وزارة التعليم العالي والبحث العلمي

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كليـــــــة العـــــــلوم

قســـــــم الكيميـــــــاء

التوافر الحيوي للأمبسيلين في أمصال دم متطوعين أصحاء

رســـالة مقدمـــة الى

مجلس كلية العلوم / جامعة ديالى

وهي جزء من متطلبات الحصول على شهادة الماجستير في علوم الكيمياء

من قبل الطالب

محمد رضا هادي داود

بكالوريوس علوم الكيمياء / جامعة بغداد 2010

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بِسْمِ ٱللَّهِ ٱلرَّحْمَٰنِ ٱلرَّحِيمِ

﴿٦٠﴾ فَمَنْ حَآجَّكَ فِيهِ مِنۢ بَعْدِ مَا جَآءَكَ مِنَ ٱلْعِلْمِ فَقُلْ تَعَالَوْا۟
نَدْعُ أَبْنَآءَنَا وَأَبْنَآءَكُمْ وَنِسَآءَنَا وَنِسَآءَكُمْ وَأَنفُسَنَا
وَأَنفُسَكُمْ ثُمَّ نَبْتَهِلْ فَنَجْعَل لَّعْنَتَ ٱللَّهِ عَلَى ٱلْكَٰذِبِينَ ﴿٦١﴾

صَدَقَ اللهُ العَلِيُّ العَظِيمُ

الاية (61)

Supervisor Certification

We certify that this thesis **(*Bioavailability of Ampicillin in Sera Healthy Volunteers)*** was carried out under our supervision in Chemistry Department, College of Science, Diyala University in partial fulfilment of the requirements for the degree of master of science in chemistry by the student (***MOHAMMED REDHA HADI***).

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Dedication

To those who made us smile, those who gave us life.

To those who I consider them my model, those who sacrificed their lives for us.

To those who made us happy

To those who are sincere in their pray, those who I pray for them in mercy and forgiveness.

To those how made us live with honor

(our righteous Iraq martyrs)

I dedicate the fruits of my humble labor to them.

Mohammad..

Acknowledgment

Praise be to Allah, the cherisher and sustainer of the world;

peace and blessing be up on the messenger who is the best among all the creatures Al-Mustafa Muhammad ﴿ peace be upon him and his family﴾and the First Imam, Ali Ibn Abi Talib, Amir Al-Mu'minin ﴿Peace be on him﴾.

I have finished my research by the help of Allah. I would like to express my sincere thanks and deepest gratitude to my great

﴿ Prof. Fadhil Muhsin Abid and Asst. Prof. Ahmed Mahdi Saeed﴾

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The researcher…

Abstract

Ampicillin is an antibiotic of β-lactam group compounds which are widely used in the treatment of infectious diseases. The bioavailability of ampicillin (500 mg) after a single dose orally administrated is investigated in twenty Iraqi healthy volunteers for both genders with different ages, weights and heights with their consents. Sera concentrations of ampicillin are determined at various times after dose administration, by High Performance Liquid Chromatography (HPLC). The new method was carried out by using [fast column C-18, (50 × 4.6 mm I.D, 3 μm particle size],sensitive (detection limit =0.02 $\mu g.ml^{-1}$),linearity (R^2=0.9999), retention time=3.307 min and can be applied to determine the drug concentration in sera and study on pharmacokinetics.

In this study ampicillin is well absorbed rapidly after single dose administration (maximum time T_{max} 1.0 hr) and without clinically adverse effects on volunteers. Absorption rate of ampicillin in males almost slightly higher than absorbed in females, but the elimination rate equal to both genders, depending upon pharmacokinetic parameters: Area under curve concertation AUC_{0-8}(19.18 ±0.51, 18.68 ±0.89)$\mu g.ml^{-1}$.hr, maximum concentration C_{max} (7.37±0.46, 6.87±0.72) $\mu g.ml^{-1}$, elimination rate constant k_e(0.52±0.03, 0.53±0.03) hr^{-1}, $T_{1/2}$(1.35±0.08, 1.31±0.08) hr, and absorption rate constant k_a (1.75±0.07, 1.69±0.06)hr^{-1} for males and females respectively

The pharmacokinetic parameters for all healthy volunteers (C_{max}) is found to be 7.15±0.63 $\mu g.ml^{-1}$ occurring T_{max} of 1.0 hr, $T_{1/2}$, AUC_{0-8} , k_a and k_e values are found to be 1.33±0.08 hr, 18.96±0.75 $\mu g. ml^{-1}$ hr, 1.73±0.07 hr^{-1} and 0.52±0.03 hr^{-1}, respectively. The effect of volunteers characteristics on bioavailability ampicillin, the results studied show there is no clear effect for weight and height but there is slight effect for age and gender for drug absorption.

Table of Contents

List of Abbreviation and Symbols

Abbreviation	Means
µ	Average
µg.ml^{-1}	Microgram per milliliter
µl	Microliter
6-APA	6 – Aminopenicillanic Acid
ACN	Acetonitrile
ADME	Absorption kinetics, Distribution, Metabolism and Elimination.
ANOVA	Analysis of variance

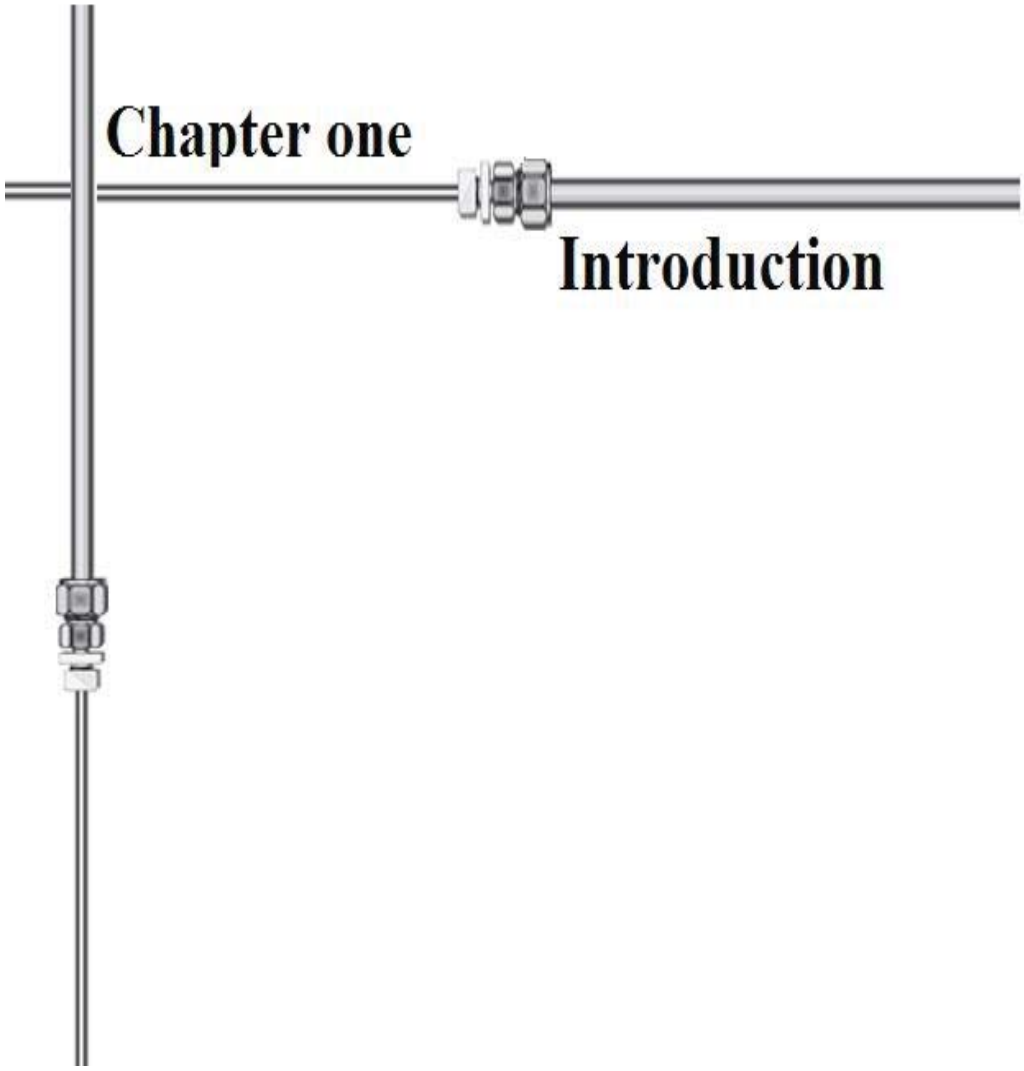
AUC	Area Under the sera curve Concentration
BDH	British Drug Houses
BP	British Pharmacopoeia
C-18	Octadecasaline packing
CD	Cyclodextrin
cm	Centimeter
cm I.D	centimeter Internal diameter
C_{max}	Maximum sera concentration
Cp	Concentration profile
Cp^0	Initial concentration profile
CT	Cold Temperature
F	Female
$g.mol^{-1}$	Gram per mole
GI	Gastrointestinal
GC	Gas Chromatography
HPLC	High Performance Liquid Chromatography
hr	hour
IV	Intravenous
k_a	Absorption rate constant
k_e , k_{el}	Elimination rate constant
kg	Kilogram
LC	Liquid Chromatography

LOD	Limit of Detection
LOQ	Limit of Quantitation
m	Slope
M	Male
M.wt	Molecular weight
Max.	Maximum
MDI	Metered – dose inhaler
MIC	Minimum inhibit concentration
min	Minute
Min.	Minimum
ml	Milliliter
$ml.min^{-1}$	Milliliter per minute
MTC	Maximum toxic concentration
nm	Nanometer
No.	Number
ns	Normal saline
PC	Program Computer
pH	Power of hydrogen
PK	Pharmacokinetic
pka	Acid dissociation constant
Psi	Pounds per square inch
PTFE	Polytetrafluoroethylene
R- LA	R- Lipoic acid

R^2	Correlation Coefficient
RBC	Red Blood Cell
Rep	Reproducibility
RP	Reversed Phase
rpm	Rotation per minute
RSD	Relative Standard Deviation
RT	Room Temperature
SD	Standard Deviation
SD	Standard Deviation
SDI	Samara Drug Industry
SE	Standard Error
SPD	Spectrophoto Diode Detector
SPSS	Statistical Package for the Social Sciences
Subj.	Subject
sw	Sterile water
$T_{1/2}$	Elimination half-life
$t_{cal.}$	$t_{calculated}$
T_{max}	Maximum time
TO	Thermostatic oven
UK	United Kingdom
uv=µv	Microvolt
UV-VIS	Ultraviolet–Visible
β	Beta

List of Figures

List of Tables



Chapter one

Introduction

1. Introduction

1. 1. Bioavailability of drug

Bioavailability can be defined as the extent and rate of drug absorption from its dosage administration into systemic circulation through blood becomes available at the site of drug action [1, 2]. Thus, the bioavailability of an intravenously injection administrated drug is rapid and complete. However, the patient convenience of most drugs are administrated orally after their final formulation in both forms capsule and table [3].

The importance of bioavailability in therapeutic determines the patient's response to the drug by effect as a function of its concentration in patient's sera. Activity of pharmacological response selected by many drugs could be directly related to the concentration or activity of the drug in vicinity of the receptor site in the blood [4]. The basic clinical bioavailability depends on the hypothesis focused on the distribution and equilibrium between drug in the receptor compartments and the blood, when the equilibrium reached optimum, the drug concentration measurement in the blood is assumed to provide an indirect measure of the receptor site [5].

The dosage form related factors include physicochemical characteristics, for example the chemical form, particle size and solubility of the drug and the type of the excipient that is used [6]. Most drugs are include as oral arrangements or extravascular infusion for the treatment of systemic disease, these drugs should be ingested and conveyed to the blood systemic and transported to the objective tissues to create their pharmacological activities [7, 8].

After oral administration, a drug to overcome a number of hurdles before reaching its sites of action;

- Liberated from its pharmaceutical form;
- Dissolved in the gastrointestinal (GI) fluid;
- Absorbed through the intestinal;

- Escaped drug molecules in the gut wall;
- Escaped excretion in the intestinal lumen by efflux pumps;
- Escaped metabolism in the liver before reaching general circulation from which it will be cleared by equilibration in tissues [8, 9].

1. 1. 1. Bio□□uival□nc□

Bioequivalence defined as two or more pharmaceutically equivalent products produce similar bioavailability characteristics in any subject, when administered in equivalent dosage. Bioequivalence studies that very important for drug development process, depending on the characteristics (pharmacokinetics parameters) and curves bioavailability of every medicine. If these properties correspond with the approved drug in the study of equivalence so as to create, efficacy and establish of new formulation, the pharmacokinetic properties of sera concentration - time curves are used to conclude that two drug formulations will give similar pharmacologic effects [10].

1. 1. □. □au□□□of lo□ bioavailability

Orally administered drugs take their route to intestinal wall and then the portal circulation to the liver; both are common sites of first pass metabolism (that occur before a drug reaches systemic circulation). Thus, many drugs may be metabolized in most common with oral dosage forms of water soluble, rapidly absorbed drugs, insufficient time for absorption in the gastrointestinal tract is the main reason of low bioavailability [11]. The drug weak ability to dissolve readily or cannot penetrate the epithelial membrane, time at the absorption site may be adequate. Therefore, bioavailability led to be highly variable. Stomach emptying, age, genetic phenotype, sex and previous gastrointestinal surgery (such as bariatric surgery) can also affect drug bioavailability [12].

1. 1. □. □□ay□d of bioavailability

For intravenous injection, the completion of drug delivered directly into the systemic circulation having bioavailability complete and the reaching maximum concentration in sera, the drug in this case a rapid clinical response is necessary of acute diseases. For other parenteral routes of intramuscular injection and under the skin, the bioavailability may still be close to bioavailability complete for many therapeutic drugs, as a result of no metabolism these drugs with time. For orally administered drugs (the most common route), their bioavailabilities are often below bioavailability complete because of incomplete absorption also differ for the different dosage forms [13, 14].

1. 1. □. Bioavailability □ub□□ct□d □ign

The subject population for bioavailability studies should be selected normally and performed with volunteers. In general, subjects should be as follow [14, 15]:

- 18 – 24 healthy volunteers;
- Selected volunteers should be distributed randomly different, to achieve a uniform distribution of the available volunteers with respect to (gender, weight, age and height);
- Should be screen for suitability by means of an extensive review of drug history and a preferable be without a history of alcohol, non – smokers and drug abuse;
- A single dose study, should be fasting at least (2 hr) before the dose, is considered acceptable;
- Subjects should not take any drug or random foods through the study;
- Males are preferred over females because lactation, menstrual cycle, menopause stages and pregnancy, that occur in females may effect of the drug level profiles in sera [16, 17].

1. 1. 5. Drug permeation through cell membranes

Many drugs need to pass through one or more cell membranes to reach their site of action, a common feature of all cell membranes is a phospholipid bilayer, there are many major mechanisms of movement the drug from one side of biological barrier to other is called biotransport for transfer of drug molecules across biological barrier by transport mechanisms, the major transport mechanisms are: passive diffusion, carrier mediated transport (a. facilitated diffusion, b. active transport), pinocytosis or phagocytosis and filtration (aqueous channels) [18, 19].

1. 1. 6. Factors affecting drug absorption and bioavailability

- Oral route

Absorption occurs when drug molecules are in the form of solutes [20]. A drug in solid form must be firstly disintegrated into smaller particles and dissolved in the medium before it traversed across the cell membrane and entered the blood stream, the rate of drug absorption depends on the relative speed of these processes [21].

- Dosage and formulation

Dosage form is basically the pharmaceutical product for use, can affect on the bioavailability and absorption of a drug. The absorption rate of different dosage forms are ordered: solution syrup > suspension > powder > capsule > tablet > coated tablet. This is because drugs in solution form would have avoided the steps of disintegration and dissolution. while covered tablets are often designed to delay the disintegration and dissolution processes until the drug reaches the small intestine where the condition may be more favorable for its absorption [22].

Other factors may affect bioavailability and absorption, for example dosage administered in a fed or fasted state, gastric emptying rate, interactions with other drugs / foods, efflux transporters of the

gastrointestinal tract, the enzymes responsible for metabolic processes, age, disease state, gender and pH [23, 24].

□. □. □. □□armaco□inetic overvie□

Pharmacokinetics is the study the rate and extent of drug movement through the body, involves kinetics of drug absorption, distribution, metabolism, and elimination (ADME) of drugs and their pharmacologic effect or therapeutic in human [25]. Applications of pharmacokinetics studies include: bioavailability measurements, evaluation of drugs interactions, dosage regulate of drugs in disease states, effects of pathological conditions on drug absorption and clinical prediction [26].

- □bsorption

It is refers to the transition of a drug from where it entered the body to the bloodstream (from the site of take dosage administration to the bloodstream). These enteral drugs are typically absorbed through the intestinal mucosa or stomach. These include any drug that is taken oral [27].

- Distribution

It is refers to the movement of a drug to various tissues of the body, after absorption stage . Therefore, the extent and rate of drug distribution are depended on: tissue components, drug concentration with plasma protein and the permeability of tissue membranes to the drug molecules [28].

- □ etabolism

It is the process of converts the drugs into chemical substances by metabolized into an active form(association with plasma proteins) by its major enzymes, these process occur in a liver [29].

- □limination

It means the irreversible loss process of removing drugs from the body. The kidney is the primary site for removal of a drug unchanged form in urine, some drugs are eliminated by excretion in the bile [30].

□. □. □. □arameters for assessment t□e p□armaco□inetics

There are some important parameters of pharmacokinetics study which have important role to evaluate bioavailability [31]:

- **□eig□t pea□ concentration** □□$_{ma}$□. It is the maximum drug concentration observed in the sera after administrate a dose of the drug. C_{max} often reach at only a single time point, referred to as time of peak concentration. It determines the toxicity and therapeutic efficacy of the drug [32].

- **□ime of □eig□t pea□ concentration** □□$_{ma}$□. It is the time after administration of a drug when the maximum sera concentration is reached and reflects the highest rate of the drug absorption in body [33].

- **□rea under t□e sera curve concentration** □□□□$_{□-t}$□. It is considered representative of the total amount of drug absorbed into the circulation after the administration of a single oral dose of the drug. The AUC_{0-t} is a measure of the extent of bioavailability whereas C_{max} and T_{max} are measures its rate [34].

- **□limination □alf-life** □□$_{□□}$□. It is undergo according to the law kinetics of a first order reaction. Also represented the time required for the amount of drug in sera to decrease by half, the half-life is completely independent on the drug concentration in sera. The concentration observed during the course of the clinical experiments [35].

- **□bsorption rate constant** □□$_{a}$□. It is the constant that relates the rate of drug absorbed into the body, according to first - order kinetics, is important to assess the bioavailability of a drug in sera, drug absorption is dependent upon dose [36].

- **□limination rate constant** □□$_{el}$ **or** □$_{e}$□. It is the first order rate constant, description of drug removal (elimination) from the body by elimination processes [37].

□. □. □ntibiotics

Antibiotics can be defined as " molecules that destroyed or reduced the growth of both fungi and bacteria", the famous used antibiotics were substances having similar mechanism of action [38].

Antibiotics can be classified to [39]:

- Tetracyclines, example (tetracycline);
- Aminoglycosides, example (amikacin, gentamicin);
- β - lactam antibiotics such as [penicillins (ampicillin, amoxicillin) carbapenems, cephalosporin];
- Sulfa antibiotics such as (sulfisoxazole);
- Macrolide antibiotics such as (erythromycin).

"β – lactam antibiotics " is group which having β –lactam core structure causing their antibacterial activity, consisting of a four membered cyclic amide with three carbon atoms and one nitrogen atom [40]. The main β – lactam antibiotics mechanism of action by inhibiting cell wall biosynthesis in the bacterial organism, this has a lethal effect on bacteria, most of all available commercially antibiotics are followed this rule [41,42].

Pencillins are β – lactam antibiotics derived from penicillium fungi and effective against infections caused by staphylococci, streptococci and syphilis. They are composed of β- lactam thiazolidine binary ring system known as 6 – Aminopenicillanic acid (6-APA) with variations in the (C-6) acylamido side chain. The nucleus, (6 – APA) consists of two amino acids valine and cysteine, twisted together biogenetically into acyclic dipeptide[43], as shown in Figure (1. 1) [44].

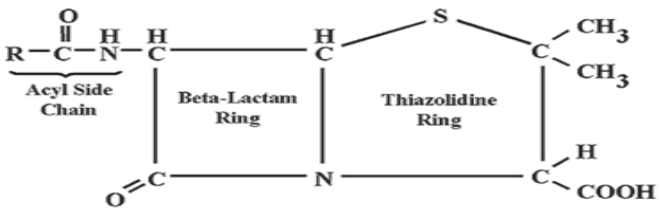


Figure (1. 1): Penicillin structure.

Different acyl chain have been chemically connected to free amino group of penicillin nucleus give various compounds which have a broader range of antimicrobial activity and reducing sensitivity to hydrolyzing β – lactam ring (penicillinases), as shown in Figure (1. 2) [45].

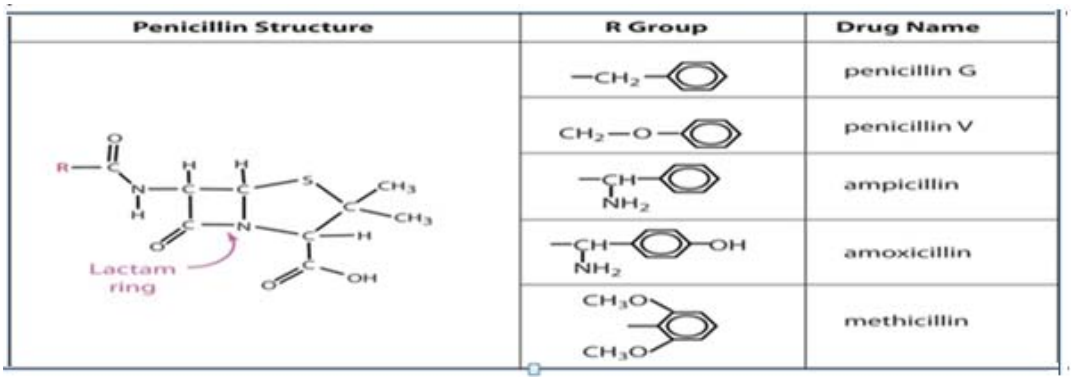


Figure (1. □) □Penicillin structure and its derivatives.

1. □. 1. □ e□□□i□□ □□□□i□□

In general β – lactam antibiotics and penicillin binding proteins which normally catalyze cross linking of bacterial cell walls. Therefore, bacteria inhibit constantly remodel their peptidoglycan cell walls [46, 47].

1. 2. 2. Ampicillin

Ampicillin is an antibiotic, a member of the penicillin family to the treatment of infection caused by bacteria, it has been synthesized first in 1961. Ampicillin is widely used in chemotherapy because of its stability in acid, rapidly absorbed, low toxicity and low minimum inhibitory concentration against bacteria, The basic structure of the ampicillin is shown below [48]:

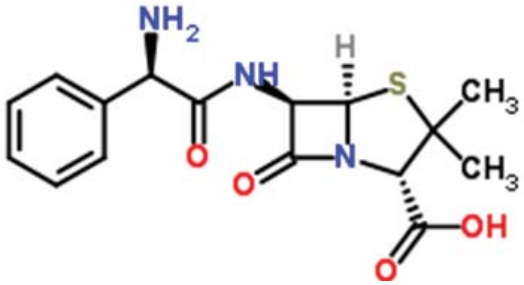


Figure (1. 3): Structure of the ampicillin.

□□□le (1. 1): Some physical and chemical characteristics of ampicillin [49].

□□me	Ampicillin, Aminobenzylpenicillin.
□ □lecul□r □□rmul□	$C_{16}H_{19}N_3O_4S$
□ □lecul□r □eig□□	349.405 $g.mol^{-1}$
□□n□n□m□	Ampicillin Sodium, Ampicillin trihydrate, Antibiotic KS R1, Omnipen, Polycillin and Amcill.
□□□ic□l □e□crip□i□n	White crystalline powder odorless, insoluble in ether, benzene and easily soluble in water.
p□	3.5 - 5.5
□ el□ing p□in□	208 ^{0}C
□□□□ili□□	Stable when it stored in a closed system at 43% and 81% relative humidity in room temperature for six weeks. Ampicillin is also stable at 35 ^{0}C in such closed systems for nine weeks.
□i□□ci□i□n c□n□□n□□	pka = 2.5, 7.3
□n□ic□i□n	For treatment of infection (Respiratory and gastrointestinal).
□□ec□□□□□	Presence of food in the gastrointestinal tract generally may effect on bioavailability and absorption of ampicillin.

1. 2. 3. □□□rm□c□l□g□□□ine□ic□□n□ □i□e e□ec□□ □□□mpicillin

□A□□□rp□i□n

Ampicillin is a β-lactam antibiotic widely used in human medicine with variability of bioavailability in humans, it is assumed that the low oral bioavailability is principally related to the hydrophilicity, this indicates; rapidly absorbed, diffuse easily into body cavities, joint spaces and very quickly excreted by the renal tubules [50].

□□i□ri□u□i□n

The concentration of an ampicillin in sera does not necessarily reflect its activity at the site of infection, to be effective the drug must reach the target organ at a therapeutic concentration by distribution with tissue [51].

□□ e□□□□li□m □n□ elimin□i□n □□□mpicillin

The process of metabolizing of ampicillin in the liver, by converted it into active substances(association with plasma proteins). Ampicillin has removed from the body to hydrophilicity substances by kidney in the urine by glomerular filtration, tubular reabsorption and renal tubular secretion [52].

-Adverse effects and toxicity of ampicillin

Ampicillin has low acute toxicity less than other antibiotics and safety available in humans that apply to the use of drug during pregnancy and early childhood [53]. The most common side effects to be expected in 10 % of users are diarrhea and rash [54].

1. 3. Liquid Chromatography (LC)

It is the separation of compounds as they pass through a column due to the differing distribution of the sample components between a particles supported stationary phase and mobile phase. LC used for ionic substances, large molecules with thermally unstable substances or low vapor pressures which cannot be vaporized without decomposing [55,56]. Thus, the distribution coefficient depend on the chemical nature of each the mobile phase and stationary phase. There are two types of LC: classical LC and High Performance Liquid Chromatography (HPLC) [57].

1. 3. 1. Classical liquid chromatography

It is a type of LC used as a sample volume in the milliliters range are often used large columns a proximately (50 – 250 mm) diameter, the deep pores of the packing which limit the mass transfer cause the separation times to be on the order of hours, the mobile phase is generally gravity fed at slow flow rates. Classical LC no special equipment, not sensitive and is usually used for organic synthesis and biochemical research[58, 59].

1. 3. 2. High Performance Liquid Chromatography (HPLC)

In 1941, Synge and Martin describe the discovery of liquid – liquid partition chromatography and also laid the foundation of HPLC and gas chromatography (GC), they also introduced the concept of height equivalent to the theoretical plate, as the measure of chromatography efficiency [60].

Since about 1969, there has been market progress of interest in the technique of liquid column chromatography because of the development of HPLC by Huber and Kirkland, they suggested high pressure systems related of operating at pressures up to (3000 psi). It has been found that separation by HPLC may be effected about 100 times faster than by the use of conventional liquid chromatography. In HPLC, small diameter columns (1- 3 mm) with support particle size in the region of (30 μm) are used and the eluent is pumped through the column at a high flow rate, during the run at high pressure, their instruments for liquid chromatography overcome the effect of higher liquid viscosities to gives the best analysis times. Sample clean up is usually much less of a problem with HPLC than GC and biological fluids can often be directly injected onto HPLC column, because of all these advantages, HPLC has already made a significant impact in forensic, clinical pharmaceutical and environmental analysis as an ideal complementary technique to GC to analysis the sample that affect by high temperature [61].

1. 3. 3. HPLC technique classified

It can be classified into the following bases [62]:

- Principle of separation: adsorption, ion exchange, partition and gel permeation;
- Modes of chromatography: Reveres or Normal phase mode;
- Elution of technique: gradient or isocratic separation;
- The scale of operation: preparative or analytical HPLC;

- The type of analysis: quantitative and qualitative analysis.

Reversed phase liquid chromatography is widely used because the column packing is nonpolar and the mobile phase is polar. So, compounds of high polarity will elute faster than compounds of low polarity giving better reproducibility and ease in solvent treatment. Mobile phase includes of aqueous – organic consists of polar solvents of varying degree of polarity [63, 64].

1. 3. 4. HPLC variables

Selective character in HPLC is often calibration by programming of some variables to achieve an efficient and good separation. For example, stationary phase properties, mobile phase installation and column switching, in addition to other variables flow programming involves initially, flow rate to better resolve the early peaks and then increasing the flow rate to elute well retained components, it can be carried out continuously or gradual if desired [65]. Isocratic system is a separation in which the mobile phase composition remains constant throughout the procedure [66].

Gradient elution or mobile phase programming is the method better effective, it involves the gradual increase of the mobile phase solvent strength with time to increase the speed of peak elution. Gradient elution, shorten the time of separation significantly without sacrifice in resolution of the early peaks. A gradient method use for samples that cannot be easily separated by isocratic, the eluent strength is increased during the separation by changing the composition of the mobile phase. As a result, the analysis time is reduced [67].

1. 3. 5. HPLC instrumentations

The mobile phase is drawn from a reservoir by a pump, which controls the flow rate and generates enough pressure to drive the mobile phase during the column. The injector is used to inject the sample in the column, which

is usually placed within a column oven. The column is one of the most important components of the HPLC because the separation of the sample components is achieved when those components pass through the column. The detector responds to changes in column effluent composition through the chromatographic run. Data system monitors the detector output and processes the data [68], as shown in Figure (2. 1).

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The detectors in HPLC are worked to continuously monitor the column eluents. Detector signal is generally processed and amplified to a potentiometric recorder to get a constant signal record with time of the analysis in the form of a chromatogram. A wide variety of HPLC detectors have been developed with universal detection requirements and high sensitivity. HPLC detectors can be generally classified as either responsive to a property of the actual solute itself or change in the property of the mobile phase, these include refractive index, UV-VIS, conductivity, electrochemical and fluorescence [69].

- **UV-VIS detector**

It is the most widely used in HPLC, because of a good stability, low cost, wide of applicability, relatively insensitive to flow changing and minor temperature change. The UV-VIS detector for measure components showing an absorption spectrum in the ultraviolet or visible region. Simply uses the different wavelength of light to assay the various compounds being eluted that absorbs light rays differently to get maximum sensitivity of the detector for each compound is eluted, it passes through the detector. The absorbance of light gives the determination of the component and the amount of light absorbed is directly related to its concentration [70].

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Kang, M. and Kang, J. (2012) reported the stabilities of two kinds of solution ampicillin sodium in sterile water (sw) and normal saline (ns) in the intravenous elastomeric device, by used HPLC - UV. Stored and assayed at a room temperature (RT) and cold temperature (CT) during 7 days. The results showed that stability of ampicillin in CT more stability than the solutions that were stored in RT [71].

Zhao et al., (2011), assayed of ampicillin in human sera by HPLC method. The results were obtained good linearity within the range of 0.14 - 11.2 μg.ml^{-1} and (r = 0.9995). Conclusions: This method is sensitive, simple and specific, which can be used to study the pharmacokinetics of ampicillin[72].

Samanidou et al., (2009), determined of ampicillin in blood, by HPLC method. The developed method was accuracy, linearity, sensitivity and stability. The detection limits in the blood were assayed as 0.02 μg.ml^{-1} for ampicillin [73].

Kumar et al., (2007), validated stability of HPLC technique for determination of ampicillin in commercials drug products. Results were obtained, that proposed single method allowed selective analysis of ampicillin in the presence of degradation products formed under stress conditions. The developed procedure was also applicable to the determine of instability of the drug in commercial products [74].

Luo et al. (1997), determined of ampicillin residues in raw and processed bovine milk, by HPLC with fluorescence detection. The limit of detection (LOD) is 1.0 μg.ml^{-1} and limit of quantification (LOQ) is 1.7μg.ml^{-1} [75].

Misic et al., (2013), determined ampicillin in human urine and pharmaceuticals by HPLC-UV. The calculated detection limit is determined at 2.58 $\mu g.ml^{-1}$. The method was good applied to assay of ampicillin in samples. The HPLC method is inexpensive, simple and efficient for the analysis of a large and small number of samples at RT in a short time [76].

Tuani et al., (2014), developed HPLC method for the determination of ampicillin in oral suspension dosage form. The retention time of ampicillin was 6.058 min. The results were showed the method was simple and rapid, it can be used for estimation of ampicillin [77].

Stepnik and Malinowska (2017), determined of ampicillin in human sera albumin, by vinylpyrrolidone owing to its ability to block protein binding with ampicillin, analysis free drug by using HPLC technique. The results were showed that the free drug concentration obtained by micellar system. This method is simple and fast for determination of free drug concentration[78].

Xie et al., (2012), determined of ampicillin in eggs by HPLC. This method used a simple liquid–liquid extraction of the samples with acetonitrile as extraction solvent, The limits of detection was 0.4 $\mu g.ml^{-1}$. This method simple, widely applicable and low-cost [79].

Credille et al., (2015), assayed of ampicillin trihydrate in sera, uterine tissue, lochial fluid and milk of cattle. Ampicillin was administrated by intramuscular injection. Concentration of ampicillin was assayed by HPLC method. Ampicillin achieves therapeutic concentrations and significantly higher in lochial fluid than uterine tissue and higher in sera and milk of cattle [80].

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Ikuta et al., (2016), reported bioavailability of R- Lipoic acid (R- LA) / γ- Cyclodextrin (CD) complex in 6 healthy volunteers, fasting, a single oral 600 mg, by HPLC. The results are; mean $AUC_{0\text{-}120min}$ (56- 121µg.ml^{-1}.min), mean C_{max} values are 1.7 - 3.4 µg.ml^{-1} for R-LA and R-LA/CD administration respectively. These results indicate that R-LA/ CD could be easily absorbed by the intestine and this CD complexation can be considered as delivering functional [81].

Tanam et al., (2014), demonstrated the bioavailability for paracetamol in human sera on 4 healthy Bangladeshi male volunteers. After oral administration of paracetamol tablet 1000 mg, by HPLC. The results are $AUC_{0\text{-}8hr}$ 31.06 µg.ml^{-1}.hr, $T_{1/2}$ are 3.9 hr and C_{max} is found to be 11.03 µg.ml^{-1} at T_{max} of 0.88 hr. The method was assayed at limit of quantification is 1.61 µg.ml^{-1} [82].

Mignot et al., (2002), work effect of food on the bioavailability of gatifloxacin gives as a single oral dose of 400 mg under fasting and fed conditions is determine in 18 healthy male volunteers. Food intake did not significantly change the C_{max}, $AUC_{0\text{-}8hr}$ and T_{max} of gatifloxacin. No clinically adverse effects or changes in clinical laboratory test results, Moreover, the rate of absorption is not affect by food intake. The results of this study indicate the drug was well tolerated in the presence or absence of food. It is suggested can be given without regard to meals [83].

Devandla et al., (2015), investigated effect of rifampicin 20 mg on the oral bioavailability of domperidone 600 mg in healthy sera human volunteers, by HPLC method. Through, rifampicin pretreatment, decreased T_{max}, $AUC_{0\text{-}24hr}$, and C_{max}. This interaction have clinical significance when domperidone (co-administrated) with rifampicin in treatment chronic

treatment conditions, for example tuberculosis and inflammation of joints[84].

Hoover et al., (2016), showed the bioavailability of delafloxacin after administrated multiple doses and oral single in 36 healthy volunteers for both genders and differ in properties, by HPLC method. No difference in pharmacokinetic parameters was observed for both genders, but in the elderlies men and women; mean delafloxacin C_{max} and AUC_{0-24hr}, are 35% higher than observed for young adults [85].

Willsie et al., (2015), studied bioavailability properties of single (IV) and repeated does sufentanil sublingual, 15 µg tablets in healthy volunteers, by HPLC method. These study results showed, the wide range of mean drug concentrations was achieved after repeated dosing at intervals 20 min., compared with a single dose to meet analgesic requirements [86].

Tian et al., (2006), used HPLC to assay bioavailability and determined of indinavir in healthy volunteers after oral administration 800 mg of drug. The results showed of each C_{max}, $T_{1/2,}$ AUC_{0-t}, T_{max} and linear correlation of indinavir concentration in the range 0.03 - 16.38 $\mu g.ml^{-1}$. The rapidity of method was very excellent and it has high validity which can be applied to determine drug concentration in sera and study pharmacokinetics [87].

Ahmed et al., (2009), analysed of nifedipine by HPLC method, in sera 6 healthy adult male volunteers. Each of subjects, received 20 mg drug orally. This study including pharmacokinetics parameters (T_{max}, $T_{1/2}$, AUC_{0-6hr} and C_{max}) that confirms the rapid absorption of nifedipine drug in sera humans [88].

Sergides et al., (2016), investigated the bioavailability and the safety of resveratrol following a single 500 mg oral dose in sera of 15 healthy male and female volunteers under fasting conditions, that analysed by HPLC

technique. Bioavailability parameters, including T_{max}, $T_{1/2}$, $AUC_{0\text{-}24hr}$ and $C_{max.}$ These pharmacokinetics of resveratrol were in agreement with those mentioned in the literatures and enhance to promote the pharmacological activities of resveratrol [89].

Abid et al., (2010), studied sensitivity HPLC method for the determined of doxycycline in sera of 20 adults healthy male volunteers with average age of (42 ±10) year, body weight 48-85 kg, body height of (160-185 cm) after a single dose of doxycycline 100 mg in form of capsules were orally administrated for both the reference drug from Pfizer and Samara drug. Both test and reference drug were show no significant difference in pharmacokinetics parameters, so they were considered to be bioequivalent [90].

Du et al., (2002), studied the relative bioavailability of salbutamol metered – dose inhaler (MDI), for 10 healthy male Chinese volunteers in sera human, by HPLC assay. The measured bioavailability parameters for salbutamol inhaled and orally 1.2 mg administrated: T_{max}, $T_{1/2}$, $AUC_{0\text{-}20}$ and C_{max}. There were significant difference in C_{max} and $AUC_{0\text{-}20hr}$ between the two dosage form, that led the absorption process of salbutamol (MDI) in volunteers was significant difference from that oral solution [91].

Mallah and Arafat (2015), studies the bioavailability of two doses 750 and 1000 mg of ciprofloxacin in 28 healthy male volunteers by using HPLC assay. Pharmacokinetic and bioavailability parameters were calculated; T_{max}, $AUC_{0\text{-}24hr}$, C_{max} and $T_{1/2}$ for two doses. The variation was significantly found in both ciprofloxacin doses and the pharmacokinetic data showed confirm relative trend in bioavailability and absorption in human sera [92].

Neto et al., (2016), described bioavailability two formulations of metformin hydrochloride 850 mg in 28 healthy volunteers (14 men and 14

women) by HPLC method. The pharmacokinetics parameters; AUC_{0-36h}, T_{max} and C_{max}, there were no significant difference for two formulations, but observed significant differences in the pharmacokinetic between the genders. These differences probably are due to less metabolism of females when compared to males [93].

Ding et al. (2005), studied bioavailability of fudosteine in sera 36 healthy volunteers after administration multiple dose (400 mg) and single dose (200, 400 and 600 mg). The drug concentrations in sera were determined by HPLC method. Results of pharmacokinetic showed that the significantly differences of C_{max} and AUC_{0-10hr} between males and females and no significant difference observed in pharmacokinetic between single dose and multi-dose [94].

Abid et al. (2010), assayed method in clinical laboratory for determination of sildenafil citrate, in sera 20 healthy male volunteers with average age of (32 ± 12) years, by using (HPLC). Received 50mg of each volunteers for two sildenafil formulations; (SDI, Samagra) and Kamagra (India). The results indicated no significant difference between the two formulations and therefore, both drugs of sildenafil are bioequivalent [95].

bb bb bbb bbb bb bbb**study**

1. The aim of the present study is to develop and valid HPLC method for determination of ampicillin in sera of healthy Iraqi volunteers which widely used to eliminate disease causing bacteria.
2. Effect the characteristics of volunteers on the drug concentration in sera and building up experience in follow up drug through the blood circulation for the purpose of bioavailability and bioequivalence by comparing any drug with high standard produced drugs.
3. Drug controlling to avoid the fake in drug industry.