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Review of *in vivo* Bioequivalence Assessment and Pharmaceutical Development in Antimicrobial Drug Research

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Abstract

The pharmaceutical development and bioequivalence assessment of antimicrobial drugs play a crucial role in ensuring therapeutic efficacy, safety, and quality in clinical practice. This review explores current methodologies, regulatory frameworks, and scientific advances in the evaluation of antimicrobial formulations and their bioequivalence with reference products. It highlights the importance of bioavailability studies, pharmacokinetic profiling, and *in vitro* dissolution testing in establishing therapeutic equivalence, particularly in the context of generic drug development. The paper also discusses key challenges associated with antimicrobial agents, such as variable absorption, resistance development, and formulation stability, which can significantly influence bioequivalence outcomes. Regulatory agencies such as the FDA and EMA have developed stringent guidelines to standardize the approval process of antimicrobial generics, ensuring consistent therapeutic performance and minimizing resistance risks. Furthermore, emerging technologies, including nano carriers, advanced drug delivery systems, and biopharmaceutical modeling, are reshaping formulation strategies and bioequivalence testing approaches. Overall, this review provides an integrated perspective on the scientific and regulatory dimensions of antimicrobial drug development, emphasizing the need for continuous innovation and harmonization to support global access to effective, high-quality antimicrobial therapies.

Keywords: Pharmaceutical Development, Bioequivalence, Antimicrobial Drugs, Nano Carriers, Chromatography

Introduction

There are a lot of published studies on bioequivalence of ceftriaxone. Most of these studies were carried out in different countries using either animals or humans. Richards *et al*, and Brogden and Ward, did an appraisal of its antibacterial activity,

pharmacokinetics properties, therapeutic uses and adverse effects in human [1, 2]. Because of the vast number of generic brands of ceftriaxone marketed in the world, there is the tendency that some may not be bioequivalent or measure up to the required quality specified by regulatory agencies [3]. It is evident that the prevalence of resistance will be high in developing countries if all the generics available are used without assessing their quality. The quality of drug assessment carried out in Nigeria indicated that 48% were out of range of the specification of British Pharmacopoeia (BP) and about 40% were manufactured in India [4]. Based on these facts, most of the health care professionals and other health workers were of the view that only innovator pharmaceutical products that are expensive are effective [3]. But this view may not always be the case because some generic versions of innovator products have been proven to be bioequivalent. Research carried out on the analysis of pharmaceutical qualities of paracetamol and ibuprofen tablets in a Nigerian market showed that some of the paracetamol and ibuprofen tablets conformed to the standard requirement while some do not [4]. The eleven (11) brands of paracetamol tested were chemically and physically equivalent to the innovator product. In order to ensure compliance, there is need for regulatory agencies to constantly monitor imported generics and those drugs manufactured locally in Nigeria.

A generic drug is defined as a pharmaceutical product that is intended to be interchangeable with an innovator product, is manufactured without a license from the innovator company and is marketed after the expiry date of the patent or other exclusive right. A generic medicine contains the same active pharmaceutical ingredients like the innovator product. It is used at the same dose, strength and route in treatment of the same disease. Because of the huge global health care expenditure, many developing countries have been encouraged to utilise generic medicines in order to cut cost. The National Health Insurance Scheme (NHIS) which is a program under the National Health Policy of Nigeria recommends the prescription of NHIS drugs to be strictly on generics, unless otherwise necessitated. The only way generic can be compared with the innovative product and the result be satisfactory is through bioequivalence studies. Some antibiotics sold in Calabar did not have the required active ingredients needed for effective therapy [5] Various methods have been used to demonstrate bioequivalence in antibiotics, either *in vitro* or *in vivo* [6]. Bioequivalence of two formulations is assessed in terms of the Area under the curve (AUC), Time to reach maximum concentration (T_{max}), and the Peak Plasma Concentration (C_{max}) If there is no statistically significant difference between the test and the reference, then the formulations are said to be bioequivalent. Bioequivalence studies for formulations can be carried out using human volunteers or animals. For example, in a two treatment cross over study, bioequivalence of two brands of cefuroxime 500mg tablets (cefuzine® and Zinnat®) in healthy human volunteers, was carried out following a single dose, two-treatment,two-period crossover design was carried out and the ratio of the test to the reference was in agreement with acceptable range of 80-125% [7]. Also, a comparative bioavailability of two intramuscular preparations of ceftriaxone in healthy Thai volunteers carried out by. The study was a randomised, double-blind two periods cross-over design involving 20 healthy volunteers. They received a 1 g intramuscular injection of a generic ceftriaxone (Cet-3) and the innovator preparation (Rocephin). Plasma ceftriaxone samples were collected and analysed using High- Performance Liquid Chromatography (HPLC) and the relevant pharmacokinetic parameters of Cet-3® and Rocephin® were compared. The result showed that Cet-3 could be used in place of the more expensive innovator product. Animal studies carried out by researchers using ceftriaxone showed that some generic formulations ceftriaxone have pharmacokinetics comparable to that of the innovator product [8], determined the pharmacokinetics of ceftriaxone in goats. In the study, they used a dose rate of 20 mg/kg. Following intramuscular administration, plasma ceftriaxone was determined using High Performance Liquid Chromatography [9]. The peak plasma concentration of ceftriaxone was 21.51± 0.6 mg/ml, Time to reach maximum concentration was 0.5 hrs, ceftriaxone concentration was determined up to 12hours, apparent volume of distribution was 0.53±0.05 l/kg, Area under the curve (AUC) was 66.78±4.9 mg.hr/ml, Elimination half-life was 2.03±0.09 hr, and the clearance was 3.04±0.34ml/min/kg, respectively. The intramuscular bioavailability was 59.0±4.0%. They concluded that at a dose of 20 mg/kg, ceftriaxone could be used to treat bacterial infections in sheep. In another research work, Tiwari and some researchers determined pharmacokinetics and bioavailability of ceftriaxone in Patanwadi sheep [8]. They concluded that a dose of 10mg/kg body weight can be used to treat various bacterial infections in Sheep.

Pharmacokinetics

Pharmacokinetics deals with the changes of drug concentration in the drug in concentration of a drug and/or its metabolite(s) in the human or animals body following administration of the drug product, i.e., the changes of drug concentration in different body fluids and tissues in the dynamic systems of liberation, absorption, distribution, body storage, binding, metabolism and excretion [10].

Non-Linear Pharmacokinetics

Non-linear kinetics or saturation kinetics refers to a change of one or more of the pharmacokinetics parameters during absorption, distribution, metabolism and excretion by saturation or overloading of the process due to increased dose sizes [10]. Most drugs like ceftriaxone, erythromycin, paracetamol follow linear pharmacokinetics or first order kinetics. Non-linear pharmacokinetics is usually due to saturation occurring in one of the pharmacokinetic mechanisms such as protein binding, hepatic metabolism or active renal transport of the drug [11].

Elimination is saturable when plasma concentration of the drug increases and the elimination rate reaches its maximum capacity. In non-linear elimination kinetics, the drug clearance decreases with increasing drug concentration [11].

For saturable protein binding, as the concentration of drug increases, the fraction of drugs that are unbound will eventually increase because all the available binding sites are saturated. Concentrations in plasma are in the range of 10s to 100s of ug/ml). For a drug that is metabolised by the liver with a low intrinsic clearance- extraction ratio, saturation of plasma binding will cause both volume of distribution and clearance to increase; $t_{½}$ may remain constant. For such drug that is cleared with high intrinsic clearance-extraction ratio, steady state concentration may remain linearly proportional to the rate of drug

administration [11].

Pharmacokinetics Parameters

The four most important pharmacokinetic parameters are clearance, volume of distribution, elimination half-life and bioavailability.

Bioavailability is the fraction of drug administered that reaches the systemic circulation. It is also the rate and extent of absorption of a drug from a dosage form. Some drugs, eg., neomycin sulphate and sulphaguanidine are either not absorbed at all or poorly absorbed following oral administration. Some factors can influence bioavailability of a drug. They include Solubility of drugs, dosage form and route of administration, first pass effect, formulation factors as well as physiological factors. Bioavailability parameters include C_{max}, T_{max} and AUC. When the AUC, C_{max} and T_{max} are the same within the statistical limit for two dosage forms, they are considered bioequivalent [11].

Clearance is the measure of the body's ability to eliminate a drug. Clearance is viewed as the single most important parameter to describe the pharmacokinetic of a drug. It is the most important concept to be considered when a rational regimen for long-term drug administration is to be designed. Clearance of a drug by several organs is additive. Drug elimination in the body is the sum total of the elimination of some important organs in the body. These organs are liver, gastrointestinal tract and kidney. The rate of elimination of each of these organs will result in their respective clearances. The total of this clearance will represent the systemic clearance [12].

CL renal +CL hepatic+ CL other = CL

Clearance (CL) = Rate of elimination/concentration

Renal clearance

Renal clearance results in the appearance of a drug in the urine. It depends on the physicochemical properties and the binding ability of the drug plasma proteins, and the physiology of the kidney. The filtration rate of a drug is dependent on the quantity of fluid that the glomerulus filters and also the concentration of the unbound drugs to the plasma. Also, the secretion rate of drugs by the kidney is dependent on active secretion of the drug as a result of clearance by the endogenous transport system. These transporters can get saturated with drugs at a certain concentration, and that can affect delivery rate to the secretory site [12].

Hepatic Clearance

For a drug that is efficiently removed from the blood by hepatic process (metabolism and/or excretion of drug into the bile), the concentration of the drug leaving the liver will be low, the extraction ratio will approach unity, and clearance of drug from the blood will become limited by hepatic blood flow (e.g., drugs with systemic clearance greater than 6ml/min/kg). They are restricted in their rate of elimination by the rate at which they can be transported in the blood to the hepatic sites of elimination and not by intrahepatic process. Such drugs include imipramine, lidocaine, morphine, chlorpromazine and propranolol. There are asome factors that can influence clearance. The rate of flow of blood to the organ and the dosage [12].

1. Albumin concentration: some drugs bind strongly to plasma albumin. Examples of these drugs are phenytoin, theophylline, and the salicylates. In disease condition, albumin level tends to be low and that can affect drug concentration.
2. Alpha 1 acid glycoprotein concentration: α_1 acid glycoprotein is an important binding protein with binding sites for drugs. Even though drug elimination may remain unchanged sometimes, the concentration of some drugs like quinidine, propranolol and lignocaine are increased in some disorders like acute inflammation that cause a change in plasma concentration.
3. Capacity-limited protein binding: the binding of drugs to plasma protein is capacity-limited. The therapeutic concentration of salicylates and prednisolone shows concentration-dependent protein binding. Because unbound drug concentration is determined by dosing rate and clearance which is not altered, in the case of these low extraction ratio drugs, by protein binding-increase in dosing rate will cause corresponding changes in the pharmacodynamically important unbound concentration. Total drug concentration will increase less rapidly than the dosing rate would suggest as protein binding approaches saturation at higher concentrations [12].

Volume of distribution (Vd) relates the amount of a drug in the body to the concentration of the drug in the blood or plasma [12]. It is the theoretical size (volume) of space necessary to contain the amount of a drug in the body given its concentration in plasma. It is useful for estimating a loading dose. It does not necessarily refer to an identifiable physiological volume, but merely to the fluid volume that would be required to contain the entire drug in the body as the same concentration as in plasma. The volume of distribution is a direct measure of the extent of distribution. It rarely corresponds to the real volume. For example, the plasma volume of an average 70 kg man is 3 litres, blood volume is 5 litres, extracellular fluid volume outside plasma is 12 litres and volume of the total body water is approximately 42 liters. However, many drugs exhibit volume of distribution far in excess of these values. For example, if 500 µg of digoxin is in the body of a 70 kg subject, as concentration of approximately 0.7 ng/ml will be observed [12].

i.e

$$\frac{500{,}000\text{ng}}{0.7\text{ ng/ml}} = 71 \text{ litres}$$

This value is almost 2 times greater than total body water of a 70 kg man. Digoxin is relatively hydrophobic. It distributes preferentially to muscle and adipose tissue and to its specific receptors, leaving a small amount of drug in the plasma. The whole blood drug concentration is used for assessing the distribution of drug into and its elimination from tissues because it is the drug in the whole blood not that restricted to plasma, which is delivered in tissues. Some drugs are strongly bound to plasma proteins but are not bound to some tissue component in such a case their volume of distribution will be equal to that of plasma. In contrast, certain drugs have a high volume of distribution even though most of the drug in the circulation is bound to albumin because such drugs are sequestered elsewhere. Changes in either tissue or plasma binding can change the apparent volume of distribution determined from plasma

concentration measurement. For elderly people, their volume of distribution is small because they have lean muscle mass. For a drug like digoxin which binds to muscle proteins, the volume of distribution may be low and not real. as in the case with tissue, the apparent volume of distribution of theophylline is proportional to body weight. For obese patients, the volume of distribution might be overestimated because of their body weight because digoxin does not bind to fat. in the obese patients. Abnormal accumulation of fluid in conditions such as oedema, ascites and pleural effusion can markedly increase the volume of distribution of drugs such as gentamycin that are hydrophilic and have a small volume of distribution. Lipophilic drugs, such as the sedative drug thiopental accumulate in fat. These agents are released slowly from the fat stores. Thus an obese person might be sedated for a greater period of time than a lean person when the same dose of thiopental is administered [12].

Elimination half-life ($t_{½}$) is the length of time required to eliminate 50% of remaining amount of drug in the body. It is useful in determining dose interval and time to reach steady state. The half-life of a drug is important when one is considering questions involving time, such as how long will it take a patient to reach steady state on a constant dosage regimen or how long will it take for all the drug to be eliminated from the body, it may also be used to estimate the appropriate dosage interval during maintenance therapy or to estimate when the steady state will be reached. It takes one half-life to reach 50% of the steady state, two half-life to reach 75%, three half-lives to reach 87.5% and four half-lives to reach 93.75% of the steady state. The difference between clearance and half-life is important in defining the underlying mechanism for the effect of disease state on drug disposition. Clearance of some drugs does not change with age. For example, the long half-life of diazepam in older patients is because of changes in the volume of distribution with age [12].

Mathematically $t_{½} = 0.698\ Vd/CL$

Where:

Vd= volume of distribution

Cl= clearance

Other secondary parameters include:

A C_{max}: This is the maximum drug concentration achieved in the systemic circulation following drug administration.

B T_{max}: It is the time required to achieve maximum drug concentration in the system circulation.

C AUC $_{o-t}$: Area Under the plasma concentration-time curve from 0 hr to the last quantifiable concentration to be calculated using trapezoidal rule.

D AUC $_{o-9}$: Area Under the plasma concentration-time curve from 0 hour to the least quantifiable concentration to be calculated using trapezoidal rule.

The reasons why bioequivalence studies are done

To compare a definitive dosage form (industrial batch) with the dosage form used in clinical trials developed and evaluated (relative bioavailability).

To evaluate two dosage forms administered by the same way, but with formulations or manufacturing process different, in the same company.

When a new product is introduced by one manufacturer and a similar product is already licensed to another (e.g. innovator).

When a drug is used to treat life-threatening diseases and assurance of therapeutic response is required, e.g. digoxin, warfarin.

When a drug has a narrow therapeutic windows/safety margin (e.g., digoxin, theophylline).

Chromatography

Chromatography is a method of separating component of a mixture by taking advantage of their different rates of movement between the mobile phase and stationary phase. It is a method used by scientists for separating organic and inorganic compounds so that they can be analysed and studied. Chromatography is an excellent physical method for observing mixtures and solvents.

The word chromatography means "colour writing" which is a way that a chemist can test liquid mixtures. While studying the colouring materials in plant life, a Russian botanist discovered chromatography in 1903. Researchers use chromatography to determine unknown substance, either solid or liquid. Law enforcement agencies also use this method in their forensic analysis to detect the presence of hard drugs like amphetamine and cocaine in the urine, alcohol in blood, heavy metals like mercury and lead in water, etc. The principle of chromatography is based on the movement of the substances across the mobile phase and the retention of others in the stationary phase. The stationary phase as the name implies is the phase that is static and does not move. The mobile phase is the phase that moves through the stationary phase by attracting the substances to be tested. Different components of the substances to be tested have different degrees of affinity to the stationary phase and the mobile phase. In paper chromatography, the paper is the stationary phase while the solvent is the mobile phase. This type of chromatography use capillary action to move the solvent through the stationary phase.

Retention Factor (RF)

This is a quantitative measure of how far a particular compound moves in a particular solvent. The RF value is a good measure of whether an unknown compound and a known compound are the same, or not the same. The retention factor, RF, is defined as RF = distance the solute (D1) moves divided by the distance traveled by the solvent front (D2).

$Rf = D1 / D2$

Where:

D1 = distance that the colour traveled, measured from the center of the band of colour to the point where the food colour was applied

D2 = total distance that solvent travelled [13].

Different Types of Chromatography

There are four main kinds of chromatography. Liquid chromatography, gas chromatography, thin-layer chromatography and paper chromatography [13].

Liquid Chromatography

It can be used to test water samples and look for toxic waste in lakes and rivers. It is also used to evaluate metal ions and organic compounds in solutions. Liquid Chromatography

uses liquids which may incorporate hydrophilic, insoluble molecules [13].

Gas Chromatography

It is used in airports to detect the explosive device. It is also used for forensics analysis in many diverse ways. It is employed also to assess blood obtained from a crime scene. Helium moves a mixture of gases through stationary phase which is a column of absorbent material.

Thin-layer Chromatography

As the name implies, it uses an absorbent material placed on a plastic or glass plates. It is a quick and simple method of checking levels of impurities of organic materials. The level of insecticides, pesticides, and other harmful organic substances can be determined in food using this means. It could also be used to provide forensic evidence especially when dye components are present in fibre.

Paper Chromatography

It is one of the most common kinds of chromatography. It uses a strip of paper as the stationary phase. Capillary action is used to push the solvents up through the paper and separate the solutes. This technique can be used in separating amino acids in the mixture of proteins and anions, performing RNA fingerprinting, as well as separating and testing for the presence of histamines, antibiotics. The paper is the stationary phase. The solvent which is the mobile phase moves along the paper which is the stationary phase through capillary action.

High-Performance Liquid chromatography (HPLC)

It is also called high-pressure chromatography. The stationary phase is usually packed with tiny particles in the range of 3 to 5µm in diameter. The mobile phase which is the liquid moves as a result of high pressure coming from the pump.

In principle, LC and HPLC work in a similar fashion except that the pace, effectiveness, sensitivity and simplicity of operation of HPLC is more superior. These components are separated from each other with the help of the column as a result of chemical and physical interactions of the particles in the column and the molecules of the substance. The components that are separated are detected at the exit point of this column by a device. What comes out of this detector is called a "**liquid chromatogram**.

HPLC has the various components with their functions**:**

The pump in HPLC is a device that forces the mobile phase which is the liquid at a determined flow rate through the liquid chromatograph. The unit in which the flow rate is expressed is millilitres per minutes (ml/min).The flow rate is 2 ml/min. The pressure of the pump can be between 6000 to 9000 PSI which is equivalent to 400 to 600 bar. During analysis of a substance, there may be a continuous delivery of the mobile phase which is called "Isocratic" or increasing delivery of the mobile phase, which is called "gradient".

The injector in HPLC is the device that introduces the liquid sample into the mobile phase. Usually, 5 to 20 microlitres volumes of sample are injected. The injector should be able to withstand the high pressures of the liquid system. An autosampler can analyse many samples. It is the automatic version of the injector which is used when manual injection is not practicable.

The column is like the heart of the chromatogram; it separates sample components of interest using different physical and chemical parameters. The small particles inside the column at normal flow rate can cause the high back pressure. The pump must overcome the pressure and resistance in order to move the mobile phase along the column.

The detector is a device that detects the individual molecules that come out (elute) from the column. A detector measures the amount of those molecules so that the sample component can be quantitatively analysed. The detector provides an output to a recorder or computer that produces the liquid chromatogram (i.e., the graph of the detector response).

The computer is also called the data system; the computer controls all the operations of the HPLC instrument. The time of elution is determined by the detector which has a signal. The time of elution is the retention time. The signal also detect the amount of sample (quantitative analysis). Page 1

HPLC can be used for the separation of biological substances and chemicals that are not volatile; non-volatile substances include pharmaceuticals like paracetamol, ibuprofen, aspirin, etc. Potassium phosphate, and sodium chloride salts; proteinous materials like blood and white egg portion; organic materials like polymers, asphalt or motor oil that are hydrocarbon in nature etc; plant extracts and herbal products, thermolabile compounds like trinitrotoluene, etc. The most important parameter for identifying a compound is dependent on its retention time. The retention time is the time taken for a compound to elute after it is injected into the column.

The HPLC uses the following principles in analysing substances:

1. Quantitative Analysis: It is the measurement of the quantity of a compound in a sample (concentration); meaning, how much is there? There are two main ways to interpret a chromatogram
   a. Determination of the peak height of a chromatogram is measured from the baseline;
   b. Determination of the peak area. For a quantitative analysis of the compound to be made the compounds will be injected and the result comes out in the form of peak, and the area can be measured from the peak. The peak area observed from the chromatogram is directly related to the concentration or the amount of substance injected [14, 15].
2. Preparation of Pure Compound(s): A pure compound can be prepared by collecting the chromatographic peak and concentrating the compound (analyte) at the exit point of the detector. This is done by evaporating the solvent. A pure substance can be prepared for future use, for example in organic synthesis, clinical studies, toxicological evaluation etc [14, 15].
3. Trace analysis: A compound whose concentration is less than 1% by weight is called a trace compound. Trace compound is important in pharmaceutical and biological analysis. This is because of the harmful effects these compounds can have on the environment or human tissue. It is often difficult to separate or detect substances in a chromatogram. It requires detectors that are very sensitive with high resolution. Four major techniques are normally employed to separate most substances. They are reverse phase column chromatography, normal or adsorption chromatography, ion exchange chromatography and size exclusion

chromatography [14, 15].

In reversed-phase column chromatography (RPC), the packing consists of nonpolar substances (e.g., C3, C18, C9, phenyl, etc.). The mobile phase consists of water-miscible organic solvent; examples include acetonitrile, methanol, ethanol, etc. More than 90% of analysts use reverse phase column chromatography. The method can be used for non-polar, polar, ionizable and ionic molecules making RPC very versatile. For samples containing different ranges of compounds, gradient elution is often used. In this method principally water-based mobile phase is started with and then organic solvents are added as a function of time. Solvent strength increases when organic solvent are used, and the compound that are eluted are strongly retained in reverse phase chromatography packing [14, 15].

(For normal or adsorption chromatography, the parking in the column could be amino-bonded, silica gel, cyanopropyl-bonded, etc. The mobile phase is non- polar. Examples of non-polar substance include ethyl acetate, isooctane, methylene chloride, etc. Normal phase separations are rarely performed, in most cases it is less than 10% performed. This method is important for compounds that are water sensitive, chiral compound, geometric isomers [14, 15].

(In ion exchange chromatography, the parking in the column are ionic groups like tetraalkylammonium and solfonic). About 20% of liquid chromatographers use this method. This method is useful for separating both organic substances that are cationic and anionic in aqueous solution. Proteins, ionic dyes and amino acid compounds that are salt in brine water can be separated by ion exchange. Examples include basic proteins on strong cation exchanger like (-SO3), RNA polymerase, proteolytic enzyme (Chymotrypsinogen) Lysozyme etc [14, 15].

(In size exclusion chromatography (SEC), there is no contact between materials in the column and the sample substance. What happens is that molecules diffuse depending on their size into pore of a porous medium. Molecules that are bigger than the pore opening cannot diffuse into the particles, but molecules smaller than the pore opening can enter the opening readily and hence easily separated. Larger molecules will elute first, then smaller molecules later. Size exclusion chromatography method is used by 10 to 15% of people using chromatography. It is mainly used for characterization of proteins and polymers. There are two methods: non-aqueous SEC [sometimes termed Gel Permeation Chromatography (GPC)] and aqueous SEC [sometimes referred to as Gel Filtration Chromatography (GFC) [14, 15].

(In HPLC temperature control is critical in achieving the desired goal. Retention in HPLC is dependent on temperature. It should be reproducible. If the temperature is not consistent, then it may be difficult to get a precise peak for compound. The peak height or area may vary [14, 15].

(Solubility is also crucial in HPLC**;** for substances that have low solubility there may be difficulty in their precipitation if they are injected into the mobile phase. Biological compounds like proteins, enzymes, hormones may be denatured by temperature. So temperature control is important. Temperature control can be achieved in three ways using oven, heater block or water bath. Basics Page 32

There are many detection methods used to detect the compounds eluting from an HPLC column.

The most common types are spectroscopic detection, refractive index detection, fluorescence detection and ultraviolet (UV) absorption. In ultraviolet absorption, a sensor measures light beam as it passes through a flow cell. The amount of light energy passing through the sensor changes if the compounds that elute from the column absorb this light energy. A recorder or data system captures the amplified electrical signal. A UV spectrum is also obtained which can aid in the detection of substances or series of compounds [14, 15].

In mass spectroscopy (MS)**,** the compound eluting from the HPLC column is detected by the sensor that ionises it. This is done by fragmenting the molecules into smaller units that are only unique to the compound or by measuring its mass. The detector in mass spectroscopy can identify compounds directly in most cases because mass spectroscopy works in a similar fashion to fingerprint and is unique only hydrogen atom of that particular compound.

In refractive index (RI), the compound or solvent ability to deflect light is a measure used for detectors. In refractive index, the ability of molecules to deflect light in the mobile phase in a flow cell used as a reference. The refractive index detector is used universally, but its sensitivity is low [14, 15].

(Fluorescence detectors are highly selective and sensitive. They can quantify impurities compounds in complex matrices even at low concentration level. Flourecence detector can only detect a substance that shows fluorescence when analysed (trace level analysis) [14, 15].

Plate 1: Picture of a typical HPLC

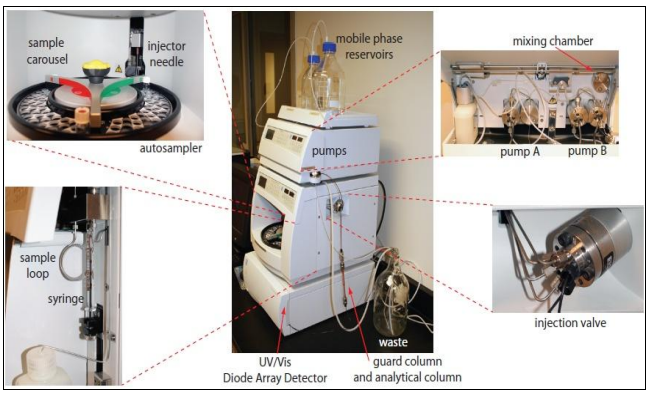

Plate 2: Picture of HPLC and its Components

Conclusion

The assessment of *in vivo* bioequivalence and pharmaceutical development of antimicrobial drugs remains essential for ensuring therapeutic consistency, patient safety, and global drug quality. Continuous advancements in formulation science, analytical techniques, and regulatory harmonization are improving the reliability of

bioequivalence evaluations. Future efforts should focus on integrating innovative technologies and addressing antimicrobial resistance challenges to enhance the efficacy, accessibility, and sustainability of antimicrobial therapies worldwide.

References

1. Richards DM, Heel RC, Brogden RN, Speight TM, Avery GS. Ceftriaxone: A review of its antibacterial activity, pharmacological properties and therapeutic use. Drugs. 1984; 27(6):469-527.
2. Brogden RN, Ward A. Ceftriaxone: A reappraisal of its antibacterial activity and pharmacokinetic properties, and an update on its therapeutic use with particular reference to once-daily administration. Drugs. 1988; 35(6):604-645.
3. Odeniyi MA, Adegoke OA, Adereti RB, Odeku OA, Itiola OA. Comparative analysis of eight brands of sulfadoxine-pyrimethamine tablets. Tropical Journal of Pharmaceutical Research. 2003; 2(1):161-167.
4. Okunlola A, Adegoke OA, Odeku OA. Generic versus innovator: Analysis of the pharmaceutical qualities of paracetamol and ibuprofen. East and Central African Journal of Pharmaceutical Sciences. 2009; 12(3).
5. Nkang AO, Okonko IO, Lennox JA, Eyarefe OD, Abubakar MJ, Ojezele MO, *et al*. Assessment of the efficacies, potencies and bacteriological qualities of some of the antibiotics sold in Calabar, Nigeria. African Journal of Biotechnology. 2010; 9(41):6987-7002.
6. Rodriguez CA, Agudelo M, Zuluaga AF, Vesga O. *In vitro* and *in vivo* comparison of the anti-staphylococcal efficacy of generic products and the innovator of oxacillin. BMC Infectious Diseases. 2010; 10(1):153.
7. Al-Said MS, Al-Khamis KI, Niazy EM, El-Sayed YM, Al-Rashood KA, Al-Bella S, *et al*. Bioequivalence evaluation of two brands of cefuroxime 500 mg tablets (Cefuzime® and Zinnat®) in healthy human volunteers. Biopharmaceutics & Drug Disposition. 2000; 21(6):205-210.
8. Tiwari S, Swati S, Bhavsar K, Patel UD, Thaker AM. Disposition of ceftriaxone in goats (Capra hircus). Online Vet. J. 2009; 4:41.
9. Hakim L, Bourne DW, Triggs EJ. High-performance liquid chromatographic assay of cefotaxime, desacetylcefotaxime and ceftriaxone in rat plasma. Journal of Chromatography B: Biomedical Sciences and Applications. 1988; 424:111-117.
10. Bauer LA. Applied clinical pharmacokinetics. New York: McGraw-Hill Medical, Jun 24, 2008.
11. Tablets A. Syllabi of Bachelor of Pharmaceutical Sciences Third Year B. Pharmacy.
12. McQuaid KR, Katzung BG. Basic and clinical pharmacology.
13. Ettre LS. Nomenclature for chromatography (IUPAC Recommendations 1993). Pure and Applied Chemistry. 1993; 65(4):819-872.
14. Ahmad HM. Separation of complex feed streams of products by layer melt crystallization.
15. Snyder LR, Kirkland JJ, Dolan JW. Introduction to modern liquid chromatography. John Wiley & Sons, 2011.