SPECTROPHOTOMETRIC ASSAY, VISUAL AND DISSOLUTION TEST OF SELECTED BRANDS OF TETRACYCLINE IN PHARMACIES IN BENIN CITY

BY

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BENIN CITY

APRIL, 2024

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A DISSERTATION SUBMITTED IN PARTIAL FUFILMENT OF THE REQUIREMENT FOR THE AWARD OF DOCTOR OF PHARMACY (PHARM.D) DEGREE OF THE UNIVERSITY OF BENIN, BENIN CITY.

APRIL, 2024

CERTIFICATION

This is to certify that this work was carried out by **ODIASE EMMANUEL AMARACHI (PHA1606826)** in the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, Benin City, Nigeria, in partial fulfillment of the requirement for the award of the Doctor of Pharmacy degree of the university.

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DR. V.O. IMIEJE **DATE**
(AG. HEAD OF DEPARTMENT)

DEDICATION

This project work is dedicated to my maker, the Almighty God, who gave me the grace and strength I needed throughout the course of this pursuit, and to my lovely parents Mr. and Mrs. Odiase, for their all-round support.

ACKNOWLEDGEMENT

I want to express my profound gratitude, and also ascribe all glory and honor to God almighty for keeping me alive and sustaining me till the end of the program.

My sincere gratitude goes to my parents Mr. and Mrs. Odiase for their support, advice and prayers towards the success of this program. Also, my profound thanks and appreciation goes to my beloved siblings for their moral and financial support.

I specially thank my project supervisor Dr.G.E. Aghayere for his commitment, intelligent criticisms and guidance throughout the course of this project research. I am also grateful to all my lecturers in the Faculty of Pharmacy for the knowledge and wisdom imparted throughout the duration of this program.

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ABSTRACT

In recent times, there have been worry over the emergence of substandard drugs in Nigeria. Tetracycline is a common POM (prescription-only medication) drug with diverse clinical and veterinary use. It is often preferred due to its low cost, comparable efficacy and ease of access. This research seeks to improve clinical decision-making, contribute to quality assurance measures and support regulatory endeavors within Benin City. Visual inspection was performed on each brand of tetracycline capsules using the World Health Organization Visual Inspection of Medicines Template. In-vitro release study was performed as outlined in the United States Pharmacopeia, 2023. A modified method of Ahmed *et al.*, 2018 was used with absorbance read at 384nm. Visual inspection of different brands of tetracycline capsules revealed that 90% of the brand analyzed met the required standard. All brands analyzed had dissolution rate in the range of 91.31 to 99.57%, 92.62 to 99.57% and 95.23 to 99.57% at 20, 40 and 60 minutes respectively. Result from spectrophotometric analysis showed that all the brands analyzed had tetracycline hydrochloride content between 91.17% and 98.58% of the label claim, with sample D7 having the least amount of tetracycline hydrochloride 91.1%. From the results above, the various brands of tetracycline available in Benin City are of good quality. However, there are concerns that the brand coded D7 may soon be substandard having failed the visual inspection test. Therefore, periodic checks need to be performed to ensure drug products available for use are of the highest quality so as to safeguard lives and ensure efficacy.

CHAPTER ONE: INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

Infectious diseases have existed as far back as man can trace history to, shaping societies and affecting civilization. These illnesses brought about by pathogenic microbes such as bacteria, viruses, fungi and parasites have had a significant impact on human health and behavior. Its key characteristic - the ability to spread from a host to another person – has led to outbreaks, epidemics and pandemics with far reaching and often disastrous effects at different points in history. For example, the Black Death or bubonic plague in the 14th century, the Spanish Flu in the 19th century and most recently the COVID-19 pandemic, all of which led to countless loss of life and near economic collapse (Glatter and Finkelman, 2021).

Our understanding, prevention, and treatment of infectious diseases have greatly improved as a result of medical advancements. Antibiotics are undeniably integral in the treatment of infectious diseases and are therefore one of the most important class of pharmaceuticals in existence today. Since the serendipitous discovery of penicillin by Sir Alexander Fleming about a 100 years ago, numerous class of antibiotics been discovered and described in literature (Kumar *et al.,* 2020). These discoveries have helped man in the war against microbes, which have been described by many scholars as a seesaw game. However, infectious diseases continue to pose a global concern despite these breakthroughs due to its dynamic nature, and certain challenges have arisen. For example, over reliance and misuse of antibiotics have led to the emergence of antibiotic resistant strains of bacteria such as methicillin-resistant Staphylococcus aureus (MRSA), vancomycin-resistant Staphylococcus aureus (VRSA) and livestock-associated methicillin-resistant Staphylococcus aureus (LA-MRSA) that pose a grave threat to global health (Mohamadi *et al.,* 2023). These resistant strains occur due to genetic mutations, acquisition of resistant genes via horizontal gene transfer (conjugation), modification of drug target site, enzymatic inactivation or the development of efflux pump. Also, the choice of broad-specrum versus narrow –spectrum antibiotics in empirical therapy raises some ethical dilemmas and may contribute to dysbiosis. Therefore, the rise of antibiotics resistance underscores the importance of the right use of these miracle agents, as highlighted by antimicrobial stewardship.

Antibiotics acts on the principle of selective toxicity – they inhibit a vital function of the invading organism(s) that differs qualitatively or quantitatively from functions of host cells. They may target specific organelles, receptor sites or pathways to disrupt bacterial growth. Antibiotics may be classified according to their mechanism of action (inhibitors of cell wall synthesis, inhibitors of protein synthesis, inhibitors of nucleic acid synthesis, inhibitors of key metabolic pathways, disruptors of cell membrane structure or function), mode of action (bactericidal or bacteriostatic), spectrum of activity (broad-spectrum or narrow spectrum), or chemical structure (Calderon and Sabundayo, 2007; Etebu and Arikekpar, 2016). Based on their chemical structure, antibiotics may be classified into many groups such as Aminoglycosides, Beta-lactams, Glycopeptides, Macrolides, Quinolones, Sulphonamides and Tetracyclines (Etebu and Arikekpar, 2016).

1.2 INCIDENCE OF ANTIMICROBIAL RESISTANCE OF TETRACYCLINES

Tetracyclines are the second most common antibiotic group, in both production and usage, worldwide (Liu et al, 2013). In Nigeria, prescription only medicines (POM) such as tetracycline is routinely sold over the counter, contributing to antimicrobial resistance. This practice have been worsened by lack of confidence in the public health sector, as well as increasing economic burden. Studies show that antimicrobial resistance (AMR) is expected to cause about 10 million deaths by 2050, with 40% of these fatalities projected to occur in Africa (Egwuenu *et al.,* 2018). Therefore, prudent use of these antibacterial agents as well as adequate prescription monitoring is required.

1.3 MECHANISM OF ACTION AND RESISTANCE OF TETRACYCLINES

Tetracyclines are broad-spectrum antibacterial agents effective against both Gram positive and Gram negative organisms. They are bacteriostatic agents that inhibit bacterial protein synthesis by binding selectively to the 30S bacterial ribosome, thus preventing attachment of aminoacyl tRNA to the aminoacyl (A) site on the mRNA-ribosome complex. This process inhibits translation, resulting in the formation of non-functional proteins (Shutter and Akhondi, 2023).

Tetracyclines enter bacteria by passive diffusion through porin channels as well as active transport via an energy-dependent system that pumps all tetracycline across the cytoplasmic membrane. Therefore, mechanism of resistance is by use of an energy-dependent efflux pathway, production of a ribosomal protection protein that displaces tetracycline from its target, or enzymatic inactivation of tetracyclines by tetracycline destructase (Arenz and Wilson, 2016; Yilmaz and Özcengiz, 2017; Durães and Sousa, 2019). In modern literature, 12 ribosome protection genes have been identified including Tet(O) and Tet(M). It is believed that these ribosomal protection proteins that occur in both Gram positive and Gram negative bacteria separate tetracycline from its binding site by directly interfering with the stacking interaction of the tetracycline D-ring and certain sites such as 16S rRNAbase present on the 30S ribosome (Grossman, 2016). This mechanism makes otherwise susceptible bacteria become resistant to typical tetracyclines such as tetracycline, minocycline, and doxycycline. However, newer tetracyclines such as tigecycline, omadacycline and eravacycline contain side chains at the C-9 position of the D-ring, thus maintaining their antibacterial property in the presence of the ribosomal protection proteins (Grossman, 2016).

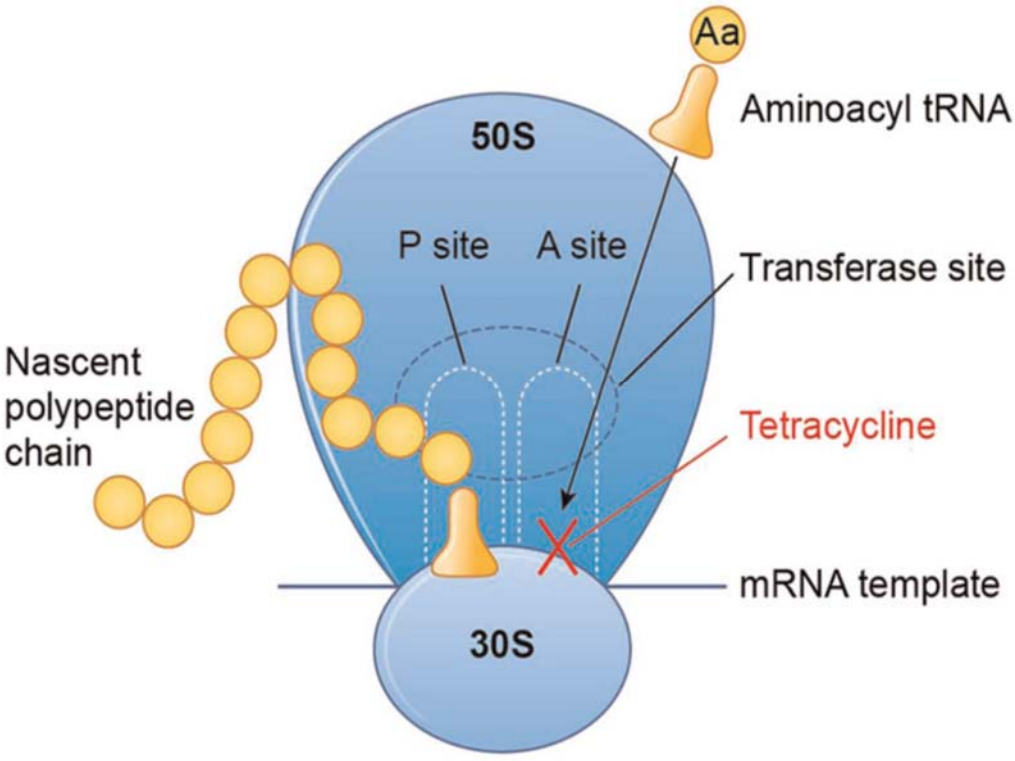


Figure 1.1: Mechanism of action of the tetracycline class of antibiotics (Graber, 2021).

1.4 CLASSIFICATION OF TETRACYCLINES

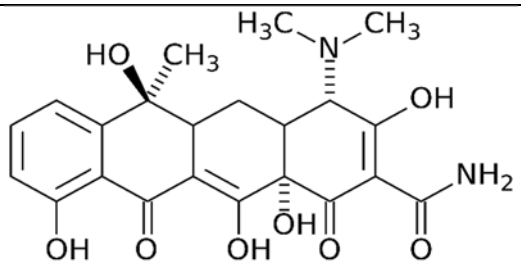
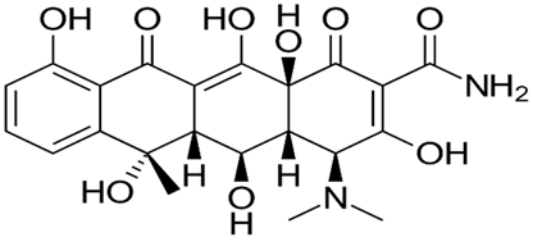
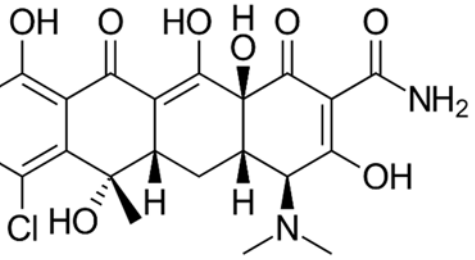
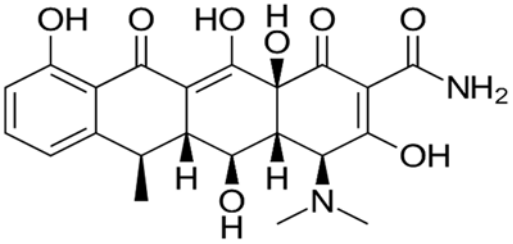
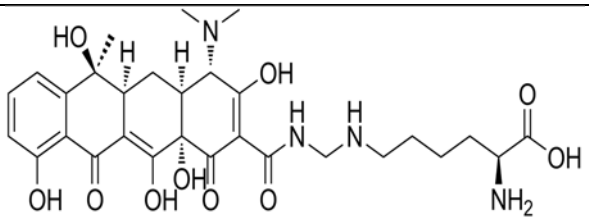
Many scholars (Fuoco, 2012; Shutter and Akhondi, 2014; Etebu and Arikekpar, 2016;) have grouped tetracyclines into classes using various criteria. However, in this paper, teteracyclines will be classified based on the following two criteria.

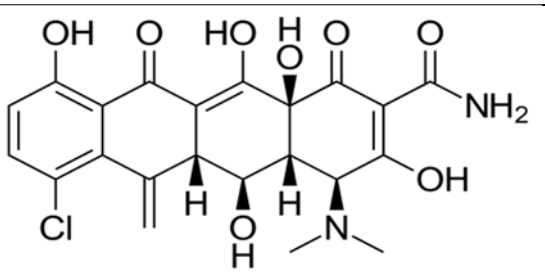
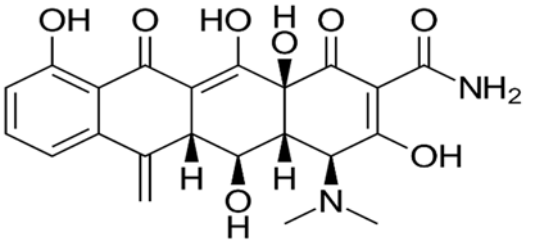
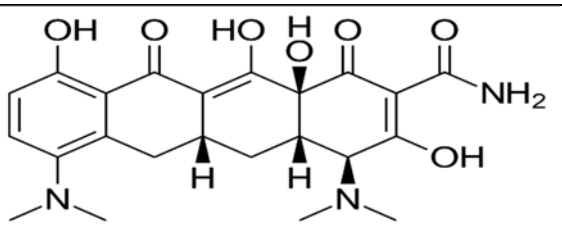
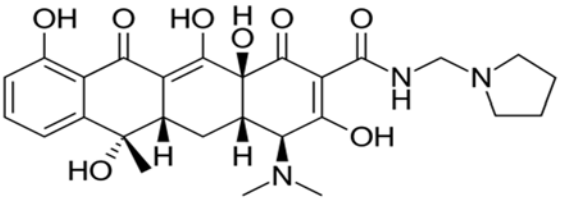
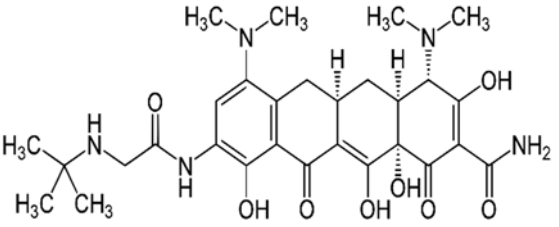
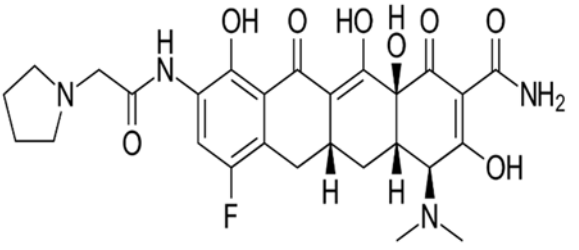
- Source
- Duration of action

1.4.1 Source

According to source, tetracyclines may be classified as natural, semi synthetic or synthetic tetracyclines (Nelson and Levy, 2011). Natural tetracyclines such as tetracycline, chlortetracycline and oxytetracycline are directly obtained from various species of *Streptomyces* bacteria (Simeis and Serra, 2021). They are also referred to as the first generation tetracyclines as they are obtained via biosynthesis (Saleha *et al.,* 2018). For example, Chlortetracycline is obtained from *S. aureofaciens*, *S. lividans* and *S. lusitanus*; Oxytetracycline is obtained from *S. alboflavus*, *S. griseus* and *S. rimosus*; and Tetracycline is obtained from *S. aureofaciens*, *S. avellaneus* and *S. lusitanus*. Semisynthetic tetracyclines although also from natural sources have been modified in a laboratory to improve their properties. They are therefore referred to as second generation tetracyclines (Saleha *et al.,* 2018). Examples of semisynthetic tetracyclines are lymecycline, meclocycline, methacycline, minocycline, rolitetracycline and doxycycline (Nelson and Levy, 2011; Saleha *et al.,* 2018). Synthetic tetracyclines such as tigecycline and eravacycline are referred to as third generation tetreacyclines as these agents are obtained via total synthesis (Saleha *et al.,* 2018, Morrissey *et al.,* 2020). However, it must be noted that newer semisynthetic tetracyclines such as omadacycline and sarecycline are considered third generation tetracyclines (Rusu and Buta, 2021). Also, many scholars classify tigecycline and its congeners as a distinct family of antibiotics referred to as glycylclines (Fuoco, 2012).

Table 1.1: Class of Tetracyclines according to source and their chemical structures

Name of Drug	Class (According to source)	Chemical structure
Tetracycline	Natural	
Oxytetracycline	Natural	
Chlortetracycline	Natural	
Doxycycline	Semisynthetic	
Lymecycline	Semisynthetic	

Meclocycline	Semisynthetic	
Methacycline	Semisynthetic	
Minocycline	Semisynthetic	
Rolitetracycline	Semisynthetic	
Tigecycline	Synthetic	
Eravacycline	Synthetic	

1.4.2 Duration of Action

Tetracyclines may be classified as short-acting, intermediate-acting or long-acting based on their serum half-life (Agwuh and MacGowan, 2006). Short-acting tetracyclines such as tetracycline, oxytetracycline and chlortetracycline generally have a half-life of 6-8 hours. Intermediate-acting tetracyclines such as demeclocyclne and methacycline have serum halflife of about 12 hours while long-acting tetracyclines such as doxycycline, minocycline and tigecycline have serum half-life of at least 16 hours.

Table 1.2: Clinical and pharmacokinetics data on tetracyclines (Saleha *et al.,* 2018)

Name of Drug	Clinical Data	Pharmacokinetic Data				
	Administration Route	Bioavailability	Protein Binding	Metabolism	Biological halflife	Excretion
Tetracycline	Oral, Topical	75%	-	Not metabolized	8-11hrs	Renal, Feces
Oxytetracycline	Oral, Topical	-	-	-	6-8hrs	Renal
Chlorotetracycline	Oral, Topical	30%	50-55%	Hepatic	5.6-9hrs	Renal, Biliary
Demeclocycline	Oral		41-50%	Hepatic	10-17hrs	Renal
Doxycycline	Oral, Intravenous	100%	90%	-	15-25hrs	Renal
Lymecycline	Oral	100%	-	-	-	Renal
Methacycline	Oral	-	-	-	-	Renal
Minocycline	Oral	100%	-	Hepatic	11-12hrs	Renal, Feces
Rolitetracycline	Oral	-	-	-	-	Renal
Tigecycline	Intravenous	100%	71-79%	Not metabolized	42.4 hrs	Renal, Biliary

1.5 THERAPEUTIC USES OF TETRACYCLINE

Despite having identical structures, tetracyclines differ greatly in their range of adverse effects and antibacterial action, which has an impact on their clinical use. Tetracycline is a dynamic drug molecule with wide utility against numerous gram-positive and gram-negative bacterial infections. It is a relatively cheap and readily available antibiotic used in the treatment of chlamydia, syphilis, gonorrhea and many other sexually transmitted infections (STIs) (Tanveer *et al.,* 2020). It is used in the treatment of ulcer when the presence of *Helicobacter pylori* is confirmed (Salmanroghani *et al.,* 2018). Tetracycline may be used in treatment of severe cholera to reduce the duration of diarrhea (Leibovici-Weissman *et al.,* 2014). It is also used in the treatment of acne due to its bacteriostatic effect on the anaerobic, gram-positive *Cutibacterium acnes* (Tanveer *et al.,* 2020). It is beneficial in the management of peridonitis (Nadig and Shah, 2016). It is also used as an alternative to penicillin in certain antibacterial therapies when the latter is contraindicated (Nelson and Levy, 2011). Species such as *Yersinia pestis, Bacillus anthracis, Francisella tularensis, Chlamydia psittaci, Chlamydia pneumonia, Mycoplasma pneumonia and Balantidium coli* have been shown to be susceptible to tetracycline when used alone or in combination. Aside its primary use as an antibiotic, tetracycline also has other clinical applications and potentials. Tetracycline facilitates wound healing by promoting the adhesion of fibroblasts, with the inhibition of pathogens at wound site as an added advantage (Lin *et al.,* 2022). Tetracycline also has slight anti-inflammatory properties by inhibiting inflammatory mediators such as reactive oxygen species (ROS). Tetracycline can therefore be used in management of inflammatory diseases mediated by IL-1β and IL-18 (Peukert *et al.,* 2021). When radio-labelled, tetracycline may be used to detect infarct or tumor, thus helping in the early detection of cancer (Saleha *et al.,* 2018). It may also be used for renal imaging to evaluate urinary pathological conditions. (Alok and Chaudhury, 2016) posits that tetracycline has radio-protective properties on DNA due to its ability to scavenge free radicals by decreasing the formation of malondialdehyde. This suggests that tetracycline may be used to reduce damage by radiations on body tissues. Tetracycline may also be used in the treatment of acute respiratory disease syndrome due to its inhibitory action on capase-1, with clinical trials ongoing (Peukert *et al.,* 2021).

1.6 SIDE EFFECTS OF TETRACYCLINE

Drug-related side effects are a major contributor to morbidity and mortality, resulting in a substantial financial burden and reduced quality of life. There are numerous side effects associated with the use of tetracycline. Tetracycline should not be used during pregnancy and in children below eight years as it may result in a permanent discoloration of teeth (Lee *et al.,* 2023). This is because tetracycline adhere to calcium ions in developing teeth during mineralization resulting in the formation of tetracycline calcium orthophosphate complex which is incorporated into teeth, cartilage and bone (Enabulele *et al.,* 2020; Yu, 2022). This permanent staining varies from yellow or gray to brown depending on the dose of drug received in relation to body weight, and may have a psychological effect on the individual. Tetracycline may also cause enamel hypoplasia of the dentition (Bloomquist *et al.,* 2021). In certain tetracycline users, photosensitivity characterized by an excessive sunburn reaction has been reported (Orylska-Ratynska *et al.,* 2022). UVA (320–400 nm) is the region of the solar spectrum that is most frequently associated with phototoxicity. Nonetheless, visible light and UVB (290–320 nm) radiation may encourage the emergence of such a response (Lee and Thompson, 2006). Susceptible patients should therefore be advised to avoid contact with sunlight and therapy with tetracycline should be stopped at the first sign skin erythema. Following exposure to light, a reaction may manifest itself within minutes or hours, depending upon skin type and pigmentation (Odorici *et al.,* 2021).

Tetracycline have also been associated with intracranial hypertension which may result in permanent vision loss (Orme *et al.,* 2020). Other side effects associated with tetracycline use include diarrhea, nausea, vomiting, rashes, black hairy tongue, white patches in the mouth, epigastric distress, anorexia and hypersensitivity (Enabulele *et al.,* 2020).

1.7 PHYSIOCHEMICAL PROPERTIES OF TETRACYCLINE

i. Tetracycline is amphoteric in nature with pKas of 3.32, 7.78 and 9.58 due to its trione, phenolic-enol and diethylamino groups respectively (Wang *et al.,* 2016). Therefore, tetracycline form stable, acidic salt when its N- diethylamino group on C4 is protonated.

Conversely, it forms unstable basic salt when sodium hydroxide or potassium hydroxide interacts with its enolic hydroxyl groups at C10-C12.

ii. Tetracycline exist in its cationic form in acidic solutions (pH < 4), as zwitterion in near neutral solutions (pH 5 – 7) and in its anionic form at high pH (Gao *et al.,* 2012).

iii. It has five stereogenic centres, with the carbon atoms at 4,4a,5a,6 and 12a being chiral.

iv. It is often encapsulated due to its bitter taste as well as to protect the drug from photodegradation (Narkhede *et al.,* 2011).

v. At lower pH, tetracycline undergoes epimerization at C4 to form 4-epitetracycline (Jia *et al.,* 2009). The 4-epitetracycline possesses less activity than tetracycline itself.

vi. In concentrated acid, tetracycline is converted into anhydrotetracycline especially on heating (Kuhne, 2001). Anhydrotetracycline may further convert to form its epimer known as 4-epi-anhydrotetracycline.

vii. Tetracycline form stable chelates with divalent metals including calcium, magnesium, aluminium and iron (Zhao *et al.,* 2022). This can result in impaired absorption of the drug in the presence of milk and antacids, as well as deposition in developing bone and teeth.

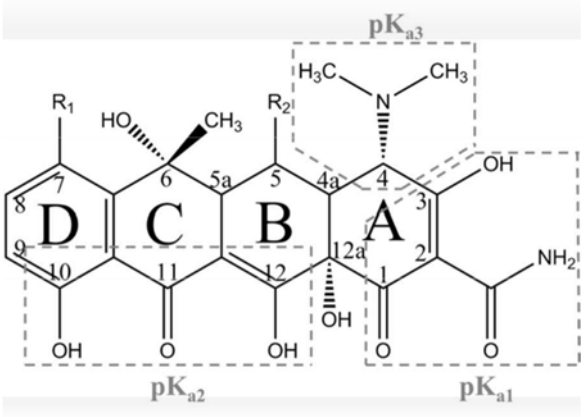


Figure 1.2: Structure of tetracycline highlighting the pKa regions (Wang *et al.,* 2016)

1.8 STRUCTURAL ACTIVITY RELATIONSHIP OF TETRACYCLINE

i. Tetracycline is a rigid, planar structure comprising of at least four fused rings (napthacene nucleus) with many substituents such as alkyl, hydroxyl and amine groups at different sides of the molecule (Saleha *et al.,* 2018; Nelson and Levy, 2011).

ii. Orientation of the DCBA napthacene ring system linearly is necessary for antibacterial activity and at least four six-membered rings is required for activity (Saleha *et al.,* 2018).

iii. Antibacterial activity also depends on the presence of an amino group at C4 and ketoenolic tautomers at C1 and C3 (Fuoco, 2012).

iv. Epimerization of the dimethylamine group at position C4 from 4S to 4R isomer results in product with much less activity against Gram negative bacteria (Ozdemir *et al.,* 2010).

v. Major alteration in the substitution at C1-C4, C10, C11, C11a and C12 will result in loss of activity. For example, modification of amide group at C2 or alkylation at position C11 causes loss of activity (Rusu and Buta, 2021).

vi. Aminoalkylation of the amide group increases the polarity of tetracycline (Ramachanderan and Schaefer, 2021).

vii. Modifications can be made at positions C5-C9 to obtain products with similar or improved antibacterial properties. For example, the presence of hydroxyl group at position C5 in doxycycline leads to better antimicrobial activity (Fuoco, 2012). Similarly, introduction of strong electron withdrawing groups such as chloro- or strong electron donating groups such as dimethylyamino at position C7 enhances activity.

viii. Tetracyclines substituted at position C9 with a glycylamido group have shown good activity against resistant bacterial strains (Tariq *et al.,* 2018).

ix. Chemical alteration of the R1, R2 and R3 groups confer better antifungal selectivity with no corresponding effect on antibacterial activity (Saleha *et al.,* 2018).

x. Chemical alteration at R4, R5 and R6 confer better antibacterial specificity as well as significant changes in pharmacokinetic parameters (Fuoco, 2012).

xi. Modifications and orientation of groups on ring A are deterministic in their clinical applications (Fuoco, 2012).

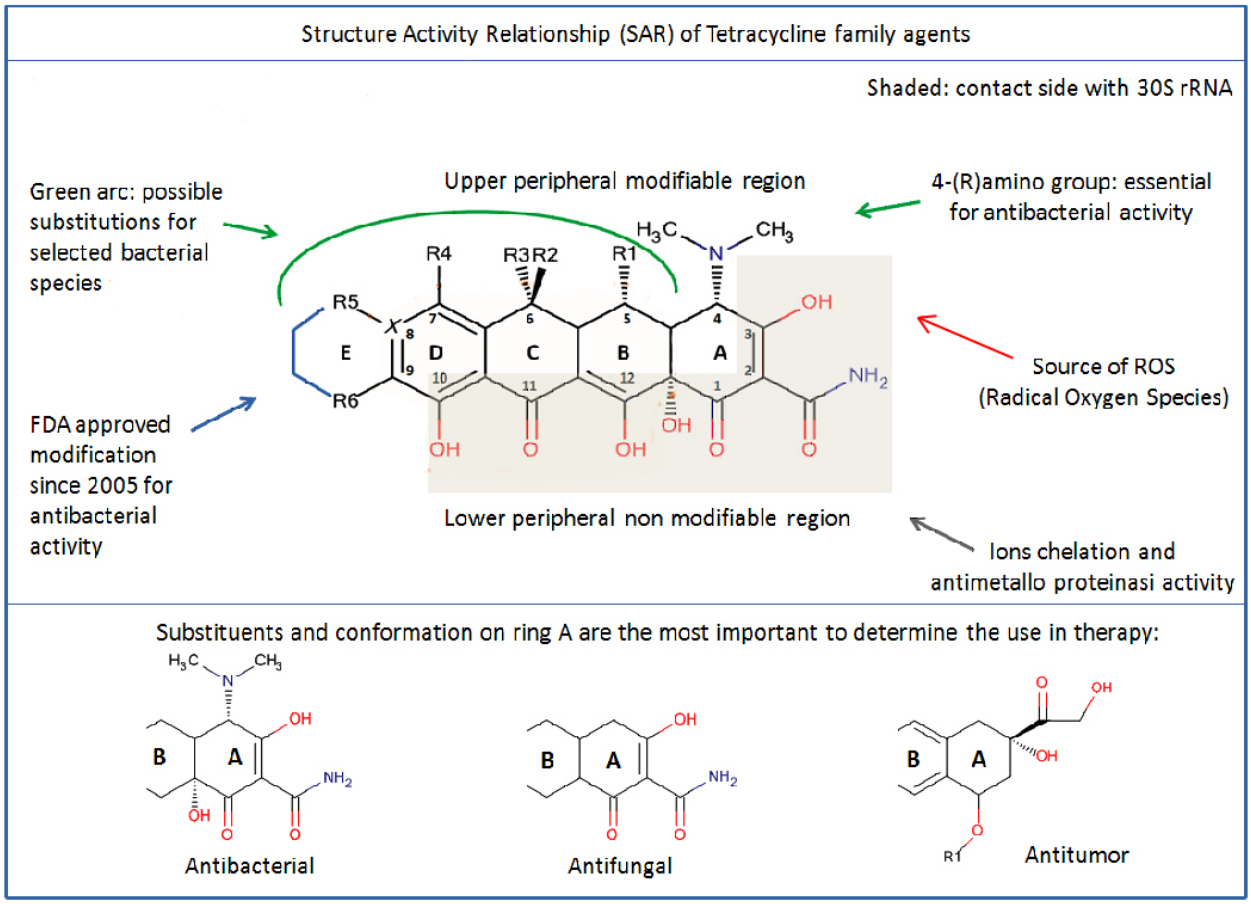


Figure 1.4: Structural activity relationship of Tetracycline (Fuoco, 2012)

1.9 PHARMACEUTICAL ANALYSIS

Pharmaceutical analysis is a broad terminology used to describe processes performed for the identification, detection, separation, qualification, quantification, purification, and structural elucidation of one or more compound(s) in a pharmaceutical product (Irshad *et al.,* 2020). Pharmaceutical analysis may be executed on active pharmaceutical ingredients (APIs), excipients, drug metabolites or drug contaminant(s) if present in a pharmaceutical formulation. The sample itself may be a finished product of any dosage form, biological sample such as urine, or pharmaceutical raw material. Pharmaceutical analysis may be broadly categorized into qualitative and quantitative analysis (Akash and Rehman, 2020). In qualitative analysis, the focus is on identifying the different components present in a sample. Here, the presence or absence of specific functional groups or element(s) in a sample is established via various

methods such as infra-red (IR) spectroscopy. Quantitative analysis however involves processes used in the determination of the amount of a substance of interest in a given sample. Examples of such methods are titrimetry and ultraviolet-visible (UV-vis) spectroscopy.

Pharmaceutical methods of analysis may be further classified into physiochemical, biological and biopharmaceutical method of analysis.

I. Physiochemical method of analysis (Akash and Rehman, 2020; Siddiqui *et al.,* 2017)

1. Classical Methods of analysis
    a. Gravimetry
    b. Titrimetry
2. Instrumental Methods of analysis
    a. Absorption of Radiation Methods
        i. UV-vis spectroscopy
        ii. Atomic absorption spectroscopy
        iii. IR spectroscopy
    b. Emission of Radiation Methods
        i. Atomic emission spectroscopy
        ii. Nuclear magnetic resonance(NMR) spectroscopy
        iii. Fluorescence spectroscopy
        iv. Flame spectroscopy
    c. Electrochemical Methods
        i. Potentiometry
        ii. Polarography
        iii. Voltammetry
        iv. Amperometry
    d. Chromatographic Methods
        i. Column chromatography
        ii. Thin layer chromatography (TLC)
        iii. Gas liquid chromatography (GLC)
        iv. High-performance liquid chromatography (HPLC)
        v. Liquid chromatography (LC)

e. Hyphenated techniques
    i. Liquid chromatography-mass spectrometry (LC-MS)
    ii. Gas chromatography-mass spectrometry (GC-MS)
    iii. High-Performance Liquid Chromatography-Ultraviolet spectrophotometry (HPLC-UV)
    iv. High-Performance Liquid Chromatography - mass spectrometry/ mass spectrometry (HPLC-MS/MS)
    v. Capillary electrophoresis-mass spectrometry (CE-MS)

3. Radiochemical Methods of analysis
    a. Ionization method
    b. Liquid scintillation method
    c. Radiometric titration
    d. Isotope dilution
4. Thermal Methods of analysis
    a. Differential thermal analysis (DTA)
    b. Thermogravimetric analysis (TGA)
    c. Differential scanning calorimetry (DSC)

II. Biological/Microbiological method of analysis
    a. Minimum inhibitory concentration (MIC)
    b. X-ray crystallography
    c. Immunohistochemistry
    d. Flow cytometry
    e. Pyrogen testing

III. Biopharmaceutical method of analysis (Adejumo *et al.*, 2021)
    a. Disintegration test
    b. Hardness test
    c. Friability test
    d. Dissolution test

1.10 SPECTROSCOPY

Spectroscopy is the study of interaction of energized radiations from the electromagnetic spectrum with matter. It involves the creation, measurement, and interpretation of spectra resulting from the interaction of electromagnetic radiation (Nielsen, 2010). These electromagnetic radiations include gamma rays, x-rays, ultraviolet rays, visible light, infrared, microwaves and radiowaves as illustrated in Figure 1.5. Numerous spectroscopic techniques are available to address a broad spectrum of analytical issues. The techniques vary depending on the species to be examined (molecular or atomic spectroscopy, for example), the kind of radiation–matter interaction to be tracked (diffraction, emission, or absorption), and the region of the electromagnetic spectrum to be used in the investigation. For both quantitative and qualitative analysis, spectroscopic approaches are highly informative and frequently utilized. The most widely used spectroscopic techniques are those that depend on the absorption or emission of radiation in the visible (Vis), infrared (IR), radio (nuclear magnetic resonance, NMR), and ultraviolet (UV) regions.

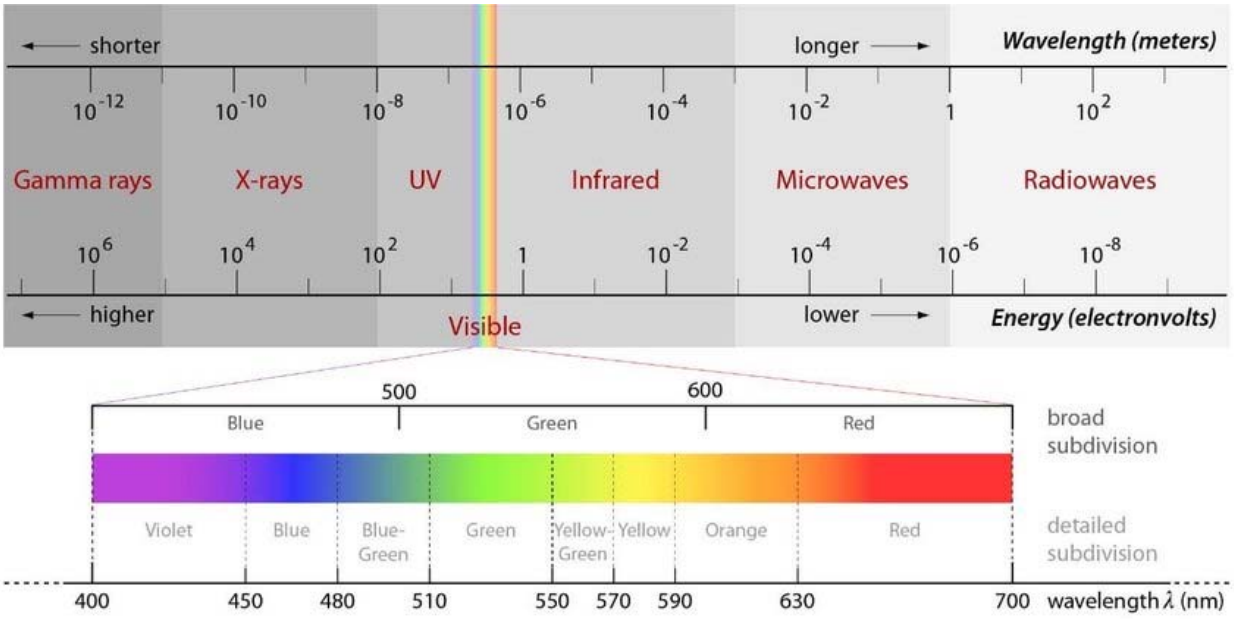


Figure 1.5: Electromagnetic Spectrum (Verhoeven, 2017)

1.10.1 ULTRAVIOLET–VISIBLE (UV–vis) SPECTROSCOPY

Ultraviolet–visible (UV–vis) spectroscopy is a technique employed in assay to determine concentration of analyte in solution. It is a type of absorption spectroscopy whereby the amount of EMR absorbed by a sample is measured. A UV-vis spectrophotometer is an instrument that performs spectroscopy in the ultraviolet-visible region to analyze a molecule of interest (Nielsen, 2010). The wavelength range of electromagnetic radiation in the UV–Vis region of the spectrum is about 190–800nm and the analyte should be able to absorb light within this range (Passos and Saraiva, 2018; Shard *et al.,* 2020). The UV range is between 200 and 350 nm while the Vis range extends from 350 to 700 nm. To the human eye, the visible spectrum has distinct colors at each wavelength, ranging from red to violet while the UV range appears colorless. UV-vis spectrophotometer is widely used in the laboratory due to its relatively low cost, versatility, availability, ease of use, and quantitative nature (Shard *et al.,* 2020). It is also a non-destructive technique. UV–vis spectrophotometry is very useful for monitoring chemical reactions in cases where the reactants and products have different absorbance features. It can therefore be used for both qualitative and quantitative pharmaceutical analysis, as well as for kinetic studies (Kalmár *et al.,* 2016; Akash and Rehman, 2020). For analytical purposes, the Beer–Lambert law which states that there is a linear relationship between how much light is absorbed (A) and the concentration (c) of the absorbing sample, is applied. Mathematically,

$A \alpha c$

$A = \varepsilon cb$

where

A = Absorbance

ε = Molar absorptivity ($L.mol^{-1}cm^{-1}$)

c = Molar concentration of the analyte in solution ($mol.L^{-1}$)

b = Path length of the sample (cm)

During experimentation, radiation of specific wavelength(s) is passed through the solution to be investigated. The sample's radiation exposure is then quantified in relation to a reference

material. The analyte concentration is estimated using the proportion of light that travels through the sample. When compared to the radiation leaving the cell's other side, I, the radiation impacting on the absorption cell, I_0, will have a substantially higher radiant power. This is due to the absorbing substance capture (absorption) of photons as the beam moves through the solution. This relationship between the power of the impacting and leaving beam may be expressed in terms of absorbance or transmittance (Nielsen, 2010). As transmittance increases, the absorbance decreases and this relationship is represented mathematically below.

$$T = I/I_0$$

$$\%T = (I/I_0) \times 100$$

$$A = - \log(I/I_0)$$

$$A = - \log T$$

$$A = 2 - \log (\%T)$$

In UV-vis spectroscopy, another important concept is the promotion of electrons in an orbital from lower to higher energy states. A chromophore is simply the light absorbing moiety of a chemical structure that gives it color. When a molecule absorbs light in the visible region and transmit or reflect other light, color is created (Shukla *et al.,* 2017). Substances such as water or ethene that do not absorb light in the visible region appears colorless. Conversely, substances that absorb visible light at all wavelength will appear black. In order to absorb visible light that strikes the chromophore, an electron must be stimulated from its ground state into an excited state. This promotion of electrons will occur first in the one that require a lower energy to excite its bonding electron to its antibonding orbital (Verma and Mishra, 2018). Also, for an organic molecule to absorb light in the visible region, it has to possess some form of conjugated π system. The wavelength of maximum absorptivity (λmax) corresponds to the point at which transition of electron from its ground state to an excited state is most probable. Increase in conjugation in a molecule is positively associated with increased wavelength of light that is absorbed. Therefore, an extensively conjugated molecule will absorb light at higher wavelength (>400nm) and therefore should have color. This is why carrots have color as they absorb light

strongly in the visible region. β-carotene absorbs blue light and reflects orange as observed by its λmax value of 453nm (Hagos *et al.,* 2022).

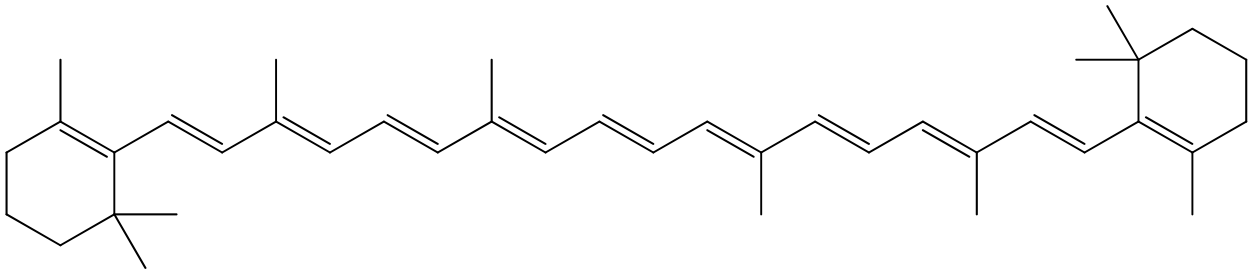
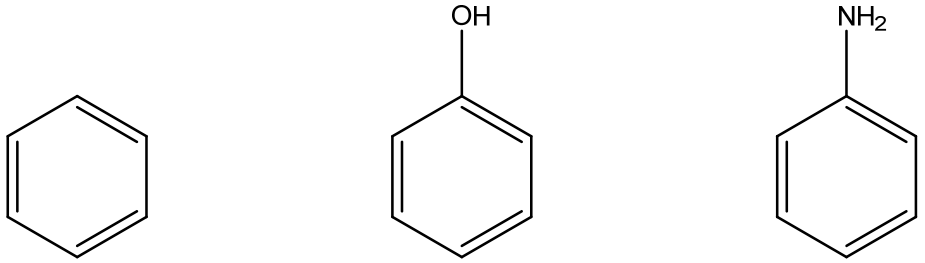


Figure 1.6: Structure of β-carotene

Auxochrome is any functional group present in a molecule that modify the chromophore's capacity to absorb light. It is the color enhancing moiety in a substance that neither absorbs light in the UV region nor produces color themselves, but when linked to a certain chromophore, increase the wavelength of absorption to intensify color initially produced by the chromophore. Examples of auxochrome include sulfhydryl group (–SH), amino group (–NH2), hydroxyl group (– OH), and halogens (Akash, 2020).



Benzene
λmax = 255nm

Phenol
λmax = 270nm

Aniline
λmax = 280nm

Figure 1.7: Structure of benzene illustrating the auxochrome effect of various substituents

Generally, substituents may impact any of the following effect on uv absorption by the chromophore viz; hyperchromic shift, hypochromic shift, bathochromic shift and hypsochromic shift (Sigurdson and Giusti, 2014). Bathochromic shift (redshift) is shift of λmax to a longer

wavelength due to the presence of an auxochrome, or change in pH. Hypsochromic (blue shift) is shift of λmax to a shorter wavelength. It is caused by the removal of conjugation or change in the solvent pH. An effect such as the introduction of an auxochrome or change in solvent that leads to an increase in absorption of light by a molecule is called hyperchromic effect. Conversely, an effect such as the introduction of a group that alters the principal chromophore or change in solvent that leads to a decrease in absorption of light by a molecule is called hypochromic effect. The various effects are illustrated below.

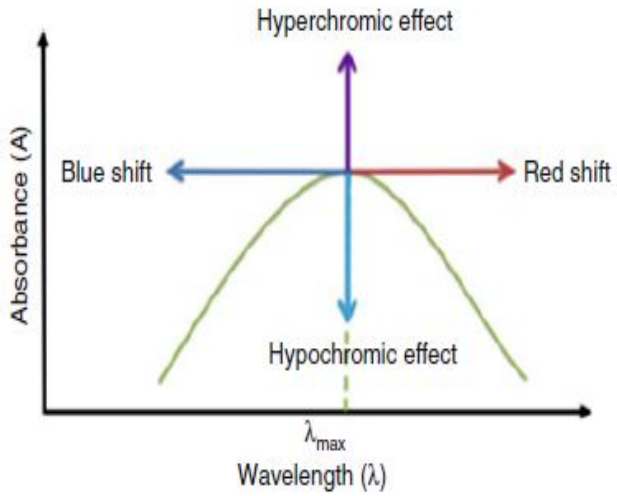


Figure 1.8: Schematic representation of the various shifts (Akash, 2020)

1.10.2 Components of Ultraviolet–visible spectrophotometer

A UV-vis spectrophotometer consists of a light source, monochromator, cell (sample and reference), detector and recording device. Broadband light sources that emit all wavelengths are typically used in UV–vis spectrometer operations. Generally, deuterium lamps are used to achieve intensity at shorter wavelengths (190-400nm), while quartz-tungsten-halogen lamps are utilized to obtain sufficient intensity at wavelengths larger than 400 nm (Shard *et al.,* 2020). A diffraction grating or prism is used to monochromate the lamp's output, which is then divided into two paths for a double-beam spectrophotometer; one travels through the sample, while the other is a reference path. The (intensities of light beam of the sample and reference is simultaneously measured) values of I and I_0 are provided, respectively, by detectors at the conclusion of each path. However, for a single beam spectrophotometer, all of the light travels

through the sample cell. In order to exclude influences from the solvent and the cell, the instrument is standardized by setting a reference in the sample holder. The intensity of light after traveling through the cell is measured and the resulting value is then deducted from subsequent sample tests. A cuvette is a transparent cell that is usually used to hold samples. It is rectangular in shape, with an interior width of 1 cm in most cases. This width represents the path length in the Beer-Lambert law as shown in the equation above. Although cuvettes are available as plastics, glass, quartz and silica, the glass and plastic cuvettes are not used for UV analysis as they absorb light in the UV region to give indiscernible spectra (Trumbo *et al.*, 2013). Light is transformed into a readable electrical signal by a detector once it has passed through the sample. The detector used should to be quick to respond and sensitive across a wide spectrum of wavelengths. Furthermore, the detector's electric signal needs to be proportionate to the intensity that is being transmitted. Typically, photoelectric coatings or semiconductors serve as the foundation for detectors. When a photoelectric coating is exposed to light, electrons are released and an electric current proportionate to light intensity is produced. One of the more popular detectors used in UV-Vis spectroscopy is the photomultiplier tube. A photomultiplier tube works on the principle of photoelectric effect, which causes electrons to be initially ejected when exposed to light. These ejected electrons then multiply successively to produce a greater electric current. Photodiodes and charge□coupled devices are examples of detectors based on semiconductor technology. The signal produced by the detector is then sent to an amplifier and the readout is displayed.

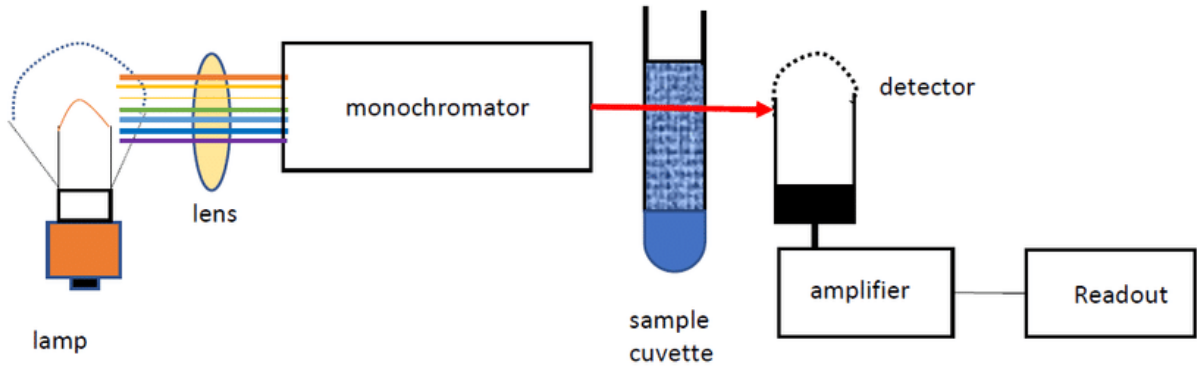


Figure 1.9: Schematic representation of a UV-vis spectrophotometer (Rahman *et al.*, 2020)

1.10.3 Limitations of Beer-Lambert Law

Since Beer-Lambert law is closely related to absorbance, it is therefore a fundamental concept in spectroscopy. Beer-Lambert law describes the relationship between light motion and the characteristics of the material that light passes through. Beer's law should ideally only be used with radiation that is actually monochromatic. It is predicated on the assumption that radiation beam is absorbed by a single molecular species independently of others; the probability of absorption is not altered by interactions between molecules; and the sample is homogeneous and does not scatter the radiation (Oshina and Spigulis, 2021). However, strict adherence to each of these requirements, however, might not be practical in practice. Also, although the relationship described by Beer-Lambert law is linear, there are circumstances in which it deviates and becomes non-linear. These deviations may be broadly classified into three classes viz; real deviation, chemical deviation and instrumental deviation (Akash, 2020). (Nielsen, 2010; Akash, 2020) highlights the following as primary reasons for this departure from Beer Lambert's law.

i. Differences in the optical properties or path lengths between the cuvettes holding the sample and the blank solutions.

ii. Concentration of a sample may affect its absorbance reading. Absorbance and molar absorptivity depends on refractive index, and the refractive index of a sample is affected by the sample concentration. Therefore, at increasing concentrations, a sample may not conform to Beer-Lambert law as its individual particles cease to interact independently with one another.

iii. When a single wavelength of light is not employed, a deviation may occur. Also, the presence of even tiny amounts of dispersed radiation may contaminate the radiation coming from a monochromator. The surface of gratings, mirrors, filters, lenses, and windows typically reflect and/or scatter light, producing stray radiation. Similar to the deviation caused by the polychromatic light, a divergence from Beer-Lambert law is observed when the analyte absorbs at the wavelength of the stray radiation.

iv. The presence of a chemical specie in a sample that results in interaction of the sample with solvent used, thus yielding a product with different absorption properties.

v. Presence of another material that absorbs at the same wavelength as the analyte.

1.11 ASSAY OF TETRACYCLINE

Pharmaceutical analysis is often required to ascertain if a pharmaceutical product has attained minimum standards for use. Generally, this minimum standard is usually that which have been specified in the monograph or other official books such as British Pharmacopoeia (BP), United States Pharmacopoeia (USP), and European Pharmacopoeia (Ph. Eur). These are legally binding books published by national or local authorities that contain a list of quality requirements and criteria for medications used in a region, as well as instructions for the identification of drug formulations (De *et al.,* 2024). Pharmaceutical analysis is also required to ensure consistency in the quality of a product is maintained. This is because production of drug molecules may differ from batch to batch in terms of color or viscosity, and therefore periodic assay is required to maintain consistency and uniformity. An assay procedure must ensure that the active ingredient of interest is fully released for analysis.

Standard requirement for tetracycline capsules are defined both in the BP and USP (BP, 2023; USP, 2023). Tetracycline is a yellow, crystalline powder that is stable to air but susceptible to sunlight. It is slightly soluble in water, freely soluble in dilute acid, alkyl carbonates and alkali

hydroxyl solutions, sparingly soluble in alcohol, and insoluble in chloroform, acetone and ether. Its IUPAC name is (4S,4aS,5aS,6S,12aS)-4-(dimethylamino)3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene -2-carboxamide with molecular formula C22H24N2O8. Tetracycline capsules must contain within 95-105% of the stated amount of tetracycline (BP, 2023) or 90-125% of the stated amount of tetracycline (USP, 2023).

Numerous methods have been employed in the assay of tetracycline from literature including fluorescence spectroscopy (Namegabe *et al.,* 2018), atomic absorption spectrophotometry (Abdulghani *et al.,* 2013), liquid chromatography (Desmarchelier *et al.,* 2018), capillary electrophoresis (Wu *et al.,* 2016) and UV-vis spectroscopy (Ali *et al.,* 2018). Assay of tetracycline capsule using UV-vis spectroscopy may be performed by the use of 1N sodium hydroxide and 0.1% sodium hypochlorite solution as solvent, with measuring wavelength of 400nm (Ahmed *et al.,* 2018). (Ahmida *et al.,* 2009) assayed tetracycline capsules using 0.01M potassium permanganate and 0.5M sodium hydroxide as solvent, with measuring wavelength of 610nm. (Ali *et al.,* 2018) assayed tetracycline capsules by using a cocktail of chemical solutions

including pure ethanol as solvent. Absorbance was measured at 403nm using a UV-vis spectrophotometer. (Hameedi, 2021) assayed tetracycline capsules using 0.01M 2,4 dinitrophenylhydrazine and 0.025M potassium preiodate at an absorbance reading of 488nm.

1.12 BACKGROUND TO THE STUDY

In recent times, there have been worry over the re-emergence of substandard drugs in Nigeria. This is due to the negative health and socio-economic impact on the populace. Such impacts include ineffective treatment, increased mortality rates, emergence of drug-resistant strains, increased healthcare cost, decreased productivity at work, pain and suffering. Substandard drugs in the pharmacy may be due to poor adherence to good manufacture practices, poor storage conditions, or mischievous producers/suppliers that want to make a quick gain. Therefore, discussions bordering around substandard drugs now occupy a center stage in academic discourse and other public fora both at the national and international levels. Addressing the problem of substandard drugs involves a multi-faceted approach, including identifying substandard drugs, strengthening regulatory frameworks, enhancing supply chain security, raising public awareness, and increasing collaboration among stakeholders to combat the production and distribution of fake medications, as well as to provide better storage conditions for drugs.

For any drug formulation, one of the most important factor often considered is its efficacy. Efficacy of the drug can be measured by assaying its content. Various tests are available to assay tetracycline such as UV-vis spectroscopy and liquid chromatography. However, the UV-vis spectroscopy method is a relatively simple, accessible and accurate method to assay tetracycline especially in a developing country like Nigeria. Although tetracycline is available in different dosage forms such as capsules and ointment, tetracycline capsules is much more common and used in Nigeria. Different brands of tetracycline are currently available in the drug market, and many more are introduced periodically. There is also an accompanying possibility of fake, substandard, or poorly stored drug. It is therefore essential to have a better information regarding the quality of tetracycline capsules available in the drug market so as to guide healthcare providers decision making.

1.13 PROBLEM STATEMENT

Tetracycline is a common POM (prescription-only medication) drug indicated for numerous conditions. In Nigeria, it is commonly used to treat acne and other bacterial infections, and is often preferred due to its low cost, comparable efficacy and ease of access. It is also used in vetinary practice to treat conditions such as contagious bovine pleuropneumonia. Due to its high ease of access in Nigeria, tetracycline is now commonly misused both in humans as well as in animals (Kehinde and Babatunde, 2013). Such indiscriminate use contributes to antimicrobial resistance with major implications for human and public health alike. Also, substandard products may exist in the pharmacy that may endanger the health of the consumer (Mukhtar *et al.,* 2023). Despite increasing awareness and numerous regulatory controls, the presence of substandard tetracycline still persists. It is therefore necessary to conduct pharmaceutical evaluations on oral tetracycline products available in the Nigerian market. Hence the need for this research.

1.14 JUSTIFICATION OF STUDY

The introduction of generic drug products from diverse source into the Nigerian healthcare system have been a double edged affair. While this action has made drugs relatively cheaper and readily available, a variety of problems have also emerged with the widespread distribution of fake and substandard drug products being a major concern. Numerous brands of tetracycline are currently available in the Nigerian drug market, and much more are being introduced periodically. These brands also vary in cost and potency. This research seeks to improve clinical decision-making, contribute to quality assurance measures and support regulatory endeavors within Benin City.

1.15 AIM AND OBJECTIVES OF THE STUDY

The aim of this study is to determine the pharmaceutical quality of tetracycline hydrochloride capsules in Benin City. To achieve this aim, specific objectives were set and include:

1. To perform visual inspection on selected brands of tetracycline hydrochloride capsules
2. To perform dissolution test on the tetracycline hydrochloride capsules
3. To perform content assay on the tetracycline hydrochloride capsules

CHAPTER TWO: MATERIALS AND METHOD

2.1 MATERIALS

Ten (10) different brand of tetracycline capsules were randomly sampled and purchased from community pharmacies with Benin City, Edo State. The brands were sampled based availability at time of visit to the community pharmacies. The products was dispensed in their original secondary container, with each pack consisting of 10 x 10 capsules. Overall, 1000 capsules were collected. All products were purchased on the same day and coded D1 – D10. They were then stored at room temperature in a dry, dark place which provided protection from contamination and damage. Samples were worked on within 72 hours of purchase. Tetracycline reference standard (Sigma, Aldrich) was used as the reference material. All weighings were performed using Ohaus digital weighing balance (Ohaus Corps, USA) with sensitivity of 0.0001g. Dissolution test was performed using Rotating Paddle dissolution apparatus (USP Type 11) and absorbance readings were measured using T80 Double Beam UV-Visible spectrophotometer with 1.0cm quartz cell cuvette.

2.2 METHODOLOGY

2.2.1 VISUAL INSPECTION OF SAMPLES

Visual inspection was performed on each brand of tetracycline according to World Health Organization Visual Inspection of Medicines Template (WHPA, 2018). This checklist contained 36 questions to evaluate the capsule packaging, labelling, active ingredient and other physical characteristics such as color uniformity. It also contained instructions on how to report faulty products. Details about each brand including manufacturer address, batch number, dates of manufacture and expiry were recorded and are presented in **Table 3.1.**

2.2.2 DISSOLUTION TEST FOR SAMPLES (USP, 2023)

In vitro release study was performed using the Rotating Paddle dissolution apparatus (USP Type 11) with three replicates. All brands were evaluated using 900ml of distilled water as medium

and maintained at 37 ± 0.5°C and 75rpm. 10ml aliquots were withdrawn at definite time intervals of 20, 40 and 60 minutes into 15ml Corning® polytetrafluoroethylene (PTFE) tubes, and replaced with 10ml distilled water after each withdrawal. All withdrawn samples were then assayed using the T80 Double Beam UV-Visible spectrophotometer at 276nm. The percentages of the drug dissolved from the capsules were calculated and presented in **Table 3.2.**

2.2.3 ASSAY OF SAMPLES (Ahmed *et al.*, 2018)

2.2.3.1 Preparation of Standard Solution of Tetracycline Hydrochloride

0.1g of tetracycline hydrochloride was quantitatively weighed and transferred into in a 100ml volumetric flask. 50ml of distilled water was added and the powder was dissolved by agitation of the flask for 20 minutes. The solution was made up to 100ml volume with additional distilled water to obtain a 1mg/ml stock solution of tetracycline hydrochloride solution. The solution was then filtered using Whatman® 42 filter paper placed in a funnel into an appropriately labelled 250ml conical flask and sealed using foil paper.

From this bulk solution, 1ml aliquot was withdrawn with the aid of a 5ml pipette into a 10ml volumetric flask. and made up to 10ml volume with additional distilled water, resulting in a 100µg/ml standard solution. Standard solution of concentration 10µg/ml, 20µg/ml, 40µg/ml and 80µg/ml were also prepared by serial dilution (C1V2=C2V2) from the 1mg/ml stock solution. The absorbance of the resulting solutions was measured at 384nm using a T80 Double Beam UV-Visible spectrophotometer. and used to develop a 5-point calibration curve.

2.2.3.2 Preparation of Tetracycline Hydrochloride Samples

10 capsules of the brand of tetracycline hydrochloride being assayed was randomly selected, opened and their content emptied into a dry, clean petri dish. An amount of homogenously mixed, fine capsule powder equivalent to 0.1g of tetracycline hydrochloride was quantitatively weighed and transferred into a 100ml volumetric flask. 50ml of distilled water was added and the powder was dissolved by agitation of the flask for 20 minutes. The solution was made up to

100ml volume with additional distilled water and agitated. The solution was then filtered using Whatman® 42 filter paper placed in a funnel into an appropriately labelled 250ml conical flask and sealed using foil paper.

From this bulk solution, 1ml aliquot was withdrawn and made up to 10ml volume with additional distilled water. This was quantitatively transferred into appropriately labelled 15ml Corning® polytetrafluoroethylene (PTFE) tubes. The absorbance of the resulting solution was measured at 384nm using a T80 Double Beam UV-Visible spectrophotometer. All determinations were done in triplicate and the results were presented as Mean ± Standard Deviation (S.D) as seen in **Table 3.3.**

CHAPTER THREE: RESULTS

A total of ten (10) tetracycline hydrochloride capsules from nine (9) different manufacturers were obtained for the study. Pertinent details from the visual inspection test performed on the different samples were summarized in **Table 3.1**. In vitro release study was performed on each brand using distilled water as medium and the results were noted in **Table 3.2**. A bar chart distribution showing percentage of different brands of tetracycline released in-vitro at 20, 40 and 60 minutes was displayed as **Figure 3.1**. Absorbance values of the various concentrations of the standard solution was recorded in **Table 3.3.** Absorption spectrum of 0.1%w/v Tetracycline hydrochloride in distilled water solution was displayed as **Figure 3.2**, and the 5-point calibration graph displayed as **Figure 3.3**. Finally, content assay was performed on all brands to determine if the label claim is within pharmacopeial specifications and the results obtained were recorded in **Table 3.4**. All absorbance values were measured using the T80 Double Beam UV-Visible spectrophotometer.

3.1 Details of the Different Brands of Tetracycline

Table 3.1: Details of the Different Brands of Tetracycline

Sample Code	Batch Number	Color of powder	Color Uniformity	Date of Manufacture	Expiry Date	NAFDAC Number
D1	B5EC826	Pale Yellow	Yes	10-2022	10-2025	B4-5290
D2	220446	Dark Yellow, Brownish Yellow	No	04-2022	04-2025	04-5819
D3	C0423015	Pale Yellow	Yes	10-2023	09-2026	A11-0404
D4	C0422019	Dark Yellow, Brownish Yellow	No	10-2022	10-2025	A11-0404
D5	210963	Dark Yellow, Brownish Yellow	No	09-2021	09-2024	04-2264
D6	KP23003	Dark Yellow, Brownish Yellow	No	03-2023	02-2026	A11-100425
D7	NDF/TCA/0048	Brownish Yellow	Yes	08-2023	07-2027	B4-1403
D8	SM230906	Pale Yellow	Yes	09-2023	09-2026	04-2991
D9	230731	Pale Yellow	Yes	07-2023	07-2026	04-3466
D10	230606	Dark Yellow, Brownish Yellow	No	06-2023	06-2026	04-5885

3.2 Dissolution Test Result of Tetracycline Hydrochloride (250mg) Capsules in Distilled Water

Table 3.2: Dissolution Test Result of Tetracycline Hydrochloride (250mg) Capsules in Distilled Water

Sample Code	Time (mins)	Absorbance	% Dissolved[a]
	20	0.210	91.31 ± 1.02
D1	40	0.223	96.96 ± 2.49
	60	0.229	99.57 ± 2.23
	20	0.211	91.75 ± 2.81
D2	40	0.213	92.62 ± 3.03
	60	0.219	95.23 ± 2.39
	20	0.213	92.62 ± 3.02
D3	40	0.227	98.70 ± 1.83
	60	0.228	99.14 ± 2.08
	20	0.228	99.14 ± 1.88
D4	40	0.229	99.57 ± 2.04
	60	0.229	99.57 ± 3.40
	20	0.215	93.49 ± 2.48
D5	40	0.227	98.70 ± 2.15
	60	0.227	98.70 ± 1.99
	20	0.217	94.36 ± 1.63
D6	40	0.225	97.83 ± 1.88
	60	0.225	97.83 ± 1.69
	20	0.214	93.05 ± 2.95
D7	40	0.221	96.09 ± 3.17
	60	0.223	96.96 ± 2.84
	20	0.227	98.70 ± 1.83
D8	40	0.229	99.57 ± 1.80
	60	0.229	99.57 ± 1.83
	20	0.229	99.57 ± 1.94
D9	40	0.224	97.40 ± 2.08
	60	0.229	99.57 ± 1.88
	20	0.224	97.40 ± 2.39
D10	40	0.224	97.40 ± 1.79
	60	0.225	97.83 ± 2.46

a: mean ± standard deviation
n = 3

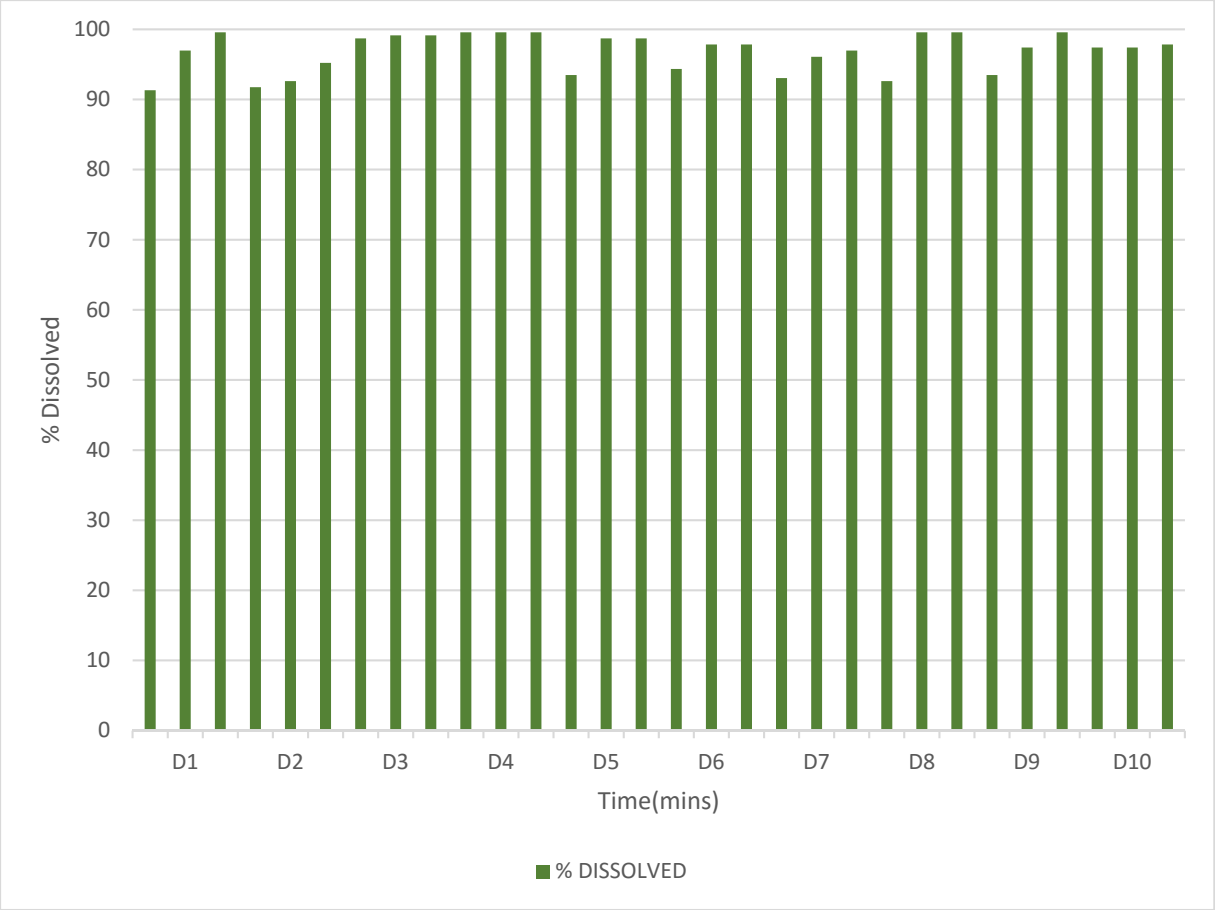
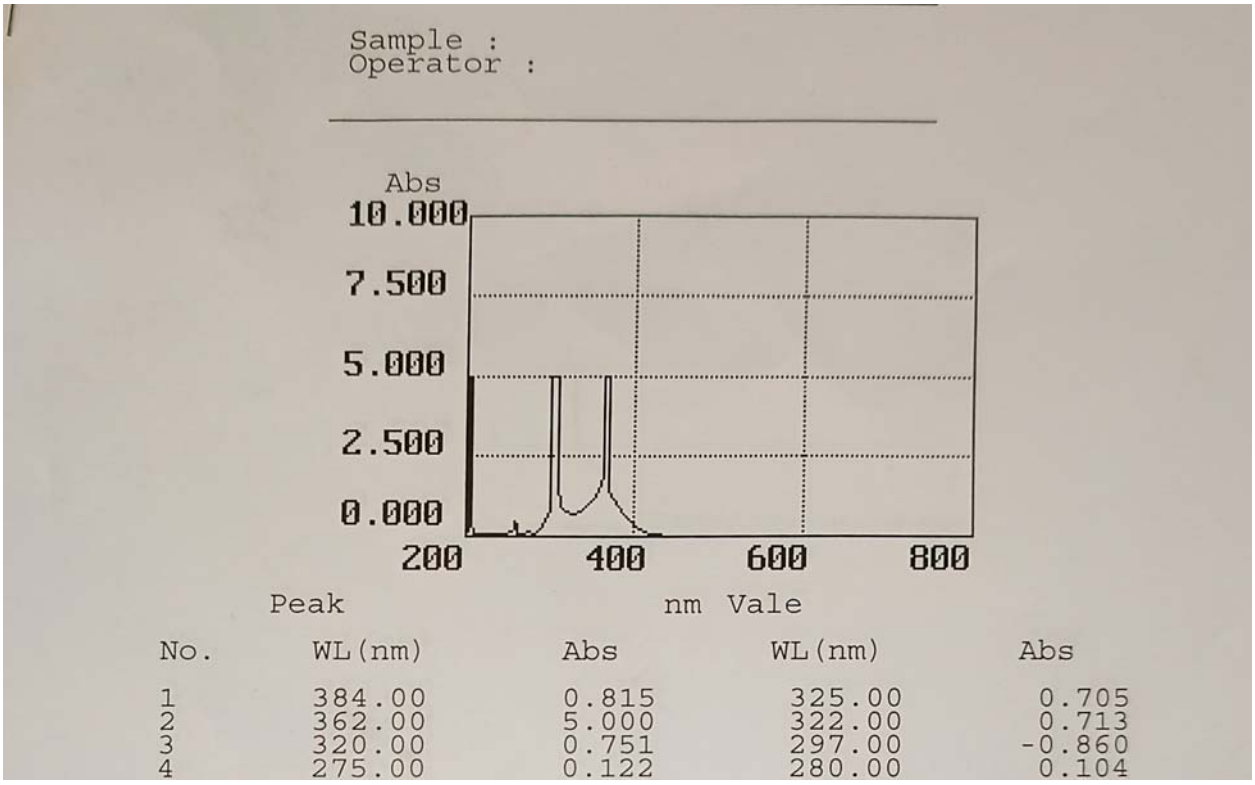


Figure 3.1: Bar Chart Distribution Showing Percentage of Different Brands of Tetracycline Released In-vitro at 20, 40 And 60 Minutes



Peak Vale

No.	WL(nm)	Abs	WL(nm)	Abs
1	384.00	0.815	325.00	0.705
2	362.00	5.000	322.00	0.713
3	320.00	0.751	297.00	-0.860
4	275.00	0.122	280.00	0.104

Figure 3.2: Absorption Spectrum of 0.1%w/v Tetracycline hydrochloride in Distilled Water

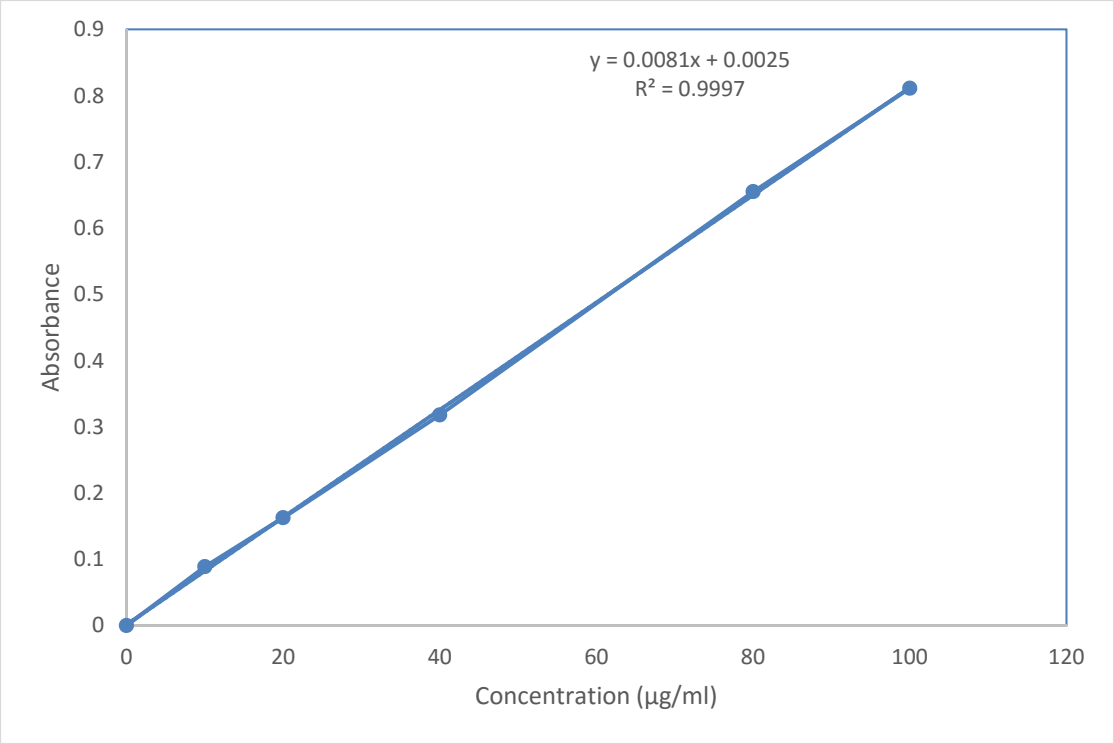


Figure 3.3: Standard Calibration Graph for Tetracycline Hydrochloride (Beer's Plot at 384nm)

3.3 Result of Concentration of Standard Tetracycline Hydrochloride Versus Absorbance

Table 3.3: Result of Concentration of Standard Tetracycline Hydrochloride Versus Absorbance

Concentration (µg/ml)	Absorbance
10	0.089
20	0.163
40	0.318
80	0.655
100	0.811

3.4 Result of Percentage Drug Content of the Tetracycline Hydrochloride Assayed

Table 3.4: Result of Percentage Drug Content of the Tetracycline Hydrochloride Assayed

Sample Code	Absorbance	Amount of API Found (mg)	Label Claim (mg)	% Recovery [a]
D1	0.793	243.98	250	97.59 ± 1.43
D2	0.747	229.78	250	91.91 ± 1.48
D3	0.781	240.28	250	96.11 ± 1.37
D4	0.794	244.29	250	97.71 ± 1.51
D5	0.774	238.11	250	95.24 ± 1.27
D6	0.768	236.26	250	94.50 ± 1.17
D7	0.741	227.93	250	91.17± 1.23
D8	0.801	246.45	250	98.58 ± 1.21
D9	0.785	241.51	250	96.60 ± 1.16
D10	0.751	231.02	250	92.40 ± 1.34

a: mean ± standard deviation
API: Active Pharmaceutical Ingredient (Tetracycline hydrochloride)
n = 3

CHAPTER FOUR: DISCUSSION

4.1 VISUAL INSPECTION

Visual inspection of capsules is a critical process in pharmaceutical quality control to ensure that a drug meets required standard and consistency. It is a basic test that can give quick, useful information on the authenticity of a drug product, and its use is self-evident. A thorough visual inspection process therefore involves inspecting various aspect of the capsule including its appearance, structure and content. In this study, a 36-question template developed by the World Health Professions Alliance (WHPA, 2018) was used to evaluate various brands of tetracycline capsules sampled. The major parameters being evaluated were the physical characteristics of the capsules and the packaging. Physical characteristics evaluated included color and color uniformity, breaks or pinholes along the capsule surface, presence of empty capsules, size uniformity and odor. Packaging characteristics evaluated included container closure, label information and package insert.

In this study, visual inspection was carried out on all ten (10) brands of tetracycline which was bought from community pharmacies within Benin City. 30% of the brands were imported brands while 70% were locally manufactured brands. On inspection of the physical appearance, all brands had consistent capsule size, no breaks or pinholes and no uniform markings. This indicated that their physical integrity was assured. However, upon examining the content of the capsules, it was discovered that all brands were of different hues of yellow, and only four (4) of the ten brands had color consistent with the reference tetracycline hydrochloride color. D2, D4, D5, D6 and D10 had non-uniform color that ranged from dark yellow to brownish yellow, indicating deterioration has begun to occur. Although sample D7 had a uniform color, it was brownish-yellow indicating that significant deterioration has also begun to occur. This may be due to errors during production, poor initial storage conditions at the storage site after production, during transportation from suppliers or at the community pharmacy. Such change may be exacerbated by the hot climatic conditions of the city even when strict packing guidelines are followed. Unfortunately, such poor physical appearance is not easily noticeable by the consumer at purchase point unless the capsule is opened and its content emptied.

All capsules were properly enclosed in their respective primary containers, and safely sealed. All had a secondary container that protected the primary container, with matching label information. Label information was legible for all brands. They all contained information regarding the active therapeutic ingredient, drug strength and form, manufacturer name, address and logo, date of manufacture, expiry date, batch number and NAFDAC (National Agency for Food and Drug Administration and Control) number. However, for brands D7 and D9, the symbol ® did not follow the trade name – a deviation from the set standard of WHPA, (2018).

4.2 DISSOLUTION TEST

For a drug to fulfil its therapeutic effect, it is necessary to be dissolved in physiological fluid and thereafter absorbed (Alves, 2017). Drug absorption and availability depends on rate of drug dissociation, disintegration and dissolution at physiological conditions. Dissolution test is therefore an important in vitro test that aims to evaluate the performance of a drug in terms of its release characteristics in vivo. Dissolution test also evaluates drug release characteristics of drug to ensure batches of said drug are similar. This is because even slight differences in performance can have significant impact on the safety and efficacy of the drug product. Dissolution rate may also affect bioavailability of the formulation.

All samples showed dissolution rate above 80% as seen in Table 3.2. This corresponded to the criteria outlined in the USP, (2023) that states that for capsules labelled to contain 250mg, percentage amount dissolved (Q) should not be less than 80% at 60 minutes. Therefore, the dissolution rates of all brands analyzed are acceptable. No dilution was required as absorbance values gotten were between 0 and 1.

4.3 CONTENT ASSAY

Content assay is an important quantitative test for capsules. This test measures the strength or amount of the active pharmaceutical ingredient (API) in the pharmaceutical capsule. Content assay gives useful information on the quality and safety of a pharmaceutical capsule. Drug quality was accessed in terms of percent content of the active pharmaceutical ingredient (API) as

compared against label claim and in compliance with the United States Pharmacopeia standard on content assay for tetracycline hydrochloride capsules (90 – 125% of the stated amount) (USP, 2023).

All brands of tetracycline hydrochloride assayed were within their shelf life at time of study. Distilled water was used as dissolution medium as tetracycline is readily soluble in distilled water. In developing countries such as Nigeria where highly technical equipment such as HPLC are not readily available, and where cost of reagents is at an all-time high, there is need to develop an easy, relatively inexpensive and accurate method to evaluate the quality of tetracycline capsules available in the drug market. This method relied on the use of simple chemicals and technique for the rapid, routine determination of tetracycline hydrochloride content in pharmaceutical capsules. The method employed was free from experimental variables such as heating. Baseline correction with the diluent (distilled water) was performed to exclude interference on the absorbance value from the diluent.

Upon analysis, all the sampled products had adequate content, i.e. contained between 90% and 125% of tetracycline hydrochloride as guaranteed by the product label and conformed with the United States Pharmacopeia standard. D7 (91.17 ± 1.23%) had the least amount of tetracycline hydrochloride when compared to others and was tending towards the lower limit of the official specifications, indicating that deterioration may soon occur. Therefore, although D7 is currently safe for use, in a short while or under critical conditions such as in humid weather or harsh environment, it is more likely to undergo significant deterioration and become unsafe for use well before its expiry date. This can lead to poor clinical outcomes and may contribute to antimicrobial resistance. This claim is reinforced by the poor visual inspection profile displayed by D7. The percentage content was determined to be in a range between 86.58 ± 1.23% and 100.99 ± 1.17% as seen in **Table 3.3**. No dilution was necessary as absorbance values gotten were between 0 and 1. Also, the sample absorbance values were within the calibration curve range.

4.4 LIMITATIONS

The study focused only on visual inspection, dissolution rate and content of the API as quality criterions. Non-compliance with other important quality specifications such as microbial load suspension, disintegration test and stability test were not evaluated. Consequently, the role of chemical processes such as oxidation or hydrolysis cannot be conclusively established or excluded. Another limitation is that only one geographic area was evaluated, therefore, the results may not be generalizable to other brands of tetracycline hydrochloride sold in the nation. Also, due to the fact that the tetracycline capsules were bought from different premises, the storage conditions differ and can impact upon the results obtained.

CHAPTER FIVE

5.1 CONCLUSION

The importance of tetracycline hydrochloride capsules cannot be overemphasized in the healthcare sector. This study has subjected different brands of tetracycline found within Benin City to many pharmaceutical evaluations. The methods used were relatively simple, accurate and affordable for the analysis of Tetracycline hydrochloride in capsules. The results obtained suggests that the various brands of tetracycline hydrochloride capsules available in Benin City are safe for use. However, certain products such as D7 was identified as having the potential to significantly deteriorate soon. Such information is beneficial to the clinician so as to make more informed decisions during drug therapy.

5.2 RECOMMENDATION

It is recommended that periodic pharmaceutical evaluations should be performed on drug products to ascertain their quality, and the information obtained should be timely communicated to the healthcare professionals. Strategies to reduce the rate and extent of deterioration should also be developed such as ensuring the drug product is transported in a closed vehicle to avoid direct contact with sunlight, and keeping the drug product away from wet walls.

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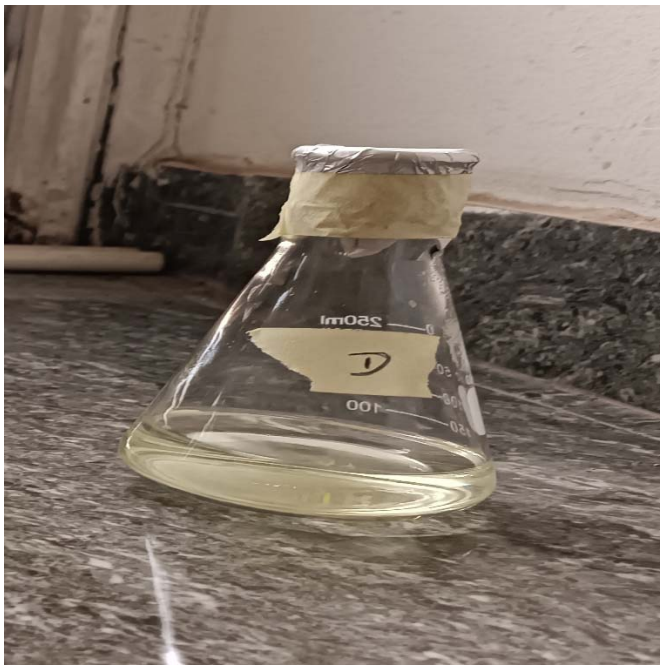
APPENDIX

DETAILS OF THE TETRACYCLINE HYDROCHLORIDE CAPSULES BRAND SAMPLED

Sample Code	Brand Name	Manufacturing Company
D1	Alben®	Alben Healthcare Industries, Nigeria
D2	Derms®	Yangzhou Norier Pharmaceuticals, China
D3	Fidson®	Fidson Healthcare Plc, Nigeria
D4	Fidson®	Fidson Healthcare Plc, Nigeria
D5	Hetrac®	Jiangxi Xierkangtai Pharmaceuticals, Nigeria
D6	Tetrakris®	Krishat Pharmaceuticals, Nigeria
D7	New Divine	New Divine Favour Pharmaceuticals, Nigeria
D8	Salmycin®	Sagar Pharmaceuticals, Nigeria
D9	Tetramac	Yangzhou Norier Pharmaceuticals, China
D10	Tetra 250®	Ningbo DHY Pharmaceutical Company, China

Sample D7 (left) and D8 (right)

Rotating Paddle dissolution apparatus (USP Type 11) and digital stop clock



1mg/ml stock solution of tetracycline hydrochloride solution in distilled water

T80 Double Beam UV-Visible spectrophotometer