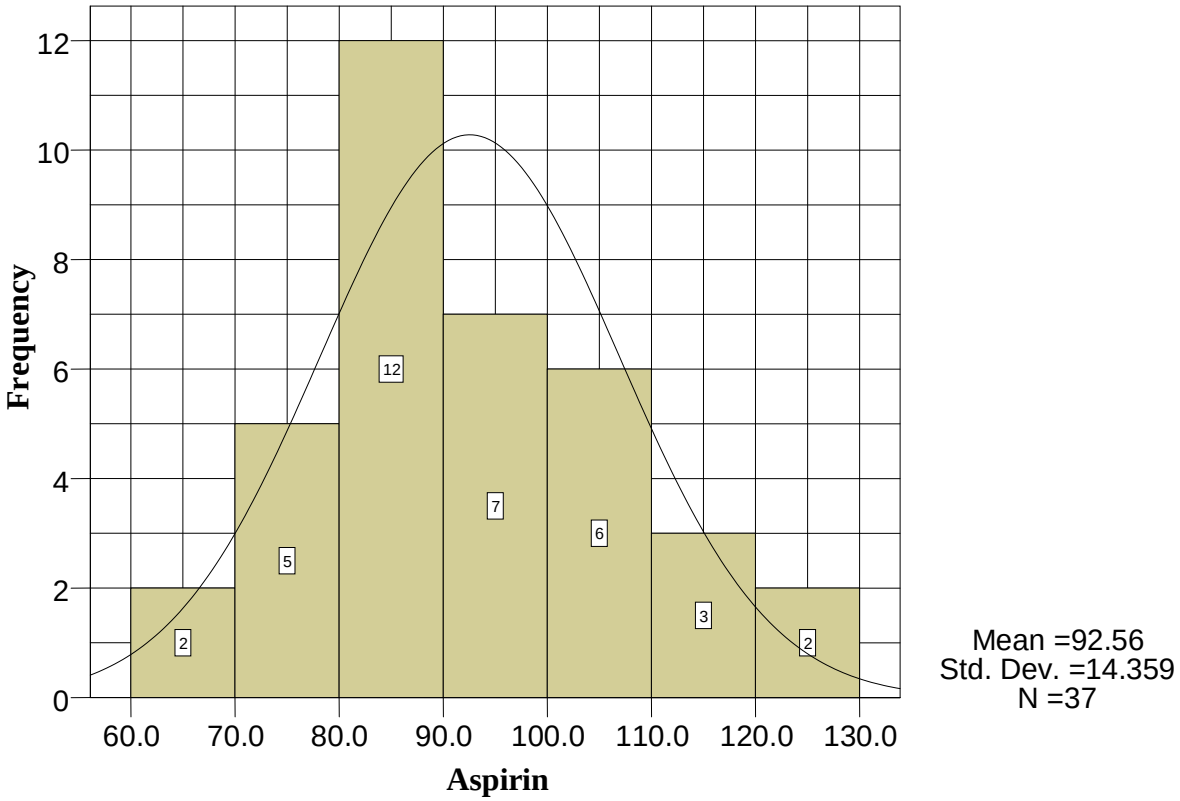


Figure 9Frequency distribution for Aspirin in the assayed batches

Table 56 Statistical data for distribution of aspirin in the assayed batches

		Aspirin
N	Valid	37
	Missing	0
Mean		92.56
Mode		84.40
Std. Deviation		14.36
Variance		206.18
Skewness		0.42
Range		55.60
Minimum		68.00
Maximum		123.60

6.7.9 Frequency distribution for Paracetamol in the assayed batches

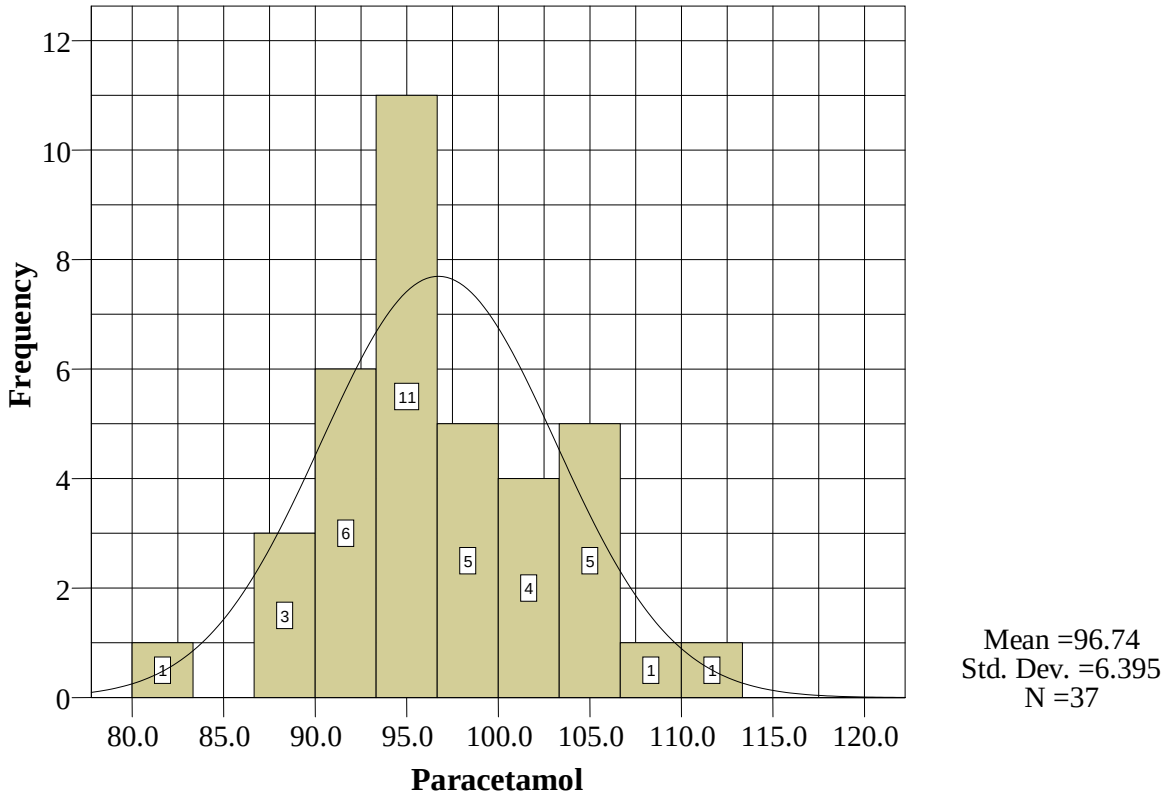


Figure 10Frequency distribution for Paracetamol in the assayed batches

Table 57 Statistical data for the distribution of paracetamol in the assayed batches

		Paracetamol
N	Valid	37
	Missing	0
Mean		96.74
Mode		94.77
Std. Deviation		6.40
Variance		40.90
Skewness		0.28
Range		32.05
Minimum		81.16
Maximum		113.21

6.7.10 Frequency distribution for Caffeine in the assayed batches

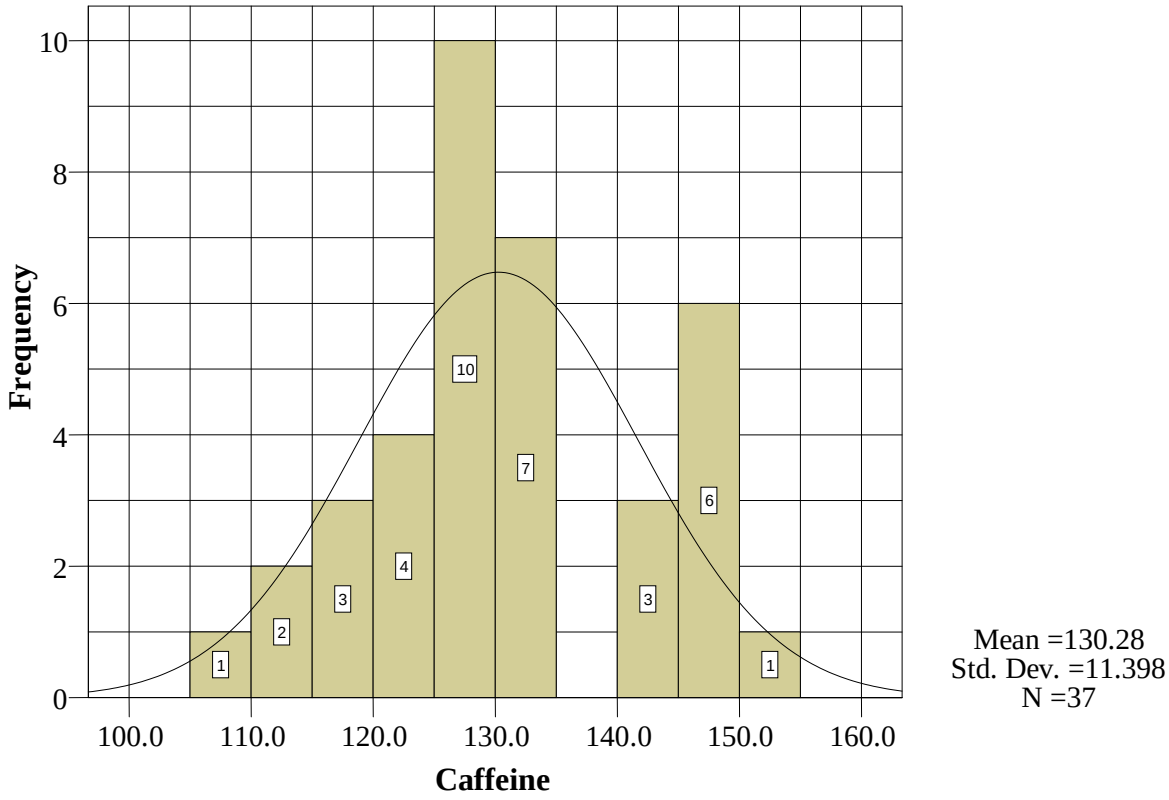
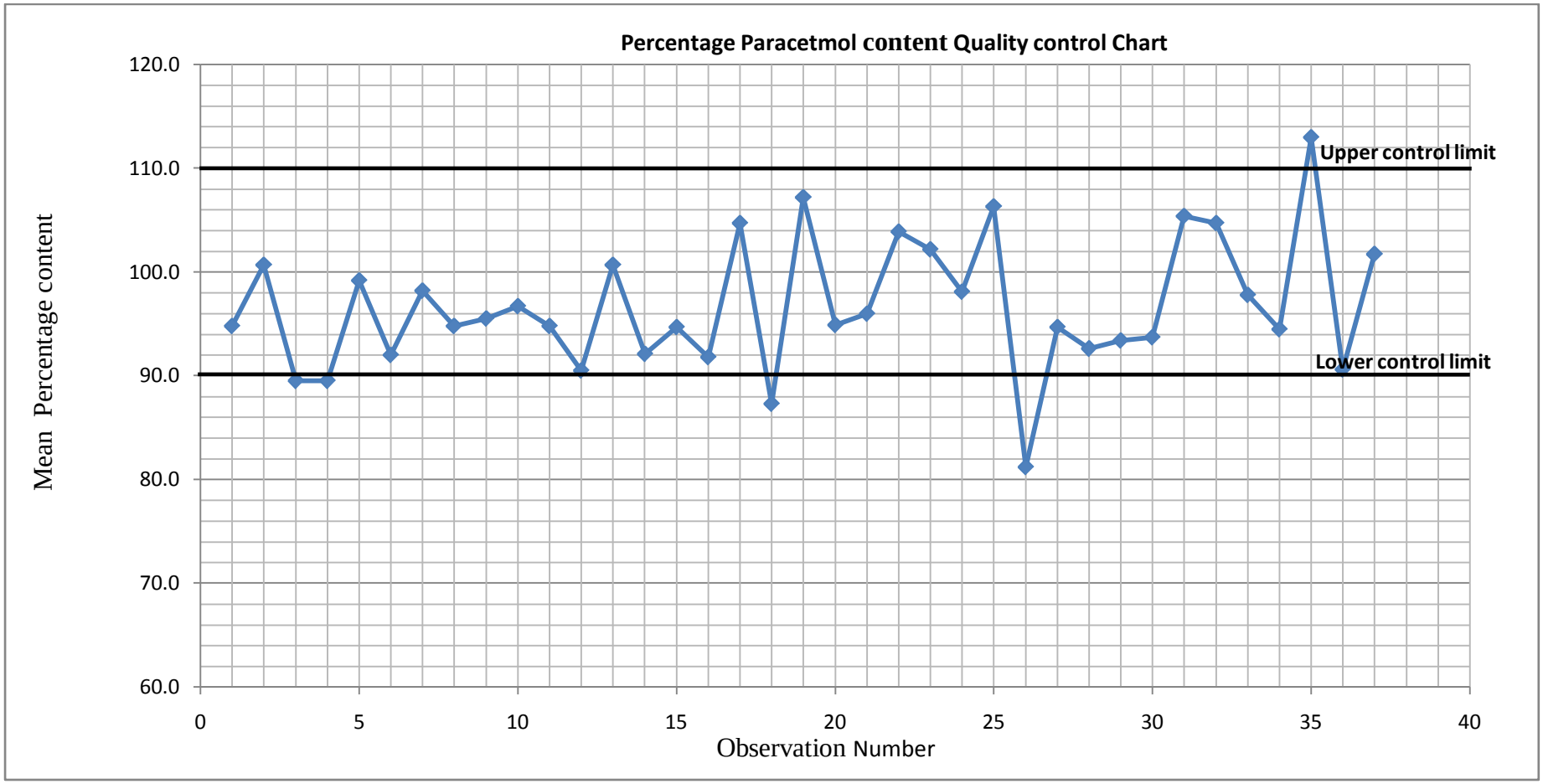


Figure 11Frequency distribution for Caffeine in the assayed batches

Table 58 Statistical data for the distribution of caffeine in the assayed batches

		Caffeine
N	Valid	37
	Missing	0
Mean		130.28
Mode		106.56
Std. Deviation		11.40
Variance		129.92
Skewness		0.06
Range		44.00
Minimum		106.56
Maximum		150.56

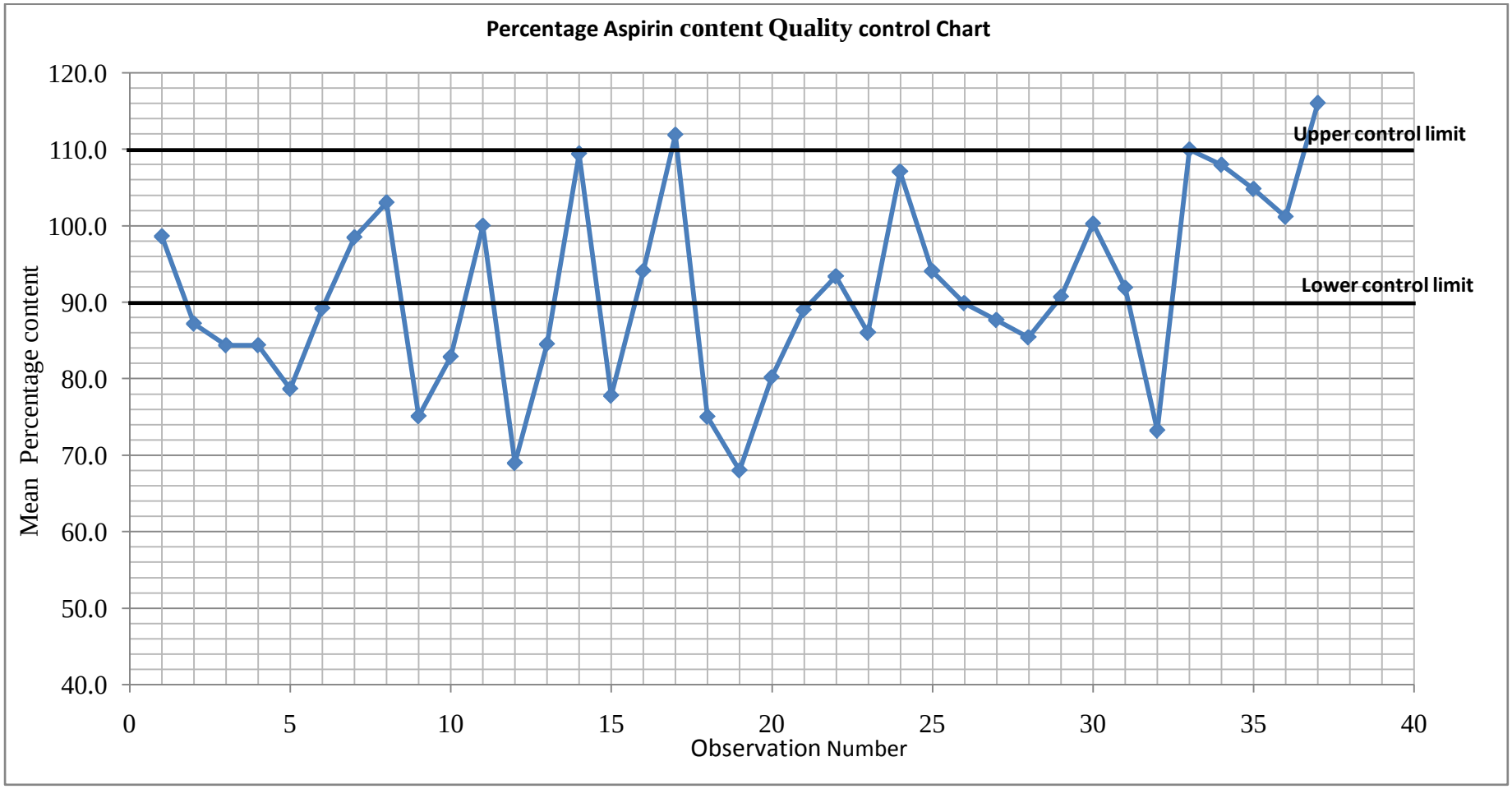
Figure 12 Quality control chart of percentage content of paracetamol in the assayed batches



Key:

——— = USP pharmacopoeia assay limits (100±10)%

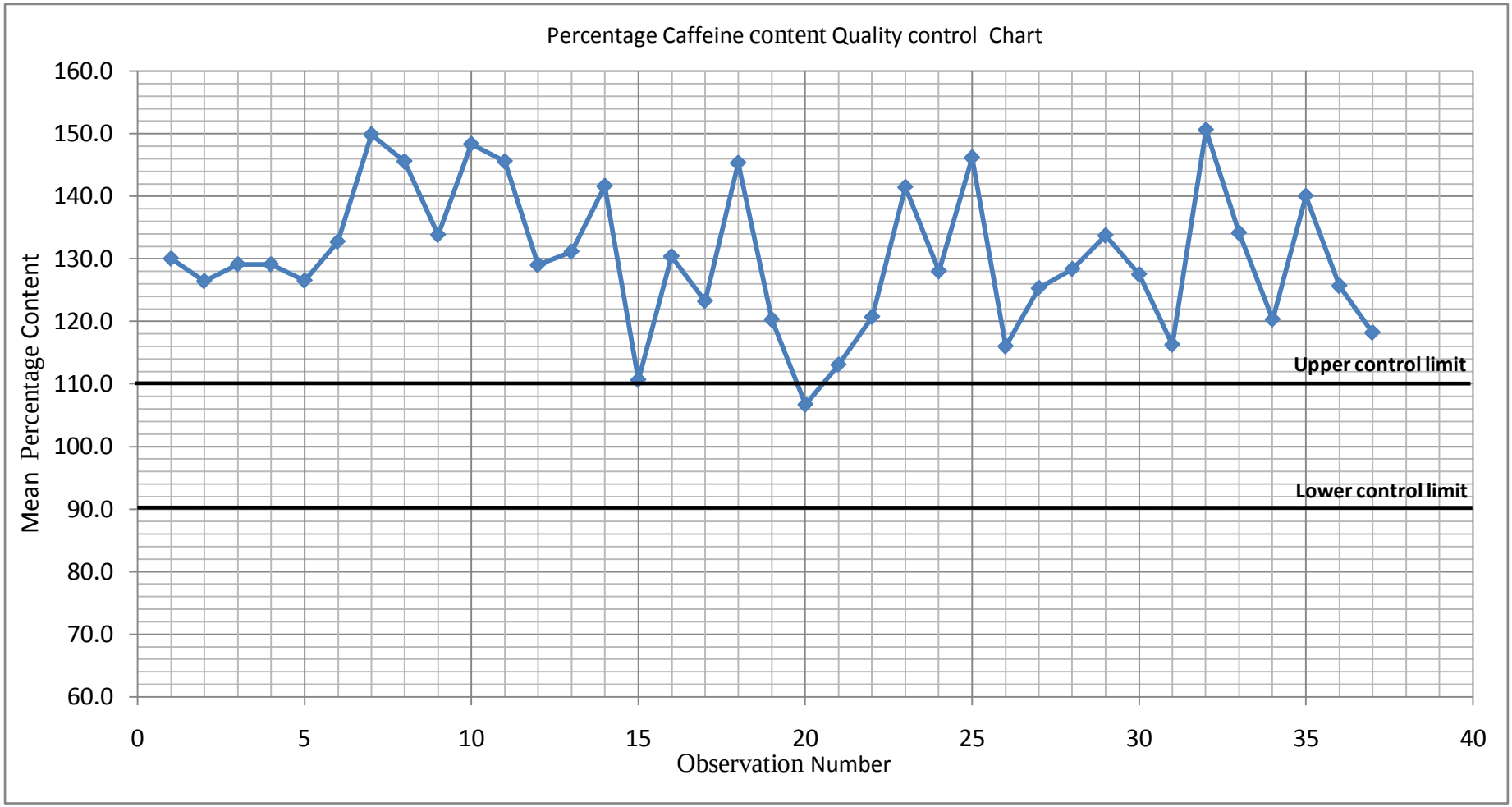
Figure 13 Quality control chart of percentage content of Aspirin in the assayed batches



Key:

——— = USP pharmacopoeia assay limits (100±10) %

Figure 14 Quality control chart of percentage content of caffeine in the assayed batches



Key:

——— = USP pharmacopoeia assay limits (100±10)%

6.8 series of calibration curve preformed with the UV/ Spectrophotometer.

6.8.1 Calibration curves for aspirin

Table 59 Calibration curve for aspirin at 229nm

volume of stock to 25ml	Concentration(%w/v)	Absorbance at 229nm
1.0	0.0009	0.435
1.2	0.0011	0.527
1.4	0.0013	0.610
1.6	0.0015	0.696
1.8	0.0017	0.779
2.0	0.0018	0.864

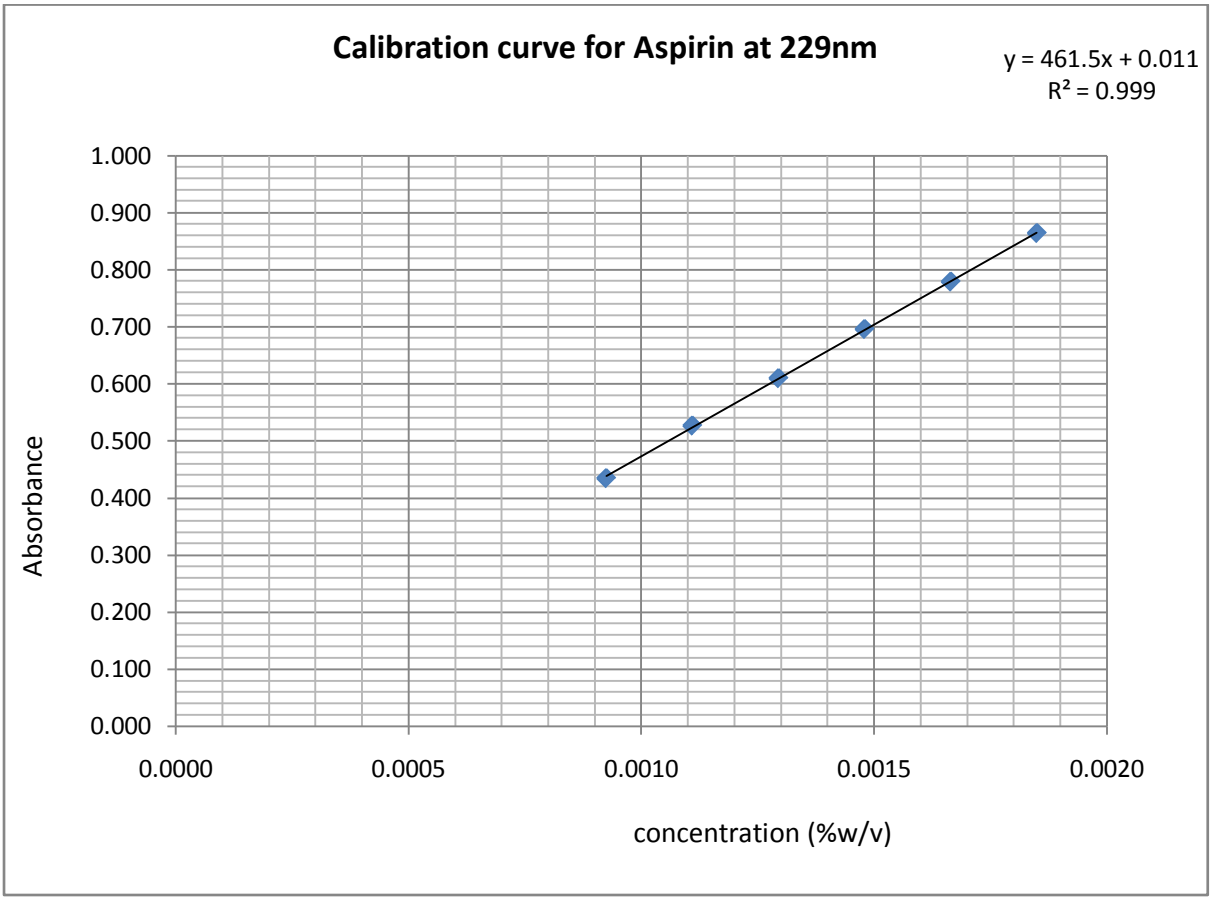


Figure 15 Calibration curve for aspirin at 229nm

Table 60 Calibration curve for aspirin at 244nm

Volume of stock to 25ml	Concentration(%w/v)	Absorbance at 244nm
2.0	0.0018	0.274
3.0	0.0028	0.406
4.0	0.0037	0.548
5.0	0.0046	0.658
6.0	0.0055	0.805

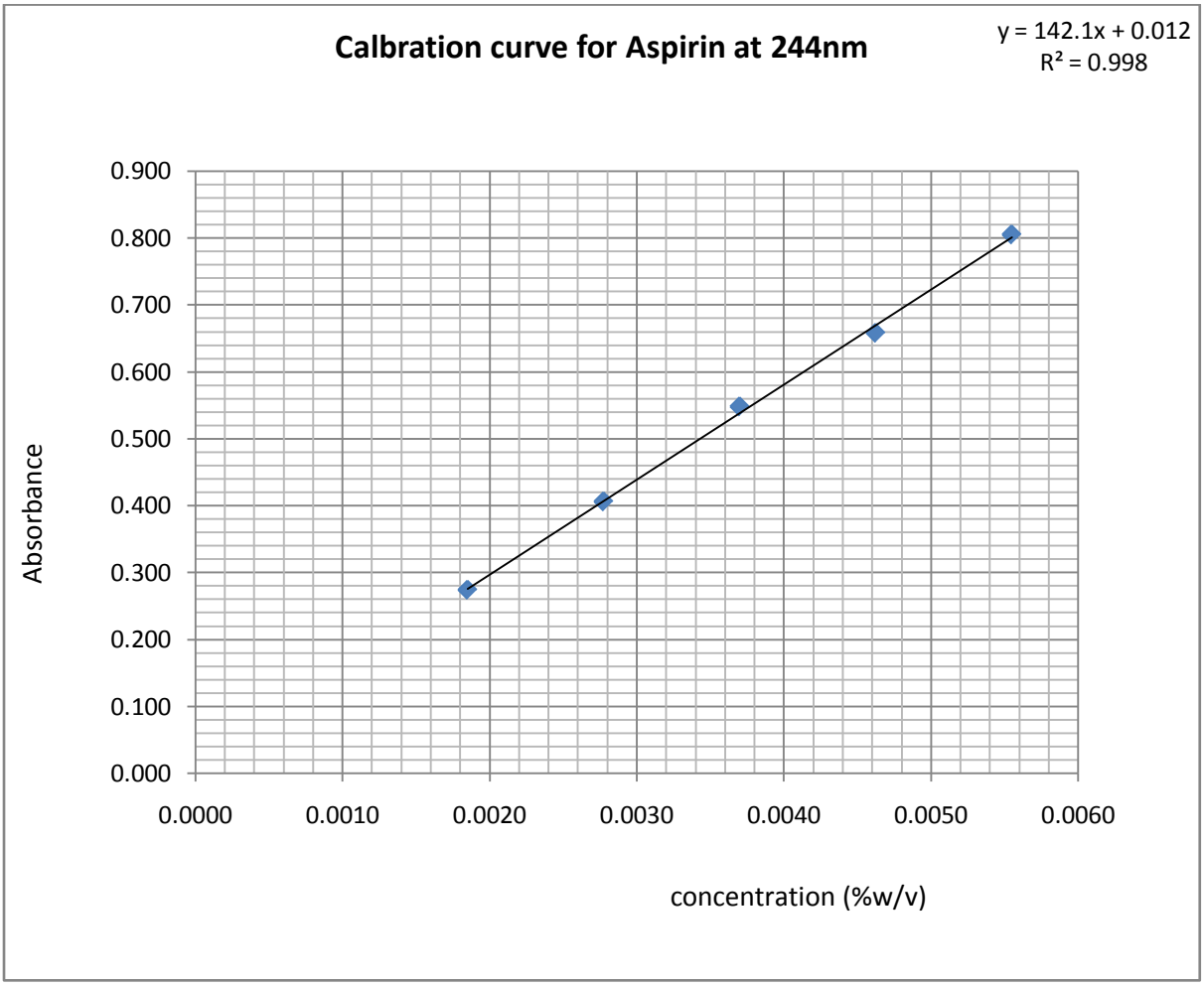


Figure 16 Calibration curve for aspirin at 244nm

Table 61 Calibration curve for aspirin at 274nm

Volume of stock to 25ml	Concentration(%w/v)	Absorbance at 274nm
4.0	0.0037	0.223
5.0	0.0046	0.270
6.0	0.0055	0.339
7.0	0.0065	0.395
8.0	0.0074	0.443
9.0	0.0083	0.501

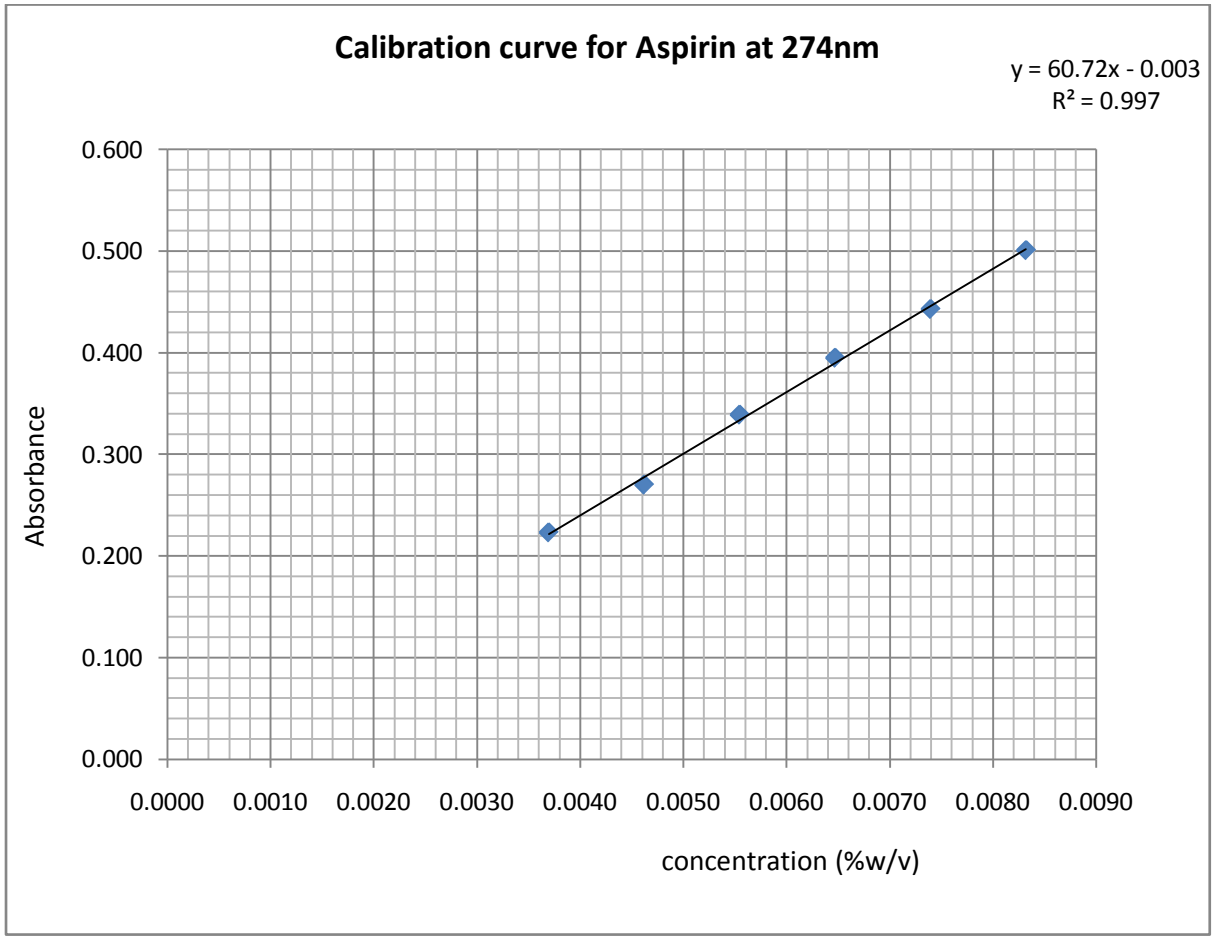


Figure 17 Calibration curve for aspirin at 274nm

6.8.2 Calibration curve for paracetamol

Table 62 Calibration curve for Paracetamol at 229nm

volume of stock to 25ml	Concentration(%w/v)	Absorbance at 229nm
0.4	0.0006	0.293
0.5	0.0007	0.366
0.6	0.0008	0.436
0.7	0.0010	0.509
0.8	0.0011	0.568
0.9	0.0013	0.638

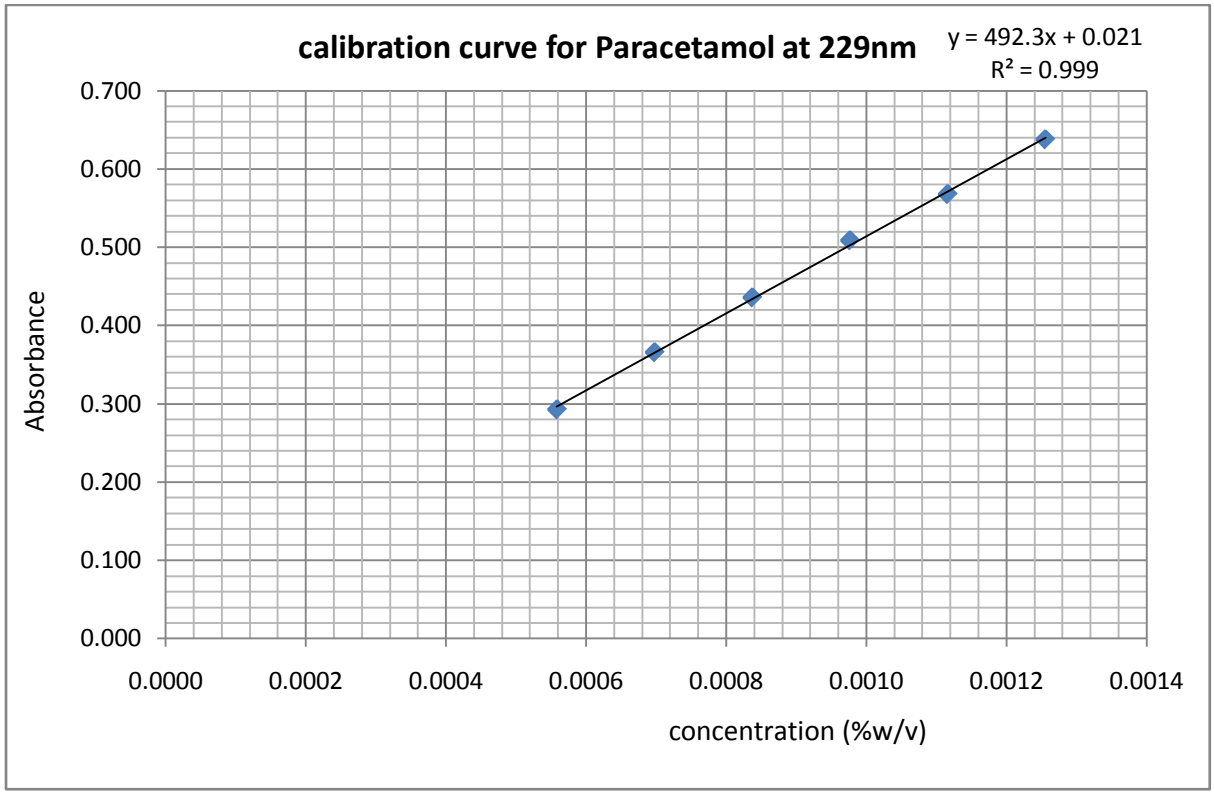


Figure 18 Calibration curve for Paracetamol at 229nm

Table 63 Calibration curve for Paracetamol at 244nm

volume of stock to 25ml	Concentration(%w/v)	Absorbance at 244nm
0.3	0.0004	0.275
0.4	0.0006	0.374
0.5	0.0007	0.477
0.6	0.0008	0.561
0.7	0.0010	0.655

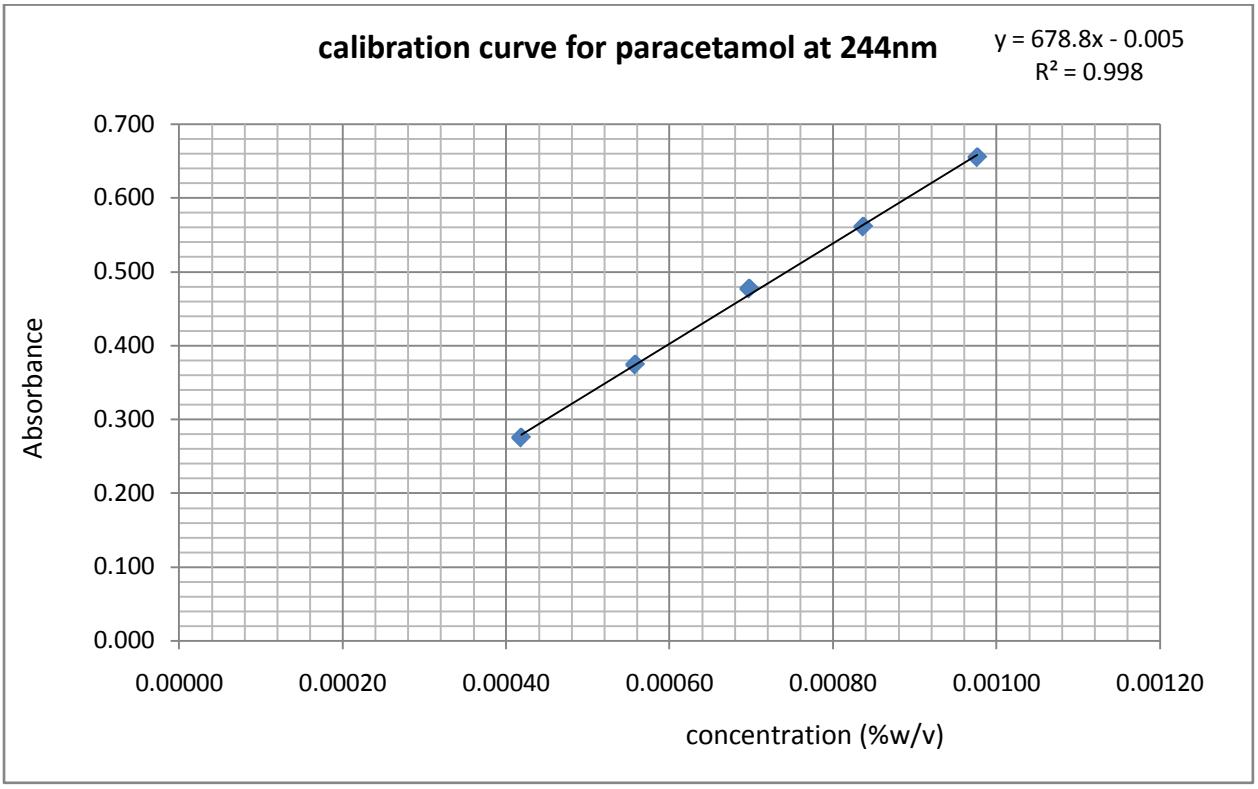


Figure 19 Calibration curve for Paracetamol at 244nm

Table 64 Calibration curve for paracetamol at 274nm

volume of stock to 25ml	Concentration(%w/v)	Absorbance at 274nm
1.2	0.0017	0.255
1.6	0.0022	0.338
2.0	0.0028	0.420
2.4	0.0033	0.509
2.8	0.0039	0.592
3.2	0.0045	0.675

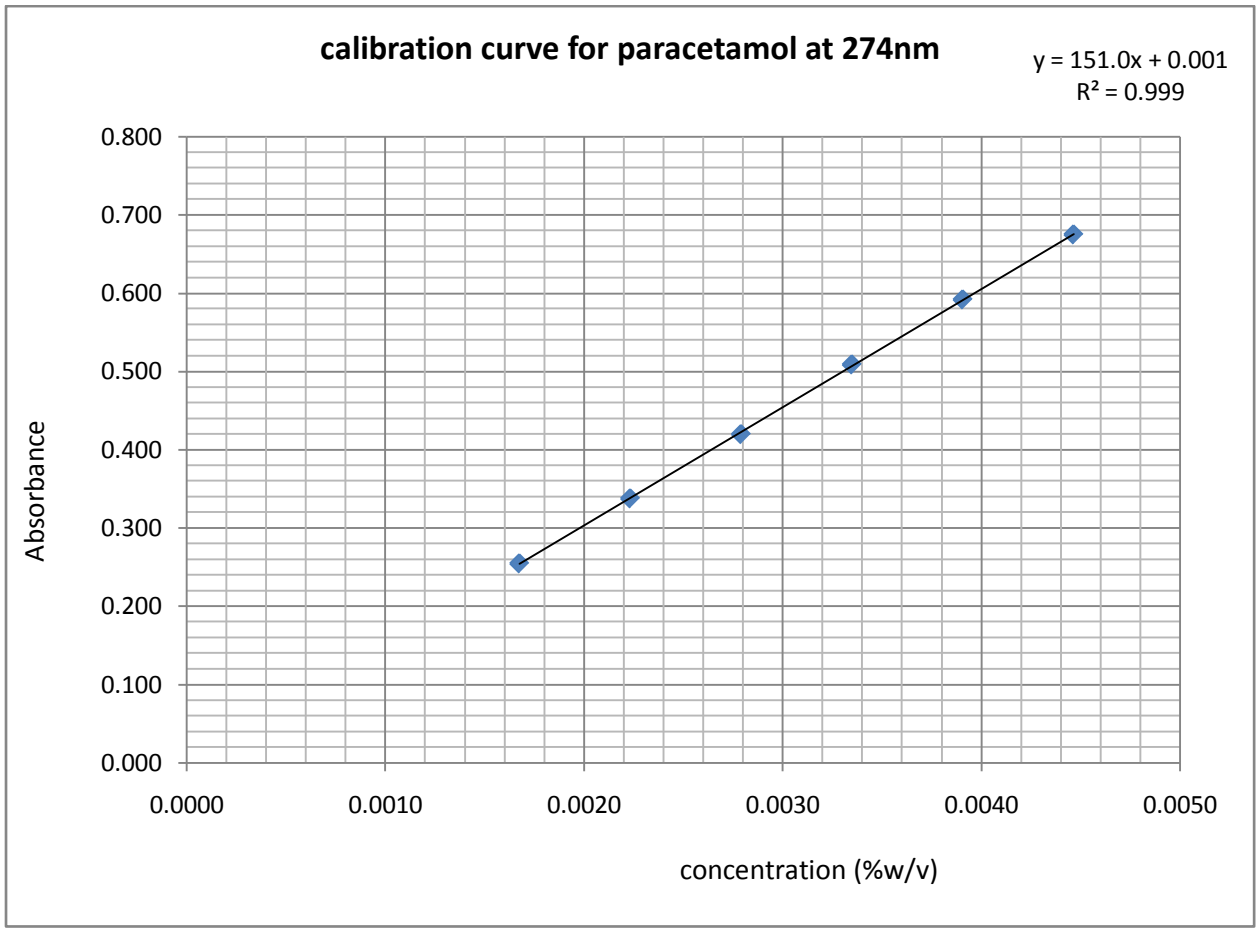


Figure 20 Calibration curve for paracetamol at 274nm

6.8.3 Calibration curves of caffeine

Table 65 Calibration curve for caffeine at 229nm

volume of stock to 25ml	Concentration(%w/v)	Absorbance at 229nm
1.2	0.00105	0.271
1.6	0.00140	0.367
2.0	0.00175	0.459
2.4	0.00210	0.567
2.8	0.00245	0.658

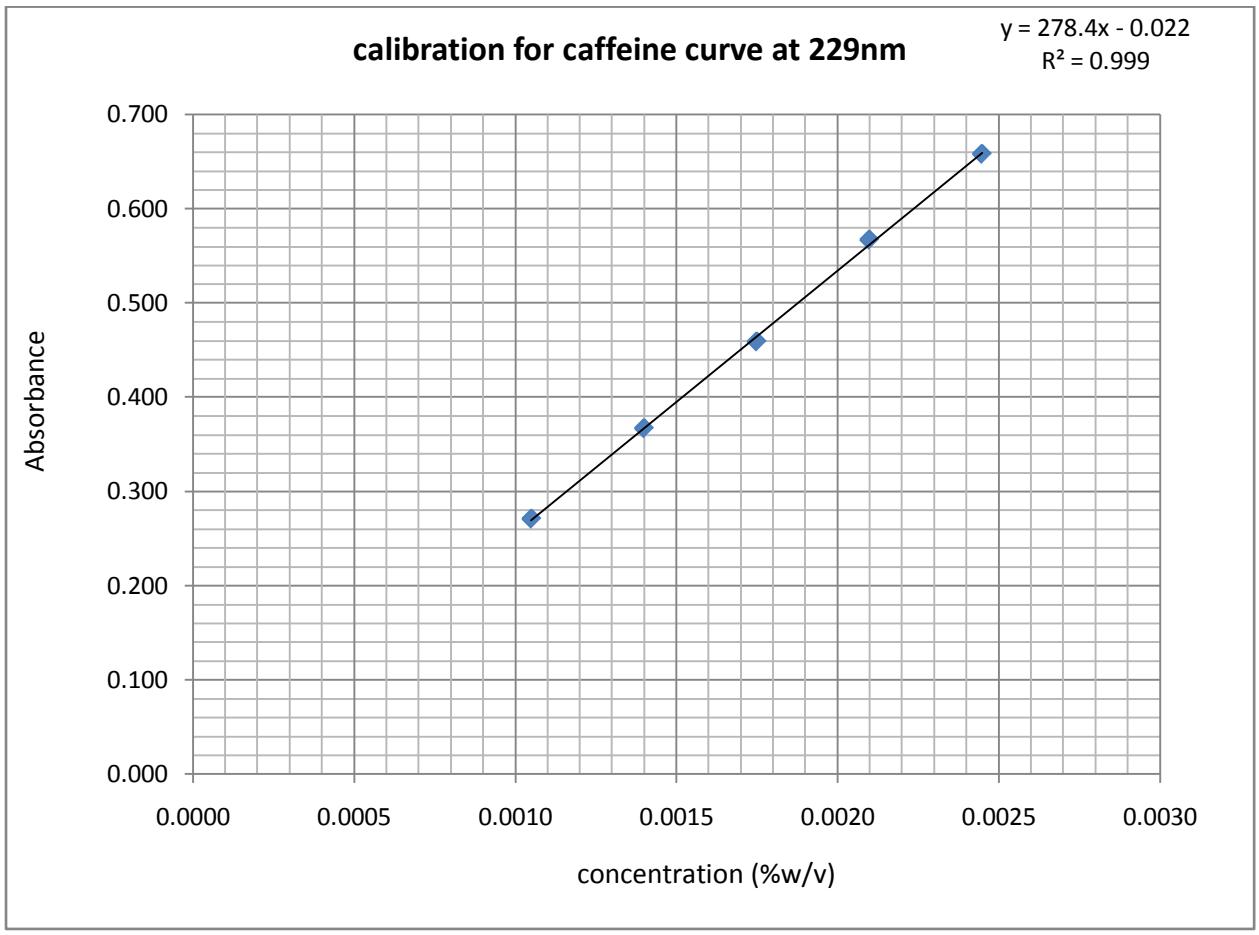


Figure 21 Calibration curve for caffeine at 229nm

Table 66 Calibration curve for caffeine at 244nm

volume of stock to 25ml	Concentration(%w/v)	Absorbance at 244nm
1.8	0.00157	0.215
2.2	0.00192	0.272
2.6	0.00227	0.320
3.0	0.00262	0.372
3.4	0.00297	0.420
3.8	0.00332	0.474

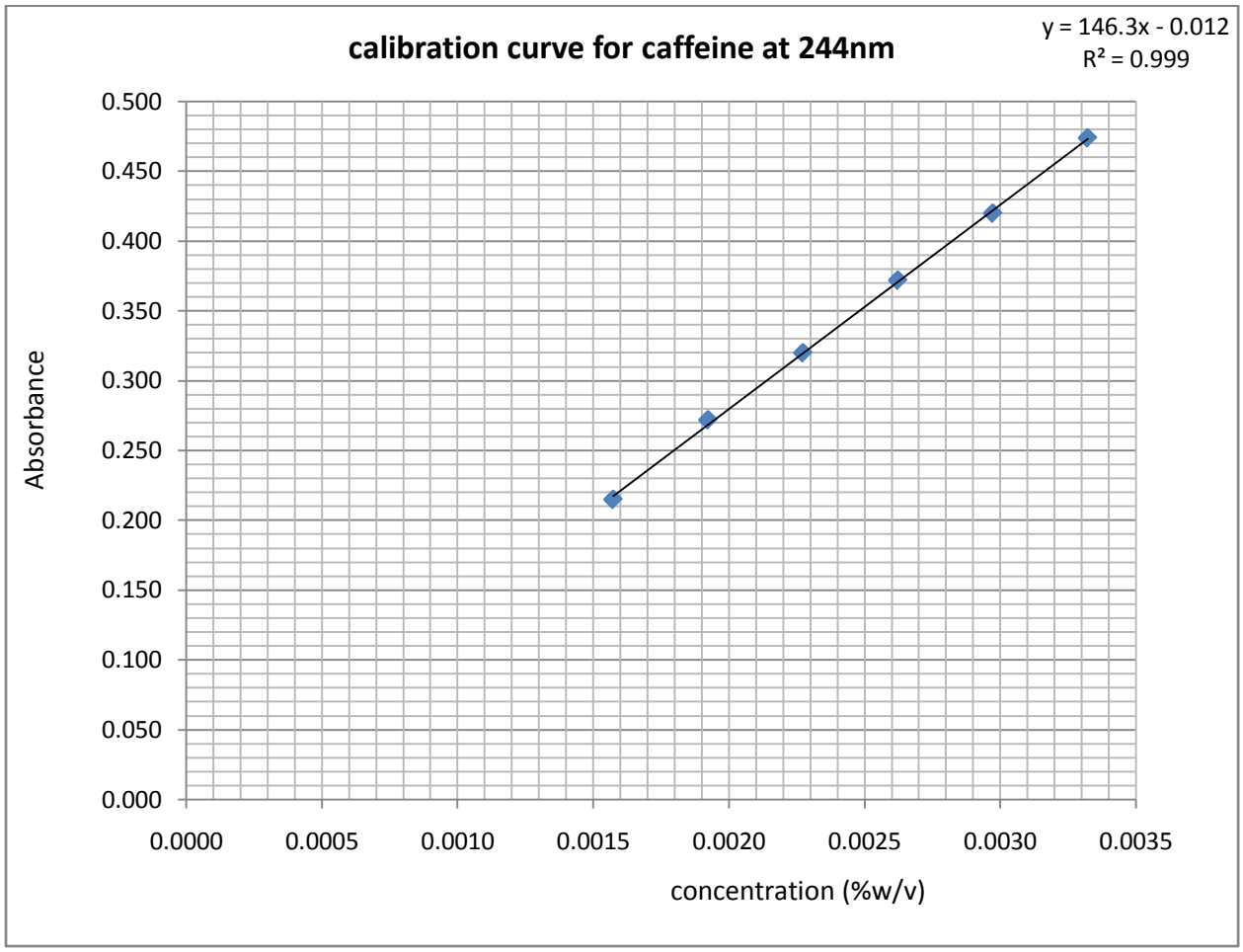


Figure 22 Calibration curve for caffeine at 244nm

Table 67 Calibration curve for caffeine at 274nm

volume of stock to 25ml	Concentration(%w/v)	Absorbance at 274nm
0.8	0.00070	0.355
1.0	0.00087	0.424
1.2	0.00105	0.525
1.4	0.00122	0.607
1.6	0.00140	0.699
1.8	0.00157	0.782

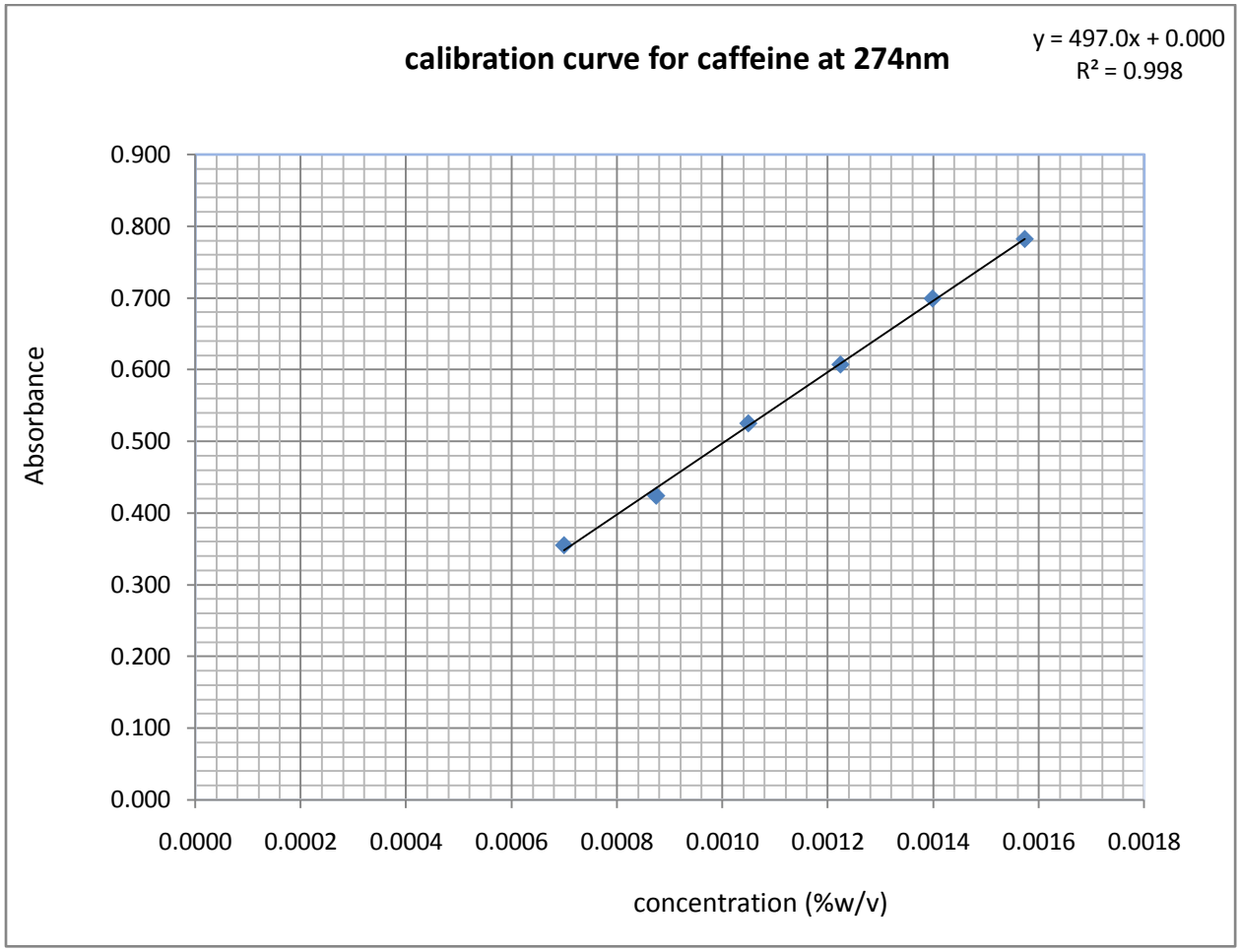


Figure 23 Calibration curve for caffeine at 274nm

Table 68 Absorbance taken after the final dilution made after the dissolution.

		Absorbance		
Observation number	Batch number	229nm	244nm	274nm
1	137367	0.649	0.604	0.201
2	137371	0.627	0.620	0.206
3	137376	0.600	0.576	0.195
4	137379	0.593	0.573	0.194
5	137381	0.622	0.623	0.198
6	137382	0.636	0.605	0.208
7	137383	0.654	0.629	0.195
8	137385	0.646	0.603	0.203
9	137387	0.651	0.623	0.209
10	137388	0.623	0.605	0.210
11	137390	0.662	0.611	0.217
12	137392	0.597	0.606	0.198
13	137398	0.623	0.635	0.203
14	137400	0.646	0.608	0.213
15	137401	0.580	0.567	0.177
16	137402	0.476	0.458	0.170
17	137404	0.665	0.641	0.214
18	137405	0.570	0.560	0.182
19	137406	0.596	0.613	0.187
20	137407	0.587	0.574	0.185
21	137409	0.636	0.608	0.200
22	137411	0.657	0.642	0.189
23	137413	0.611	0.609	0.208
24	137414	0.672	0.651	0.215
25	137422	0.682	0.676	0.216
26	137423	0.576	0.528	0.182
27	137424	0.609	0.578	0.196
28	137425	0.577	0.573	0.198

29	137428	0.652	0.628	0.211
30	137429	0.652	0.623	0.209
31	137430	0.645	0.619	0.197
32	137433	0.640	0.663	0.211
33	137434	0.658	0.613	0.184
34	137436	0.641	0.592	0.201
35	137438	0.675	0.685	0.230
36	137439	0.617	0.580	0.192
37	137441	0.665	0.645	0.209

Table 69 Percentage content of APIs in tablets released after dissolution

		Percentage content		
Observation number	Batch number	Aspirin	paracetamol	caffeine
1	137367	98.9	92.3	114.0
2	137371	79.5	97.4	123.0
3	137376	82.8	89.2	120.1
4	137379	80.0	89.0	121.0
5	137381	77.1	98.7	104.6
6	137382	90.0	93.1	132.0
7	137383	94.0	98.0	90.0
8	137385	97.2	92.1	120.0
9	137387	91.5	96.2	125.7
10	137388	82.0	94.0	138.0
11	137390	100.7	92.5	144.1
12	137392	69.2	96.4	115.7
13	137398	72.1	101.1	113.0
14	137400	93.6	93.0	140.0
15	137401	78.1	89.0	87.0
16	137402	60.3	70.9	129.0
17	137404	92.1	99.3	127.0
18	137405	74.0	87.9	103.0
19	137406	68.1	98.4	89.3
20	137407	78.0	90.0	101.0
21	137409	90.5	94.1	112.0
22	137411	92.1	100.9	73.3
23	137413	74.0	95.7	134.7
24	137414	91.9	101.2	124.1
25	137422	88.3	106.3	116.3
26	137423	89.0	80.2	112.0
27	137424	87.0	89.0	120.0
28	137425	70.0	90.0	131.0

29	137428	90.0	97.3	126.3
30	137429	92.1	96.2	124.7
31	137430	92.1	96.2	101.0
32	137433	70.0	106.3	115.7
33	137434	104.6	94.3	71.7
34	137436	98.9	90.1	119.0
35	137438	77.9	108.5	143.7
36	137439	91.8	89.0	108.3
37	137441	91.5	100.5	113.4

Table 70 A sample dissolution profile table for batch number 137428

	Mean Percentage released			Absorbance		
Time(minutes)	Aspirin	Paracetamol	Caffeine	229nm	244nm	274nm
10	77.1	92.0	64.0	0.583	0.580	0.169
20	84.7	94.2	116.7	0.624	0.605	0.200
30	86.1	96.0	121.0	0.636	0.617	0.205
40	89.5	96.8	126.0	0.649	0.625	0.210
50	89.4	97.3	126.7	0.651	0.627	0.211
60	89.8	97.3	127.3	0.652	0.628	0.211

Figure 24 A sample dissolution profile for EFPAC

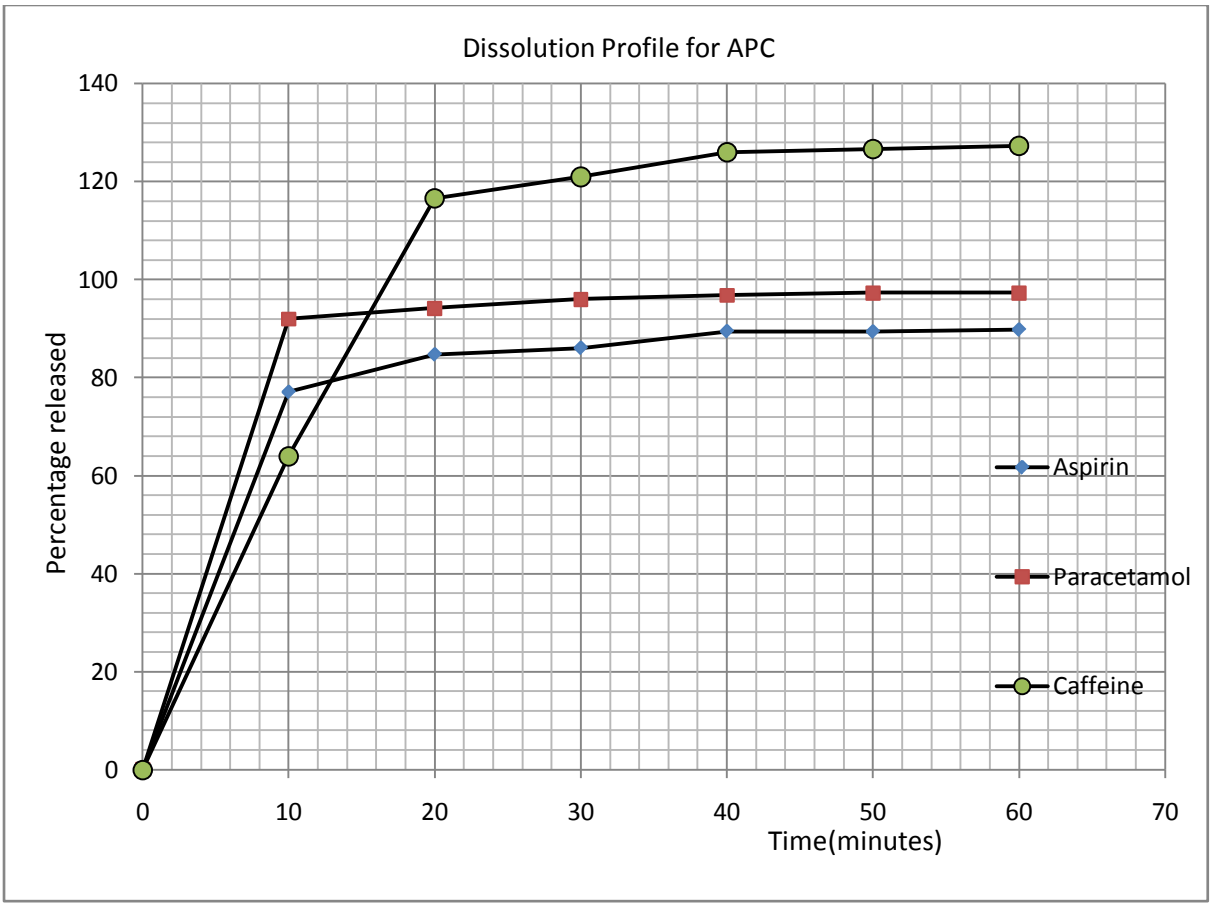


Table 71 Comparison of aspirin content in tablets using various methods:

Aspirin assay from tablet									
Batch Number	137379	137385	137390	137401	137406	137425	137434	Mean (%)	Standard deviation
BP Method	85.5%	123.9%	126.10%	78.01%	67.80%	85.67%	111.9%	96.98	0.2333
HPLC	84.40%	123.40%	123.60%	77.83%	68%	85.40%	110%	96.09	0.2262
UV. Sec	84.60%	124.20%	124.8%	77.10%	69.1%	85.55%	111.8%	96.74	0.2305

Table 72 Anova table for aspirin assay

Percentage content					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.986	2	1.493	0.003	0.997
Within Groups	9524.783	18	529.155		
Total	9527.770	20			
There is no significant difference between the actual mean and the means from the assy. $F(2,18)=0.001, p>0.05$					

Table 73 Comparison of caffeine content in tablets using various methods:

caffeine from tablet									
Batch Number	137379	137385	137390	137401	137406	137425	137434	Mean (%)	Standard deviation
BP Method	129.20%	146.10%	145.91%	109.89%	121.71%	128.40%	135.10%	130.90	0.1297
HPLC	129.13%	145.56%	145.59%	110.56%	120.28%	128.26%	134.21%	130.51	0.1276
UV. Sec	129.40%	145.90%	144.97%	110.10%	120%	127.54%	135%	130.46	0.1295

Table 74 Anova table for caffeine assay

Percentage content					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.63	2	0.31	0.002	0.998
Within Groups	2972.98	18	165.17		
Total	2973.60	20			
There is no significant difference between the actual mean and the means from the assy. $F(2,18) = 0.002, p>0.05$					