KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI

COLLEGE OF HEALTH SCIENCES

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DEPARTMENT OF PHARMACEUTICAL CHEMISTRY

STABILITY STUDIES ON FLUCLOXACILLIN AND THE DESIGN OF AN HPLC METHOD FOR THE ASSAY OF FLUCLOXACILLIN IN CAPSULE DOSAGE FORMS

By

Mahmood Brobbey Oppong (B. Pharm. Hons)

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DECLARATION

I, Mahmood Brobbey Oppong, declare that this work was done at Department of Pharmaceutical Chemistry, KNUST and that any reference material consulted has been duly acknowledged. This work has not been submitted anywhere for the award of any degree.

HEAD OF DEPARTMENT

Signature……..

Date……..

PROF. R.K. ADOSRAKU

SUPERVISOR

Signature…….

Date……

PROF. J.S.K. AYIM

CANDIDATE

Signature……

Date……

MAHMOOD BROBBEY OPPONG

ABSTRACT

Flucloxacillin is extensively used in the treatment of various infections caused by susceptible organisms. It breaks down easily in the presence of moisture and the breakdown products are responsible for the hypersensitivity reactions in susceptible individuals.

Stability studies on flucloxacillin sodium for capsule formulations were carried out. Fixed amounts of flucloxacillin sodium (250mg) were mixed with varying amounts of dried starch and dried plain carboxymethyl cellulose (plain cmc). The mixtures were put in well closed containers and kept in a room for two and half months. Iodimetry was used to monitor the amounts of flucloxacillin in the mixtures over the stated period.

It was noticed after the period that the mixtures with the dried plain cmc were more stable than mixtures with dried starch. The percentage breakdown for the dried starch mixtures were 28.47, 24.67, 27.32 and 25.20% respectively for 75, 125, 150 and 250mg of dried starch respectively. The percentage breakdown of the dried plain cmc mixtures were 27.40, 22.63, 23.07 and 20.57% respectively for 75, 125, 150 and 250mg of the dried plain cmc. The breakdown process followed pseudo-first order kinetics. The rate constants for the dried starch mixtures were 0.0336, 0.0305, 0.0345 and 0.0297week^{-1} for 75, 125, 150 and 250mg of dried starch respectively. The rate constants for the dried plain cmc mixtures were 0.0317, 0.0291, 0.0268 and 0.0231week^{-1} for 75, 125, 150 and 250mg of dried plain cmc respectively.

A reversed phase high performance liquid chromatography (RP- HPLC) was also designed to assay a mixture of flucloxacillin sodium, amoxicillin trihydrate, ampicillin trihydrate, benzylpenicillin sodium and phenoxymethylpenicillin sodium. The mobile phase used was 40%v/v redistilled methanol and 60%v/v0.02M potassium dihydrogen phosphate, KH_2PO_4 (pH = 3.70) on a Phenomenex ® LICHROSORB 10 RP-1 C18 column (250×4.60mm I.D., 5µm particle size). The flow rate was 1.00ml/min with UV detection at 225nm. Mean

retention times of 3.14 ± 0.01, 4.36 ± 0.02, 8.50 ± 0.04, 13.57 ± 0.06 and 21.18 ± 0.08minutes (**n=18**) were recorded for amoxicillin trihydrate (AXT), ampicillin trihydrate (APT), benzylpenicillin sodium (BPS), phenoxymethylpenicillin sodium (PMS) and flucloxacillin sodium (FXS) respectively.

The calibration plots for linearity gave straight lines with coefficient of correlation, r^2 values of 0.9989, 0.9987, 0.9988, 0.9984 and 0.9995 for AXT, APT, BPS, PMS and FXS respectively in the concentration range of0.005% to 0.10%w/w for BPS, FXS and PMS and 0.003% to 0.06%w/w for AXT and APT.

The limit of Detection (LOD) of the method were 0.002578, 0.002830, 0.004397, 0.005184 and 0.003029%w/v for AXT, APT, BPS, PMS and FXS respectively.

The Limit of Quantitation (LOQ) of the method were 0.007811, 0.008576, 0.013325, 0.015708 and 0.009178%w/v for AXT, APT, BPS, PMS and FXS respectively.

The method was precise with relative standard deviations (RSD) obtained for the intra-day precision/repeatability being 1.75, 1.64, 1.62, 4.03 and 1.75% and the inter-day/intermediate precision being 1.22, 2.23 1.90, 3.97 and 2.25% for AXT, APT, BPS, PMS and FXS respectively in the concentration range stated above. The method was also found to be robust since deliberate alteration of flow rate and wavelength of detection in the range of 1.00±0.20ml/min and 225±2nm respectively did not affect the precision of the method.

The accuracy of the method was confirmed with mean percentage recoveries (n=3) of 99.88, 100.66, 100.30, 100.48 and 101.33%w/w at 80% concentration level, 100.57, 100.01, 100.66, 100.48 and 100.51%w/w at 100% concentration level and 99.10, 99.48, 100.79, 100.41 and 100.40% w/w at 120% concentration level obtained for AXT, APT, BPS, PMS and FXS respectively.

The method can assay any of these penicillins individually either in single or combined formulations. It assays all five penicillins simultaneously and also detects breakdown products of flucloxacillin.

Key words: Amoxicillin trihydrate (AXT), ampicillin trihydrate (APT), benzylpenicillin sodium (BPS), phenoxymethylpenicillin sodium (PMS) flucloxacillin sodium (FXS), dried plain cmc, dried starch, iodimetry and RP-HPLC.

DEDICATION

I dedicate this work first and foremost to Allah almighty and secondly to my family especially my sweet mother Mrs. Mariam Brobbey and lastly to my dear father of blessed memory, Mr. Kofi Issah Oppong.

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ABBREVIATIONS

APT	:	Ampicillin sodium
AXT	:	Amoxicillin trihydrate
BNF	:	British National Formulary
BTS	:	British Thoracic Society
BP	:	British Pharmacopoeia
BPX	:	Benzylpenicillin sodium
CMC	:	Carboxymethyl cellulose
FXS	:	Flucloxacillin sodium
HPLC	:	High Performance Liquid Chromatography
ICH	:	International Conference on Harmonization
LOD	:	Limit of Detection
LOQ	:	Limit of Quantitation
MHRA	:	Medicines and Healthcare products Regulatory Agency
ODS	:	Octadecylsilane
PMS	:	Phenoxymethyl penicillin sodium
RP-HPLC	:	Reverse Phase HPLC
RSD	:	Relative Standard Deviation
SD	:	Standard Deviation
STG	:	Standard Treatment Guidelines
TLC	:	Thin Layer Chromatography
UV	:	Ultra-Violet
WHO	:	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

Antibiotics are compounds of natural, semi-synthetic, or synthetic origin which inhibit growth of microorganisms without significant toxicity to the human or animal host. **(Vaillemin, 1890)**.

They are generally defined as chemicals, produced by one microorganism, that are capable of killing or inhibiting the growth of other microorganisms. Penicillins are a class of antibiotic produced by some members of the genus Penicillium. In brief, in 1929, Fleming discovered penicillin and recognized its antibacterial properties. Penicillin and derivatives were produced by fermentation by the end of the 1940s. **(Moore & Nygren, 2004)**

Penicillins can be classified in several ways. Either as natural or synthetic, or with regard to their resistance to acids or penicillinases, or as having narrow or broad spectrum properties **(Remers et al., 1998).**

Penicillins are used for treatment of a large number of bacterial infections in humans and animals. The primary mechanism of action is inhibition of the bacterial cell wall synthesis. Penicillins have a structural analogy of the ß-lactam ring with D-alanyl-D-alanine, which terminates free N-acetylmuramic acid, a part of the glycan structure of the bacterial cell wall. Gram-positive bacteria are more sensitive to penicillin than gram-negative bacteria as they have higher glycan content. **(Van Krimpen & Van Bennekom, 1987**).

Flucloxacillin is a narrow-spectrum beta-lactam antibiotic of the penicillin class. It is used to treat infections caused by susceptible Gram-positive bacteria. Unlike other penicillins, flucloxacillin has activity against beta-lactamase-producing organisms such as *Staphylococcus aureus*. It is insensitive to beta-lactamase (also known as penicillinase) enzymes secreted by many penicillin-resistant bacteria owing to its bulky isoxazolyl group on

the side-chain of the penicillin nucleus. It is more acid-stable than many other penicillins and can be given orally, in addition to parenteral routes but it is however, less potent than benzylpenicillin against non-β-lactamase-producing Gram-positive bacteria. It is commercially available as the sodium salt flucloxacillin sodium, in capsules, oral suspensions, and injections (powder for reconstitution) dosage forms.**(Sutherland et al., 1970)**

Flucloxacillin may be used alone or in combination with other penicillins in the treatment of infections caused by susceptible organisms. Specific approved indications include: Staphylococcal skin infections and cellulitis – including impetigo, otitis externa, folliculitis, boils, carbuncles, and mastitis, adjunct in pneumonia management, osteomyelitis, septic arthritis septicaemia, empirical treatment for endocarditis and surgical prophylaxis**. (BNF, 2009).**

Amoxicillin – an acid stable, semi-synthetic drug – belongs to a class of antibiotics called the Penicillins (β-lactam antibiotics). It is effective against a wide range of infections caused by wide range of Gram -positive and Gram- negative bacteria in both human and animals. **(Kaur et al., 2011.)**

Flucloxacillin may be administered in combination with other penicillins including amoxicillin (in a proportion of 1:1 known as flumocin or flomoxin) to produce a wider spectrum of activity. The standard prophylactic regimen for patients undergoing cardiac surgery is flucloxacillin and amoxicillin. **(Danton et al., 2004)**

According to the British Thoracic Society guidelines for the management of community acquired Pneumonia in childhood, 2002, combination of flucloxacillin and amoxicillin is recommended if staphylococcus aureus is the likely pathogen. **(BTS guidelines, 2002)**

In Ghana, flucloxacillin is clinically used alone in the treatment of boils (furuncle) or carbuncles, impetigo, bacterial super-infection of skin lesions in chicken pox, post-partum pyrexia in mastitis and acute septic arthritis. It is also used in combination with amoxicillin in the treatment of cellulitis and erysipelas, human and dog bites**. (STG, 2010)**

Flucloxacillin, just like any other penicillin is an unstable drug which can be broken down by moisture, acids, bases and heavy metal ions. **(Ayim & Rapson, 1972**). This implies that formulations of the drug ought to be kept away from these. It is practically possible to keep them away from acids, bases and heavy metals but very difficult to totally keep formulations away from moisture because of the high humidity that persists in our environment.

This chemically unstable nature of flucloxacillin and related penicillins is a major cause of poor drug quality in tropical countries **(Hogerzeil et al., 1991)** and also a contributor to substandard drugs especially in countries with high humidity and temperatures like Ghana due to excessive decomposition of active moiety. Furthermore, chemical instability is a key cause of antibiotic resistance. These lead to treatment failure and consequently increased in mortality and morbidity**. (Kelesidis et al., 2007).**

Frequent recalls of flucloxacillin capsule dosage forms from the market are as a result its chemically unstable nature. On August 28, 2003, UK Generics limited (a Pharmaceutical company) had to recall 14 batches of 500mg flucloxacillin capsule dosage forms which had three more years ahead of its expiry due to concerns with stability of the finished products.**(MHRA, 2003)**

Again on August 21, 2007, Milpharm and Arrow Generics (a pharmaceutical company in UK) recalled two batches flucloxacillin 500mg capsule dosage forms from the market due to concerns with stability of the finished product.**(MHRA, 2007)**

Amoxicillin and phenoxymethyl penicillin had also been recalled on few occasions due to poor microbial quality. **(MHRA, 2003)**

Again, though the penicillins are closely related structurally, (i.e., all of them having the β-lactam ring), they differ in their activity, thus have different range or spectrum of activity, some being penicillinase stable others being penicillinase unstable, acid stable and acid unstable owing to their different side chains. **(Olaniyi, 2005)**. They are not interchangeable thus one cannot completely substitute one for the other. Substituting one penicillin for the other during manufacture constitutes drug counterfeiting**.(WHO, 2013)**

Price variation among the penicillins including flucloxacillin (being more expensive than the other penicillins) makes it a potential target of drug counterfeiting in the penicillin industry. **(WHO, 2013)**

The ever increasing production and marketing of products containing the same active ingredient by myriad of companies under different brand names once the patent has expired and manufacturing failures resulting in legitimate drugs with no or wrong active ingredients (complete substitution) and partial substituted actives are rampant on the market and pose difficulties to the regulatory bodies with respect to their regulations.

One major problem associated with the use of flucloxacillin like other penicillin antibiotics is the hypersensitivity reactions some susceptible individuals have when they use them. **(Stewart, 1973)**. Breakdown products such as penicilloic acids have been implicated in these reactions**.(Deshpande et al., 2004).** The penicilloic acid among other breakdown products comes about when the drug is exposed to acids, bases, alcohols, enzyme sand moisture. It is much easier to keep the drug away from all these factors compared to keeping it away from moisture as it is present everywhere owing to the very humid nature of our atmosphere. Thus flucloxacillin still breaks down when kept in airtight packaging materials.

Another problem with flucloxacillin use is the development of resistant strains of bacteria towards it. Failure of patients to take complete courses of antibiotics is a major cause. Another major cause is the issue of under-dosage because if a patient took a complete course of a low dose medication, levels attained in the body would be well below therapeutic levels to kill off any bacteria. This low dosage can come about when one takes a medication in which has significant amount of the drug has broken down even prior to the expiration of the product or issuance of sub-standard/counterfeit medications as a result of either intentional or accidental partial/complete substitution of the active principles on the part of the manufacturers.

Moreover the frequent recall of flucloxacillin from the market as indicated earlier puts a heavy financial burden on the manufacturing companies involved. Ghana being a tropical country with high temperature and humidity, there is therefore the possibility of this happening to our local companies engaged in Flucloxacillin production.

It is therefore very important that stability studies be conducted on flucloxacillin to investigate the effects of excipients (starch and carboxymethyl cellulose) on it and possibly find out which excipient and in which amounts with the active drug will render it most stable when formulated with it.

Again it is also prudent that a simple, rapid and cheaper HPLC method that could detect and assay simultaneously various penicilins and other related impurities when formulated with flucloxacillin be developed and validated as this will help in the regulation of counterfeit/substandard flucloxacillin formulations. It would also help in the monitoring of the stability of the product. Earlier method like iodimetry is tedious and not selective or specific for flucloxacillin and its related impurities. UV/visible spectroscopic analysis of the drug have similar challenges.

This work therefore seeks to carry out the above stated investigations and the HPLC method development and validation.

1.1 JUSTIFICATION

The continuous use of flucloxacillin sodium in medicine for the treatment of various infections caused by susceptible bacteria both in Ghana and many other parts of the world, the hypersensitivity experienced by susceptible individuals caused by breakdown products of the drug, frequent recalls of the drug from the markets due to stability concerns and related financial burden on the manufacturing companies and resistance development towards the drug partly due to breakdown of the drug on storage call for concerns for investigating into the stability of the drug.

Also lack of rapid, simple and inexpensive analytical methods that can simultaneously assay, detect possible adulteration and breakdown products of the drug warrants the need to design one such method.

1.2 OBJECTIVES

The objectives of the research work are:

- To determine the rate of reaction for the breakdown of flucloxacillin by moisture.
- To investigate the effect of starch and plain carboxymethyl cellulose (cmc) on flucloxacillin stability.
- To determine the amount of starch and plain cmc that retards/slows down the decomposition of flucloxacillin.
- To design and validate a simple HPLC method for the simultaneous detection and assay of flucloxacillin and some related penicillins in single/combined flucloxacillin dosage forms.

1.3 LITERATURE REVIEW

1.3.1 HISTORY AND DEVELOPMENT OF PENICILLIN

Penicillin is one of the earliest antibiotics discovered by man. In 1929, Sir Alexander Fleming observed that colonies of the bacterium *Staphylococcus aureus* were destroyed by the mold *Penicillium notatum,* proving that there was an antibacterial agent there in principle. Fleming then conducted several experiments to investigate the antimicrobial activity of this mould. The result was that the mould destroyed the harmful bacteria used and upon further experiments he proved that the mould was nontoxic to man.

In 1940s, the Australian Howard Florey and German refugee Ernst Chain isolated *Penicillium notatum* as a brown powder. The brown powder was found to retain its antibacterial activity for some few days. They did some studies on the powder and proved that it was safe to be used in humans.

Mass production of penicillin then started in the 1940s. Penicillin saved many lives that would have been lost during the Second World War as a result of bacterial infestation of small cuts and wounds.

Fleming is credited for the discovery of penicillin and Florey and Chain rendered the agent usable. As a result, all three were awarded a Nobel Prize in Physiology or Medicine in 1945. Fleming's discovery led the way in the discovery of other antibiotics that killed bacteria. **(http://history1900s.about.com/od/medicaladvancesissues/a/penicillin_2.htm)**

Penicillin's use was limited by its narrow spectrum of activity, short duration of action, acid and penicillinase sensitivity. This stimulated a search for derivatives that would demonstrate a broad spectrum of activity against bacteria as well as being acid and penicillinase stable/resistant coupled with patients' tolerance. **(Olaniyi, 2005 & http://www. News medical. net/health/Penicillin-Developments.aspx)**

The structural elucidation of penicilin and confirmation using X-ray crystallography by Sheehan and Henry-Logan and the isolation of the penicillin nucleus, 6-Aminopenicillanicacid (6-APA) by Batchelor and co-workers in 1959 constituted a major break-through in penicillin synthesis.**(Olaniyi, 2005).**

Ampicillin was the first semi-synthetic derivative and it was shown to have a broad spectrum of activity and its modification led to amoxicillin, which in addition to having broad spectrum of activity, has a longer duration of action.

Further advancements led to the production of penicillins that are beta-lactamase resistant (penicillinase resistant) like flucloxacillin, cloxacillin, dicloxacillin and methicillin. These showed activity against bacteria that produced the enzyme, beta-lactamase that destroys penicillin but are inactive against methicillin resistant *Staphylococcus aureus* which developed with time. Antipseudomonal penicillins like ticarcillin, carbenicillin and piperacillin were also later discovered and they showed activity against gram negative bacteria**. (Olaniyi, 2005)**

1.3.2 THIN LAYER CHROMATOFGRAPHY (TLC) OF SOME PENICILLINS

Penicillin G and cloxacillin have been detected and quantitated in milk using TLC. The milk was treated to remove proteins with 2 volumes of acetonitrile and then shaken with buffers and organic solvents; first with a buffer of pH 2.2 and then with a buffer of pH 7. The antibiotics were extracted into methylene chloride and spotted on silica gel plates and developed with chloroform/ acetone/ glacial acetic acid in the ratio of 10:9:1. Spraying the plates with 1N HCl and starch and final exposure to iodine vapour aided the location of the spots which appeared blue- black. **(Moats et al., 1983)**

Amoxicillin, ampicillin, cloxacillin, cephalexin, streptomycin, tetracycline, gentamicin, erythromycin and co-trimoxazole have been separated in a mixture on a titanic inorganic ion exchanger successfully. Erythromycin and tetracycline were well separated from the other

antibiotics when a solvent system of chloroform: methanol: ammonium hydroxide in the ratio of 1:1:1 was used as mobile phase. Several other systems of solvent as mobile phases gave other separations. A 1% w/v ninhydrin in ethanol was used to locate amoxicillin, ampicillin, cloxacillin, cephalexin, gentamicin and co-trimoxazole spots while 5% (w/v) potassium dichromate in concentrated sulphuric acid was used in locating streptomycin, erythromycin, tetracycline and doxycycline spots**. (Husain et al., 2004)**

A mixture of penicillamine, ampicillin, amoxicillin and benzyl penicillin have been successfully separated using various mobile phase systems like acetonitrile/water and butanol/acetic acid/water respectively in ratios of 3:5 and 4:1:1. Other mobile phase systems were also used successfully. A cation exchange stannic arsenate cellulose support material was used. Iodine vapour was employed to aid spot visualization. **(Nabi et al., 2006)**

Amoxicillin trihydrate and ampicillin trihydrate have been identified using a mixture of equal amounts of amoxicillin trihydrate and ampicillin trihydrate in sodium bicarbonate solution as reference and a mobile phase made of 10volume of acetone and 90volume of 154g/l solution of ammonium acetate adjusted to a pH of 5 with glacial acetic acid. The support material was silica gel and iodine vapour again was used for spot visualization**. (BP, 2007)**

Benzylpenicillin and phenoxymethyl penicillin have also been identified using a mixture of equal amounts of Benzylpenicillin and phenoxymethyl penicillin in distilled water as reference and a mobile phase made of 30volume of acetone and 70volume of 154g/l solution of ammonium acetate adjusted to a pH of 5 with glacial acetic acid. The support material was silica gel and iodine vapour again was used for spot visualization**. (BP, 2007)**

Flucloxacillin and cloxacillin have the same method of identification. They have the same reference solutions made of equal amounts of cloxacillin, dicloxacillin and flucloxacillin in distilled water. The mobile phase used was 30volumes of acetone and 70volumes of 154g/l

ammonium acetate adjusted to a pH of 5 with glacial acetic acid. The support material was silica gel and iodine vapour was used for spot visualization. **(BP, 2007)**

The chromatographic behavior of penicillins, cephalosporins and carbapenems has been studied on the thin layers of transition-metal ion (*viz.* $Ni^{2+}/Zn^{2+}/Cu^{2+}/Co^{2+}$) silicate modified silica with transition-metal silicate (3.92%) and silica (96.08%) being found to be optimum as it resulted in spherical-compact spots and improved resolution of the analytes. Various mobile phases were employed in the study. The chromatograms were visualized as yellow spots using iodine vapour. **(Butler, 2012)**

13.3 IODINE TITRATIONS OF PENICILLINS

Penicillins have been assayed by iodimetry because the penicilloic acids they produce after degradation in acid or alkaline media takes up iodine which aids their quantification.

Penicillins have been shown to be quantifiable in culture fluids. The penicillin was extracted from the culture fluid using amyl acetate at a pH of 2 from the aqueous phase followed by a reextraction from the amyl acetate using a known volume of phosphate buffer of pH 7. The penicillin in the phosphate buffer was then assayed using standard solutions of sodium thiosulphate and iodine using sodium starch glycollate as indicator. A correction factor was introduced for those substances that were extracted with the penicillin from the culture fluid and took up iodine during the assay. **(Beloff-Chain &D'accadia, 1952)**

The sodium and potassium salts of benzyl penicillin have been assayed by iodimetry. A solution of the drug of either the sodium or potassium salt was hydrolyzed by 1M sodium hydroxide to give the penicilloic acid which was stabilized in acetate buffer which also created a desired pH for the titration. The excess hydroxide solution was neutralized with 1M hydrochloric acid so as not to alter the pH of the medium. A standard solution of iodine of concentration 0.01M was added to the mixture and the vessel stoppered and kept away from light. After twenty minutes the excess iodine was titrated against a standard solution of

thiosulphate using starch mucilage as indicator. A blank determination in which no sodium hydroxide was added to the penicillin was carried out to rule out other impurities that could absorb iodine in addition to the penicilloic acid generated so as to enable the exact quantification of the amount of benzyl penicillin present. The blank determination proceeded with an addition of acetate buffer and standard iodine solution to a solution of the drug. The vessel was stoppered, kept away from light and titrated against a standard solution of thiosulphate using starch mucilage as indicator after twenty minutes. The difference between the two determinations aided in the quantification of benzyl penicillin. **(BP, 1980)**

The sodium and potassium salts of phenoxymethyl penicillin have also been assayed like benzyl penicillin with the only difference being the preparation of the samples. Aliquots for the assay were obtained from the resulting solution obtained by: adding an amount of phenoxymethyl penicillin to a saturated solution of sodium hydrogen carbonate, followed addition of some water and 1M hydrochloric acid with occasional shaking and adjustment to the desired volume again with water. **(BP, 1980)**

The USP also describes a similar method for the assay of various penicillins including diclocxacillin, nafcillin, methicillin, cyclacillin, phenethicillin, amoxicillin, ampicillin and oxacillin. The differences lie in the use of distilled water or phosphate buffer as a solvent for the preparation of drug solution and the use of starch iodide paste as indicator. **(http://www.pharmacopeia.cn/v29240/usp29nf24s0_c425.html)**

Phenoxymethyl penicillin has been assayed using the method described above in body fluids during an in-vitro study on the pharmaceutical equivalence of phenoxymethyl penicillin in tablet formulations available in Bangladesh. **(Apu et al., 2011)**

1.3.4 FLUCLOXACILLIN SODIUM

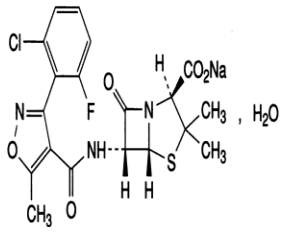

Figure 1: Structure of flucloxacillin sodium

The chemical name is

(2S, 5R, 6R)-6-((3-(2-chloro-6-fluorophenyl)-5-methylisoxazol-4-yl) carbonyl) amino)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo(3.2.0)heptanes-2-carboxylate. It has an empirical formula of $C_{19}H_{16}ClFN_3NaO_5S.\ H_20$ and has a molecular mass of 493g/mol.

Flucloxacillin sodium is a white or almost white powder crystalline in nature and hygroscopic. It is soluble in 96% ethanol and freely soluble in methanol and in water. The absorbance of10%w/v solution of flucloxacillin sodium taken at 430nm should not exceed 0.04. The Ph obtainable for such a solution is from 5.0 to 7.0.The specific optical rotation of 1% w/v solution determined using the anhydrous substance as a reference is +158 to +168.

Flucloxacillin can be assayed by high performance liquid chromatography (HPLC), iodimetric titration and ultraviolet-visible spectroscopy and the content of the drug must not be below 95.0% and should not exceed 101.0%. Flucloxacillin should be kept in an airtight container and its storage temperature should not exceed 25°C. **(BP, 2007).** Reconstituted suspensions must be kept refrigerated at temperatures between 2-8°C. **(http://www.mhra.gov.uk/home/group/1-unit/documents/websiteresource/con2033471.pdf).**

Flucloxacillin is well absorbed with about 79% of the total amount of the drug being absorbed after oral administration. It diffuses well into body tissues: amounts that have been recovered in spongy bone and compact bone are 11.6mg/l and 15.6mg/ml respectively with a mean level of 8.9mg/ml in serum. About 95% of the drug is bound to proteins following administration. Only small quantities diffuse into the cerebrospinal fluid of subjects whose

meninges are not inflamed. Approximately 10% of flucloxacillin is metabolized to penicilloic acid when administered by the oral route to normal subjects. It has an elimination half-life of 53 minutes. Excretion is mainly renal with about 65.5% and 76.1% of the dose administered remaining unchanged in urine within 8 hours respectively by the oral and parenteral routes. A small amount is however excreted in the bile and breast milk. There is therefore a reduced clearance of flucloxacillin in cases of renal impairment. Probenecid, aspirin and anti-inflammatory agents compete for renal excretion of flucloxacillin and other penicillins thereby leading to toxicity when co-administered. Flucloxacillin like other penicillins reduces the effectiveness of oral contraceptives. It also reduces excretion of cytotoxic drugs like methotrexate when given together. Side effects of flucloxacillin include hepato-biliary disorders e.g. cholestatic jaundice and hepatitis, immune system disorders e.g. hypersensitive reactions and rarely anaphylactic shock often with oral administration and angioneurotic edema. Gastrointestinal disorders range from nausea, diarrhea, sore mouth or tongue to pseudomembranous colitis. Blood and lymphatic system disorders like neutropenia, agranulocytosis and thrombocytopenia have also been reported. Skin and subcutaneous tissue disorders include urticarial, rash, and Stephen Johnson's syndrome. Musculoskeletal and connective tissue disorders reported are arthralgia and myalgia. Interstitial nephritis has also been documented as renal and urinary system disorders of flucloxacillin **(http://www.mhra.gov.uk/home/group/1-unit/documents/websiteresource/con2033471.pdf).** It is contraindicated in penicillin hypersensitivity **(BNF, 2009)**

1.3.5 STABILITY OF FLUCLOXACILLIN SODIUM

Flucloxacillin, like any other penicillin has a very unique structure: having a beta lactam ring which is fused to a thiazolidine ring. The strained beta lactam ring can be cleaved by nucleophiles, acids, bases, moisture, alcohol, heavy metals and enzymes. Flucloxacillin

though a penicillin is not liable to enzymatic cleavage because of its bulky isoxazolyl side chain that hinders this form of cleavage.

Flucloxacillin is hydrolyzed in aqueous solutions by degrading the beta lactam ring. The hydrolysis is promoted by an increase in temperature or in acids or bases. The breakdown products include penillic, penicillenic acid and penicilloic acids which reduce the pH and further promote the degradation process. Minimal amounts of penicillamine have also been detected. This degradation process is insignificant at a pH of about 6.8 and the inclusion of a buffer like citrate buffer retards the breakdown. **(Sweetman, 2009)**

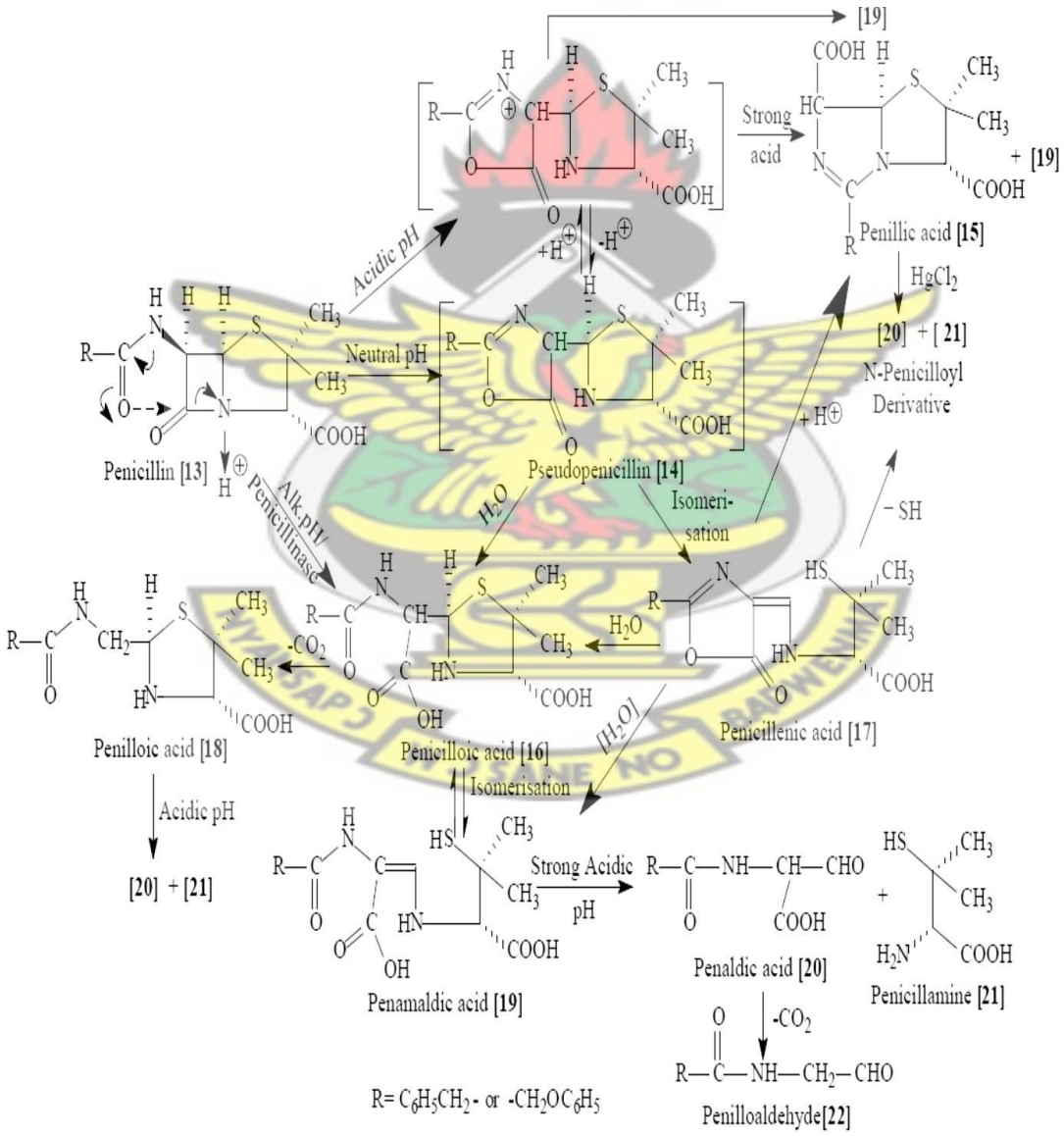

Figure 2: Scheme for the breakdown of penicillins in different media. (Deshpande et al., 2004)

1.3.6 REVIEW OF ANALYTICAL METHODS USED TO ASSAY FLUCLOXACILLIN

It is a quality control requirement that drug products not only contain the actual active ingredients that are stated on them but also in their right amounts in order to render them acceptable and desirable to be used by humans and sometimes animals. The rates at which substandard products are released unto the markets and their usage have led to a lot of problems. Some patients have had their health conditions deteriorated as a result of this. There is also the development of resistant strains of bacteria when poor quality antibiotic products are used.

To prevent this, analysts have designed various analytical procedures to assay drugs in formulations, body fluids and tissues and other matrices. Flucloxacillin have been assayed using titrimetry, ultraviolet-visible spectroscopy and HPLC and these can be found in the British Pharmacopoeia (BP) and published articles.

1.3.6.1 ASSAY OF FLUCLOXACILLIN USING TITRIMETRY

The **BP (1968)** and **BP (1973)** have described an acid-base titrimetric method for the assay of cloxacillin. The procedure simply involved hydrolyzing the drug in a neutralized solution with sodium hydroxide and the excess alkaline solution was titrated with a standard solution of hydrochloric acid. A blank was performed and the difference in readings represented the amount of sodium hydroxide required. The theory behind the titration makes it applicable to flucloxacillin as it will react in like manner as cloxacillin to yield penicilloic acid upon hydrolysis with sodium hydroxide. The procedure is satisfactory for pure flucloxacillin powder, capsules and injections.

Iodimetric titrations have also been described for the assay of benzylpenicillin and phenoxymethylpenicillin (described earlier) which are also applicable to flucloxacillin as the

principle upon which method work is applicable to all penicillins. The major limitation to these titrimetric methods is the lack for selectivity for these penicillins in combined formulations.

1.3.6.2 ASSAY OF FLUCLOXACILLIN USING UV- VISIBLE SPECTROSCOPY

Direct UV analysis has been described for flucloxacillin involving the use of double distilled water as solvent and 219nm as the maximum wavelength of absorption. **(Dey et al., 2010).**

A similar method involving the dissolution of flucloxacillin and measurement of the absorbance at 273nm has also been reported. **(Prakash et al., 2012).**

Direct UV analysis of flucloxacillin like the other penicillins may yield results that may interfere with accuracy and precision because penicilloic acids and other degradation products interfere with the results.**(Jun et al., 1985).**

To overcome this problem, Jun et al., employed the use of a derivatizing agent 1, 2, 4 – triazole and Mercury (II) chloride as the derivatizing agents. This was warmed with the drug at 60°C for 10minutes to give a derivative which absorbed more strongly at a detection wavelength ranging from 323-346nm where all interferences due to penicilloic acid and other substances were eliminated.

In the **BP (1993)**, imidazole mercury reagent as also been employed as a derivatizing agent. This was warmed with the drug at 60°C for 25minutes to give penicillenic acid- mercury (II) mercaptide that absorbed more strongly and at a longer wavelength of detection of 364nm eliminating interferences due to penicilloic acid and other substances.

A UV method has also been described where iodine solution was added to methanolic solutions of the drug and the absorbance measured at 362 nm against the reagent blank. The

method was reported to be based on charge complexation reaction of iodine with flucloxacillin. **(Gujral et al., 2009)**

The methods are suitable for flucloxacillin pure powder, capsules, injection, oral solution and suspension but still lack selectivity when analyzing combined formulation.

1.3.6.3 ASSAY OF FLUCLOXACILLIN BY HPLC

HPLC having the advantage being sensitive, accurate and precise also shows selectivity that the above mentioned methods lack. Several HPLC methods have been outlined for the assay of flucloxacillin with UV detection.

An isocratic ion exchange has been developed for the simultaneous determination of flucloxacillin and amoxicillin. A ZORBAX 3000-SCX column and 0.025M ammonium dihydrogen phosphate (adjusted to pH 2.6 with phosphoric acid) – acetonitrile (95:5) as mobile phase, flow rate of 1.50ml/min and 225nm as detection wavelength were employed.**(Liu et al., 2005)**

A method has been developed and validated for the determination of the concentration of the drug in plasma with dicloxacillin as internal standard using RP –HPLC with ODS as the stationary phase and a mobile phase composition of 64.5% potassium dihydrogen phosphate and 35.5% acetonitrile.**(Zhou et al., 2007)**

The **BP 2007** and **BP 2009** describe another reverse phase method involving C-18 Column. The mobile phase system was made of 25 volumes of acetonitrile and 75 volumes of 2.7g/l of potassium dihydrogen phosphate adjusted to pH 5 with sodium hydroxide. The flow rate was 1ml/min and detection wavelength was 225nm.

Flucloxacillin has also been assayed in the presence of amoxicillin also in the reversed phase using a C-18 column and 0.020M potassium dihydrogen orthophosphate - acetonitrile (75:25)

as mobile phase. Detection was made at 225nm with a flow rate of 1.5ml/min.**(Nikam et al., 2009)**

Flucloxacillin has also been assayed in the presence of amoxicillin by using a mobile phase made of a mixture of buffer (prepared from 0.001M diammonium hydrogen orthophosphate and 0.04M tetrabutylammonium bromide, pH adjusted to 7.0±0.1 with ortho phosphoric acid) and acetonitrile in the ratio (99:10v/v), on a strong cation exchange column, (LUNA SCX, 250mm x 4.6mm I.D. 5 µm particles). Detection was made at 254nm with a flow rate of 1.00ml/min **(Shanmugasundaram et al., 2009)**

Another reverse phase involving ODS column as stationery and 40% methanol and 60% buffer (9mM sodium heptanesulfonate, 30mM potassiumdihydrogen phosphate and 0.04% triethylamine adjusted to pH 5 with 1M phosphoric acid) as mobile phase with penicillin-G as internal standard has been described for the determination of flucloxacillin in the presence of amoxicillin, ampicillin, cloxacillin, dicloxacillin and phenoxymethylpenicillin (penicillin V) in single or combined pharmaceutical formulations.
(http://shodh.inflibnet.ac.in/bitstream/123456789/806/3/03_literature%20review.pdf.)

CHAPTER TWO

2.0 INSTRUMENTS AND MATERIALS

- Adam – analytical weighing balance, WA 210; 210/ 0.0001g
- Beakers (25ml, 250ml, 1000ml)
- Boiling tubes
- Burette
- Buchi heating bath B-419
- Buchirota vapor R- 210
- Buchi distillation chiller B- 741
- Conical flasks
- Clean white sheets of A4 paper
- Delivery pipettes (1ml, 2ml,5ml, 10ml, 20ml and 25ml)
- Drying oven
- Eutech instruments pH meter
- Fisher Scientific FS28H Sonicator
- Fourier Transform Infrared Spectrometer, Module 200-X, Serial - 200043
- Graduated pipettes (1ml and 5ml)
- Hanna instruments pH 211 microprocessor pH meter
- Measuring cylinder
- No. 1 Whatmann filter papers
- Petri dishes
- Phenomenex ® LICHROSORB 10 RP-1 C18 column (250×4.60mm I.D., 5µm particle size)
- Plastic funnel
- Spatula

- Syringe
- Volumetric flasks (10ml, 25ml, 50ml, 100ml, 500ml and 1000ml)

2.1 REAGENTS AND SAMPLES USED

- Benzylpenicillin sodium powder
- Capsule brands from different manufacturing companies
- Capsule excipients (starch and carboxymethyl cellulose)
- Chloroform (BDH, Poole, England)
- Copper sulphate penta- hydrate (Fissons Scientific Equipment)
- Distilled water
- Disodium hydrogen orthophosphate (BDH, Poole, England)
- Glacial acetic acid (Philip Harris plc, Shaneson, England
- Formaldehyde (BDH, Poole, England)
- Hydrochloric acid (36% w/w) (Philip Harris plc, Shaneson, England)
- Iodine (Breckland Scientific Supplies, UK)
- Methanol
- Phenoxymethyl penicillin sodium
- Potassium iodide (Finkem Laboratory Reagents)
- Potassium iodate (Fissons Scientific Equipment)
- Potassium dihydrogen orthophosphate (BDH, Poole, England)
- Pure flucloxacillin powder (assay: 96.0%)
- Pure Amoxicillin powder (assay: 98.80%)
- Pure Ampicillin trihydrate (assay: 98.20%)
- Sulphuric acid (98% w/w) (BDH, Poole, England)
- Sodium hydroxide (99%) (BDH, Poole, England)
- Sodium acetate (BDH, Poole, England)

- Sodium thiosulphate (BDH, Poole, England)
- Starch mucilage powder (Finkem Laboratory Reagents)

Table 1: Data on pure samples used.

Material	Source	Batch number	Manufacturing date	Expiry date
Benzylpenicillin sodium	Troge Medical GMBH	1871-01	NOVEMBER,2010	NOVEMBER., 2013
Amoxicillin trihydrate	Ernest Chemist Ltd	AMX 11070991	JULY, 2011	JUNE, 2015
Ampicillin trihydrate	Ernest Chemist Ltd	KAT 06120351	JUNE, 2012	MAY, 2015
Carboxymethyl cellulose (plain)	Ernest Chemist Ltd	H1430	NOVEMBER, 2010	NOVEMER, 2015
Phenoxymethyl penicillin sodium	Letap Pharmaceuticals	089049	20013	APRIL 2015
Flucloxacillin sodium	Letap Pharmaceuticals	VCLFCLXL	NOVEMBER, 2010	OCTOBER, 2013
Maize starch	Ernest Chemist Ltd	STEX 1011338	NOVEMBER, 2010	OCTOBER, 2013

Table 2: Data on the brands of flucloxacillin used.

Brand code	Batch number	Manufacturing date	Expiry date	Source/country of origin
Sample A	110925	09/2011	09/2013	Medreich pharmaceuticals Ltd, England
Sample B	5310M	10/2011	10/2013	Ernest Chemist Ltd, Ghana
Sample C	0230152	09/2011	09/2013	Letap pharmaceuticals, Ghana

2.2 METHODS

2.2.1 PREPARATION OF SOLUTIONS

2.2.1.1 PREPARATION OF PRIMARY STANDARD, KIO_3 SOLUTION, TO STANDARDIZE 0.01M $Na_2S_2O_3 .5H_2O$

About 0.1779g of potassium iodate crystals was weighed in a small beaker on an analytical balance and transferred quantitatively into 500ml volumetric flask using distilled water. The flask was stoppered, shaken vigorously to ensure complete dissolution and homogeneity. The solution was further adjusted to the 500ml mark with distilled water, stoppered, shaken and labelled accordingly.

2.2.1.2 PREPARATION OF 2M H_2SO_4

About 11ml of the stock sulphuric acid was measured with a measuring cylinder and poured in a thin stream into a beaker containing about 50ml of distilled water. The mixture was stirred and allowed cool after which it was quantitatively transferred into 100ml volumetric flask and made to the mark with distilled water. The flask was stoppered, shaken and labelled appropriately.

2.2.1.3 PREPARATION OF STARCH MUCILAGE

About 5g of starch mucilage powder was mixed with about 100ml of water in a beaker. This was gently heated over a Bunsen flame with stirring for about five minutes to give the starch mucilage.

2.2.1.4 PREPARATION AND STANDARDIZATION OF 250ml OF 0.01M $Na_2S_2O_3 .5H_2O$

0.6209g of sodium thiosulphate was weighed in a small beaker on an analytical balance and transferred quantitatively using distilled water into 250ml volumetric flask. The flask was

stoppered, shaken vigorously to ensure complete dissolution and homogeneity. The solution was further adjusted to the 250ml mark with distilled water, stoppered, shaken and labeled accordingly. The prepared thiosulphate solution was filled into a clean burette. 20ml of the primary standard was pipette into a conical flask. About 2g of potassium iodide and 5ml of 2M sulphuric acid were added and titrated against the titrant until the analyte turned pale brown. 5ml of starch mucilage added and titration continued until the blue- black colour is discharged. The factor of the primary standard and the thiosulphate were calculated.

2.2.1.5 PREPARATION OF 0.01M IODINE (I_2) SOLUTION

About 5g of potassium iodide was dissolved in about 10ml of water in a glass mortar. 2.5380g of iodine was added and allowed to dissolve. The undissolved portion was triturated with a glass pestle to yield a homogenous solution. This was transferred quantitatively into a 1000ml flask and made to the mark with distilled water. The flask was stoppered, shaken and labelled accordingly.

2.2.1.6 PREPARATION OF MIXED PHOSPHATE BUFFER pH 4

About 1.008g of disodium hydrogen orthophosphate and 0.6020g of potassium dihydrogen orthophosphate were dissolved in sufficient distilled water to produce 200ml and pH was adjusted with drops of glacial acetic acid to 4**.(BP, 1988)**

2.2.1.7 PREPARATION OF MIXED PHOSPHATE BUFFER PH 7

About 0.1000g of anhydrous disodium hydrogen orthophosphate and 0.0602g of potassium dihydrogen orthophosphate were dissolved in sufficient water to produce 200ml**.(BP, 1988)**

2.2.1.8 …..PREPARATION OF FORMALDEHYDE- SULPHURIC ACID SOLUTION

2ml of formaldehyde solution was mixed with 100ml of sulphuric acid (96%w/w). **(BP, 2007)**

2.2.1.9 PREPARATION OF 1M NaOH

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

2.2.1.10 PREPARATION OF 1M HCl

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

2.2.1.11 PREPARATION OF 2.40% w/v GLACIAL ACETIC ACID

About 5.80ml of the stock glacial acetic acid was measured with a measuring cylinder and poured in a thin stream into a beaker containing about 50ml of distilled water. The mixture was stirred and allowed cool after which it was quantitatively transferred into 250ml volumetric flask and made to the mark with distilled water. The flask was stoppered, shaken and labeled appropriately.

2.2.1.12 PREPARATION OF 5.44% w/v SODIUM ACETATE BUFFER

13.6000g of sodium acetate was weighed accurately with a small beaker and quantitatively transferred into a 250mlvolumetric flask with the 2.40% w/v glacial acetic acid. More of the glacial acetic acid was added, stoppering and shaking the flask upon every addition for thorough dissolution until the volume of the solution reached the 250ml mark.

2.2.1.13 PREPARATION OF PRIMARY STANDARD, KIO_3 SOLUTION, TO STANDARDIZE 0.02M $Na_2S_2O_3$.$5H_2O$

About 0.1785g of potassium iodate crystals was weighed in a small beaker on an analytical balance and transferred quantitatively into 250ml volumetric flask using distilled water. The flask was stoppered, shaken vigorously to ensure complete dissolution and homogeneity. The solution was further adjusted to the 250ml mark with distilled water, stoppered, shaken and labelled accordingly.

2.2.1.14 PREPARATION AND STANDARDIZATION OF 0.02M $Na_2S_2O_3$.$5H_2O$

About 4.9636g of sodium thiosulphate heptahydrate was weighed in a small beaker on an analytical balance and transferred quantitatively using distilled water into 1000ml volumetric flask. The flask was stoppered, shaken vigorously to ensure complete dissolution and homogeneity. The solution was further adjusted to the 1000ml mark with distilled water, stoppered, shaken and labeled accordingly. The prepared thiosulphate solution was filled into a clean burette. 20ml of the primary standard was pipetted into a conical flask. About 2g of potassium iodide and 5ml of 2M sulphuric acid were added and titrated against the titrant until the analyte turned pale brown. 5ml of starch mucilage added and titration continued until the blue- black colour is discharged. The factor of the primary standard and hence the thiosulphate were calculated.

2.2.2 PREPARATION OF DRY STARCH AND ITS MOISTURE CONTENT DETERMINATION

A known weight of starch powder was put into a pre-weighed petri dish and weight taken and noted. The weight of the starch and petri dish was taken again after the dish with the starch had been heated in an oven to a constant weight. The difference in weight (weight loss) was expressed as a percentage to give the moisture content of the starch. The starch obtained thereafter was the dried starch.

2.2.3 PREPARATIONS OF PURE FLUCLOXACILLIN AND DRIED STARCH MIXTURES

2.2.3.1 250mg FLUCLOXACILLIN AND 75mg DRIED STARCH

7.5000g of pure flucloxacillin powder and 2.2500g of dried starch were weighed into a glass mortar and triturated to obtain a homogenous mixture and transferred into clean airtight container and closed tightly. The container was then labeled and kept for 12 weeks at room temperature and samples drawn and assayed periodically.

2.2.3.2 250mgFLUCLOXACILLIN and 125mg DRIED STARCH

7.5000g of pure flucloxacillin powder and 3.7500g of dried starch were used as described above.

2.2.3.3 250mgFLUCLOXACILLIN and 150mg DRIED STARCH

7.500g of pure flucloxacillin powder and 4.5300g of dried starch were used as described above.

2.2.3.4 250mg FLUCLOXACILLIN and 250mg DRIED STARCH

7.500g of pure flucloxacillin powder and 7.5000g of dried starch were used as described above.

2.2.4 PREPARATION OF DRY PLAIN CMC AND ITS MOISTURE CONTENT DETERMINATION

A known weight of plain carboxymethyl cellulose powder was put into a pre-weighed petri dish and weight taken and noted. The weight of the plain cmc and petri dish was taken again after the dish with the plain cmc had been heated in an oven to a constant weight. The difference in weight (weight loss) was expressed as a percentage to give the moisture content of the plain cmc. The plain cmc obtained thereafter was the dried plain cmc.

2.2.5 PREPARATIONS OF PURE FLUCLOXACILLIN AND DRIED PLAIN CARBOXYMETHYL CELLULOSE (CMC) MIXTURES

2.2.5.1 250mg FLUCLOXACILLIN and 75mg PLAIN CARBOXYMETHYL CELLULOSE

7.5000g of pure flucloxacillin powder and 2.2500g of plain cmc powder were used.

2.2.5.2 250mg FLUCLOXACILLINAND 125mg PLAIN CARBOXYMETHYL CELLULOSE

7.5000g of pure flucloxacillin powder and 3.7500g of plain cmc powder were used.

2.2.5.3 250mg FLUCLOXACILLINAND 150mg PLAIN CARBOXYMETHYL CELLULOSE

7.5000g of pure flucloxacillin powder and 4.5000g of plain cmc powder were used.

2.2.5.4 250mg FLUCLOXACILLINAND 250mg PLAIN CARBOXYMETHYL CELLULOSE

7.5000g of pure flucloxacillin powder and 7.5000g of plain cmc powder were used. The various combinations of flucloxacillin and plain carboxymethyl cellulose (cmc) were made as described for dried starch and flucloxacillin.

2.2.6 CONTROL – FLUCLOXACILLIN ONLY

7.5000g of the flucloxacillin sodium was weighed into an air-tight container and kept at room temperature. Samples were taken periodically on same days of analyses of the flucloxacillin-excipient samples.

2.2.7 MONITORING OF MOISTURE ABSORBED BY EXCIPIENTS (DRIED STARCH AND DRIED PLAIN CMC) DURING STABILITY STUDIES.

5.0000g of dried starch and plain cmc were put in a pre-weighed airtight container and kept at room temperature similar to samples prepared for stability studies. The new weight of the

container was taken periodically on same days of analyses of the flucloxacillin-excipient samples.

2.2.8 UNIFORMITY OF CONTENT FOR CAPSULES

20 capsules were randomly selected from a batch of each brand and collectively weighed. The capsules were then individually weighed, opened, emptied into an airtight container and the weight of the shells of each capsule taken. This was done for all twenty capsules and the weight of the shells for the twenty capsules collectively taken. The average weight of the capsules was calculated and the weight of the content of each capsule realized by the difference between the weight of the capsule and its shells. The deviation and hence percentage deviation were calculated for each capsule. **(BP, 2007)**

2.2.9 IDENTIFICATION TESTS

2.2.9.1 IDENTIFICATION TESTS FOR FLUCLOXACILLIN SODIUM

A small amount of the pure powder was placed in a clean test- tube with the aid of a spatula. The sample was moistened with a small amount of distilled water after which 2ml of sulphuric acid-formaldehyde reagent was added and heated in a water-bath for a minute. The colour of the solution turned yellowish. For the capsules, an amount of the contents was taken and mixed with water, filtered and the filtrate was treated as described above. **(BP, 2009)**

2.2.9.2 IDENTIFICATION TEST FOR AMOXICILLIN TRIHYDRATE

The pure amoxicillin powder was identified as for pure flucloxacillin.

2.2.9.3 IDENTIFICATION TEST FOR AMPICILLIN TRIHYDRATE

The pure ampicillin powder was identified as for pure flucloxacillin.

2.2.9.4 IDENTIFICATION TEST FOR BENZYLPENICILLIN SODIUM

A small amount of the pure powder was placed in a clean test- tube with the aid of a spatula. The sample was moistened with a small amount of distilled water after which 2ml of sulphuric acid-formaldehyde reagent was added and heated in a water-bath for a minute. The colour of the solution turned reddish-brown. **(BP, 2009)**

2.2.9.5 IDENTIFICATION TEST FOR PHENOXYMETHYL PENICILLIN SODIUM

A small amount of the pure powder was placed in a clean test- tube with the aid of a spatula. The sample was moistened with a small amount of distilled water after which 2ml of sulphuric acid-formaldehyde reagent was added and heated in a water-bath for a minute. The colour of the solution turned dark reddish-brown. **(BP, 2009)**

2.2.9.6 IDENTIFICATION TEST FOR MAIZE STARCH

To a small amount of the starch in a boiling tube, a small amount of the iodine solution was added and the tube swirled. The development of a blue- black colour confirmed the sample to be starch. It was also tested for in the capsules by taking a small amount of the capsule content and adding a small amount of distilled followed by a small amount of iodine solution. **(BP, 2009)**

2.2.9.7 IDENTIFICATION TEST FOR PLAIN CARBOXYMETHYL CELLULOSE (CMC)

A 0.5%w/v solution of sodium cmc was made by dissolving about 0.2500g in distilled water and making to 50ml in a volumetric flask with distilled water. A 5% solution of copper sulphate penta-hydrate was also made by dissolving 2.5000g and making to 50ml in a volumetric flask with distilled water. 5ml of both solutions were mixed in a boiling tube and the formation of a precipitate indicated the presence of sodium cmc. **(FAO, 1984)**

2.2.9.8 IDENTIFICATION TEST FOR MAGNESIUM STEARATE

About 0.1000g of the capsule content was taken and mixed with about 10ml of distilled water in a boiling tube and thoroughly stirred to effect homogeneity. Similar volume of a 1M

NaOH was then added to the suspension obtained. Formation of a white precipitate of magnesium hydroxide indicates the presence of magnesium stearate. **(Vogel, 1979)**

2.2.10 IDENTIFICATION OF PENICILLIN SAMPLES BY IR SPECTROSCOPY

200mg of spectroscopic grade KBr was weighed into a clean agate mortar, 2mg of sample was also weighed and added to the KBr and ground thoroughly. The mixture was then placed in the pressing chamber of the mould such that it was held between the polished surfaces of the lower and upper pressing dies and compressed manually. KBr disc was then removed from the compressing mould and fixed in position in the sample holder of the precalibrated IR Spectroscopy and runs were made.

2.2.11 DETERMINATION OF IODINE ABSORBING IMPURITIES

0.1250g of pure flucloxacillin sodium powder was dissolved in sufficient mixed phosphate buffer pH7.0 to produce 25ml. To 10ml, 10ml of mixed phosphate buffer pH 4.0 and 10ml of 0.01M iodine were added and titrated immediately with 0.01M sodium thiosulphate using starch mucilage, added towards the end of the titration, as indicator. The titration was repeated without the substance being examined. The difference between the titrations represented the amount of iodine- absorbing substances present. Each ml of 0.01M sodium thiosulphate VS is equivalent to 0.524mg of iodine- absorbing substances. **(BP, 1993).**

For the capsules the weight of powder equivalent to 0.1250g flucloxacillin was taken and extracted with the buffer (pH =7) and the filtrate made to 25ml same buffer and assayed as described above.

2.2.12 ASSAY OF FLUCLOXACILLIN SODIUM

0.1000g of flucloxacillin powder was dissolved in sufficient water to produce 100ml. 10ml was transferred to a stoppered flask; 5ml of 1M NaOH was added and allowed to stand for 20 minutes. 20ml of freshly prepared buffered solution containing 5.44 per cent w/v of glacial acetic acid, 5ml of 1M HCl and 25ml of 0.01M iodine were added. The flask was stoppered

and allowed to stand for 20minutes protected from light and then titrated with 0.02M sodium thiosulphate using starch solution added towards the end of the titration, as indicator.

To a further 10ml of the initial solution, 20ml of the buffer solution and 25ml of iodine VS were added and allowed to stand for 20minutes protected from light and then titrated with 0.02M sodium thiosulphate VS using starch solution added towards the end of the titration as indicator. The difference between the titrations represented the volume of 0.01M iodine VS equivalent to the total flucloxacillin present. **(BP, 1980)**

For the capsules and mixtures, the weight of powder equivalent to 0.1000g of flucloxacillin was used. The drug was extracted with distilled water and the filtrate was made to 100ml and assayed as described above.

2.3 HPLC METHOD DEVELOPMENT AND VALIDATION

2.3.1 CHROMATOGRAPHIC CONDITIONS

- Column : Phenomenex ® LICHROSORB 10 RP-1 , (250×4.60mm I.D., 5µm particle size)
- Flow rate: 1.00ml/ min
- Mobile phase: 40% Methanol : 60% 0.02M KH_2PO_4(pH = 3.70)
- Wavelength of detection : 225nm
- Temperature : ambient
- Injection volume: 20µl

2.3.2 PREPARATION OF THE MOBILE PHASE

The 0.02M $KH_2PO_4$was made by weighing 2.7200g of KH_2PO_4 and quantitatively transferring it into a 1000ml volumetric flask and making to volume with distilled water. The flask was shaken to ensure complete dissolution of the salt and a homogenous solution.

300ml of this solution was taken with a measuring cylinder and mixed thoroughly with 200ml of redistilled methanol to obtain 500ml of the mobile phase.

2.3.3 COLUMN CONDITIONING AND EQUILIBRATION

Before samples were injected, a water- methanol mixture of a ratio of 50:50 was pumped through the column for about an hour followed by the mobile phase for about 60 minutes to ensure that good results were obtained. This was done each time before work started.

2.3.4 METHOD DEVELOPMENT

The choice of mobile phase was guided by extensive reading of a myriad of published works on the assay of flucloxacillin. Several of those methods employed the aforementioned solution and varied combinations of acetonitrile. A few however used completely different systems of solvent. Several combinations of methanol and 0.02M KH_2PO_4were tried beginning with a 50: 50 combination until a ratio of methanol to 0.02M KH_2PO_4 of 40: 60 was settled on as it gave a neat chromatogram with all five(5)penicillins(amoxicillin trihydrate, ampicillin trihydrate, benzylpenicillin sodium, phenoxymethylpenicillin sodium and flucloxacillin sodium) well separated within a reasonable retention time.

2.3.5 METHOD VALIDATION

- Linearity

30.0000mg each of amoxicillin trihydrate and ampicillin trihydrate and 50.0000mg of benzylpenicillin sodium, phenoxymethylpenicillin sodium and flucloxacillin sodium were accurately weighed and transferred into a 50.00ml volumetric flask. Distilled water was added, shaken to ensure complete dissolution and homogeneity, then topped to the mark with more distilled water to give concentrations of 0.06%w/v for amoxicillin trihydrate and ampicillin trihydrate and 0.10%w/v for benzylpenicillin sodium, phenoxymethylpenicillin sodium and flucloxacillin sodium. From this concentration, serial dilutions were made to

obtain concentrations of 0.005, 0.0075, 0.01, 0.02, 0.04, 0.06, and 0.08% for benzylpenicillin sodium, phenoxymethylpenicillin sodium and flucloxacillin sodium. Similar serial dilutions were made to obtain concentrations of 0.003, 0.0045, 0.006, 0.012, 0.024, 0.036, and 0.048% for amoxicillin trihydrate and ampicillin trihydrate. Each of these concentrations was injected and their peak areas plotted against their respective concentrations to obtain their calibration curves. The coefficient of correlation, the limits of detection and quantification were all calculated from the calibration curves. **(http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf)**

- Selectivity

A solution containing a mixture of amoxicillin trihydrate, ampicillin trihydrate, benzylpenicillin sodium, phenoxymethylpenicillin sodium and flucloxacillin sodium as described above was injected three times and their retention times noted. **(http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf)**

- Accuracy

1.0000g each of benzylpenicillin sodium, phenoxymethylpenicillin sodium, flucloxacillin sodium, magnesium stearate, plain carboxymethyl cellulose, starch and lactose and 0.6000g each of amoxicillin trihydrate and ampicillin trihydrate were accurately weighed into a clean glass mortar and triturated to ensure a uniform and homogeneous mixture. An amount of mixture equivalent to 40.0000mg, 50.0000mg and 60.0000mg of flucloxacillin sodium were separately weighed accurately and transferred into a 50.00ml volumetric flask. Distilled water was added, shaken to ensure complete dissolution and homogeneity, then topped to the mark with more distilled water, shaken and then labelled appropriately. Each of these solutions was injected three times and their mean percentage contents calculated as their mean recoveries.**(**

(http://www.ich. org/fileadmin/ Public_Web_Site/ICH_Products/ Guidelines/Quality/ Q2_R1/ S tep4/Q2_R1 __Guideline .pdf)

- Precision

A solution made of 0.10%w/v of benzylpenicillin sodium, phenoxymethylpenicillin sodium, flucloxacillin sodium and 0.06% of amoxicillin trihydrate and ampicillin trihydrate were injected to obtain six determinations for the intra-day precision. For the inter-day precision samples of the same concentration were injected on three consecutive days to obtain six different determinations for each of the three days. The percentage purities were calculate and hence their relative standard deviations.

(http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_ R1/S tep4/Q2_R1__Guideline.pdf)

- Robustness

Using solution described for precision above, injections were made at varying flow rates while holding other parameters constant. The detection wavelength was also varied while keeping other parameters constant.

(http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_ R1/S tep4/Q2_R1__Guideline.pdf)

2.4 DETECTION OF BREAKDOWN PRODUCTS OF FLUCLOXACILLIN SODIUM

Flucloxacillin sodium solution of concentration 0.10%w/v was prepared. Sample solution was injected and remaining kept for 4 weeks. Samples were drawn from it and injected weekly till the fourth week.

For penicilloic acid detection, 5.00ml of 1M NaOH was added to 10.00ml of 0.10%w/v flucloxacillin sodium, allowed to stand for 20minutes followed by addition of 5.00ml of 1M

HCl to neutralize the NaOH initially added. Samples from this solution were injected and chromatogram noted.

CHAPTER THREE

3.0 RESULTS FOR IDENTIFICATION TESTS

3.0.1 FOR FLUCLOXACILLIN IN SAMPLES USED

Table 3: Results for flucloxacillin identification in capsule brands

SAMPLE	OBSERVATION	INFERENCE
PURE FLUCLOXACILLIN	Intense yellow coloration observed	Sample passed
A	Intense yellow coloration observed	Sample passed
B	Intense yellow coloration observed	Sample passed
C	Intense yellow coloration observed	Sample passed
D	Intense yellow coloration observed	Sample passed

* All capsules of various brands employed in experiment were well sealed in their blisters, clean and intact and had no physical defects upon a thorough examination.

3.0.2 TEST FOR OTHER SAMPLES USED

Table 4: Results for tests for other sample used

SAMPLE	OBSERVATION	INFERENCE
Amoxicillin trihydrate	Intense yellow colouration observed	Sample passed
Ampicillin trihydrate	Intense yellow coloration observed	Sample passed
Benzylpenicillin sodium	A reddish-brown colouration observed	Sample passed
Phenoxymethylpenicillin sodium	A dark reddish-brown colouration observed	Sample passed
Plain carboxymethyl celllulose	A precipitate was formed	Sample passed
Magnesium stearate	A white precipitate was formed	Sample passed
Starch	Blue black colouration observed	Sample passed

3.0.3 TEST FOR SOME EXCIPIENTS IN CAPSULE BRANDS

Table 5: Results for the test of some excipients in capsule brands

SAMPLE	APPEARANCE OF CONTENT	TEST FOR STARCH	TEST FOR PLAIN CMC	TEST FOR MAGNESIUM STEARATE
A	Powdery white content	No blue black colouration	Formation of a precipitate	White precipitated was formed
B	Powdery white content	No blue black colouration	Formation of a precipitate	White precipitated was formed
C	Powdery white content	No blue black colouration	Formation of a precipitate	White precipitated was formed
D	Yellowish cake with a whitish tinge	Blue black colouration	Formation of a precipitate	White precipitated was formed

3.1 UNIFORMITY OF CONTENT FOR CAPSULES

Table 6: Results for uniformity of content for capsule brands.

SAMPE	RESULT
A	PASSED
B	PASSED
C	FAILED
D	PASSED

3.2 DETERMINATION OF AMOUNT OF MOISTURE IN EXCIPIENTS USED FOR STABILITY STUDIES

Table 7: Results for moisture content determination in excipients before start of stability studies

SAMPLE	WT. OF EMPTY PETRI-DISH(g)	WEIGHT OF DISH + SAMPLE(g)	WEIGHT OF SAMPLE BEFORE HEATING TO CONSTANT WEIGHT(g)	WEIGHT OF SAMPLE AFTER HEATING TO CONSTANT WEIGHT(g)	MOISTURE CONTENT(%w/w)
STARCH	53.3700	78.3600	24.9900	75.2700	12.36
PLAIN CMC	50.9000	80.9200	30.0200	77.5200	11.33

3.3 MONITORING OF MOISTURE ABSORBED BY EXCIPIENTS (DRIED STARCH AND DRIED PLAIN CMC) DURING STABILITY STUDIES

3.3.1 RESULTS FOR MOISTURE MONITORING IN DRIED STARCH

Table 8: Results for moisture monitoring in dried starch

TIME/WEEKS	WEIGHT OF EMPTY CONTAINER (g)	WEIGHT OF CONTAINER + CONTENT (g)	WEIGHT OF CONTENT (g)	MOISTURE CONTENT (%w/w)
0	19.9400	24.9400	5.0000	0.00
1	19.9400	24.9500	5.0100	0.20
2	19.9400	25.0200	5.0800	1.60
3	19.9400	25.0500	5.1100	2.20
4	19.9400	25.0800	5.1400	2.80
6	19.9400	25.200	5.2600	5.20
10	19.9400	25.3000	5.3600	7.20

3.3.2 RESULTS FOR MOISTURE MONITORING IN DRIED PLAIN CMC

Table 9: Results for moisture monitoring in dried plain

TIME/WEEKS	WEIGHT OF EMPTY CONTAINER (g)	WEIGHT OF CONTAINER + CONTENT (g)	WEIGHT OF CONTENT (g)	MOISTURE CONTENT (%w/w)
0	19.9300	24.9300	5.0000	0.00
1	19.9300	24.9400	5.0100	0.10
2	19.9300	25.9900	5.0600	1.20
3	19.9300	25.0300	5.1000	2.00
4	19.900	25.0600	5.1300	2.60
6	19.9300	25.1400	5.2100	4.20
10	19.9300	25.2500	5.3200	6.40

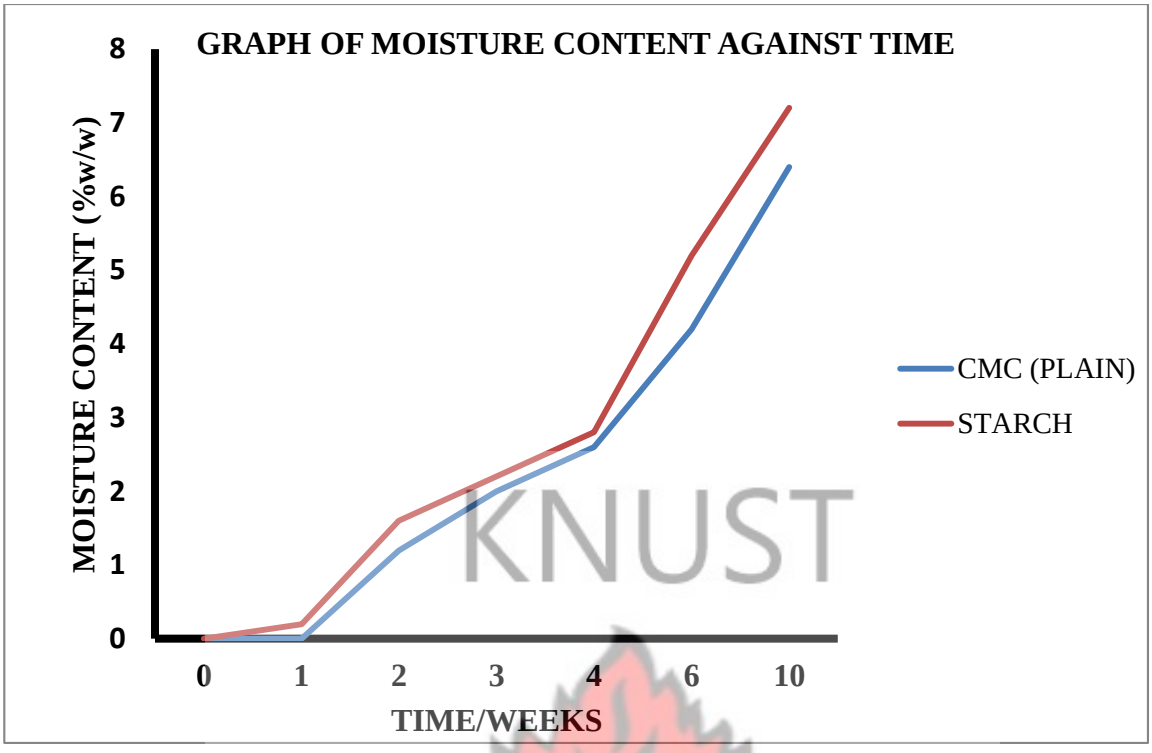

Figure 3: A graph showing percentage moisture absorbed by starch and plain cmc after 10weeks

3.4 RESULTS FOR VARIOUS SAMPLES KEPT AND ASSAYED FOR 10 WEEKS

3.4.1 SAMPLES AND CONTROL

Table 10: Percentage content and percentage breakdown of flucloxacillin in capsule brands over 10 weeks

SAMPLES	A	B	C	CONTROL
WEEK 0				
%CONTENT(%w/w)	99.40	104.45	97.49	96.60
%BREAKDOWN(%w/w)	-	-	-	-
WEEK 1				
%CONTENT(%w/w)	92.50	101.65	96.70	95.95
%BREAKDOWN(%w/w)	6.94	2.68	0.81	0.67
WEEK 2				
%CONTENT(%w/w)	91.12	98.70	95.45	92.56
%BREAKDOWN(%w/w)	8.33	5.51	2.09	4.18
WEEK 3				
%CONTENT(%w/w)	81.45	96.16	86.26	85.40
%BREAKDOWN(%w/w)	18.05	7.94	11.52	11.59
WEEK 4				
%CONTENT(%w/w)	80.40	94.70	84.80	83.20
%BREAKDOWN(%w/w)	19.11	9.33	13.03	13.87
WEEK 6				
%CONTENT(%w/w)	77.10	91.25	80.15	80.15
%BREAKDOWN(%w/w)	22.43	12.64	17.79	17.03
WEEK 10				
%CONTENT(%w/w)	68.00	82.49	73.68	72.30
%BREAKDOWN(%w/w)	31.59	12.64	24.42	25.16

3.4.2 DRIED STARCH AND 250MG FLUCLOXACILLIN COMBINATIONS

Table 11: Percentage content and percentage breakdown of flucloxacillin in various proportions with dried Starch

SAMPLES	75mg DRIED STARCH	125mg DRIED STARCH	150mg DRIED STARCH	250mg DREIED STARCH
SWEEK 0				
%CONTENT(%w/w)	98.27	96.60	97.90	94.92
%BREAKDOWN(%w/w)	-	-	-	-
WEEK 1				
%CONTENT(%w/w)	94.70	96.50	97.10	94.10
%BREAKDOWN(%w/w)	3.63	0.10	0.82	0.86
WEEK 2				
%CONTENT(%w/w)	92.33	95.55	95.55	93.70
%BREAKDOWN(%w/w)	6.04	1.09	2.40	1.29
WEEK 3				
%CONTENT(%w/w)	82.24	88.06	84.63	87.70
%BREAKDOWN(%w/w)	16.31	8.84	13.55	7.61
WEEK 4				
%CONTENT(%w/w)	81.50	86.80	82.70	85.90
%BREAKDOWN(%w/w)	17.07	10.14	15.53	9.50
WEEK 6				
%CONTENT(%w/w)	78.80	81.50	78.30	81.60
%BREAKDOWN(%w/w)	19.81	15.63	20.02	14.03
WEEK 10				
%CONTENT(%w/w)	70.29	72.77	71.15	71.00
%BREAKDOWN(%w/w)	28.47	24.67	27.32	25.20

3.4.3 DRIED PLAIN CMC AND 250MG FLUCLOXACILLIN COMBINATIONS

Table 12: Percentage content and percentage breakdown of flucloxacillin in various proportions with dried Plain cmc

SAMPLES	75mg DRIED PLAIN CMC	125mg DRIED PLAIN CMC	150mg DRIED PLAIN CMC	250mg DRIED PLAIN CMC
WEEK 0				
%CONTENT(%w/w)	97.80	97.71	98.27	98.17
%BREAKDOWN(%w/w)	-	-	-	-
WEEK 1				
%CONTENT(%w/w)	97.77	97.20	98.23	95.30
%BREAKDOWN(%w/w)	0.03	0.52	0.04	2.98
WEEK 2				
%CONTENT(%w/w)	90.98	93.99	92.61	92.61
%BREAKDOWN(%w/w)	6.97	3.81	5.76	5.66
WEEK 3				
%CONTENT(%w/w)	86.47	88.47	89.75	89.75
%BREAKDOWN(%w/w)	11.58	9.46	8.67	8.58
WEEK 4				
%CONTENT(%w/w)	86.85	87.10	87.90	87.90
%BREAKDOWN(%w/w)	11.20	10.86	10.55	10.46
WEEK 6				
%CONTENT(%w/w)	83.18	83.00	84.30	84.05
%BREAKDOWN(%w/w)	14.95	15.05	14.22	14.38
WEEK 10				
%CONTENT(%w/w)	71.00	75.60	75.60	77.98
%BREAKDOWN(%w/w)	27.40	22.63	23.07	20.57

3.5 IODINE ABSORBONG IMPURITIES IN PURE FLUCLOXACILLIN AND CAPSULE SAMPLES

Table 13: Contents of I_2 absorbing substances in pure flucloxacillin and capsule samples.

SAMPLE	IODINE ABSORBING IMPURITIES(%w/w)
A	7.72
B	5.08
C	8.35
D	25.57
CONTROL(PURE FLUCLOXACILLIN)	7.60

3.6 DETERMINATION OF REACTION ORDER

- Zero order

The zero order equation is given as:

$c = c_o - kt$ and comparing with $y = mx + c$

c= concentration after a certain period of time, t, c_o = initial concentration and k = rate constant

Thus a plot of c against time, t is a straight line with intercept, c_o and gradient,-k

- Pseudo-first order

The derived equation is given as:

lna – ln(a-x) = kt

ln(a/a-x) = kt and comparing with y= mx +c

y= ln (a/a-x), x=t, m= k

a = initial concentration, (a-x) = concentration after a certain period of time, t, and k = rate constant

Hence a plot of ln(a/a-x) against time (t) is a straight line with gradient equals to k.

3.6.1 VALUES OBTAINED FOR ZERO AND PSEUDO-FIRST ORDER PLOTS FOR SAMPLESUSED

3.6.1.1 DRIED STARCH AND 250MG FLUCLOXACILLIN COMBINATIONS

Table 14: Values plotted for zero and pseudo-first orders for dried starch/flucloxacillin combinations

TIME/ WEEKS	75mg DRIED STARCH		125mg DRIED STARCH		150mg DRIED STARCH		250mg DRIED STARCH	
	Conc.	ln(a/a-x)	Conc.	ln (a/a-x)	Conc.	ln (a/a-x)	Conc.	ln (a/a-x)
0	98.27	0	96.6	0	97.9	0	94.92	0
1	94.7	0.0370047	96.5	0.0010357	97.1	0.0082051	94.1	0.0086763
2	92.33	0.0623496	95.7	0.0093604	95.55	0.0242968	93.7	0.0129362
3	82.24	0.1780769	88.06	0.0925603	84.63	0.1456577	87.7	0.0791125
4	81.5	0.1871157	86.8	0.1069721	82.7	0.1687269	85.9	0.0998506
6	78.8	0.2208057	81.5	0.1699757	78.3	0.2233989	81.6	0.1512051
10	70.29	0.33508925	72.77	0.28327495	71.15	0.319156	71.42	0.284456

3.6.1.2 DRIED PLAIN CMC AND 250MG FLUCLOXACILLIN COMBINATIONS

Table 15: Values plotted for zero and pseudo-first orders for dried starch/flucloxacillin combinations

TIME/ WEEKS	75mg DRIED PLAIN CMC		125mg DRIED PLAIN CMC		150mg DRIED PLAIN CMC		250mg DRIED PLAIN CMC	
	Conc.	ln (a/a-x)	Conc.	ln (a/a-x)	Conc.	ln (a/a-x)	Conc.	ln (a/a-x)
0	97.80	0	97.71	0	98.27	0	98.27	0
1	97.77	0.0003067	97.20	0.0052331	98.23	0.0004071	95.30	0.0306889
2	90.98	0.0722848	93.99	0.0388155	92.61	0.0593216	93.41	0.0507203
3	86.47	0.1231270	88.47	0.0993403	89.75	0.0906907	89.04	0.0986330
4	86.85	0.1187420	87.10	0.1149470 2	87.90	0.1115189	87.20	0.1195144
6	83.18	0.1619176 43	83.00	0.1631633	84.30	0.1533369	84.05	0.1563069
10	71.00	0.3202447	74.30	0.2779388 15	75.60	0.2622625 09	77.98	0.2312664 09

3.6.1.3 CONTROL AND CAPSULE BRANDS

Table 16: Values plotted for zero and pseudo-orders for control and capsule brands

TIME/ WEEKS	A		B		C		CONTROL	
	Con.	ln (a/a-x)	Conc.	ln (a/a-x)	Con.	ln (a/a-x)	Con.	ln (a/a-x)
0	99.40	0	104.45	0	97.49	0	96.60	0
1	92.50	0.071943469	101.65	0.027172948	96.70	0.008136406	95.94	0.006855745
2	91.12	0.0869747	98.70	0.0566235	95.45	0.0211472	92.56	0.0427216
3	81.45	0.1991627	96.16	0.0826950	86.26	0.1223838	85.4	0.1232326
4	80.40	0.2121379	94.70	0.0979944	84.80	0.1394542	82.10	0.1626407
6	77.10	0.2540488	91.25	0.1351054	80.15	0.1958499	80.15	0.1866788
10	68.00	0.3796444	82.49	0.2360314	73.68	0.2800184	72.30	0.2897546

3.6.2 SAMPLE GRAPHS FOR REACTION ORDER PLOTS

3.6.2.1 zero order graph of 150mg plain cmc and 250mg flucloxacillin sodium

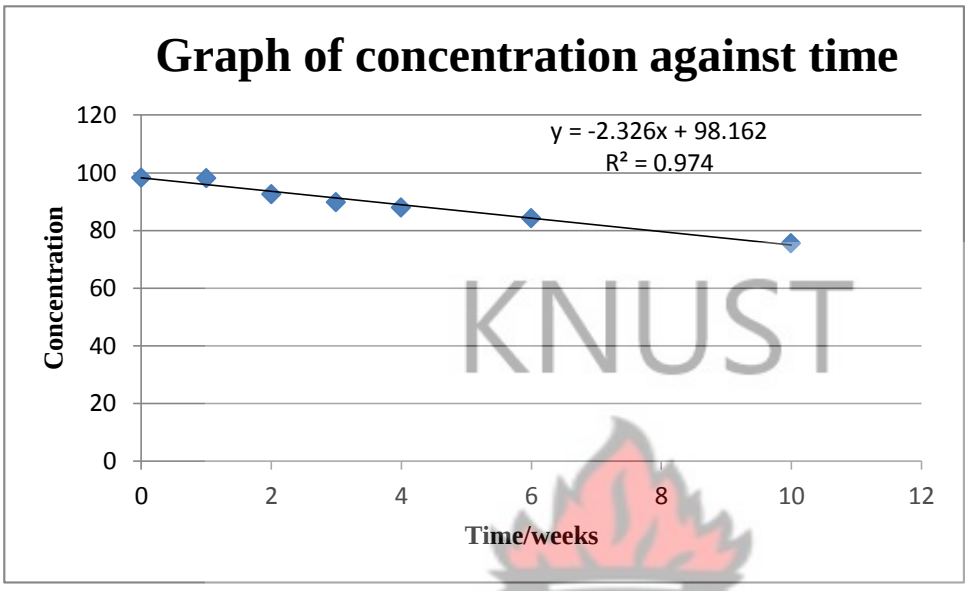

Figure 4: Zero order graph of 150mg plain cmc and 250mg flucloxacillin sodium

3.6.2.2 pseudo-first order graph of 150mg plain cmc and 250mg flucloxacillin sodium

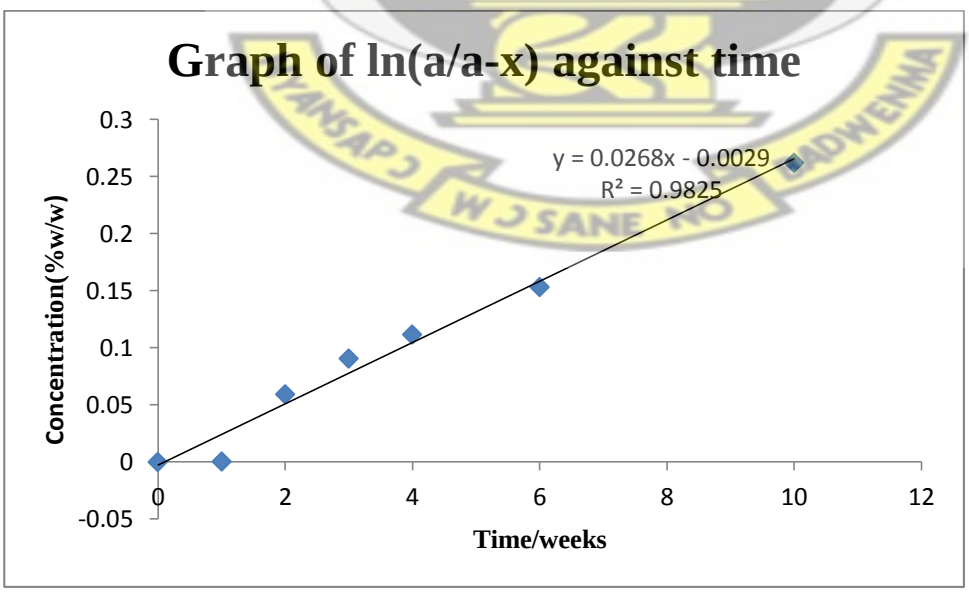

Figure 5: Pseudo-first order graph of 150mg plain cmc and 250mg flucloxacillin sodium

3.6.2.3 Zero order graph of sample B

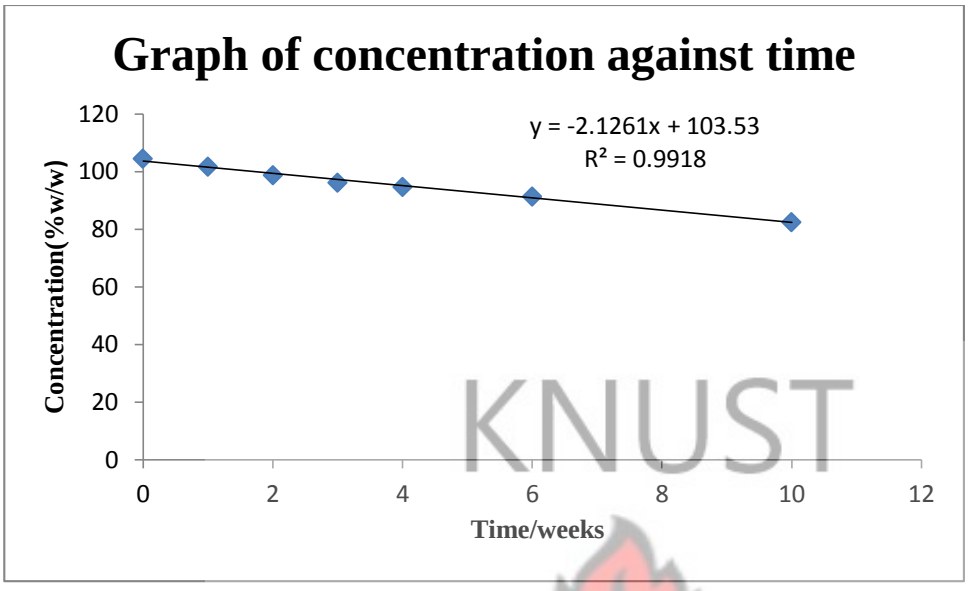

Figure 6: Zero order graph of sample B

3.6.2.4 Pseudo-first order graph of sample B

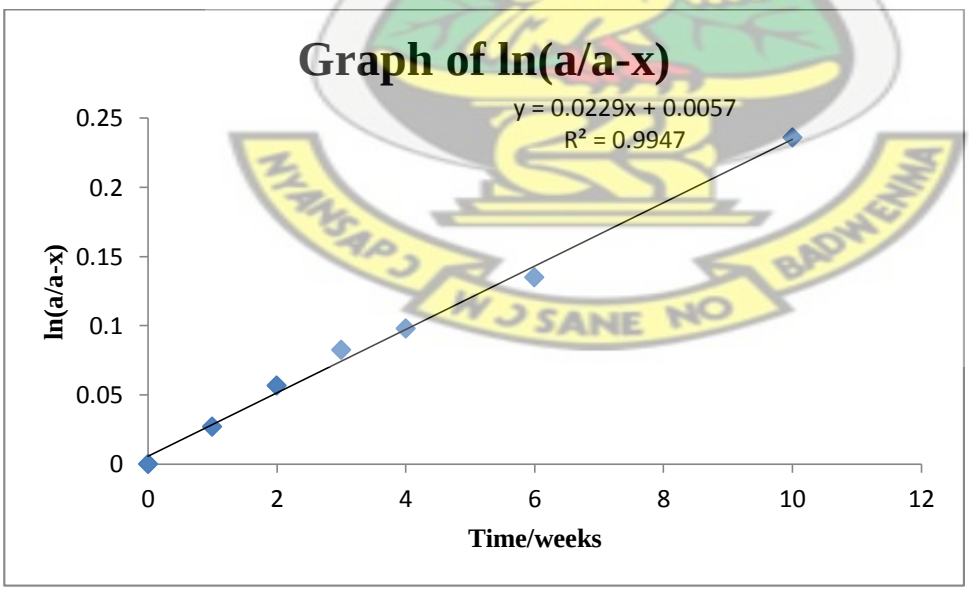

Figure 7: Pseudo-first order graph of sample B

3.6.3 COMPARISONS OF RATE CONSTANTS (K) AND COEFFICIENT OFCORRELATION (R^2) VALUES FOR PSEUDO-FIRST AND ZERO ORDERS FOR THESAMPLES USED.

3.6.3.1 DRIED STARCH AND 250MG FLUCLOXACILLIN COMBINATIONS

Table 17: Comparison of rate constants of the reaction orders and correlation coefficients for the various combinations of dried starch with flucloxacillin

AMOUNT OF DRIED STARCH	RATE CONSTANT (K)		COEFFEICIENT OF CORRELATION (R^2)	
	ZERO ORDER	PSEUDO-1ST ORDER	ZERO ORDER	PSEUDO-1ST ORDER
75mg	2.7920	0.0336	0.9063	0.9383
125mg	2.5809	0.0305	0.9622	0.9699
150mg	2.9038	0.0345	0.9083	0.9288
250mg	2.4667	0.0297	0.9788	0.9791

3.6.3.2 DRIED PLAIN CMC AND 250MG FLUCLOXACILLIN COMBINATIONS

Table 18: Comparison of rate constants of the reaction orders and correlation coefficients for the various combinations of dried starch with flucloxacillin

AMOUNT OF DRIED PLAIN CMC	RATE CONSTANT (K)		COEFFEICIENT OF CORRELATION (R^2)	
	ZERO ORDER	PSEUDO-1ST ORDER	ZERO ORDER	PSEUDO-1ST ORDER
75mg	2.6666	0.0317	0.9573	0.9640
125mg	2.4679	0.0291	0.9700	0.9801
150mg	2.3260	0.0268	0.9740	0.9825
250mg	2.0196	0.0231	0.9647	0.9758

3.6.3.3 CONTROL AND CAPSULE BRANDS

Table 19: Comparison of rate constants of the reaction orders and correlation coefficients for control and capsule brands

SAMPLE	RATE CONSTANT (K)		COEFFEICIENT OF CORRELATION (R^2)	
	ZERO ORDER	PSEUDO-1ST ORDER	ZERO ORDER	PSEUDO-1ST ORDER
A	2.9963	0.0366	0.9134	0.9392
B	2.1261	0.0229	0.9918	0.9947
C	2.5819	0.0303	0.9240	0.9404
CONTROL	2.5605	0.0305	0.9282	0.9450

3.7 HPLC METHOD VALIDATION

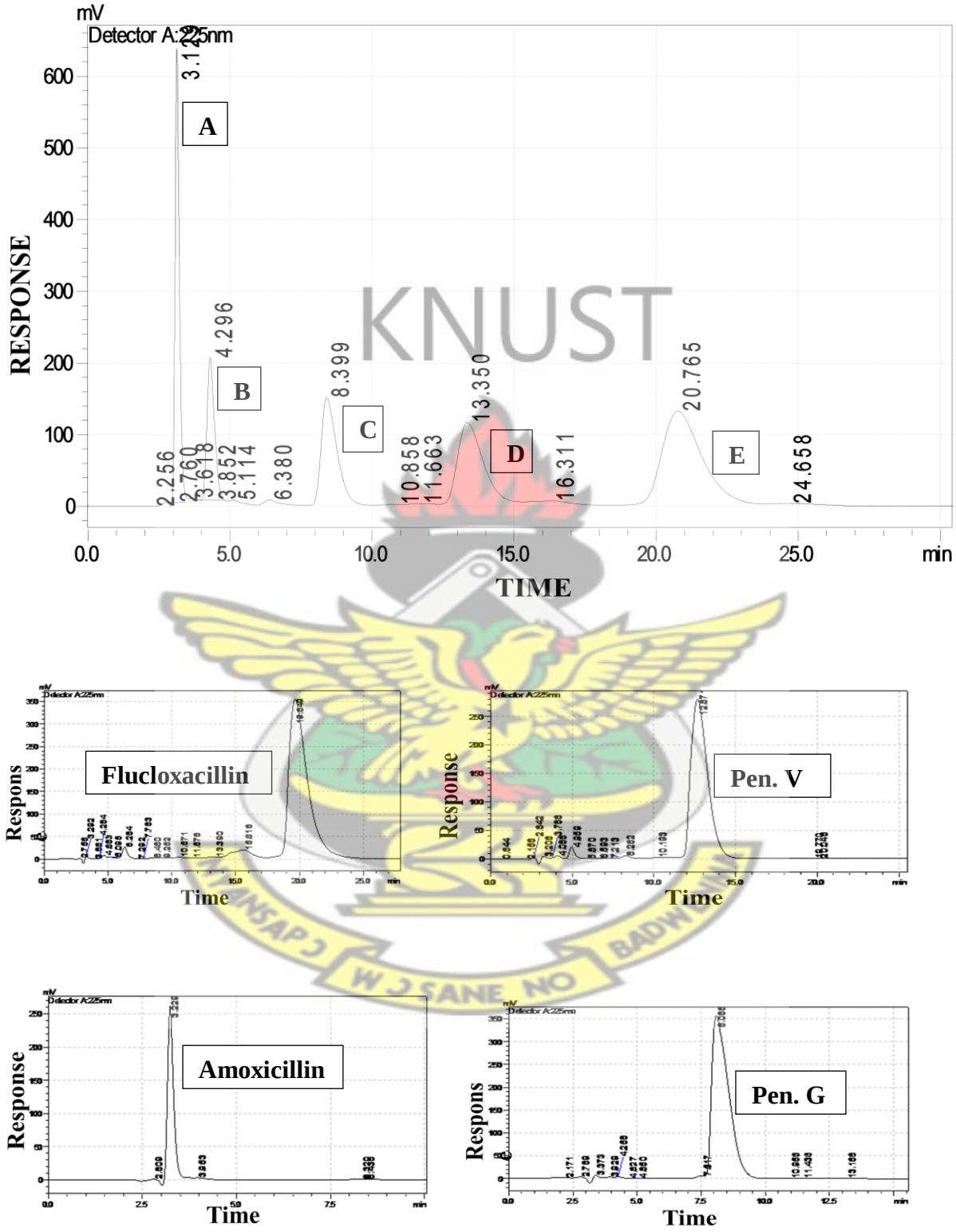

Figure 8: Chromatogram of individual penicillins and a mixture of penicillins: amoxicillin trihydrate (A), ampicillin trihydrate (B), benzylpenicillin sodium (C) and phenoxymethyl penicillin sodium (D) and flucloxacillin sodium (E)

3.7.1 LINEARITY

3.7.1.1 CALIBRATION FOR FLUCLOXACILLIN SODIUM

Table 20: Concentrations of flucloxacillin and their corresponding peak and residual peak are as

CONCETRATION (%w/w)	PEAK AREA	RESIDUAL PEAK AREA
0.005	2982076	473561.200
0.0075	3138970	-162949.500
0.01	3719625	-375699.300
0.02	7418808	149864.800
0.04	13553304	-62877.140
0.06	19750657	-212763.000
0.08	26520227	209571.000
0.1	32639187	-18708.040

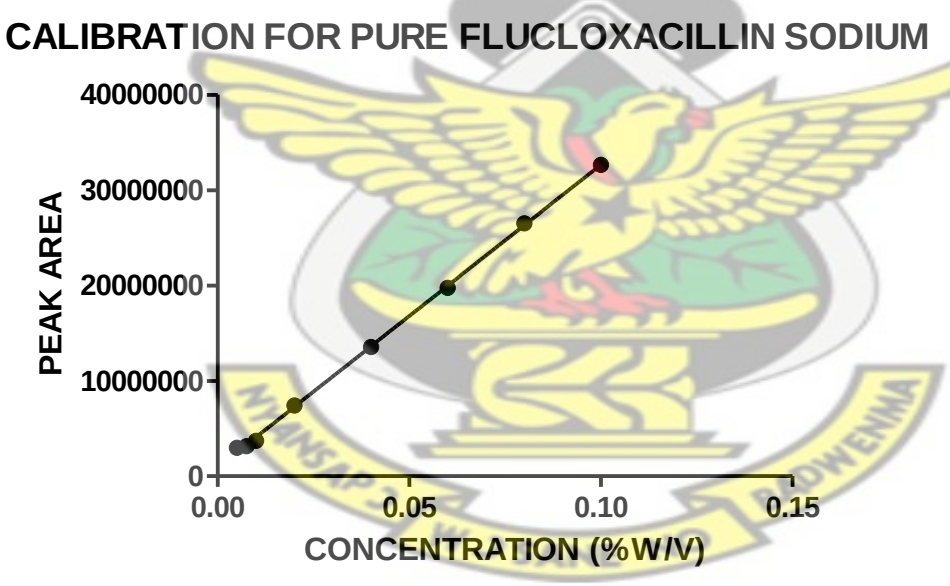

Figure 9: Calibration curve for pure flucloxacillin sodium

Table 21: Data from calibration curve for flucloxacillin sodium

Best-fit values	
Slope	317362000 ± 3036600
Y-intercept when X=0.0	921705 ± 159895
X-intercept when Y=0.0	-0.00290427
1/slope	0.000000003151
95% Confidence Intervals	
Slope	309931000 to 324792000
Y-intercept when X=0.0	530442 to 1312970
X-intercept when Y=0.0	-0.00421421 to -0.00164174
Goodness of Fit	
r^2	0.999451
Sy.x	290951
Is slope significantly non-zero?	
F	10922.8
DFn, DFd	1.00000, 6.00000
P value	< 0.0001
Deviation from zero?	Significant
Data	
Number of X values	8
Maximum number of Y replicates	1
Total number of values	8
Number of missing values	0
Runs test	
Points above line	3
Points below line	5
Number of runs	6
P value (runs test)	0.9286
Deviation from linearity	**Not Significant**

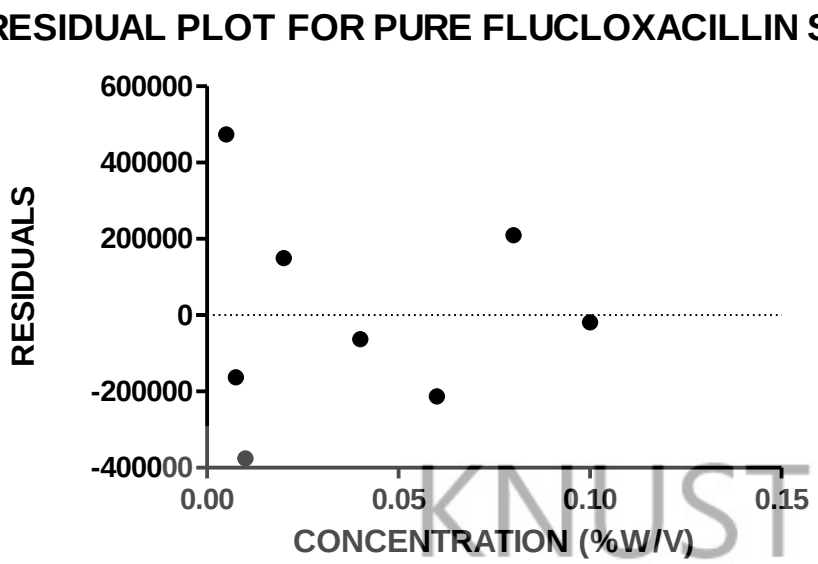

Figure 10: Residual plot for flucloxacillin sodium

3.7.1.2 LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTIFICATION (LOQ)

LOD = 3.3σ / S

LOQ = 10σ / S where; σ = standard deviation of the response

S = slope of the calibration curve

Table 22: Limit of detection (LOD) and Limit of Quantitation (LOQ) for the various penicillins

Sample	Slope Of Calibration Curve	Standard Deviation Of Response	Limit Of Detection (LOD)/%w/v	Limit Of Quantitation (LOQ)/%w/v
Amoxicillin trihydrate	2.88E+08	224959.9	0.002578	0.007811
Ampicillin trihydrate	1.79E+08	153502	0.002830	0.008576
Benzylpenicillin sodium	9.43E+7	125651	0.004397	0.013325
Phenoxymethyl penicillin	1.85E+08	290595	0.005184	0.015708
Flucloxacillin sodium	3.17E+08	290951	0.003029	0.009178

3.7.2 PRECISION

3.7.2.1 INTRA-DAY PRECISION/REPEATABILITY/WITHIN-RUN PRECISION FOR FLUCLOXACILLIN SODIUM

Table 23: Results for intra-day precision for flucloxacillin sodium

DETERMINATION	% PURITY
1	99.38
2	99.53
3	100.25
4	99.73
5	99.60
6	96.77
7	95.26
8	100.50
9	98.88

Table 24: Statistical analysis of results for intra-day precision for flucloxacillin sodium

PARAMETER	RESULTS
Number of values	9
Mean	98.8775
Std. Deviation	1.73066
Std. Error	0.576886
Lower 95% CI of mean	97.5472
Upper 95% CI of mean	100.208
Coefficient of variation	**1.75%**

Table 25: Relative standard deviations for repeatability for the various penicillins

SAMPLE	MEAN %PURITY(%w/w)	RELATIVE STANDARD DEVIATION (%)
Amoxicillin trihydrate	96.10	1.75
Ampicillin trihydrate	101.21	1.64
Benzylpenicillin sodium	98.75	1.62
Phenoxymethyl penicillin	100.16	4.03
Flucloxacillin sodium	98.88	1.75

3.7.2.2 INTER-DAY PRECISION/INTERMEDIATE PRECISION FOR FLUCLOXACILLIN SODIUM

Table 26: Results for inter-day precision for flucloxacillin sodium

DETERMINATION	% PURITY
1	99.38
2	99.53
3	100.23
4	99.73
5	99.60
6	96.77
7	95.26
8	100.50
9	98.88
10	98.18
11	99.33
12	99.45
13	101.06
14	104.90
15	96.30
16	99.83
17	95.52
18	99.53

Table 27: Statistical analysis of results for inter-day precision for flucloxacillin sodium

PARAMETER	RESULTS
Mean	99.1109
Std. Deviation	2.22642
Std. Error	0.524773
Lower 95% CI of mean	98.0037
Upper 95% CI of mean	100.218
Coefficient of variation	**2.25%**

Table 28: Relative standard deviations for inter-day precision for the various penicillins

SAMPLE	MEAN %PURITY (%w/w)	RELATIVE STANDARD DEVIATION
Amoxicillin trihydrate	98.17	1.22
Ampicillin trihydrate	99.23	2.23
Benzylpenicillin sodium	99.13	1.90
Phenoxymethyl penicillin	101.22	3.97
Flucloxacillin sodium	99.11	2.25

3.7.3 ACCURACY FOR FLUCLOXACILLIN SODIUM

Table 29: Results for accuracy for flucloxacillin sodium

DETERMINATION	PERCENTAGE RECOVERY		
	80%	100%	120%
1	99.75	99.15	99.48
2	101.81	101.89	100.59
3	102.45	100.49	101.13

One-way analysis of variance				
P value	0.6232			
P value summary	ns			
Are means signif. different? (P < 0.05)	**No**			
Number of groups	3			
F	0.5122			
R squared	0.1458			
ANOVA Table	SS	df	MS	
Treatment (between columns)	1.563	2	0.7815	
Residual (within columns)	9.155	6	1.526	
Total	10.72	8		

Newman-Keuls Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.01?	Summary
Column C vs Column A	**-0.9340**	**1.310**	**No**	**Ns**
Column C vs Column B	**-0.1104**	**---**	**No**	**Ns**
Column B vs Column A	**-0.8236**	**---**	**No**	**Ns**

Table 30: Mean percentage recovery for the various penicillins at their various concentration levels

SAMPLE	MEAN PERCENTAGE RECOVERY		
	80% conc. level	100%conc. Level	120%conc. Level
Amoxicillin trihydrate	99.88	100.57	99.10
Ampicillin trihydrate	100.66	100.01	99.48
Benzylpenicillin sodium	100.30	100.66	100.79
Phenoxymethyl penicillin	100.48	100.48	100.41
Flucloxacillin sodium	101.33	100.51	100.40

3.7.4 ROBUSTNESS

3.7.4.1 FLOW RATE VARIATION FOR FLUCLOXACILLIN SODIUM

Table 31: Percentage purity for 0.10%w/v flucloxacillin sodium upon flow rate variation

DETERMINATION	PERCENTAGE PURITY (%w/w)		
	0.80ml/min	STANDARD(1.00ml/min)	1.20ml/min
1	101.09	100.25	100.73
2	100.10	99.83	99.14
3	101.71	100.50	100.11

One-way analysis of variance				
P value	0.2606			
P value summary	ns			
Are means signif. different? (P < 0.05)	No			
Number of groups	3			
F	1.696			
R squared	0.3612			
ANOVA Table	SS	df	MS	
Treatment (between columns)	1.598	2	0.799	
Residual (within columns)	2.826	6	0.471	
Total	4.424	8		
Newman-Keuls Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary
1.20ml/min vs 0.80ml/min	-0.9773	2.467	No	ns
1.20ml/min vs STANDARD (I.00ml/min)	-0.2011	---	No	ns
STANDARD (I.00ml/min) vs 0.80ml/min	-0.7761	---	No	ns
1.20ml/min vs 0.80ml/min	-0.3662	1.158	No	ns
1.20ml/min vs STANDARD (I.00ml/min)	-0.1164	---	No	ns
STANDARD (I.00ml/min) vs 0.80ml/min	-0.2498	---	No	ns

3.7.4.2 WAVELENGTH VARIATION FOR FLUCLOXACILLIN SODIUM

Table 32: Percentage purity of 0.10%w/v flucloxacillin sodium upon wavelength variation

DETERMINATION	PERCENTAGE PURITY (%w/w)		
	223nm	STANDARD(225nm)	227nm
1	99.75	100.25	99.15
2	101.81	99.83	101.89
3	102.45	100.50	100.49

One-way analysis of variance				
P value	0.4985			
P value summary	ns			
Are means signif. different? (P < 0.05)	**No**			
Number of groups	3			
F	0.7836			
R squared	0.2071			
ANOVA Table	SS	df	MS	
Treatment (between columns)	2.08	2	1.04	
Residual (within columns)	7.963	6	1.327	
Total	10.04	8		
Newman-Keuls Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary
STANDARD (225nm) vs 223nm	**-1.141**	**1.715**	**No**	**ns**
STANDARD (225nm) vs 227nm	**-0.3171**	**---**	**No**	**ns**
227nm vs 223nm	**-0.8236**	**---**	**No**	**ns**

3.8.5 SELECTIVITY

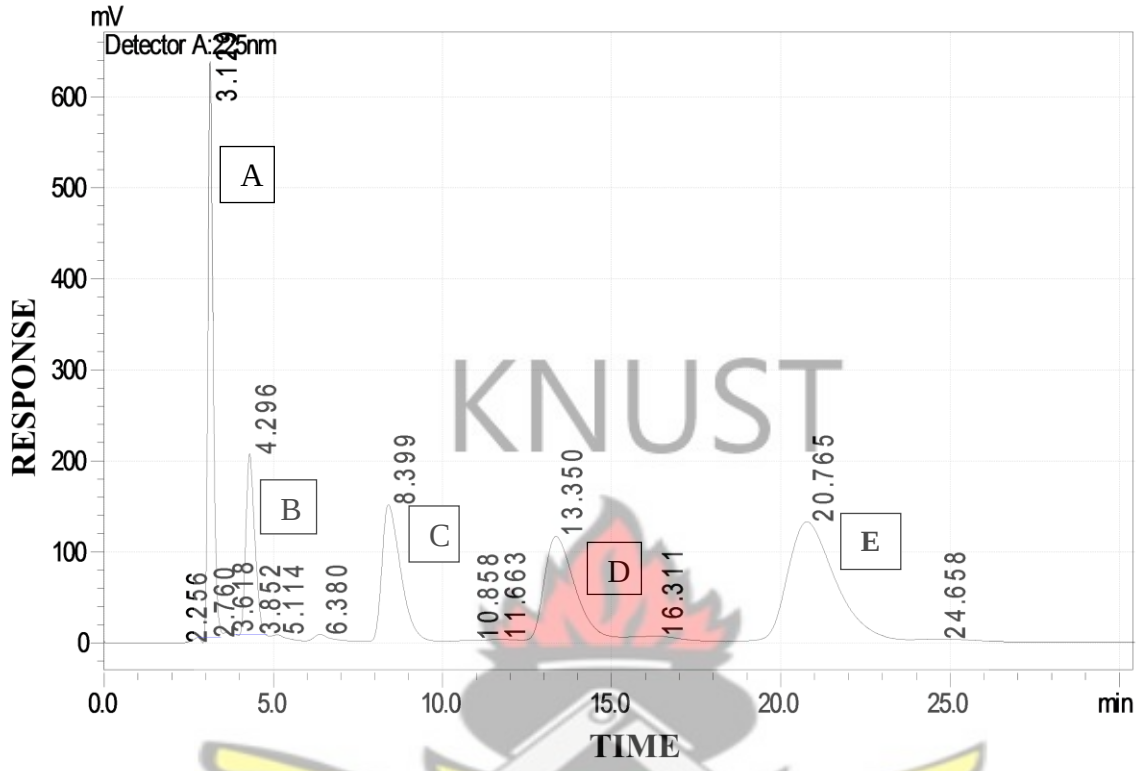

Figure 11: Chromatogram of a mixture of amoxicillin trihydrate (A), ampicillin trihydrate (B), benzylpenicillin sodium (C) and phenoxymethylpenicillin sodium (D) and flucloxacillin sodium (E).

Table 33: Retention times for the various penicillins used for specificity

SAMPLE	RETENTION TIME (MINUTES)	TAILING FACTOR	CAPACITY FACTOR
Amoxicillin trihydrate (A)	3.14 ± 0.01	1.05	1.05
Ampicillin trihydrate (B)	4.36 ± 0.02	1.07	1.07
Benzylpenicillin sodium (C)	8.50 ± 0.04	1.13	1.13
Phenoxymethyl penicillin (D)	13.57 ± 0.06	1.33	1.33
Flucloxacillin sodium (E)	21.18 ± 0.08	1.25	1.25

3.9 ……DETECTION OF BREAKDOWN PRODUCTS OF FLUCLOXACILLIN SODIUM

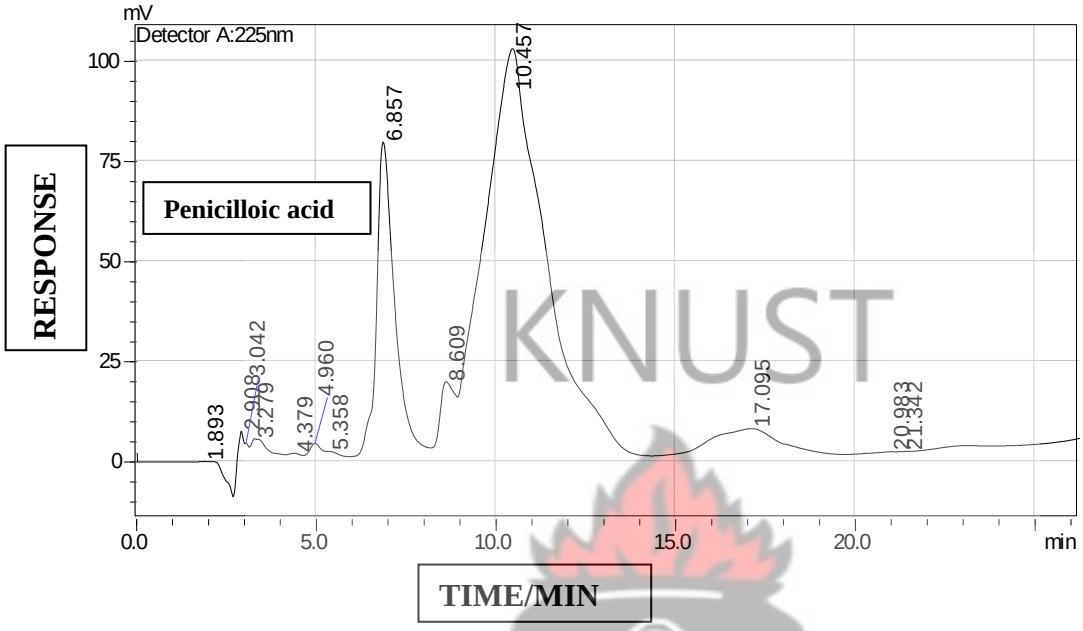

Figure 12: Chromatogram of a mixture of flucloxacillin sodium and 0.1M NaOH, allowed to stand for 20minutes followed by neutralization with 0.1M HCl

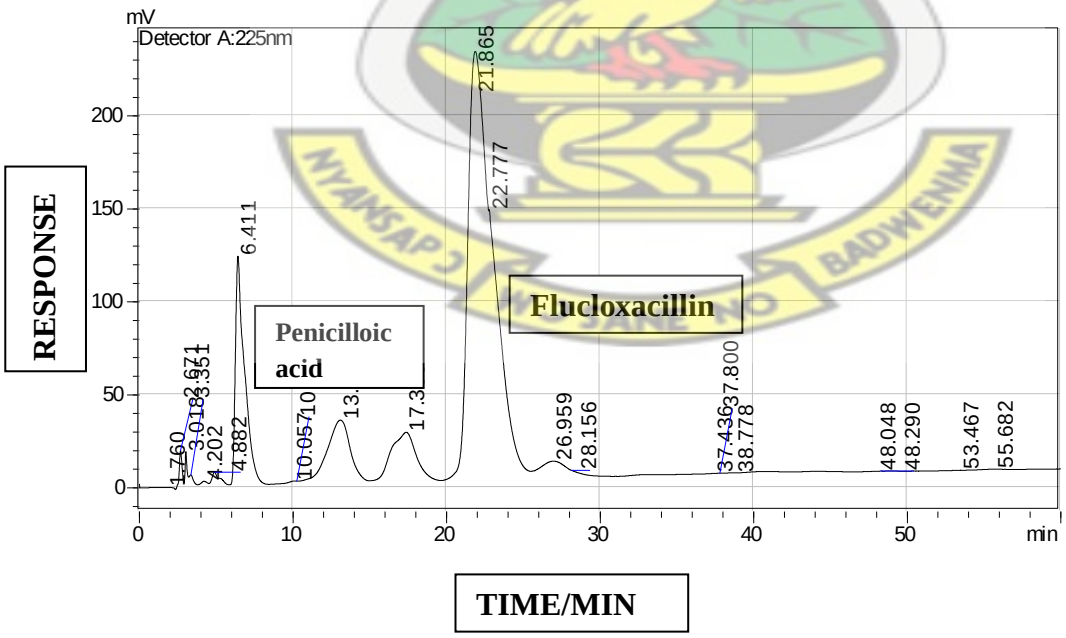

Figure 13: Chromatogram of flucloxacillin sodium solution kept for 14days.

CHAPTER FOUR

4.0 DISCUSSION

4.0.1 IDENTIFICATION OF SAMPLES

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

4.0.1.1 PURE PENICILLINS

The samples responded positively to the identification test described in the BP 2009 by giving an intense yellowish colour for Pure flucloxacillin sodium , pure amoxicillin trihydrate and pure ampicillin trihydrate and a reddish brown colour for pure benzylpenicillin sodium and pure and phenoxymethylpenicillin sodium upon treatment with sulphuric acid-formaldehyde reagent and heating in a water- bath.

Also IR spectra obtained from the samples showed specific bands specified by the BP 2007.The HPLC chromatograms of each also indicated one major peak showing that flucloxacillin, amoxicillin, ampicillin, benzylpenicillin and phenoxymethylpenicillin were largely present.

4.0.1.2 FLUCLOXACILLIN CAPSULE BRANDS

The extracted active flucloxacillin from the capsule brands also tested positive by giving an intense yellow colour with sulphuric acid-formaldehyde reagent after warming in a water-bath for a minute. IR spectrum showed specific bands typical of the flucloxacillin on comparison to flucloxacillin reference spectrum. The HPLC chromatograms also showed major peaks indicating the presence of flucloxacillin in the capsule brands.

4.0.1.3 EXCIPIENTS USED FOR STABILITY STUDIES

The excipients employed tested positive for their identification tests; maize starch gave a blue-black colour with iodine solution and plain carboxymethyl cellulose gave a precipitate with copper sulphate solution.

4.0.2 UNIFORMITY OF CONTENT

Uniformity of content is one of many in-process controls that are performed during manufacturing. It is designed to ensure that every capsule within a batch contains the amount of drug substance intended with very little variation among the capsules within the batch. It also gives a clue on the variation of doses within the batch and to a greater extent whether patients would be under-dosed or over-dosed upon taking such capsules.

The **BP 2009** states that for capsules with an average weight of more than 300mg, not more than two should have deviations greater than 7.5% and none should deviate by more than 15%.The average weight of capsule brands A, B, and D were more than 300mg. Capsule brand C had an average weight of less than 300mg. For capsules whose average weights are below 300mg the **BP 2009** states that not more than two should have deviations greater than 10% and none should deviate by more than 20%. Based on these observations only brand C failed the test.

4.0.3 MOISTURE CONTENT DETERMINATION

Starch and plain carboxymethyl cellulose are hygroscopic substances and are used as a disintegrant and a binder respectively in capsule and tablet formulation. In formulating moisture sensitive drugs, example flucloxacillin, it is very important that starch of low moisture content is used in order to prevent premature degradation of the drug. Also in capsule formulations, the capsule shell can take up water from starch that has a high moisture content, swell and distort resulting in compromise of product quality. The maize starch used for the stability studies had a moisture content of 12.36% which is less than the 15% stated in the **BP (2009)** implying that the starch sample passed the test. The plain cmc also had a moisture content of 11.33% which was also satisfactory.

4.0.4 MONITORING OF MOISTURE ABSORBED BY EXCIPIENTS (DRIED STARCH AND DRIED PLAIN CMC) DURING STABILITY STUDIES

Keeping pharmaceuticals from moisture is practically an impossible task not even when they are kept in air-tight containers. This is due to the fact that the containers "breathe" and therefore allow exchange of air (moist/dry) between them and their environment**. (Olaniyi, 2010)**. Monitoring the moisture absorbed by the excipients gives an insight as to the amount of moisture to which the formulations/mixtures were exposed to. It also gives an indication of how much moisture they can absorb when formulated with flucloxacillin in order to prevent the drug from moisture induced degradation. After the 10 week period of the study, the moisture contents of the starch and the plain cmc were 7.20 and 6.40% respectively.

4.0.5 DETERMINATION OF IODINE ABSORBING IMPURITIES

Iodine absorbing impurity can also be used as an index of penicillin breakdown. A very high level is therefore indicative of an appreciable breakdown of the drug. Most of the breakdown products react with iodine.

One major issue associated with the use of penicillin antibiotics is the occurrence of allergic reactions/hypersensitivity in sensitive individuals. These reactions are caused by degradation products of the penicillins and are quantifiable because they can react with iodine. A penicillin product like flucloxacillin is likely to cause such a reaction in a sensitive individual if the levels of these impurities are high.

The limit for iodine absorbing impurities in flucloxacillin is 5% according to the BP 2003. All the samples failed the test. It can be said that a high amount of the flucloxacillin in samples had broken down and usage of such products could cause an allergic reaction in susceptible persons and will also constitute under-dosage that could consequently lead to bacterial resistance development.

Very high iodine absorbing impurity of 25.57% in sample D could be due to inappropriate storage conditions for example, exposure to acidic and basic substances, and moisture from the humid environment or inappropriate packaging of the product. Again it could also be due to the fact that the product has expired since expired product has a minimum of 10% decomposition. Sample D was used as a positive control for the test.

4.0.6 ASSAY OF FLUCLOXACILLIN IN CAPSULE AND PURE FLUCLOXACILLIN

The percentage content of flucloxacillin sodium in capsules should be 92.5 to 101.0% and 95 to 101% for the pure powder as stated in the **BP 2007**.All the capsule brands used and the pure powder passed the test as their contents were within their respective ranges with the exception of Brand B.

All the samples (A, B and C) failed the test for iodine absorbing impurities implying significant level of breakdown but on the contrary Brand A and B passed the assay with percentage contents of 99.40 and 97.46% respectively. Brand C on the other hand failed the

assay with percentage content of 104.45%. These imply that these brands might have contained very high levels of the flucloxacillin sodium or greater quantities of the flucloxacillin sodium than stated amounts were put into these capsules during manufacture. These practices are sometimes carried out so that at any point within the expiry period of the product, assay of the product would show good contents and to demonstrate stability of the product.

Brand C after failing test for iodine absorbing impurities (8.35%) also failed the assay test with a high percentage content of 104.45% showing clearly that greater amount than the stated amount of flucloxacillin sodium was used in its manufacture.

All samples were also within their expiration period. Brands A and C expire on 09/2013 and Brand B also expires on 10/2013. This implies that these products would be or are still available to consumers. Though they have adequate amount of flucloxacillin needed for its therapeutic purposes (with the exception of Brand C, which contains more than necessary and can cause over-dosing and its associated problems) they still contain very significant or high levels of breakdown products and therefore could cause hypersensitivity in susceptible individuals.

The pure powder passed the content test but failed for the iodine absorbing impurity content. High iodine absorbing impurities could be attributed to breakdown as a result of poor storage conditions or inefficient purification during manufacturing as any oxidizable substances present could also react with the iodine used during its determination.

4.0.7 STABILITY STUDIES

4.0.7.1 PREPARATION OF FLUCLOXACILLIN/EXCIPIENT MIXTURES

Various proportions of 75mg, 125mg, 150mg and 250mg of excipient and 250mg flucloxacillin mixtures were chosen using the average weight of the capsules as a guide. Though capsules did not appear to contain so much of the excipients from the estimate made from the difference between the capsule content and the amount of flucloxacillin contained, 150mg and 250mg of excipients were also considered to find out their effects on the product stability should they be used during formulation with some other excipients that are often used in small quantities.

4.0.7.2 DETERMINATION OF THE ORDER OF THE REACTION

The samples were assayed over a period of ten weeks and their percentage contents converted to concentration terms suitable to plot graphs for zero and first order reactions.

The main aim of the experiment was to find out how best the excipients used could stabilize the drug (flucloxacillin) in the presence of moisture. Moisture is always in the air and more so in high amounts because of the high humidity of our atmosphere (tropical country).Flucloxacillin sodium being a pharmaceutical agent, its degradation was likely to follow a zero order, first order or pseudo first order though it could also degrade by complicated mechanisms. **(Lachman et al., 1976)**

Consideration was thus given to a possible pseudo-first order because the amount of moisture is in far excess of the drug as it is always present in air and its amount will not change even after reaction with the drug (amount maintained at a constant compared to drug) and the reaction taken to be dependent only on the drug amounts present. Thus the amount of

moisture does not exhibit any significant change during the degradation process. **(Lachman et al., 1976)**

A zero order was also seen as a possibility because it was also thought that irrespective of the amounts of water and drug that may be present, the rate of the degradation reaction could be independent on them.**(Lachman et al., 1976)**

Another reason for ruling out possible second order kinetics was due to the fact that pseudo first order was considered and a single reaction cannot be pseudo first and second order at the same time. **(Lachman et al., 1976)**

After obtaining zero and pseudo-first order graphs for the samples it was seen that all of the samples followed pseudo first order kinetics. The co-efficient of correlation obtained from the pseudo-first order graphs for the samples were higher than those obtained for zero order. The coefficient of correlation shows the strength of correlation between y (concentration terms) and x (time) values but does not necessarily confirm linearity. **(Vogel's, 1989).** These showed that there were stronger degrees of correlation between the concentration terms and the time for pseudo first order. The linear graphs for these samples also looked better than the zero order graphs.

Again all the capsule brands including the control (pure flucloxacillin only) also decomposed according to pseudo first order kinetics as their co-efficient of correlation were higher for pseudo first order compared to that for zero order kinetics.

4.0.7.3 COMPARING REACTION RATE CONSTANTS AND PREDICTING THE MOST STABLE FORMULATION

The reaction rate constant, k, is a very useful measure and indicates how fast or slow a reaction proceeds with time. A high k value therefore denotes a faster reaction rate which for

a degradation reaction represents a very rapid breakdown and implies an unstable product. The opposite is true for a small rate constant. **(Lachman et al, 1976)**

The concentration of flucloxacillin in the capsules dosage forms, those combined with varying proportions of dried starch and dried plain cmc and control without any excipient added fell with time upon exposure to moisture in air when stored at room temperature. A confirmation that flucloxacillin readily breaks down with time when it comes into contact with moisture.

A critical look at the k (rate constant) values revealed that the rate constants for any amount of dried plain cmc were lower than those obtained for the dried starch. It is worth noting that the value for the rate constant for pure flucloxacillin ($0.0305week^{-1}$) was higher than all these except that for 75mg dries starch ($0.0336week^{-1}$), 75mg dried plain cmc ($0.0317week^{-1}$) and 150mg dried starch($0.0345week^{-1}$). However it has the same rate constant as that of 125mg dried starch. t was observed that in general dried plain cmc formed a more stable product with flucloxacillin compared to dried starch. It was also observed that 250mg dried plain cmc gave the most stable formulation with a rate constant of $0.0231\ week^{-1}$, followed by 250mg dried starch(rate constant of $0.0297week^{-1}$) with 150mg dried starch giving the least stable formulation (rate constant of $0.0345\ week^{-1}$).

Starch and sodium cmc are hygroscopic substances and it was thought that their inclusion would make flucloxacillin stable as they would selectively take up any moisture present in the formulation first thereby protecting the drug from moisture induced hydrolysis. This protection is thought to be possible because both substances have hydroxyl groups projecting from their backbones and can link up with water molecules through hydrogen bonding.

Over the period of study, dried starch absorbed more moisture than dried plain cmc: moisture absorbed being 7.20 and 6.40% for dried starch and dried plain cmc respectively. This implies than dried starch is more hygroscopic that dried plain cmc.

The higher rate constant (lower stability) for pure flucloxacillin (control) compared to dried plain cmc and dried starch combinations could be explained on the grounds that the dried plain cmc and starch absorbed most of moisture present in their formulations and hence prevented the moisture from getting to the flucloxacillin to cause water hydrolysis.

There was also a trend of reducing rate constants with increase in the amount of the excipients used. This means that the more excipient used the higher the protection offered to flucloxacillin and the more stable the product. This was made evident as there was a decrease in rate constant as excipients were increased from 75mg to 250mg, with 250mg excipient-flucloxacillin combinations recording the least rate constants. However 150mg starch – flucloxacillin combination was an exception as it gave the highest rate constant.

For the capsule formulations, sample B was the most stable, followed by sample C with sample A being the least stable. Samples A and B had rate constants lower than that for the pure flucloxacillin and this could be attributed to the fact that they contained plain cmc.

Sample C being the least stable and also with rate constant greater than that for pure flucloxacillin could be attributed to one or a combination of the following reasons: the absence of starch, formulation with moist or hydrated excipients and the presence of chemical substances that promoted breakdown.

Notwithstanding the protection offered to the flucloxacillin sodium by the 250mg dried starch and the 250mg dried plain cmc against the decomposition of the drug by moisture, the percentage breakdown of the drug were very high over the 10 week period. The percentage

breakdowns were 20.57 and 25.20% for 250mg dried plain cmc and 250mg dried starch respectively.

4.0.8 HPLC METHOD DEVELOPMENT AND VALIDATION

4.0.8.1 METHOD DEVELOPMENT

Flucloxacillin has been assayed in literature with several mobile phase systems involving combinations of solvents like acetonitrile which is expensive and various buffers some of which are not readily available. This work therefore sought to use mobile phase system that is relatively inexpensive and readily available but would still give comparable results to the earlier stated systems if not better. The mobile phase system of methanol (redistilled) and 0.02M potassium dihydrogen orthophosphate (KH_2PO_4) was used.

The choice of methanol (redistilled) and KH_2PO_4 was made because they are cheap and readily obtainable. Methanol redistilled is a general purpose methanol that has been freshly distilled repeatedly to rid of any impurities. It is cheaper than the conventional HPLC grade methanol. Potassium dihydrogen orthophosphate (KH_2PO_4) is also a common and readily available salt compared to other salts. A detection wavelength of 225nm was chosen from literature **(BP, 2007)** and as it also gave sharp and nice peaks of the drug.

The trial begun with a 50:50 mixture of the two solvents at a flow rate of 1.00ml/min which could not resolve completely all the five penicillins. There was therefore the need to increase the inorganic component of the mobile phase system in order to increase the retention time so as to prevent overlap of the combined penicillins since a reverse phase column was being used. **(Beckett and Stenlake, 1988).**

The phosphate component was increased to 60% and the methanol component reduced to 40% which gave well resolved peaks for the penicillins within a reasonable period of time of

approximately 22 minutes. A further increase in the phosphate component resulted in broadening of the peaks with an undue increase in the retention times. Change in flow rate from 1.00ml/min to 0.50ml/min resulted in peak broadening and longer retention times. Therefore, 40% : 60% combination of methanol (redistilled) : 0.02M KH_2PO_4(pH = 3.70) as mobile phase and 1.00ml/min as flow rate was considered optimum for the HPLC method.

4.0.8.2 METHOD VALIDATION

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

The parameters considered when validating an analytical procedure include linearity, accuracy, precision, limit of detection, limit of quantitation, selectivity, and robustness/ruggedness. Data obtained during the validation process were analyzed using graph pad prism version 5 statistical software.

Linearity is the ability of analytical procedure to produce test results or responses which are proportional to the concentration (amount) of analyte in samples within a given concentration range, either directly or by means of a well-defined mathematical transformation. **(http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/S tep4/Q2_R1__Guideline.pdf).** The calibration plots for linearity gave straight lines with a coefficient of correlation, r^2 values of 0.9989, 0.9987, 0.9988, 0.9984 and 0.9995 for amoxicillin trihydrate, ampicillin trihydrate, benzylpenicillin sodium, phenoxymethylpenicillin sodium and flucloxacillin sodium respectively. The linearity of a

method is established by visual inspection of a plot of analytical response as a function of analyte concentration and a correlation co-efficient greater than 0.99 suggest a non-significant deviation from linearity and also a strong correlation between response and analyte concentration **.(http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/S tep4/Q2_R1__Guideline.pdf)**

The R^2 values obtained for flucloxacillin and the other penicillins were all greater than 0.99 implying that the concentration terms and the peak areas had a strong correlation. Again the residuals plots for the various penicillins also confirmed the linearity by giving equal distribution between their positive and negative residual values.

The responses to flucloxacillin sodium, benzylpenicillin sodium and phenoxymethylpenicillin sodium are linear with concentration over the range of0.005 to 0.10%. Responses to amoxicillin trihydrate and ampicillin trihydrate are also linear with concentration over the range 0.003 to 0.06%.

The limit of detection (LOD) of an analytical procedure is the lowest amount of an analyte in a sample that can be detected, but not necessarily quantitated as an exact value. **(http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/S tep4/Q2_R1__Guideline.pdf.)** The LOD calculated from the various calibration curves were 0.002578, 0.002830, 0.004397, 0.005184 and 0.003029%w/v for amoxicillin trihydrate, ampicillin trihydrate, benzylpenicillin sodium, phenoxymethylpenicillin sodium and flucloxacillin sodium respectively. This means that concentrations below the above would not give signals or cannot be detected using the chromatographic conditions developed.

The limit of quantitation (LOQ) is the lowest amount of the analyte in the sample that can be quantitatively determined with defined/acceptable level of precision and accuracy under the

stated experimental conditions**. (http://www.standardbase.com/tech/HPLC validation PE.pdf).** The LOQ calculated from the various calibration curves were 0.007811, 0.008576, 0.013325, 0.015708 and 0.009178%w/v for amoxicillin trihydrate, ampicillin trihydrate, benzylpenicillin sodium, phenoxymethylpenicillin sodium and flucloxacillin sodium respectively. The limit of quantitation is a parameter of quantitative assays for low levels of compounds in sample matrices and is used particularly for the determination of impurities and/or degradation products or low levels of active constituent in a product. Though values looked quite high it is satisfactory as the method is designed to assay these penicillins in formulations with sufficiently high contents.

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. **http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf.)** It therefore in effect measures how reproducible the analytical procedure is under ordinary circumstances of operation. The relative standard deviations obtained for the intra-day precision/repeatability determinations were 1.75, 1.64, 1.62, 4.03 and 1.75% for amoxicillin trihydrate, ampicillin trihydrate, benzylpenicillin sodium, phenoxymethylpenicillin sodium and flucloxacillin sodium respectively.

The inter-day/intermediate precision determinations also gave relative standard deviations of 1.22, 2.23 1.90, 3.97 and 2.25% for amoxicillin trihydrate, ampicillin trihydrate, benzylpenicillin sodium, phenoxymethylpenicillin sodium and flucloxacillin sodium respectively.

The acceptance criteria for precision depend on the amount/concentration of the component being measured in the sample with the higher the amount of component being measured, the lower the relative standard deviation calculated should be as shown in the table below;

Table 24: Reference table for precision

Component measured in sample	**Recommended precision**
≥10.0%	≤ 2%
1.0 up to 10.0%	≤ 5%
0.1 up to 1.0%	≤ 10%
< 0.1%	≤ 20%

(http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/S tep4/Q2_R1__Guideline.pdf)

For amoxicillin trihydrate and ampicillin trihydrate, a concentration of 0.06%w/v was employed for the determinations which are below 0.1% and therefore the relative standard deviations should not be greater than 20%. The results obtained showed that none of them was greater than the stated value.

Again for benzylpenicillin sodium, phenoxymethylpenicillin sodium and flucloxacillin sodium a concentration of 0.1%w/v was employed and there the relative standard deviation should not be greater than 10%. The result obtained showed that none them for both repeatability and intermediate precision was greater than 10%. The method designed can therefore be described as precise which means that determinations of a particular concentration of the analyte under the chromatographic conditions will yield results that are reproducible or very close.

The accuracy of an analytical method is defined as the degree to which the determined value of analyte in a sample corresponds to the true value.

(http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf). The mean percentage recoveries at 80% concentration level were 99.88, 100.66, 100.30, 100.48 and 101.33%w/w for amoxicillin trihydrate, ampicillin trihydrate, benzylpenicillin sodium, phenoxymethylpenicillin sodium and flucloxacillin sodium respectively.

The mean percentage recoveries at 100% concentration level were 100.57, 100.01, 100.66, 100.48and 100.51% w/w for amoxicillin trihydrate, ampicillin trihydrate, benzylpenicillin sodium, phenoxymethylpenicillin sodium and flucloxacillin sodium respectively.

The mean percentage recoveries at 120% concentration level were 99.10, 99.48, 100.79, 100.41and 100.40% w/w for amoxicillin trihydrate, ampicillin trihydrate, benzylpenicillin sodium, phenoxymethylpenicillin sodium and flucloxacillin sodium respectively.

The acceptance criteria for the mean recovery depend on the sample matrix, the sample processing procedure and on the analyte concentration. The mean % recovery should be within the following ranges as shown in the table below;

Table 25: Reference table for accuracy

% Active/impurity content	**Acceptable mean recovery**
≥10.0	98 –102%
1.0 - 10.0	90 –110%
0.1 - 1.0	80 – 120%
< 0.1	75 – 125%

(http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf)

For amoxicillin trihydrate and ampicillin trihydrate, a concentration of 0.06%w/v was employed for the determinations which are below 0.1% and therefore the mean percentage

recovery should not fall outside the range of 75 – 125% The results obtained showed all the mean percentage recoveries at the various concentration levels were within the stated range.

Again for benzylpenicillin sodium, phenoxymethylpenicillin sodium and flucloxacillin sodium a concentration of 0.1%w/v was employed and therefore the mean percentage recovery should not fall outside the range of 80 – 120%. The results obtained showed all the mean percentage recoveries at the various concentration levels were within the stated range.

Furthermore, the means obtained was statistically analyzed using the graph pad prism version 5 and were found to be not significantly different from each other at all concentration levels at 95% confidence interval. The method can therefore be described as accurate.

Selectivity of a method refers to the extent to which it can determine particular analyte(s) in a complex mixture without interference from other components in the mixture**.(http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf).**The method gave distinct and well resolved peaks for all the 5 penicillins that were combined without any interference. The mean retention times (n=18), recorded for the penicillin combined were 3.14 ± 0.01, 4.36 ± 0.02, 8.50 ± 0.04, 13.57 ± 0.06 and 21.18 ± 0.08minutes for amoxicillin trihydrate, ampicillin trihydrate, benzylpenicillin sodium, phenoxymethylpenicillin sodium and flucloxacillin sodium respectively.

Again the method was able to determine the analytes (penicillins) without any interference when they were mixed with excipients during the accuracy determination. The method was also able to discriminate between breakdown products of flucloxacillin sodium (penicilloic acid) and flucloxacillin sodium by giving two distinct peaks with retention times of 6.41 and 21.18minutes respectively. Since the method provides responses for a number of chemical entities with all responses being distinguishable, the method is then said to be selective**.**

(http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/S tep4/Q2_R1__Guideline.pdf). This means that flucloxacillin or any of the penicillins can be conveniently assayed in the presence of one another with the method.

Robustness is the ability of an analytical procedure to give reproducible results for the same material or sample under varying conditions: different analysts or operators, instruments, reagents, assay temperatures, slight changes in mobile phases and so on. **(http://www.standardbase.com/tech/HPLC validation PE.pdf)** the method was tested for robustness by intentionally varying the flow rate (0.80ml/min and 1.20ml/min) and varying the detection wavelength of detection (223nm and 227 nm). For all the 5 penicillins and at all the various conditions, the mean %purities obtained were found not to be significantly different at 95% confidence interval using the graph pad prism version 5. This implies that the method is robust. It was however realized that slight changes in retention time were observed when flow rate was varied. Broad peaks were also obtained when flow rate was changed from 1.00ml/min to 0.80ml/min.

4.0.9 DETECTION OF BREAKDOWN PRODUCTS OF FLUCLOXACILLIN SODIUM

Flucloxacillin sodium breaks down readily as stated earlier and these breakdown products are associated with allergies experienced by susceptible individuals. The designed method was able to distinguish between the flucloxacillin and its breakdown products by showing peaks for both flucloxacillin and its breakdown products at different retention times.

4.1 CONCLUSION

All samples used their qualification tests with the capsule brands containing starch, carboxymethyl cellulose and magnesium stearate except brand D which contained no starch. Starch is more hygroscopic than cmc. All brands passed both uniformity of content assay except brand C. All the capsule brands including pure flucloxacillin powder failed test for iodine absorbing impurities.

Flucloxacillin breaks down according to pseudo – first order kinetics in the presence of moisture. Starch and plain cmc had no significant effect on retarding flucloxacillin decomposition (Flucloxacillin breaks down irrespective of the amount of starch or plain cmc added). Dry plain cmc –flucloxacillin mixtures were more stable than that of starch – flucloxacillin mixtures at all comparable weight combinations. (250mg plain cmc mixture being most stable).

The proposed HPLC method is suitable for the assay of flucloxacillin sodium in flucloxacillin only dosage forms and also for the simultaneous assay of flucloxacillin sodium, amoxicillin trihydrate, ampicillin trihydrate, benzylpenicillin sodium and phenoxymethylpenicillin sodium in combined dosage forms. The proposed method can also be used to detect breakdown products of flucloxacillin sodium and monitor its breakdown profile. It can also be used to detect any adulteration of any of the above penicillins with one another.

4.2 RECOMMENDATIONS

Stability studies should be carried out with combination of two or more excipients in differing quantities.

A hygrometer should be used to investigate effect of humidity changes on flucloxacillin stability.

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APPENDICES

MILLIEQUIVALENT CALCULATIONS

Preparation of 250ml of 0.01M $Na_2S_2O_3.5H_2O$

248.1800g in 1000ml ≡ 1M Na2S2O3.5H2O

2.4818g in 1000ml ≡ 0.01M

0.6205g in 250ml ≡ 0.01M

Preparation of primary standard, KIO_3 solution for 0.01M $Na_2S_2O_3.5H_2O$

214.0000g in 1000ml ≡ 6M $Na_2S_2O_3$

35.6667g in 1000ml ≡ 1M

0.3566g in 1000ml ≡ 0.01M

0.1783g in 500ml ≡ 0.01M

Preparation of 0.01M iodine, I_2 solution

2(126.9000g) in 1000ml ≡ 1M I2

2.5380g in 1000ml ≡ 0.01M

0.5076g in 200ml ≡ 0.01M

Preparation of 2M H_2SO_4

98.0700g in 1000ml ≡ 1M

196.1400g in 1000ml ≡ 2M

19.6140g in 100ml ≡ 2M

But assay of H2SO4 is 98%

98% = 19.6140g

100% = 100% × 19.6140g

98%

= 20.0000g

Specific gravity of H_2SO_4 = 1.835g/ml

1.835g = 1ml

20.0000g = 20.0000g × 1ml

1.835g

= 10.91ml

Preparation of 1M NaOH

40.0000g in 1000ml ≡ 1M NaOH

4.0000g in 100ml ≡ 1M NaOH

% purity of NaOH = 99%

99% = 4.0000g

100% = 100% × 4.0000g

99%

= 4.0404g

Preparation of 1M HCl

36.4500g in 1000ml ≡ 1M HCl

3.6450g in 1000ml ≡ 1M HCl

Assay = 36%

36% = 3.6450g

100% = 100% × 3.6450g

36%

= 10.1250g

Specific gravity = 1.18g/ml

1.18g = 1ml

10.1250g = 10.1250g × 1ml

1.18g

= 8.58 ml

Preparation of 2.40% w/v Glacial acetic acid

This implies 2.40g made to 100ml

Specific gravity = 1.048g/ml- 1.0511g/ml

Average value = 1.0495g/ml

Assay = 99%

99% = 2.4000g

100% = 100% × 2.4000g

99%

= 2.4242g

1.0495g = 1ml

2.4242g = 2.4242g/1.0495g

× 1ml

= 2.3099ml ≈ 2.31ml

But for 250ml = 250ml/100ml

× 2.31ml

= 5.78ml of glacial acetic acid.

Preparation of 0.02M $Na_2S_2O_3.5H_20$

248.1800g in 1000ml ≡ 1M $Na_2S_2O_3.5H_20$

4.9636g in 1000ml ≡ 0.02M

2.4818g in 500ml ≡ 0.02M

Preparation of primary standard, KIO_3 solution for 0.02M $Na_2S_2O_3.5H_20$

214.0000g in 1000ml ≡ 6M Na2S2O3

35.6667g in 1000ml ≡ 1M

0.7133g in 1000ml ≡ 0.01M

0.3567g in 500ml ≡ 0.01M

UNIFORMITY OF CONTENT TABLES

SAMPLE A

Capsule number	Weight of capsule(g)	Weight of shell(g)	Weight of content(g)	Deviation (g)	% deviation (%)
1	0.6700	0.1000	0.5700	-0.0130	-2.33
2	0.6600	0.0900	0.5700	-0.0130	-2.33
3	0.6400	0.1000	0.5400	0.0170	3.05
4	0.6400	0.0900	0.5500	0.0070	1.26
5	0.6400	0.0900	0.5500	0.0070	1.26
6	0.6400	0.0900	0.5500	0.0070	1.26
7	0.6400	0.1000	0.5400	0.0170	3.05
8	0.6500	0.0900	0.5600	-0.0030	-0.54
9	0.6500	0.1000	0.5500	0.0070	1.26
10	0.6400	0.0900	0.5500	0.0070	1.26
11	0.6500	0.1000	0.5500	0.0070	1.26
12	0.6300	0.1000	0.5300	0.0270	4.85
13	0.6600	0.0800	0.5800	-0.0230	-4.13
14	0.6600	0.0900	0.5700	-0.0130	-2.33
15	0.6600	0.1000	0.5600	-0.0030	-0.54
16	0.6600	0.0900	0.5700	-0.0130	-2.33
17	0.6400	0.0900	0.5500	0.0070	1.26
18	0.6500	0.0900	0.5600	-0.0030	-0.54
19	0.6500	0.0900	0.5600	-0.0030	-0.54
20	0.6500	0.1000	0.5500	0.0070	1.26

Weight of 20 shells = 1. Weight of 20 capsules = 13.0100g

SAMPLE B

Capsule number	Weight of capsule(g)	Weight of shell(g)	Weight of content(g)	Deviation (g)	% deviation (%)
1	0.4700	0.0700	0.4000	-0.0170	-4.44
2	0.4600	0.0800	0.3800	0.0030	0.78
3	0.4500	0.0900	0.3600	0.0230	6.01
4	0.4600	0.0700	0.3900	-0.0070	-1.83
5	0.4400	0.0800	0.3600	0.0030	0.78
6	0.4700	0.0700	0.4000	-0.0176	-4.44
7	0.4600	0.0700	0.3900	-0.0070	-1.83
8	0.4800	0.0700	0.4100	-0.0027	-7.05
9	0.4600	0.0700	0.3900	-0.0070	-1.83
10	0.4700	0.0800	0.3900	-0.0070	-1.83
11	0.4400	0.0700	0.3700	0.0130	3.39
12	0.4600	0.0800	0.3800	0.0030	0.78
13	0.4400	0.0700	0.3800	0.0030	0.78
14	0.4800	0.0700	0.4100	-0.0270	-7.05
15	0.4600	0.0700	0.3900	-0.0070	-1.83
16	0.4200	0.0800	0.3400	0.0430	11.23
17	0.4700	0.0800	0.3900	-0.0070	-1.83
18	0.4400	0.0700	0.3700	0.0130	3.39
19	0.4300	0.0700	0.3600	0.0230	6.01
20	0.4400	0.0700	0.3700	0.0130	3.39

Weight of 20 shells = 1.4800g Weight of 20 capsules = 9.1400g

SAMPLE C

Capsule number	Weight of capsule(g)	Weight of shell(g)	Weight of content(g)	Deviation (g)	% deviation (%)
1	0.3400	0.0800	0.2600	0.0145	5.28
2	0.3600	0.0700	0.3000	-0.2550	-9.29
3	0.3200	0.0800	0.2400	0.0345	12.57
4	0.3600	0.0800	0.2800	-0.0055	-2.00
5	0.3900	0.0800	0.3100	-0.0355	-12.93
6	0.3400	0.0700	0.2700	0.0045	1.64
7	0.3300	0.0700	0.2600	0.0145	5.28
8	0.3500	0.0700	0.2800	-0.0055	-2.00
9	0.3500	0.0800	0.2700	0.0045	8.93
10	0.3600	0.0800	0.2800	-0.0055	-5.65
11	0.3300	0.0800	0.2500	0.0245	8.93
12	0.3600	0.0700	0.2900	-0.0155	-5.65
13	0.3300	0.0800	0.2500	0.0245	8.93
14	0.3500	0.0700	0.2800	-0.0055	-2.00
15	0.3300	0.0800	0.2500	0.0245	8.93
16	0.3500	0.0700	0.2800	-0.0055	-2.00
17	0.3400	0.0700	0.2700	0.0045	1.64
18	0.3400	0.0800	0.2600	0.0145	5.28
19	0.3600	0.0700	0.2900	-0.0155	-5.65
20	0.3400	0.0700	0.2700	0.0045	1.64

Weight of 20 shells = 1.5300g Weight of 20 capsules = 7.0200g

SAMPLE D

Capsule number	Weight of capsule(g)	Weight of shell(g)	Weight of content(g)	Deviation (g)	% deviation (%)
1	0.4486	0.0801	0.3685	0.0063	1.68
2	0.4354	0.0750	0.3604	0.0144	3.84
3	0.4630	0.0768	0.3586	0.0162	4.32
4	0.4442	0.0806	0.3636	0.0112	2.99
5	0.4726	0.0751	0.3975	-0.0227	-6.06
6	0.4419	0.0817	0.3602	0.0146	3.90
7	0.4646	0.0818	0.3838	-0.009	-2.40
8	0.4805	0.0802	0.4003	-0.0255	-6.80
9	0.4530	0.0835	0.3695	0.0053	1.41
10	0.4517	0.0778	0.3739	0.0009	0.24
11	0.4507	0.0767	0.374	0.0008	0.21
12	0.4929	0.0763	0.4166	-0.0418	-11.15
13	0.4408	0.0753	0.3655	0.0093	2.48
14	0.4534	0.0778	0.3756	-0.0008	-0.21
15	0.4627	0.0776	0.3851	-0.0103	-0.54
16	0.4347	0.0759	0.3588	0.016	4.27
17	0.4465	0.0761	0.3704	0.0044	1.17
18	0.4377	0.0752	0.3625	0.0123	3.28
19	0.4411	0.0812	0.3599	0.0149	3.98
20	0.4432	0.0778	0.3654	0.0094	2.51

Weight of 20 shells = 1.5625g Weight of 20 capsules = 9.0592g

MIXTURES

Flucloxacillin + starch (250:75)

7.5000g 2.2700g

CW = 20.1600g

Flucloxacillin + CMC (250 : 75)

7.5000g 2.2500g

CW = 20.0200g

Flucloxacillin +starch (250:150)

7.5000g 4.5000g

CW = 19.9400g

Flucloxacillin +CMC (250:125)

7.5000g 3.7500g

CW = 20.2500g

Flucloxacillin +starch (250:125)

7.5000g 3.7500g

CW = 20.1400g

Flucloxacillin +CMC (250:150)

7.5000g 4.5000g

CW = 20.1100g

Flucloxacillin + starch (250:250)

7.5000g 7.500g

CW = 19.7600g

Flucloxacillin +CMC (250:250)

7.5000g 7.500g

CW = 19.4200g

Control starch = 5.0000g

Weight of container = 19.9400g

Control CMC = 5.0000g

Weight of container = 19.9300g

CW = WEIGHT OF EMPTY CONTAINER.

WEIGHT OF MIXTURE TAKEN FOR ANALYSIS

250mg flucloxacillin + 75mg Exp. Mixture

9.97g mixture = 7.5g flucloxacillin

? = 0.1

$\frac{0.1}{7.5}$x 9.97 = 0.1329g

250mg flucloxacillin : 150mg Exp. Mixture

13.43g mixture = 7.5g flucloxacillin

? = 0.1

$\frac{0.1}{7.5}$x 12.00 = 0.1600g

250mg flucloxacillin: 125mg Exp. mixtures

12.68g mixture = 7.5g flucloxacillin

? = 0.1

$\frac{0.1}{7.5}$x 11.25 = 0.1500g

250mg flucloxacillin: 250mg Exp. mixtures

16.43g mixture = 7.5g flucloxacillin

? = 0.1

$\frac{0.1}{7.5}$x 15 = 0.2000g

MOISTURE CONTENT DETERMINATION.

STARCH

Weight of petri dish = 53.3700g

Weight of petri dish and starch = 78.3600g

Weight of petri dish and starch after heating to constant weight = 75.2700g

Weight loss = 78.3600g – 75.2700g = 3.0900g

Weight used = 78.3600g – 53.3700g = 24.9900g

Moisture content = $\frac{3.09}{24.99}x100$ = 12.36%$\frac{w}{w}$

CMC

Weight of petri dish = 50.9000g

Weight of petri dish and CMC = 80.9200g

Weight of petri dish and CMC after heating to constant weight = 77.5200g

Weight loss = 80.9200g – 77.5200g = 3.4000g

Weight used = 80.9200g – 50.9000g = 30.0200g

Moisture content = $\frac{3.40}{30.02}x100$ = 11.33%$\frac{w}{w}$

IODINE ABSORBING IMPURITIES

STANDARDIZATION OF 0.01M THIOSULPHATE SOLUTION

Titrations	**1**	**2**	**3**
Final(ml)	20.00	40.10	20.00
Initial(ml)	0.00	20.00	0.00
Vol.used(ml)	20.00	20.10	20.00

Average = 20.03ml

F_{KIO3}= 0.1779 / 0.1788

= 0.9966

Factor of thiosulphate

= o.9966 × 20 / 20.03

= 0.9951

PURE FLUCLOXACILLIN

TEST

TITRATIONS	**1**	**2**	**3**
FINAL VOL/ML	10.80	21.50	32.10
INITIAL VOL/ML	0.00	10.80	21.50
VOL. USED /ML	10.80	10.70	10.60

AVERAGE = 10.70ML

BLANK

TITRATIONS	**1**	**2**	**3**
FINAL VOL/ML	18.00	36.10	17.90
INITIAL VOL/ML	0.00	18.00	0.00
VOL. USED /ML	18.00	36.10	17.90

AVREAGE = 18.00ml

SAMPLE A

TEST

TITRATIONS	**1**	**2**	**3**
FINAL VOL/ML	10.70	21.20	31.80
INITIAL VOL/ML	0.00	10.70	21.20
VOL. USED /ML	10.70	10.50	10.60

AVERAGE = 10.60ml

BLANK

TITRATIONS	**1**	**2**	**3**
FINAL VOL/ML	18.00	36.10	17.90
INITIAL VOL/ML	0.00	18.00	0.00
VOL. USED /ML	18.00	36.10	17.90

AVERAGE = 18.00ml

SAMPLE B

TEST

TITRATIONS	1	2	3
Final vol/ml	13.00	26.50	39.40
Initial vol/ml	0.00	13.00	26.50
Vol. Used /ml	13.00	13.00	12.90

AVERAGE = 13.13ml

BLANK

TITRATIONS	1	2	3
Final vol/ml	18.00	36.10	17.90
Initial vol/ml	0.00	18.00	0.00
Vol. Used /ml	18.00	36.10	17.90

AVERAGE = 18.00ml

SAMPLE C

TEST

TITRATIONS	1	2	3
Final vol/ml	10.00	20.00	30.10
Initial vol/ml	0.00	10.00	20.00
Vol. Used /ml	1.00	10.00	11.20

AVERAGE = 11.20ml

BLANK

TITRATIONS	1	2	3
Final vol/ml	18.00	36.10	17.90
Initial vol/ml	0.00	18.00	0.00
Vol. Used /ml	18.00	36.10	17.90

AVERAGE = 18.00ml

SAMPLE D

TEST

TITRATIONS	1	2	3
Final vol/ml	11.20	22.40	33.60
Initial vol/ml	0.00	11.20	24.40
Vol. Used /ml	11.20	11.20	11.20

AVERAGE = 11.20ml

BLANK

TITRATIONS	1	2	3
Final vol/ml	37.70	35. 70	35.70
Initial vol/ml	2.00	0.00	0.00
Vol. Used /ml	35.70	35.70	35.70

AVERAGE = 35.70ml

SAMPLE CALCULATION

PURE FLUCLOXACILLIN

Volume of thiosulphate equivalent to iodine absorbed by impurities = (blank – test/assay) × Factor of thiosulphate

= (18.00 – 10.70) ×0.9960

= 7.27ml

1ml 0.01M $Na_2S_2O_3.5H_2O$ = 0.524mg I_2 absorbing impurities

7.27ml = 3.8099mg

25ml of solution contains 125mg of Flucloxacillin

10ml → 50mg

% I_2 absorbing impurities = 3.8099/50 ×100%

= 7.6198%

= 7.62%

ASSAY OF FLUCLOXACILLIN SODIUM BY IODIMETRY

WEEK O

STANDARDIZATION OF THIOSULPHATE SOLUTION

Titrations	**1**	**2**	**3**
Final(ml)	20.70	41.40	20.80
Initial(ml)	0.00	20.70	0.00
Vol.used(ml)	20.70	20.40	20.80

Average = 20.70ml

F_{KIO3} = 0.1779 / 0.1788

= 0.9966

Factor of thiosulphate = 0.9966×20/20.70 =0.9629

CMC MIXTURES

250:125

ASSAY

Titrations	**1**	**2**	**3**
Final(ml)	15.90	31.50	15.70
Initial(ml)	0.00	15.90	0.00
Vol.used(ml)	15.90	15.60	15.70

BLANK

Titrations	**1**	**2**	**3**
Final(ml)	25.60	25.40	25.40
Initial(ml)	0.00	0.00	0.00
Vol.used(ml)	25.60	25.40	25.40

CMC MIXTURES

250:150

ASSAY

Titrations	**1**	**2**	**3**
Final(ml)	15.90	31.80	15.90
Initial(ml)	0.00	15.90	0.00
Vol.used(ml)	15.90	15.90	15.90

BLANK

Titrations	1	2	3
Final(ml)	23.90	47.80	23.90
Initial(ml)	0.00	23.90	0.00
Vol.used(ml)	23.90	23.90	23.90

CMC MIXTURES

250:75

ASSAY

Titrations	1	2	3
Final(ml)	15.40	30.80	15.30
Initial(ml)	0.10	15.40	0.00
Vol.used(ml)	15.30	15.40	15.30

BLANK

Titrations	1	2	3
Final(ml)	24.20	24.20	24.20
Initial(ml)	0.00	0.00	0.00
Vol.used(ml)	24.20	24.20	24.20

CMC MIXTURES: 250:250

ASSAY

Titrations	1	2	3
Final(ml)	15.50	30.60	15.40
Initial(ml)	0.00	15.50	0.10
Vol.used(ml)	15.50	15.10	15.30

BLANK

Titrations	1	2	3
Final(ml)	24.10	48.30	24.20
Initial(ml)	0.00	24.10	0.10
Vol.used(ml)	21.10	24.20	24.10

Pure flucloxacillin – control

ASSAY

Titrations	1	2	3
Final(ml)	15.80	31.50	16.20
Initial(ml)	0.00	15.80	0.30
Vol.used(ml)	15.80	15.70	15.90

BLANK

Titrations	1	2	3
Final(ml)	24.40	24.50	24.40
Initial(ml)	0.00	0.00	0.00
Vol.used(ml)	24.40	24.50	24.40

STARCH MIXTURES

250:75

ASSAY

Titrations	**1**	**2**	**3**
Final(ml)	16.80	33.20	16.50
Initial(ml)	0.20	16.80	0.00
Vol.used(ml)	16.60	16.40	16.50

250:125

ASSAY

Titrations	**1**	**2**	**3**
Final(ml)	15.40	30.50	15.40
Initial(ml)	0.00	15.40	0.00
Vol.used(ml)	15.40	15.10	15.40

BLANK

Titrations	**1**	**2**	**3**
Final(ml)	23.90	24.00	23.90
Initial(ml)	0.00	0.00	0.00
Vol.used(ml)	23.90	24.00	23.90

BLANK

Titrations	**1**	**2**	**3**
Final(ml)	24.60	24.50	24.50
Initial(ml)	0.20	0.00	0.00
Vol.used(ml)	24.40	24.50	24.50

250:150

ASSAY

Titrations	**1**	**2**	**3**
Final(ml)	15.30	30.80	15.50
Initial(ml)	0.00	15.30	0.00
Vol.used(ml)	15.30	15.50	15.50

BLANK

Titrations	**1**	**2**	**3**
Final(ml)	24.20	24.10	24.10
Initial(ml)	0.00	0.00	0.00
Vol.used(ml)	24.20	24.10	24.10

250:250

ASSAY

Titrations	1	2	3
Final(ml)	39.80	15.70	15.60
Initial(ml)	24.10	0.00	0.00
Vol.used(ml)	15.70	15.70	15.60

BLANK

Titrations	1	2	3
Final(ml)	24.20	24.20	39.90
Initial(ml)	0.00	0.00	15.20
Vol.used(ml)	24.20	24.20	24.20

FORMULATIONS

WEEK 0

Sample A

ASSAY

Titrations	1	2	3
Final(ml)	15.90	36.00	15.90
Initial(ml)	0.00	20.00	0.00
Vol.used(ml)	15.90	16.00	15.90

BLANK

Titrations	1	2	3
Final(ml)	24.10	45.30	24.20
Initial(ml)	0.00	21.10	0.00
Vol.used(ml)	24.10	24.20	24.20

Sample B

ASSAY

Titrations	1	2	3
Final(ml)	15.80	35.70	15.90
Initial(ml)	0.00	20.00	0.00
Volume(ml)	15.80	15.70	15.90

BLANK

Titrations	1	2	3
Final(ml)	24.50	24.50	24.40
Initial(ml)	0.00	0.00	0.00
Volume(ml)	24.50	24.50	24.40

Sample C

ASSAY

Titrations	1	2	3
Final(ml)	16.20	23.00	16.00
Initial(ml)	0.00	17.00	0.00
Volume(ml)	16.200	16.00	16.10

BLANK

Titrations	1	2	3
Final(ml)	24.00	48.10	23.90
Initial(ml)	0.00	24.00	0.00
Volume(ml)	24.00	24.10	23.90

WEEK 1

STANDARDISATION OF 0.02M $Na_2S_2O_3.5H_20$

Titrations	1	2	3
Final(ml)	26.40	25.50	25.50
Initial(ml)	1.00	0.10	0.00
Vol.used(ml)	25.40	25.40	25.50

Factor of KIO_3 = 0.9966

Factor of $Na_2S_2O_3.5H_20$

= 0.9965×25 / 25.4

=0.9808

FLUCLOXACILLIN + STARCH (MIXTURE)

250mg + 125mg

ASSAY

Titrations	1	2	3
Final(ml)	14.60	29.20	14.60
Initial(ml)	0.00	14.60	0.00
Vol.used(ml)	14.60	14.60	14.60

BLANK

Titrations	1	2	3
Final(ml)	22.50	45.00	22.50
Initial(ml)	0.00	23.00	0.00
Vol.used(ml)	22.50	22.50	22.50

250mg + 150mg

ASSAY

Titrations	1	2	3
Final(ml)	14.70	29.30	14.70
Initial(ml)	0.00	14.70	0.00
Vol.used(ml)	14.70	14.60	14.70

BLANK

Titrations	1	2	3
Final(ml)	22.70	27.70	22.50
Initial(ml)	0.00	5.00	0.00
Vol.used(ml)	22.70	22.70	22.50

250mg + 75mg

ASSAY

Titrations	1	2	3
Final(ml)	14.50	29.00	14.50
Initial(ml)	0.00	14.50	0.00
Vol.used(ml)	14.50	14.50	14.50

BLANK

Titrations	1	2	3
Final(ml)	22.50	22.40	47.90
Initial(ml)	0.00	0.00	22.40
Vol.used(ml)	22.50	22.40	22.50

250mg + 250mg

ASSAY

Titrations	1	2	3
Final(ml)	14.80	30.00	14.80
Initial(ml)	0.00	15.00	0.00
Vol.used(ml)	14.80	15.00	14.80

BLANK

Titrations	1	2	3
Final(ml)	22.60	47.60	22.60
Initial(ml)	0.00	25.00	0.00
Vol.used(ml)	22.60	22.60	22.60

FLUCLOXACILLIN + CMC (PLAIN) MIXTURES

250mg + 75mg

ASSAY

Titrations	1	2	Blank
Final(ml)	14.60	29.00	14.40
Initial(ml)	0.00	14.60	0.00
Vol.used(ml)	14.60	14.40	14.40

BLANK

Titrations	1	2	3
Final(ml)	22.50	22.40	47.90
Initial(ml)	0.00	0.00	22.40
Vol.used(ml)	22.50	22.40	22.50

250mg + 125mg

ASSAY

Titrations	1	2	Blank
Final(ml)	14.40	14.60	28.80
Initial(ml)	0.00	0.00	14.60
Vol.used(ml)	14.40	14.60	14.20

BLANK

Titrations	1	2	3
Final(ml)	22.60	47.60	22.60
Initial(ml)	0.00	25.00	0.00
Vol.used(ml)	22.60	22.60	22.60

250mg + 250mg

ASSAY

Titrations	1	2	Blank
Final(ml)	13.50	27.20	40.90
Initial(ml)	0.00	13.50	27.20
Vol.used(ml)	13.50	13.70	13.70

BLANK

Titrations	1	2	Blank
Final(ml)	21.70	21.70	43.30
Initial(ml)	0.00	0.00	21.70
Vol.used(ml)	21.70	21.70	21.60

250mg + 150mg

ASSAY

Titrations	1	2	3
Final(ml)	13.80	27.70	13.70
Initial(ml)	0.00	13.80	0.00
Vol.used(ml)	13.80	13.90	13.70

BLANK

Titrations	1	2	3
Final(ml)	22.10	44.30	22.10
Initial(ml)	0.00	22.10	0.00
Vol.used(ml)	22.10	22.20	22.10

Control

ASSAY

Titration	1	2	3
Final(ml)	12.90	26.00	12.90
Initial(ml)	0.00	13.00	0.00
Vol.used(ml)	12.90	13.00	12.90

BLANK

Titrations	1	2	3
Final(ml)	21.50	21.30	42.60
Initial(ml)	0.00	0.00	21.30
Vol.used(ml)	21.50	21.30	21.30

FORMULATIONS

Sample A

ASSAY

Titrations	1	2	3
Final(ml)	12.30	24.70	12.20
Initial(ml)	0.00	12.30	0.00
Vol.used(ml)	12.30	12.40	12.20

BLANK

Titrations	1	2	3
Final(ml)	19.80	39.50	19.80
Initial(ml)	0.00	19.80	0.00
Vol.used(ml)	19.80	19.70	19.80

Sample B

ASSAY

	1	2	Blank
Final(ml)	16.10	32.30	16.20
Initial(ml)	0.00	16.10	0.00
Volume(ml)	16.10	16.2	16.20

BLANK

Titrations	1	2	3
Final(ml)	24.60	24.60	24.50
Initial(ml)	0.00	0.00	0.00
Volume(ml)	24.60	24.60	24.50

Sample C

ASSAY

Titrations	1	2	3
Final(ml)	14.60	29.20	14.60
Initial(ml)	0.00	14.60	0.00
Vol.used(ml)	14.60	14.60	14.60

BLANK

Titrations	1	2	3
Final(ml)	22.50	45.00	22.50
Initial(ml)	0.00	23.00	0.00
Vol.used(ml)	22.50	22.50	22.50

WEEK 2

FLUCLOX + STARCH MIXTURES

250mg + 75mg

ASSAY

Titrations	1	2	3
Final(ml)	12.30	24.70	12.20
Initial(ml)	0.00	12.30	0.00
Vol.used(ml)	12.30	12.40	12.20

BLANK

Titrations	1	2	3
Final(ml)	19.80	39.50	19.80
Initial(ml)	0.00	19.80	0.00
Vol.used(ml)	19.80	19.70	19.80

250mg + 125mg

ASSAY

Titrations	1	2	3
Final(ml)	14.20	28.20	14.00
Initial(ml)	0.00	14.20	0.00
Vol.used(ml)	14.20	14.00	14.00

BLANK

Titrations	1	2	3
Final(ml)	21.60	43.30	21.70
Initial(ml)	0.00	21.60	0.00
Vol.used(ml)	21.60	21.70	21.70

250mg + 150mg

ASSAY

Titrations	1	2	3
Final(ml)	13.80	27.70	13.90
Initial(ml)	0.00	13.80	0.00
Vol.used(ml)	13.80	13.90	13.90

BLANK

Titrations	1	2	3
Final(ml)	21.50	23.00	21.50
Initial(ml)	0.00	21.50	0.00
Vol.used(ml)	21.50	21.50	21.50

250mg + 250mg

ASSAY

Titrations	1	2	3
Final(ml)	12.80	25.70	12.80
Initial(ml)	0.00	12.80	0.00
Vol.used(ml)	12.80	12.90	12.80

BLANK

Titrations	1	2	3
Final(ml)	20.50	40.80	20.30
Initial(ml)	0.00	20.50	0.00
Vol.used(ml)	20.50	20.30	20.30

FLUCLOX + CMC (PLAIN) MIXTURES

250mg + 75mg

ASSAY

Titrations	1	2	3
Final(ml)	12.10	24.30	26.40
Initial(ml)	0.00	12.10	24.30
Vol.used(ml)	12.10	12.20	12.10

BLANK

Titrations	1	2	3
Final(ml)	19.20	38.50	19.20
Initial(ml)	0.00	19.20	0.00
Vol.used(ml)	19.20	19.30	19.20

250mg + 125mg

ASSAY

Titrations	1	2	3
Final(ml)	12.80	25.70	12.80
Initial(ml)	0.00	12.80	0.00
Vol.used(ml)	12.80	12.90	12.80

BLANK

Titrations	1	2	3
Final(ml)	20.50	40.80	20.30
Initial(ml)	0.00	20.50	0.00
Vol.used(ml)	20.50	20.30	20.30

250mg + 150mg

ASSAY

Titrations	1	2	3
Final(ml)	13.20	26.40	39.70
Initial(ml)	0.00	13.20	26.40
Vol.used(ml)	13.20	13.20	13.30

BLANK

Titrations	1	2	Blank
Final(ml)	20.90	41.60	20.80
Initial(ml)	0.00	20.90	0.00
Vol.used(ml)	20.90	20.70	20.80

250mg + 250mg

ASSAY

Titrations	1	2	3
Final(ml)	12.80	25.70	12.80
Initial(ml)	0.00	12.80	0.00
Vol.used(ml)	12.80	12.90	12.80

BLANK

Titrations	1	2	3
Final(ml)	20.50	40.80	20.30
Initial(ml)	0.00	20.50	0.00
Vol.used(ml)	20.50	20.30	20.30

Control

ASSAY

Titrations	1	2	3
Final(ml)	13.00	26.00	39.10
Initial(ml)	0.00	13.00	26.00
Vol.used(ml)	13.00	13.00	13.10

BLANK

Titrations	1	2	Blank
Final(ml)	20.90	41.60	20.80
Initial(ml)	0.00	20.90	0.00
Vol.used(ml)	20.90	20.70	20.80

FORMULATIONS

Sample A

ASSAY

Titrations	1	2	3
Final(ml)	12.10	24.30	26.40
Initial(ml)	0.00	12.10	24.30
Vol.used(ml)	12.10	12.20	12.10

BLANK

Titrations	1	2	3
Final(ml)	19.20	38.50	19.20
Initial(ml)	0.00	19.20	0.00
Vol.used(ml)	19.20	19.30	19.20

Sample B

ASSAY

Titrations	1	2	3
Final(ml)	13.80	27.70	13.70
Initial(ml)	0.00	13.80	0.00
Vol.used(ml)	13.80	13.90	13.70

BLANK

Titrations	1	2	3
Final(ml)	22.10	44.30	22.10
Initial(ml)	0.00	22.10	0.00
Vol.used(ml)	22.10	22.20	22.10

Sample C

ASSAY

Titrations	1	2	3
Final(ml)	13.80	27.70	13.90
Initial(ml)	0.00	13.80	0.00
Vol.used(ml)	13.80	13.90	13.90

BLANK

Titrations	1	2	3
Final(ml)	21.50	23.00	21.50
Initial(ml)	0.00	21.50	0.00
Vol.used(ml)	21.50	21.50	21.50

WEEK 3

FLUCLOX + STARCH MIXTURES

250mg + 75mg

ASSAY

Titrations	1	2	3
Final(ml)	11.50	22.90	34.40
Initial(ml)	0.00	11.50	22.90
Vol.used(ml)	11.50	11.40	11.50

BLANK

Titrations	1	2	3
Final(ml)	18.40	36.90	18.50
Initial(ml)	0.00	18.40	0.00
Vol.used(ml)	18.40	18.50	18.50

250mg + 125mg

ASSAY

Titrations	1	2	3
Final(ml)	12.50	24.90	37.40
Initial(ml)	0.00	12.50	24.90
Vol.used(ml)	12.50	12.40	12.50

BLANK

Titrations	1	2	3
Final(ml)	19.00	38.00	19.10
Initial(ml)	0.00	19.00	0.00
Vol.used(ml)	19.00	19.00	19.10

250mg + 150mg

ASSAY

Titrations	1	2	3
Final(ml)	11.70	23.40	35.10
Initial(ml)	0.00	11.70	23.40
Vol.used(ml)	11.70	11.70	11.70

BLANK

Titrations	1	2	3
Final(ml)	18.40	36.80	18.40
Initial(ml)	0.00	18.40	0.00
Vol.used(ml)	18.40	18.40	18.40

250mg + 250mg

ASSAY

Titrations	1	2	3
Final(ml)	12.50	24.90	37.40
Initial(ml)	0.00	12.50	24.90
Vol.used(ml)	12.50	12.40	12.50

BLANK

Titrations	1	2	3
Final(ml)	19.00	38.00	19.10
Initial(ml)	0.00	19.00	0.00
Vol.used(ml)	19.00	19.00	19.10

FLUCLOX + CMC (PLAIN) MIXTURES

250mg + 75mg

ASSAY

Titrations	1	2	3
Final(ml)	12.50	24.90	37.40
Initial(ml)	0.00	12.50	24.90
Vol.used(ml)	12.50	12.40	12.50

BLANK

Titrations	1	2	3
Final(ml)	19.00	38.00	19.10
Initial(ml)	0.00	19.00	0.00
Vol.used(ml)	19.00	19.00	19.10

250mg + 125mg

ASSAY

Titrations	1	2	3
Final(ml)	12.50	24.90	37.40
Initial(ml)	0.00	12.50	24.90
Vol.used(ml)	12.50	12.40	12.50

BLANK

Titrations	1	2	3
Final(ml)	19.20	38.50	19.30
Initial(ml)	0.00	19.20	0.00
Vol.used(ml)	19.20	19.30	19.30

250mg + 150mg

ASSAY

Titrations	1	2	3
Final(ml)	12.30	24.50	37.10
Initial(ml)	0.00	12.30	24.90
Vol.used(ml)	12.30	12.20	12.20

BLANK

Titrations	1	2	3
Final(ml)	19.00	38.20	19.40
Initial(ml)	0.00	19.00	0.00
Vol.used(ml)	19.00	19.20	19.40

250mg + 250mg

ASSAY

Titrations	1	2	3
Final(ml)	12.10	24.30	36.50
Initial(ml)	0.00	12.10	24.30
Vol.used(ml)	12.10	12.20	12.20

BLANK

Titrations	1	2	3
Final(ml)	19.00	38.20	19.20
Initial(ml)	0.00	19.00	0.00
Vol.used(ml)	19.10	19.20	19.20

Control

ASSAY

Titration	1	2	Blank
Final(ml)	10.70	21.50	32.30
Initial(ml)	0.00	10.70	21.50
Vol.used(ml)	10.70	10.80	10.80

BLANK

Titration	1	2	Blank
Final(ml)	18.30	36.50	18.20
Initial(ml)	0.00	18.30	0.00
Vol.used(ml)	18.30	18.20	18.20

FORMULATIONS

Sample A

ASSAY

Titrations	1	2	3
Final(ml)	11.50	23.20	34.50
Initial(ml)	0.00	11.50	22.90
Vol.used(ml)	11.50	11.70	11.60

BLANK

Titrations	1	2	3
Final(ml)	18.00	36.00	18.10
Initial(ml)	0.00	18.00	0.00
Vol.used(ml)	18.00	18.00	18.10

Sample B

ASSAY

Titrations	1	2	3
Final(ml)	14.60	29.20	14.60
Initial(ml)	0.00	14.60	0.00
Vol.used(ml)	14.60	14.60	14.60

BLANK

Titrations	1	2	3
Final(ml)	22.50	45.00	22.50
Initial(ml)	0.00	23.00	0.00
Vol.used(ml)	22.50	22.50	22.50

Sample C

ASSAY

Titration	1	2	3
Final(ml)	10.70	21.50	32.30
Initial(ml)	0.00	10.70	21.50
Vol.used(ml)	10.70	10.80	10.80

BLANK

Titration	1	2	3
Final(ml)	18.30	36.60	18.30
Initial(ml)	0.00	18.30	0.00
Vol.used(ml)	18.30	18.30	18.30

WEEK 4

FLUCLOX + STARCH MIXTURES

250mg + 75mg

ASSAY

Titrations	1	2	3
Final(ml)	11.50	23.20	34.50
Initial(ml)	0.00	11.50	22.90
Vol.used(ml)	11.50	11.70	11.60

BLANK

Titrations	1	2	3
Final(ml)	18.00	36.00	18.10
Initial(ml)	0.00	18.00	0.00
Vol.used(ml)	18.00	18.00	18.10

250mg + 125mg

ASSAY

Titration	1	2	3
Final(ml)	10.70	21.50	32.30
Initial(ml)	0.00	10.70	21.50
Vol.used(ml)	10.70	10.80	10.80

BLANK

Titration	1	2	3
Final(ml)	18.30	36.60	18.30
Initial(ml)	0.00	18.30	0.00
Vol.used(ml)	18.30	18.30	18.30

250mg + 150mg

ASSAY

Titrations	1	2	3
Final(ml)	11.30	22.70	34.00
Initial(ml)	0.00	11.30	22.70
Vol.used(ml)	11.30	11.40	11.30

BLANK

Titrations	1	2	3
Final(ml)	18.00	36.10	18.10
Initial(ml)	0.00	18.00	0.00
Vol.used(ml)	18.00	18.10	18.10

250mg + 250mg

ASSAY

Titration	1	2	3
Final(ml)	10.70	21.50	32.30
Initial(ml)	0.00	10.70	21.50
Vol.used(ml)	10.70	10.80	10.80

BLANK

Titration	1	2	3
Final(ml)	18.30	36.50	18.20
Initial(ml)	0.00	18.30	0.00
Vol.used(ml)	18.30	18.20	18.20

FLUCLOX + CMC (PLAIN) MIXTURES

250mg + 75mg

ASSAY

Titrations	1	2	3
Final(ml)	11.00	21.90	32.90
Initial(ml)	0.00	11.00	21.90
Vol.used(ml)	11.00	10.90	11.00

BLANK

Titrations	1	2	3
Final(ml)	18.30	36.60	18.30
Initial(ml)	0.00	18.30	0.00
Vol.used(ml)	18.30	18.30	18.30

250mg + 125mg

ASSAY

Titrations	1	2	3
Final(ml)	11.10	22.30	33.50
Initial(ml)	0.00	11.10	22.30
Vol.used(ml)	11.10	11.20	11.20

BLANK

Titrations	1	2	3
Final(ml)	18.50	36.90	18.50
Initial(ml)	0.00	18.50	0.00
Vol.used(ml)	18.50	18.40	18.50

250mg + 150mg

ASSAY

Titrations	1	2	3
Final(ml)	11.00	22.10	33.00
Initial(ml)	0.00	11.00	22.00
Vol.used(ml)	11.00	11.10	11.00

BLANK

Titrations	1	2	3
Final(ml)	18.50	36.90	18.50
Initial(ml)	0.00	18.50	0.00
Vol.used(ml)	18.50	18.40	18.50

250mg + 250mg

ASSAY

Titrations	1	2	3
Final(ml)	11.10	22.30	33.50
Initial(ml)	0.00	11.10	22.30
Vol.used(ml)	11.10	11.20	11.20

BLANK

Titrations	1	2	3
Final(ml)	18.50	36.90	18.50
Initial(ml)	0.00	18.50	0.00
Vol.used(ml)	18.50	18.40	18.50

Control

ASSAY

Titration	1	2	Blank
Final(ml)	10.90	20.70	31.90
Initial(ml)	0.00	10.90	20.70
Vol.used(ml)	10.90	10.80	11.20

BLANK

Titration	1	2	Blank
Final(ml)	17.90	36.00	18.10
Initial(ml)	0.00	17.90	0.00
Vol.used(ml)	17.90	18.10	18.10

FORMULATIONS

WEEK 4

Sample A

ASSAY

Titrations	1	2	3
Final(ml)	9.70	19.50	29.20
Initial(ml)	0.00	9.70	19.50
Vol.used(ml)	9.70	9.80	9.70

BLANK

Titrations	1	2	3
Final(ml)	16.40	32.80	16.30
Initial(ml)	0.00	16.40	0.00
Vol.used(ml)	16.40	16.40	16.30

Sample B

ASSAY

Titrations	1	2	3
Final(ml)	14.60	29.50	14.50
Initial(ml)	0.00	15.00	0.00
Vol.used(ml)	14.60	14.50	14.50

BLANK

Titrations	1	2	3
Final(ml)	22.60	47.60	22.60
Initial(ml)	0.00	25.00	0.00
Vol.used(ml)	22.60	22.60	22.60

Sample C

ASSAY

Titrations	1	2	3
Final(ml)	11.50	23.20	35.00
Initial(ml)	0.00	11.50	23.40
Vol.used(ml)	11.50	11.70	11.60

BLANK

Titrations	1	2	3
Final(ml)	18.40	36.80	18.40
Initial(ml)	0.00	18.40	0.00
Vol.used(ml)	18.40	18.40	18.40

WEEK 6

FLUCLOX + STARCH MIXTURES

250mg + 75mg

ASSAY

Titrations	1	2	3
Final(ml)	9.10	18.10	27.20
Initial(ml)	0.00	9.10	18.10
Vol.used(ml)	9.10	9.00	9.10

BLANK

Titrations	1	2	3
Final(ml)	15.80	31.40	15.80
Initial(ml)	0.00	15.80	0.00
Vol.used(ml)	15.80	15.60	15.80

250mg + 125mg

ASSAY

Titrations	1	2	3
Final(ml)	11.50	23.20	34.50
Initial(ml)	0.00	11.50	22.90
Vol.used(ml)	11.50	11.70	11.60

BLANK

Titrations	1	2	3
Final(ml)	18.00	36.00	18.10
Initial(ml)	0.00	18.00	0.00
Vol.used(ml)	18.00	18.00	18.10

250mg + 150mg

ASSAY

Titrations	1	2	3
Final(ml)	9.10	18.10	27.20
Initial(ml)	0.00	9.10	18.10
Vol.used(ml)	9.10	9.00	9.10

BLANK

Titrations	1	2	3
Final(ml)	15.80	31.40	15.80
Initial(ml)	0.00	15.80	0.00
Vol.used(ml)	15.80	15.60	15.80

250mg + 250mg

ASSAY

Titrations	1	2	3
Final(ml)	11.50	23.20	34.50
Initial(ml)	0.00	11.50	22.90
Vol.used(ml)	11.50	11.70	11.60

BLANK

Titrations	1	2	3
Final(ml)	18.00	36.00	18.10
Initial(ml)	0.00	18.00	0.00
Vol.used(ml)	18.00	18.00	18.10

FLUCLOX + CMC (PLAIN) MIXTURES

250mg + 75mg

ASSAY

Titration	1	2	3
Final(ml)	11.00	20.80	31.90
Initial(ml)	0.00	11.00	20.80
Vol.used(ml)	11.00	10.80	11.10

BLANK

Titration	1	2	3
Final(ml)	17.90	36.00	18.10
Initial(ml)	0.00	17.90	0.00
Vol.used(ml)	17.90	18.10	18.10

250mg + 125mg

ASSAY

Titration	1	2	3
Final(ml)	10.90	20.70	31.90
Initial(ml)	0.00	10.90	20.70
Vol.used(ml)	10.90	10.80	11.20

BLANK

Titration	1	2	3
Final(ml)	17.90	36.00	18.10
Initial(ml)	0.00	17.90	0.00
Vol.used(ml)	17.90	18.10	18.10

250mg + 150mg

ASSAY

Titrations	1	2	3
Final(ml)	7.10	14.30	28.60
Initial(ml)	0.00	7.10	14.30
Vol.used(ml)	7.10	7.20	14.30

BLANK

Titrations	1	2	3
Final(ml)	18.20	36.40	18.30
Initial(ml)	0.00	18.20	0.00
Vol.used(ml)	18.20	18.20	18.30

250mg + 250mg

ASSAY

Titrations	1	2	3
Final(ml)	12.30	24.50	37.10
Initial(ml)	0.00	12.30	24.90
Vol.used(ml)	12.30	12.20	12.20

BLANK

Titrations	1	2	3
Final(ml)	19.00	38.20	19.40
Initial(ml)	0.00	19.00	0.00
Vol.used(ml)	19.00	19.20	19.40

Control

ASSAY

Titrations	1	2	3
Final(ml)	9.70	19.50	29.20
Initial(ml)	0.00	9.70	19.50
Vol.used(ml)	9.70	9.80	9.70

BLANK

Titrations	1	2	3
Final(ml)	16.40	32.80	16.30
Initial(ml)	0.00	16.40	0.00
Vol.used(ml)	16.40	16.40	16.30

FORMULATIONS

WEEK 6

Sample A

ASSAY

Titrations	1	2	3
Final(ml)	8.90	17.70	26.60
Initial(ml)	0.00	8.90	17.70
Vol.used(ml)	8.90	8.80	8.90

BLANK

Titrations	1	2	3
Final(ml)	15.60	31.20	15.60
Initial(ml)	0.00	15.60	0.00
Vol.used(ml)	15.60	15.50	15.60

Sample B ASSAY

Titrations	1	2	3
Final(ml)	12.10	24.30	26.40
Initial(ml)	0.00	12.10	24.30
Vol.used(ml)	12.10	12.20	12.10

BLANK

Titrations	1	2	3
Final(ml)	19.20	38.50	19.40
Initial(ml)	0.00	19.20	0.00
Vol.used(ml)	19.20	19.30	19.40

Sample C

ASSAY

Titrations	1	2	3
Final(ml)	9.70	19.50	29.20
Initial(ml)	0.00	9.70	19.50
Vol.used(ml)	9.70	9.80	9.70

BLANK

Titrations	1	2	3
Final(ml)	16.40	32.80	16.30
Initial(ml)	0.00	16.40	0.00
Vol.used(ml)	16.40	16.40	16.30

WEEK 10

FLUCLOX + STARCH MIXTURES

250mg + 75mg

ASSAY

Titrations	1	2	3
Final(ml)	9.60	19.40	29.20
Initial(ml)	0.00	9.60	19.40
Vol.used(ml)	9.60	9.80	9.80

BLANK

Titrations	1	2	3
Final(ml)	15.80	31.10	15.80
Initial(ml)	0.00	15.30	0.00
Vol.used(ml)	15.80	15.80	15.80

250mg + 125mg

ASSAY

Titrations	1	2	3
Final(ml)	10.60	21.30	31.90
Initial(ml)	0.00	10.60	21.30
Vol.used(ml)	10.60	10.70	10.60

BLANK

Titrations	1	2	3
Final(ml)	16.70	33.20	16.70
Initial(ml)	0.00	16.70	0.00
Vol.used(ml)	16.70	16.50	16.70

250mg + 150mg

ASSAY

Titrations	1	2	3
Final(ml)	10.30	20.70	10.40
Initial(ml)	0.00	10.30	0.00
Vol.used(ml)	10.30	10.40	10.40

BLANK

Titrations	1	2	3
Final(ml)	16.40	32.40	16.30
Initial(ml)	0.00	16.40	0.00
Vol.used(ml)	16.40	16.00	16.30

250mg + 250mg

ASSAY

Titrations	1	2	3
Final(ml)	10.40	20.90	31.30
Initial(ml)	0.00	10.40	20.90
Vol.used(ml)	10.40	10.50	10.40

BLANK

Titrations	1	2	3
Final(ml)	15.80	31.40	15.80
Initial(ml)	0.00	15.80	0.00
Vol.used(ml)	15.80	15.60	15.80

BLANK

Titrations	1	2	3
Final(ml)	16.40	33.10	16.50
Initial(ml)	0.00	16.40	0.00
Vol.used(ml)	16.40	16.70	16.50

FLUCLOX + CMC (PLAIN) MIXTURES

250mg + 75mg

ASSAY

Titrations	1	2	3
Final(ml)	10.40	20.70	31.10
Initial(ml)	0.00	10.40	20.70
Vol.used(ml)	10.40	10.30	10.40

BLANK

Titrations	1	2	3
Final(ml)	16.50	32.90	16.40
Initial(ml)	0.00	16.50	0.00
Vol.used(ml)	16.50	16.40	16.40

250mg + 125mg ASSAY

Titrations	1	2	3
Final(ml)	9.80	22.90	35.90
Initial(ml)	0.00	9.80	22.90
Vol.used(ml)	9.80	13.10	13.00

BLANK

Titrations	1	2	3
Final(ml)	16.30	36.70	16.20
Initial(ml)	0.00	16.30	0.00
Vol.used(ml)	16.30	16.40	16.20

250mg + 150mg

ASSAY

Titrations	1	2	3
Final(ml)	9.90	20.10	30.20
Initial(ml)	0.00	9.90	20.10
Vol.used(ml)	9.90	10.20	10.10

BLANK

Titrations	1	2	3
Final(ml)	16.30	32.70	16.40
Initial(ml)	0.00	16.30	0.00
Vol.used(ml)	16.30	16.40	16.40

250mg + 250mg

ASSAY

Titrations	1	2	3
Final(ml)	8.90	17.70	26.60
Initial(ml)	0.00	8.90	17.70
Vol.used(ml)	8.90	8.80	8.90

BLANK

Titrations	1	2	3
Final(ml)	15.60	31.20	15.60
Initial(ml)	0.00	15.60	0.00
Vol.used(ml)	15.60	15.50	15.60

Control

ASSAY

Titrations	1	2	3
Final(ml)	10.60	21.30	31.90
Initial(ml)	0.00	10.60	21.30
Vol.used(ml)	10.60	10.70	10.60

BLANK

Titrations	1	2	3
Final(ml)	16.70	33.20	16.70
Initial(ml)	0.00	16.70	0.00
Vol.used(ml)	16.70	16.50	16.70

FORMULATIONS

SAMPLE A

ASSAY

Titrations	1	2	3
Final(ml)	10.10	20.30	30.20
Initial(ml)	0.00	10.10	20.30
Vol.used(ml)	10.10	10.20	9.90

BLANK

Titrations	1	2	3
Final(ml)	14.90	30.10	45.30
Initial(ml)	0.00	14.90	30.10
Vol.used(ml)	14.90	15.20	15.20

SAMPLE B

ASSAY

Titration	1	2	3
Final(ml)	10.90	20.70	31.90
Initial(ml)	0.00	10.90	20.70
Vol.used(ml)	10.90	10.80	11.20

BLANK

Titration	1	2	3
Final(ml)	17.90	36.00	18.10
Initial(ml)	0.00	17.90	0.00
Vol.used(ml)	17.90	18.10	18.10

SAMPLE C

ASSAY

Titrations	1	2	3
Final(ml)	9.80	22.90	35.90
Initial(ml)	0.00	9.80	22.90
Vol.used(ml)	9.80	13.10	13.00

BLANK

Titrations	1	2	3
Final(ml)	16.30	36.70	16.20
Initial(ml)	0.00	16.30	0.00
Vol.used(ml)	16.30	16.40	16.20

SAMPLE CALCULATION

WEEK O

250mg flucloxacillin: 125mg dried cmc

Average titre = 15.75ml

Volume used = (blank – assay)× Factor of thiosulphate

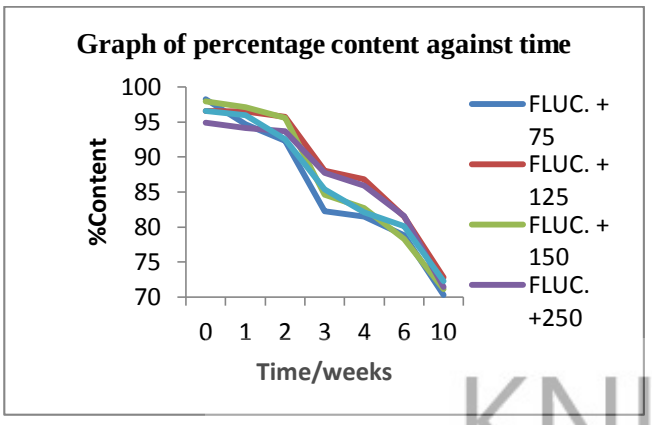
Volume = (24.40ml – 15.75ml)×0.9987

Weight = volume used x milliequivalent

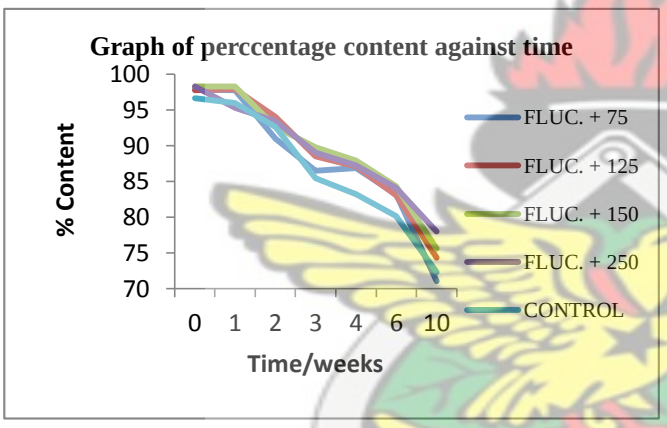
Weight = 8.738 x 0.00123 = 0.0107g

Percentage content = $\frac{0.0107}{0.0110}$x100= 97.71% $\frac{w}{w}$

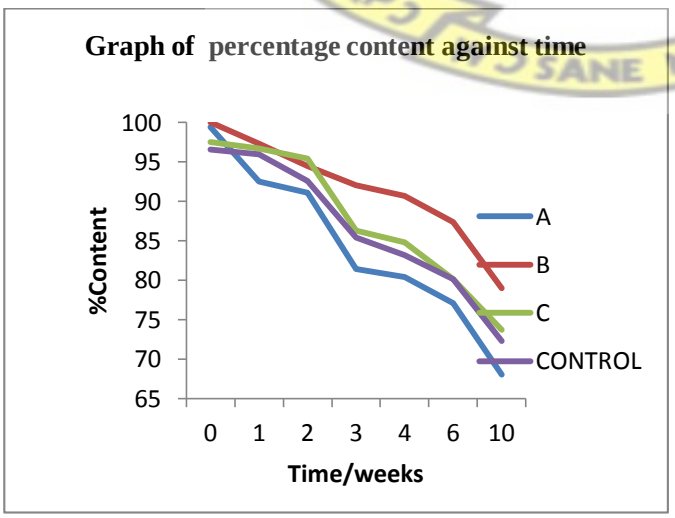
FLUCLOXACILLIN - DRIED STARCH MIXTURES



FLUCLOXACILLIN – DRIED CMC MIXTURES

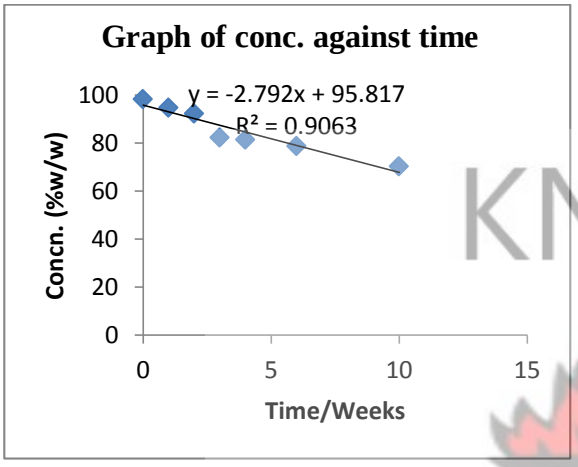


SAMPLE FORMULATIONS

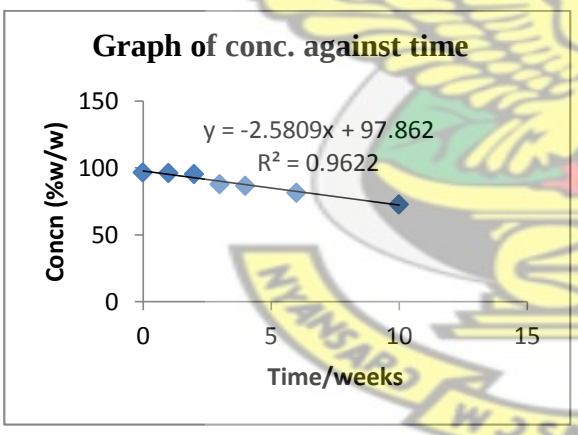


ZERO ORDER GRAPHS

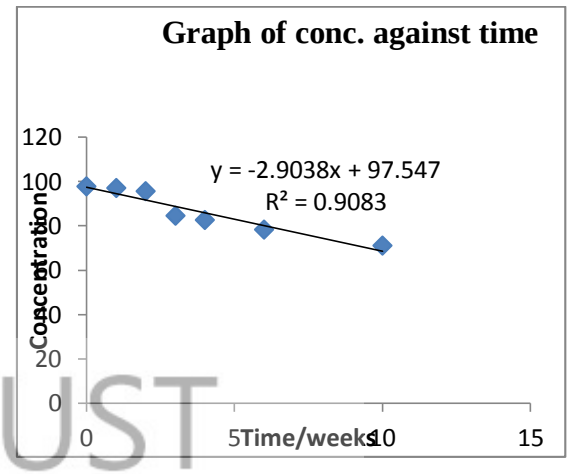
FLUCLOX - 75MG DRIED STARCH



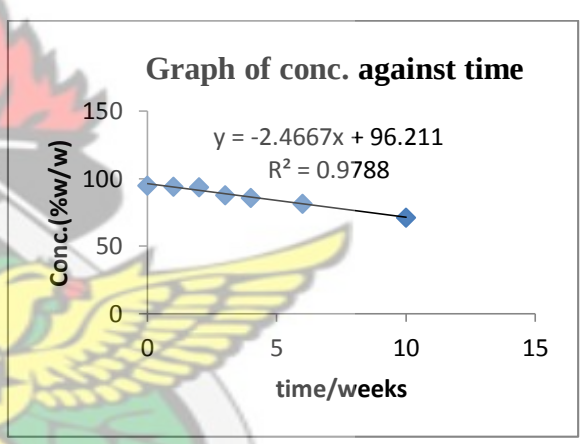
FLUCLOX - 125MG DRIED STARCH



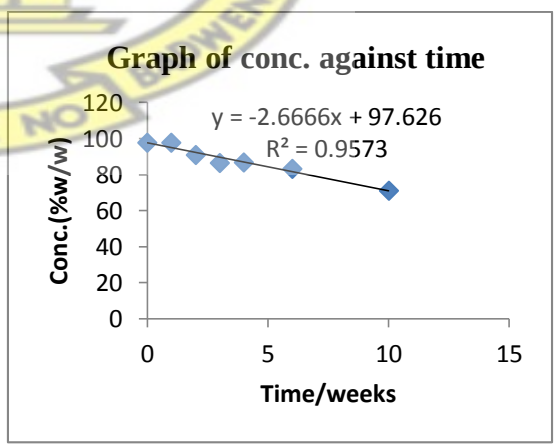
FLUCLOX - 150MG DRIED STARCH



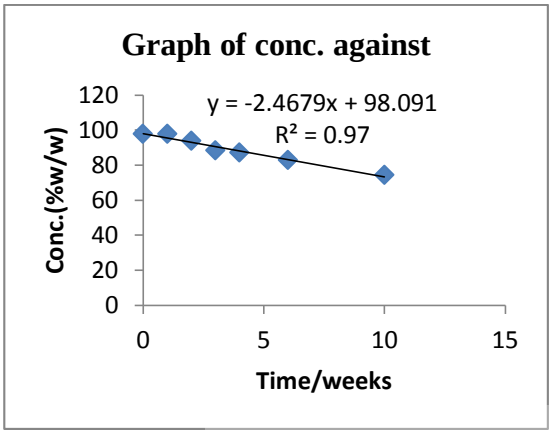
FLUCLOX - 250MG DRIED STARCH



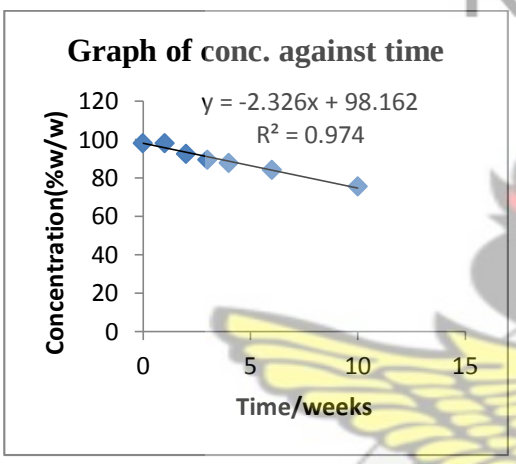
FLUCLOX - 75MG DRIED CMC



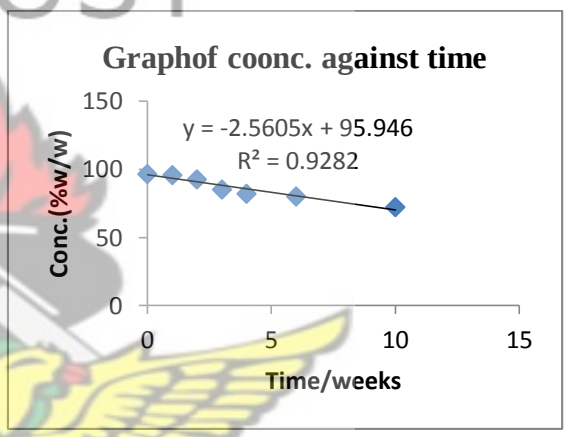
FLUCLOX - 125MG DRIED CMC



FLUCLOX - 150MG DRIED CMC

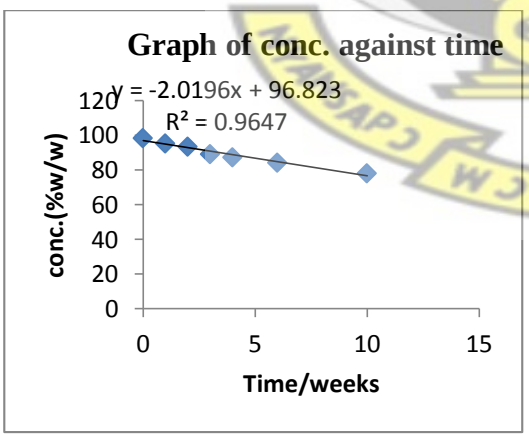


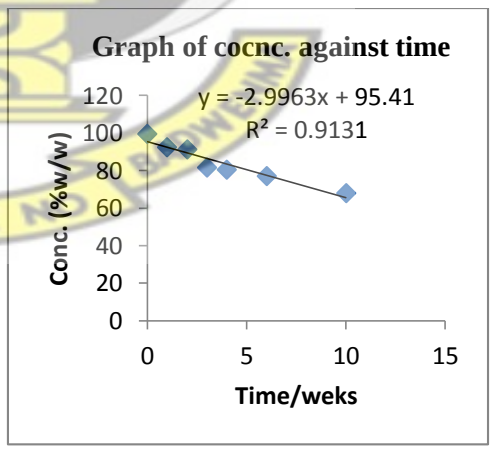
CONTROL/FLUCLOX ONLY



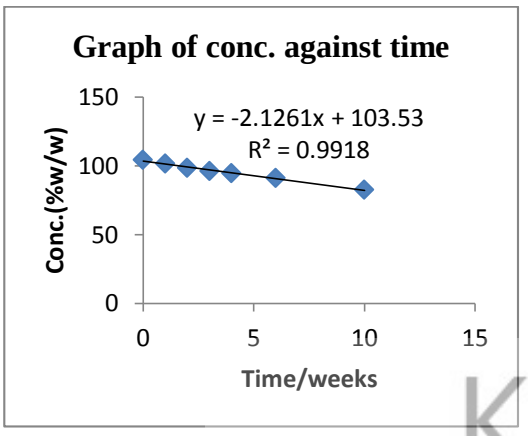
SAMPLE A

FLUCLOX - 250MG DRIED CMC

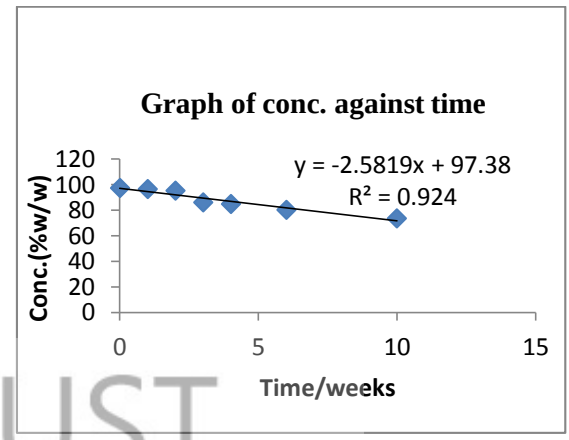




SAMPLE B

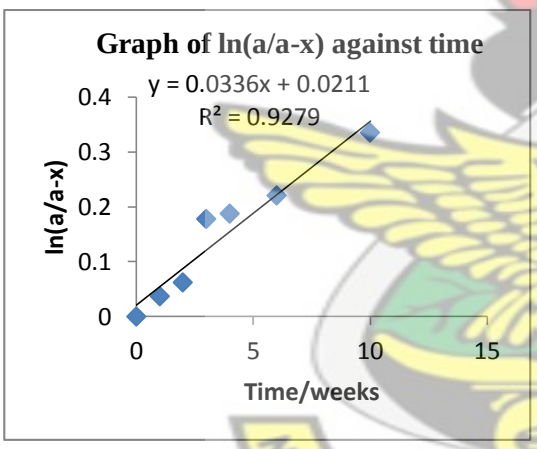


SAMPLE C

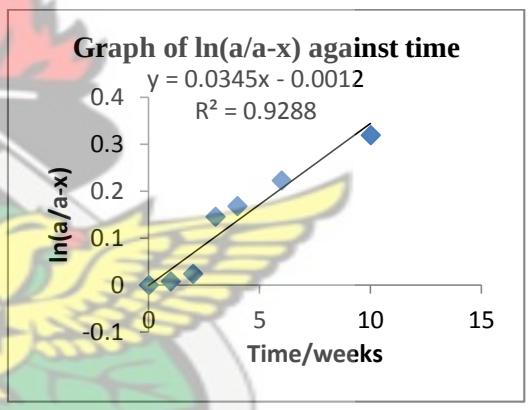


PSEUDO – FIRST ORDER GRAPHS

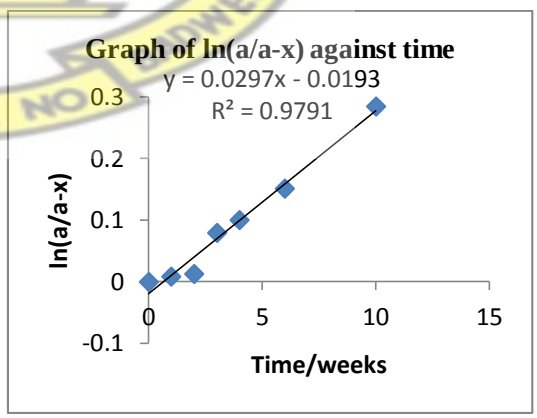
FLUCLOX - 75MG DRIED STARCH



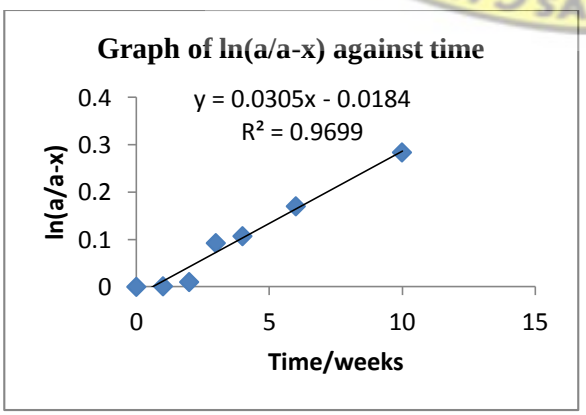
FLUCLOX - 150MG DRIED STARCH



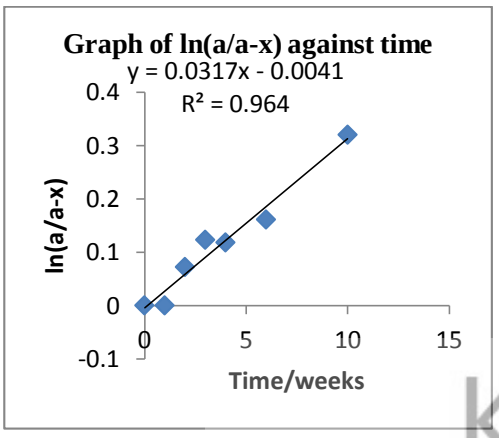
FLUCLOX - 250MG DRIED STARCH



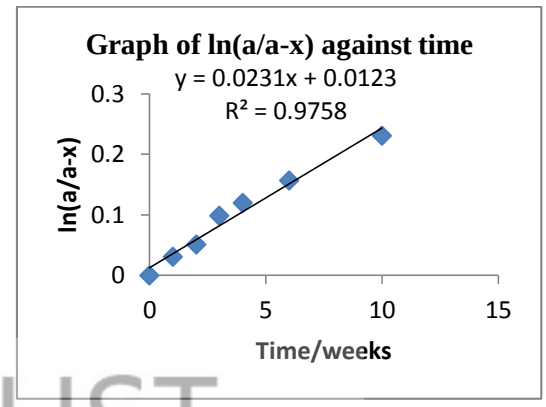
FLUCLOX - 125MG DRIED STARCH



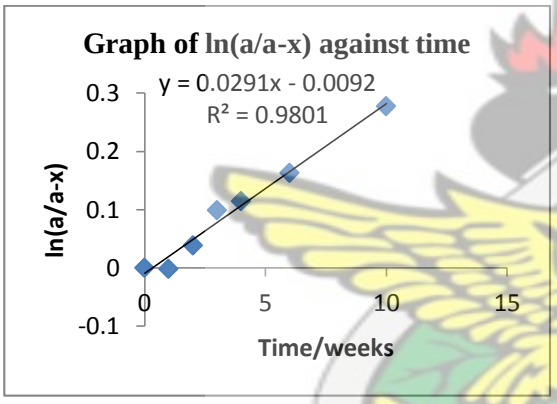
FLUCLOX - 75MG DRIED CMC



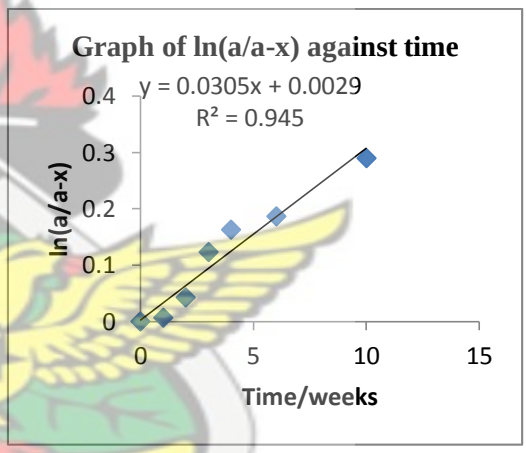
FLUCLOX - 250MG DRIED CMC



FLUCLOX - 125MG DRIED CMC

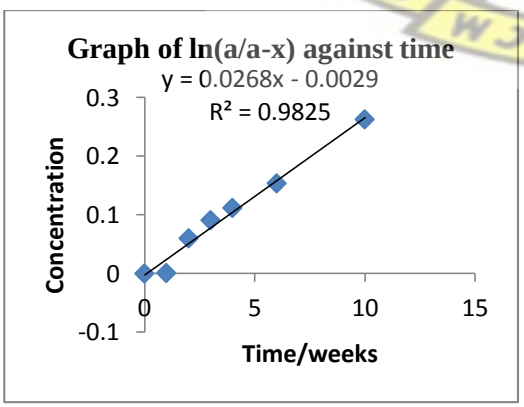


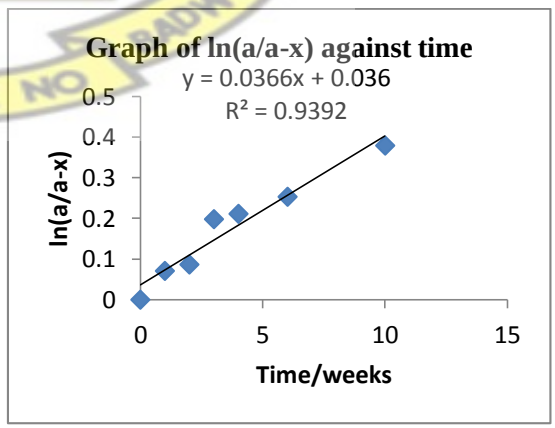
CONTROL/FLUCLOX ONLY



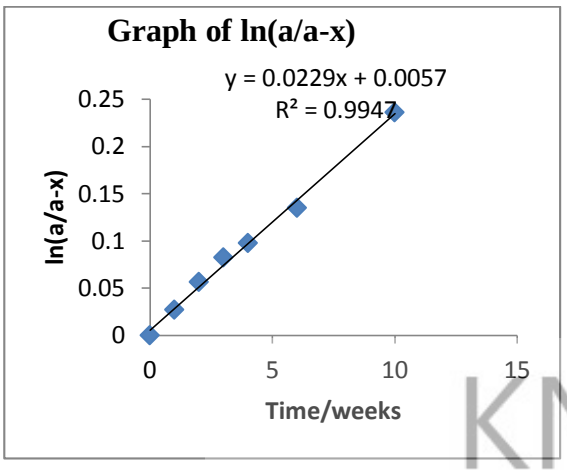
SAMPLE A

FLUCLOX - 150MG DRIED CMC

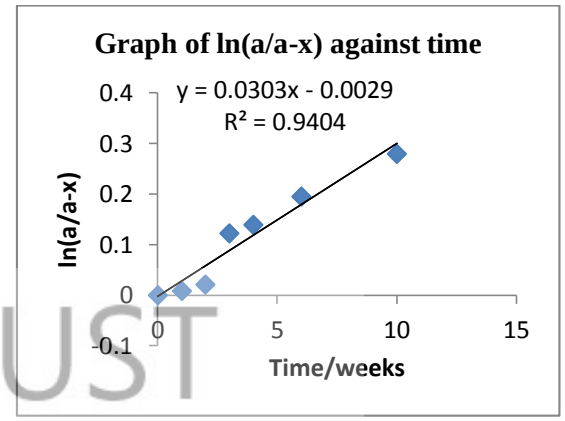




SAMPLE B

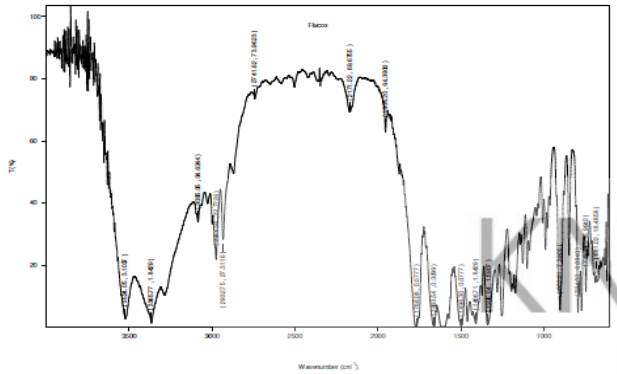


SAMPLE C

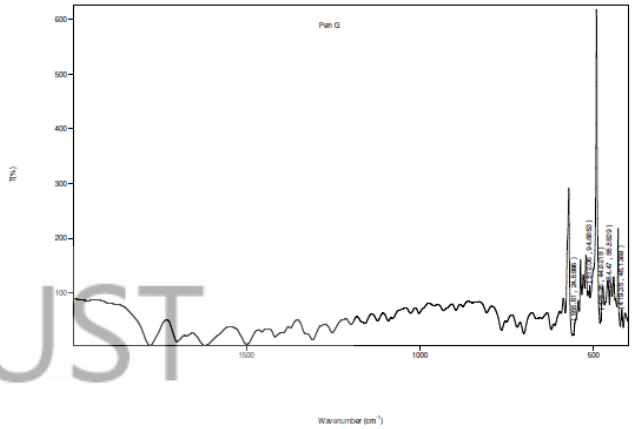


IR SPECTRA FOR THE PENICILLINS

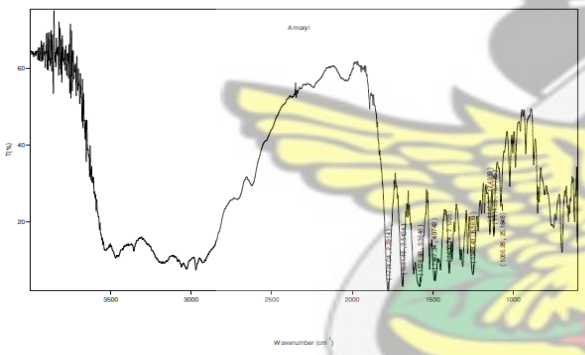
IR spectra of flucloxacillin sodium



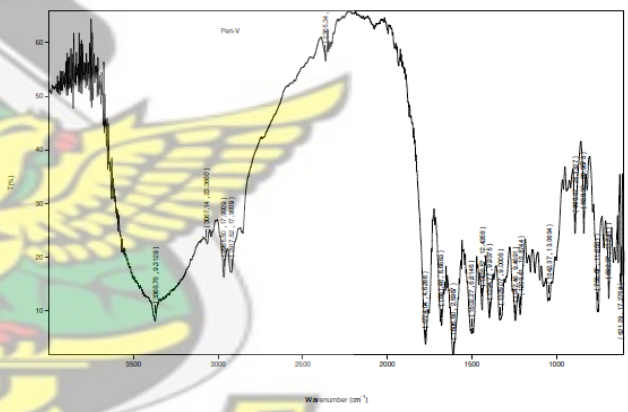
IR spectra of benzylpenicillin sodium



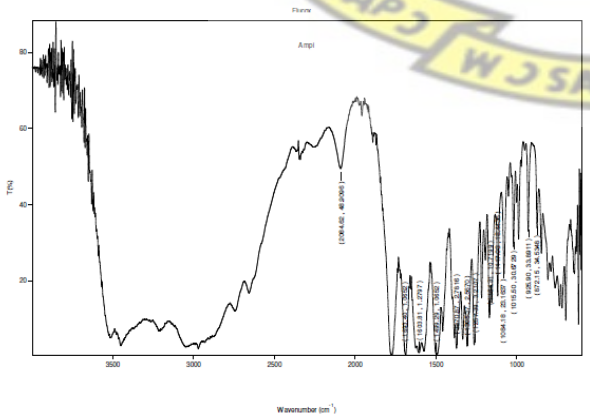
IR spectrum amoxicillin trihydrate



IR spectra of phenoxymethyl penicillin



IR spectra of ampicillin trihydrate



CONCENTRATIONS AND PEAK AREAS OF PENICILLINS

AMOXICILLIN TRIHYDRATE

Conc. (%w/w)	Peak area	Residual peak area
0.003	1738821	338042.800
0.0045	1851362	18869.210
0.006	2255990	-8217.530
0.012	3876207	-114859.200
0.024	7176758	-268025.600
0.036	10650916	-247584.400
0.048	14454906	102687.700
0.06	17985022	179086.900

AMPICILLIN TRIHYDRATE

Conc. (%w/w)	Peak area	Residual peak area
0.003	1310158	247341.200
0.0045	1315409	-16218.760
0.006	1576974	-23465.380
0.012	2623167	-52516.190
0.024	4654510	-171662.600
0.036	6807034	-169626.300
0.048	9197190	70040.700
0.06	11393745	116107.400

BENZYLPENICILLIN SODIUM

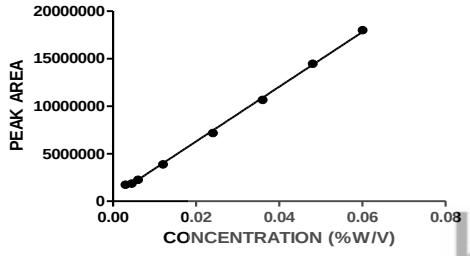
Conc. (%w/w)	Peak area	Residual peak area
0.005	708176	57450.450
0.0075	781040.7	-105395.600
0.01	1008429	-113718.400
0.02	2155665	90675.100
0.04	3971099	20422.470
0.06	6045185	208823.100
0.08	7606733	-115316.000
0.1	9564794	-42941.030

PHENOXYMETHYLPENICILLIN SODIUM

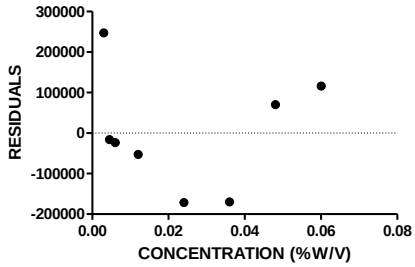
Conc. (%w/w)	Peak area	Residual peak area
0.005	1508066	478085.800
0.0075	1418021	-74956.200
0.01	1834122	-121852.200
0.02	3530466	-277496.300
0.04	7655370	143431.600
0.06	10911232	-304682.500
0.08	14833046	-86844.660
0.1	18868182	244314.500

CALIBRATION CURVES FOR PENICILLINS

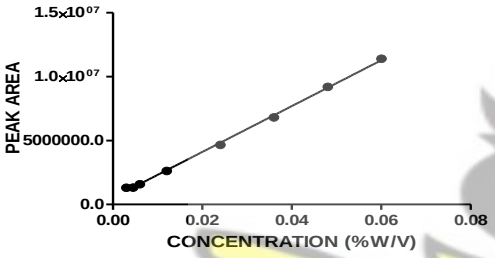
CALIBRATION FOR PURE AMOXICILLIN TRIHYDRATE



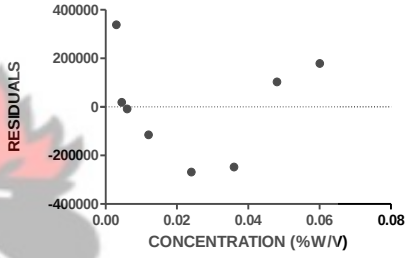
RESIDUAL PLOT FOR PURE AMPICILLIN TRIHYDRATE



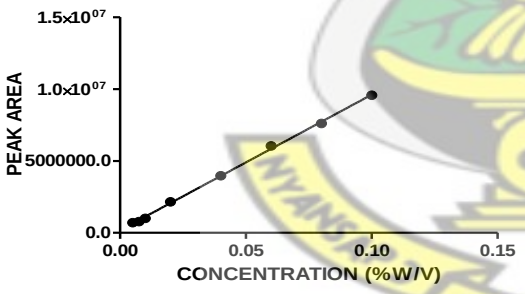
CALIBRATION FOR PURE AMPICILLIN TRIHYDRATE



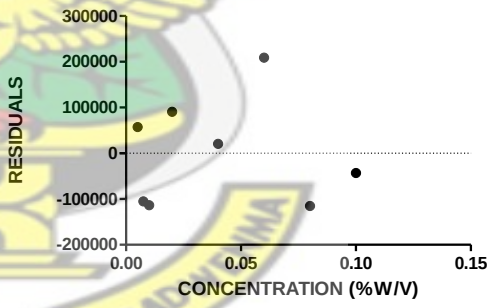
RESIDUAL PLOT FOR PURE AMOXICILLIN TRIHYDRATE



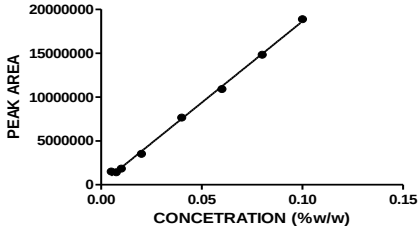
CALIBRATION FOR BENZYLPENICILLIN



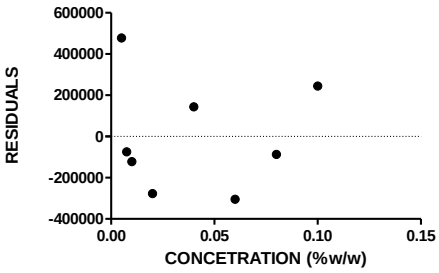
RESIDUAL PLOT FOR BENZYLPENICILLIN



CALIBRATION FOR PURE PHENOXYMETHYL PENECILLIN



RESIDUAL PLOT FOR PHENOXYMETHYL PENICILLIN



PRECISION FOR PENICILLINS

INTRA-DAY PRECISION

AMOXICILLIN TRIHYDRATE

DETERMINATION	%PURITY
1	98.42
2	95.27
3	95.65
4	95.15
5	94.39
6	93.64
7	97.78
8	97.91
9	96.65

AMPICILLIN TRHYDRATE

DETERMINATION	%PURITY
1	99.30
2	102.12
3	102.12
4	99.58
5	98.79
6	98.57
7	102.47
8	98.44
9	100.46

BENZYLPENICILLIN SODIUM

DETEMINATION	%PURITY
1	100.8978
2	96.74717
3	97.8059
4	97.84836
5	98.17504
6	97.74502
7	98.52348
8	99.38843
9	101.6211

PHENOXYMETHYL PENICILLIN SODIUM

DETERMINATION	%PURITY
1	98.50
2	97.87
3	97.82
4	97.13
5	105.10
6	96.64
7	96.42
8	106.95
9	96.03

INTER-DAY PRECISION

AMOXICILLIN TRIHYDRATE

DETERMINATION	%PURITY
1	98.31
2	97.92
3	96.92
4	98.42
5	97.79
6	96.84
7	97.44
8	97.79
9	96.46
10	100.46
11	98.95
12	98.77
13	100.16
14	97.32
15	97.72
16	97.72
17	97.56
18	100.48

AMPICILLIN TRHYDRATE

DETERMINATION	%PURITY
1	99.30
2	102.12
3	102.12
4	99.57
5	98.79
6	98.57
7	102.47
8	98.44
9	100.46
10	98.37
11	98.74
12	96.72
13	97.60
14	104.07
15	96.93
16	96.81
17	96.34
18	98.79

BENZYLPENICILLIN SODIUM

DETEMINATION	%PURITY
1	100.90
2	96.75
3	97.81
4	97.85
5	98.18
6	97.75
7	98.52
8	99.39
9	101.62
10	98.55
11	104.29
12	100.18
13	99.53
14	100.35
15	98.55
16	97.60
17	96.76
18	99.73

PHENOXYMETHYL PENICILLIN SODIUM

DETERMINATION	%PURITY
1	98.50
2	97.87
3	97.82
4	97.13
5	105.10
6	96.64
7	96.42
8	106.95
9	96.03
10	104.6194
11	102.55
12	101.38
13	103.47
14	104.62
15	105.83
16	105.37
17	96.34
18	105.37

ACCURACY FOR PENICILLINS

AMOXICILLIN TRIHYDRATE

		RECOVERY	
DETERMINATION	80%	100%	120%
1	99.80	99.69	99.15
2	99.69	100.33	98.90
3	100.16	101.50	98.90

AMPICILLIN TRIHYDRATE

		RECOVERY	
DETERMINATION	80%	100%	120%
1	98.95	99.40	99.22
2	101.14	100.55	99.51
3	101.88	100.08	99.70

BENZYLPENICILLIN SODIUM

		RECOVERY	
DETERMINATION	80%	100%	120%
1	99.87	99.91	99.92
2	99.34	100.96	100.67
3	101.69	101.12	101.79

PHENOXYMETHYL PENICILLIN SODIUM

		RECOVERY	
DETERMINATION	80%	100%	120%
1	99.98	99.092	100.37
2	100.35	101.23	101.80
3	101.09	101.12	102.07

REBOSTNESS FOR PENICILLINS

FLOW RATE VARIATION

AMOXICILLIN TRIHYDRATE

STANDARD (I.00ML/MIN)	0.80ML/MIN	1.20ML/MIN
100.47	100.90	99.89
100.15	99.839	100.98
100.46	101.10	99.87

AMPICILLIN TRIHYDRATE

STANDARD (I.00ML/MIN)	0.80ML/MIN	1.20ML/MIN	
100.4568	99.83871	99.51677	
99.57894	100.6981	99.13596	
102.1239	100.505	101.268	

BENZYLPENICILLIN SODIUM

STANDARD (I.00ML/MIN)	0.80ML/MIN	1.20ML/MIN	
100.89	102.41	101.14	
99.388	99.94	100.25	
101.62	99.18	99.56	

AMPICILLIN TRIHYDRATE

STANDARD (225nm)	223nm	227nm
100.45	98.95	99.39
99.57	101.14	100.54
102.12	101.88	100.08

PHENOXYMETHYL PENICILLIN SODIUM

STANDARD (I.00ML/MIN)	0.80ML/MIN	1.20ML/MIN
101.37	99.45	99.03
102.55	99.03	99.19
98.50	101.59	101.86

BENZYLPENICILLIN SODIUM

STANDARD (225nm)	223nm	227nm
100.89	99.92	99.91
99.38	100.67	100.95
101.62	101.78	101.11

WAVELENGTH VARIATION

AMOXICILLIN TRIHYDRATE

STANDARD (225nm)	223nm	227nm
100.4798	99.68947	99.80078
100.1555	100.3328	99.68923
100.4645	101.4971	100.1604

PHENOXYMETHYL PENICILLIN SODIUM

STANDARD (225nm)	223nm	227nm
101.37	99.98	99.09
102.55	100.35	101.22
98.50	101.092	101.11

DATA FROM THE CALIBRATION CURVES OF THE PENICILLINS

Penicillins	Correlation co-efficient (R^2)	Slope	Y-Intercept	Range
Amoxicillin trihydrate	0.9988921	287809800 ± 3913105	537348.9 ± 123629.1	0.003 – 0.06%w/v
Ampicillin trihydrate	0.998670	179207000 ± 2670120	525195 ± 84358.6	0.003 – 0.06%w/v
Benzylpenicillin sodium	0.998841	94284300 ± 1311390	179304 ± 69052.7	0.005 – 0.1%w/v
Phenoxymethyl penicillin sodium	0.998394	185199000 ± 3032880	103986 ± 159700	0.005 – 0.1%w/v
Flucloxacillin sodium	0.999451	317362000 ± 3036600	921705 ± 159895	0.005 – 0.1%w/v