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and Scientific Research
Diyala University
College of Science
Department of Chemistry

Bioavailability of Ampicillin in Sera Healthy Volunteers

A Thesis Submitted to the
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In Partial Fulfillment of the Requirements for the Degree
of Master in Chemistry Sciences
by

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سورة آل عمران

بِسْمِ ٱللَّهِ ٱلرَّحْمَٰنِ ٱلرَّحِيمِ

﴿٦٠﴾ فَمَنْ حَآجَّكَ فِيهِ مِنۢ بَعْدِ مَا جَآءَكَ مِنَ ٱلْعِلْمِ فَقُلْ تَعَالَوْا۟
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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..

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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.
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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.

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List of Abbreviation and Symbols

Abbreviation	Means
µ	Average
$\mu g.ml^{-1}$	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

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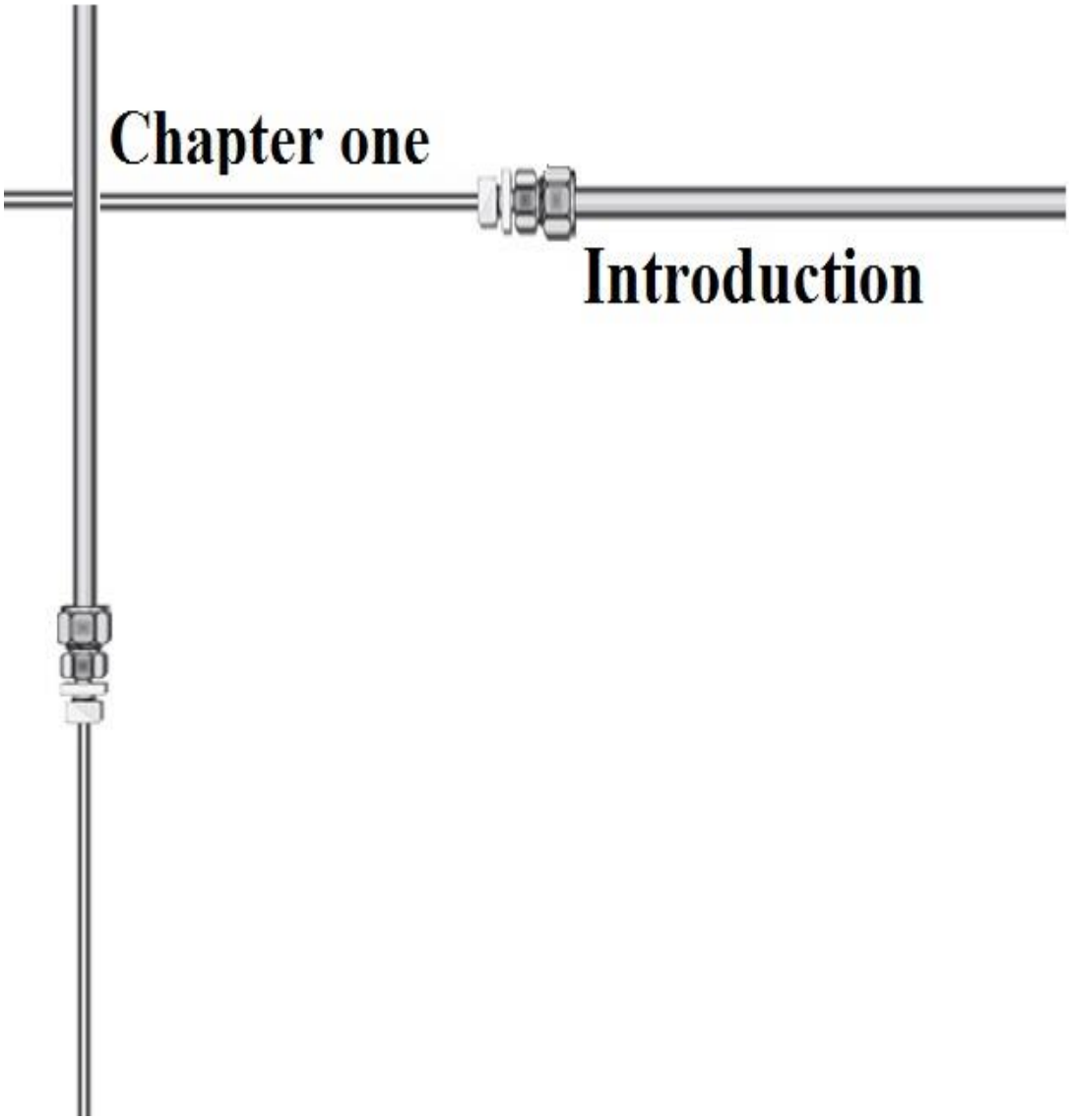


Chapter one

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. Bioequivalence

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. 2. Causes of low 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. 3. Assayed 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. 4. Bioavailability subjects design

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].

1. 1. 7. Pharmacokinetic overview

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].

- Absorption

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].

- Metabolism

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].

- Elimination

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].

1. 1. 8. Parameters for assessment the pharmacokinetics

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

- **Height peak concentration (C_{max}).** 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].

- **Time of height peak concentration (T_{max}).** 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].

- **Area under the sera curve concentration (AUC_{0-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].

- **Elimination half-life ($T_{1/2}$).** 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].

- **Absorption rate constant (k_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].

- **Elimination rate constant (k_{el} or k_e).** It is the first order rate constant, description of drug removal (elimination) from the body by elimination processes [37].

1. 2. Antibiotics

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].

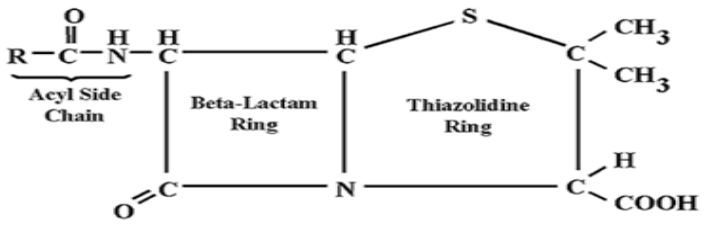


Figure (1. 1): Penicillin structure.

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].

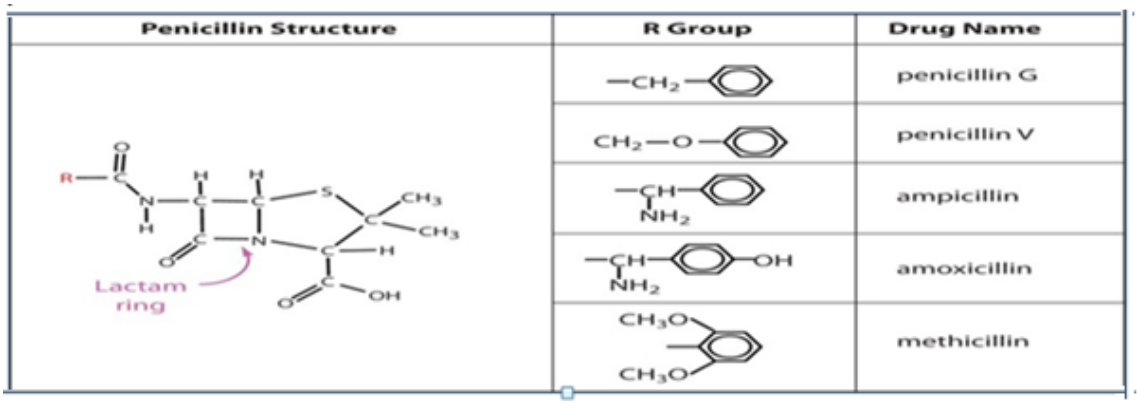


Figure (1. 2): Penicillin structure and its derivatives.

1. 2. 1. Mechanism of action

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]:

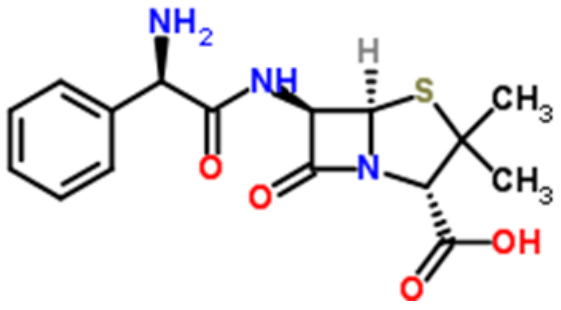


Figure (1. 3): Structure of the ampicillin.

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

Name	Ampicillin, Aminobenzylpenicillin.
Molecular formula	$C_{16}H_{19}N_3O_4S$
Molecular weight	349.405 $g.mol^{-1}$
Synonyms	Ampicillin Sodium, Ampicillin trihydrate, Antibiotic KS R1, Omnipen, Polycillin and Amcill.
Physical description	White crystalline powder odorless, insoluble in ether, benzene and easily soluble in water.
pH	3.5 - 5.5
Melting point	208 ^0C
Stability	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 ^0C in such closed systems for nine weeks.
Dissociation constants	pka = 2.5, 7.3
Indication	For treatment of infection (Respiratory and gastrointestinal).
Effect food	Presence of food in the gastrointestinal tract generally may effect on bioavailability and absorption of ampicillin.

1. 2. 3. Pharmacology, kinetics and side effects of ampicillin

- Absorption

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].

- Distribution

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].

- Metabolism and elimination of ampicillin

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).

1. 3. 6. Detection system

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].

1. 4. Review of literatures

1. 4. 1. HPLC method of ampicillin analysis

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 $\mu g.ml^{-1}$ 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 $\mu g.ml^{-1}$ 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 $\mu g.ml^{-1}$ and limit of quantification (LOQ) is 1.7$\mu g.ml^{-1}$ [75].

Misic et al., (2013), determined ampicillin in human urine and pharmaceuticals by HPLC-UV. The calculated detection limit is determined at 2.58 μg.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 μg.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].

1. 4. 2. Review literatures for bioavailability of drugs

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

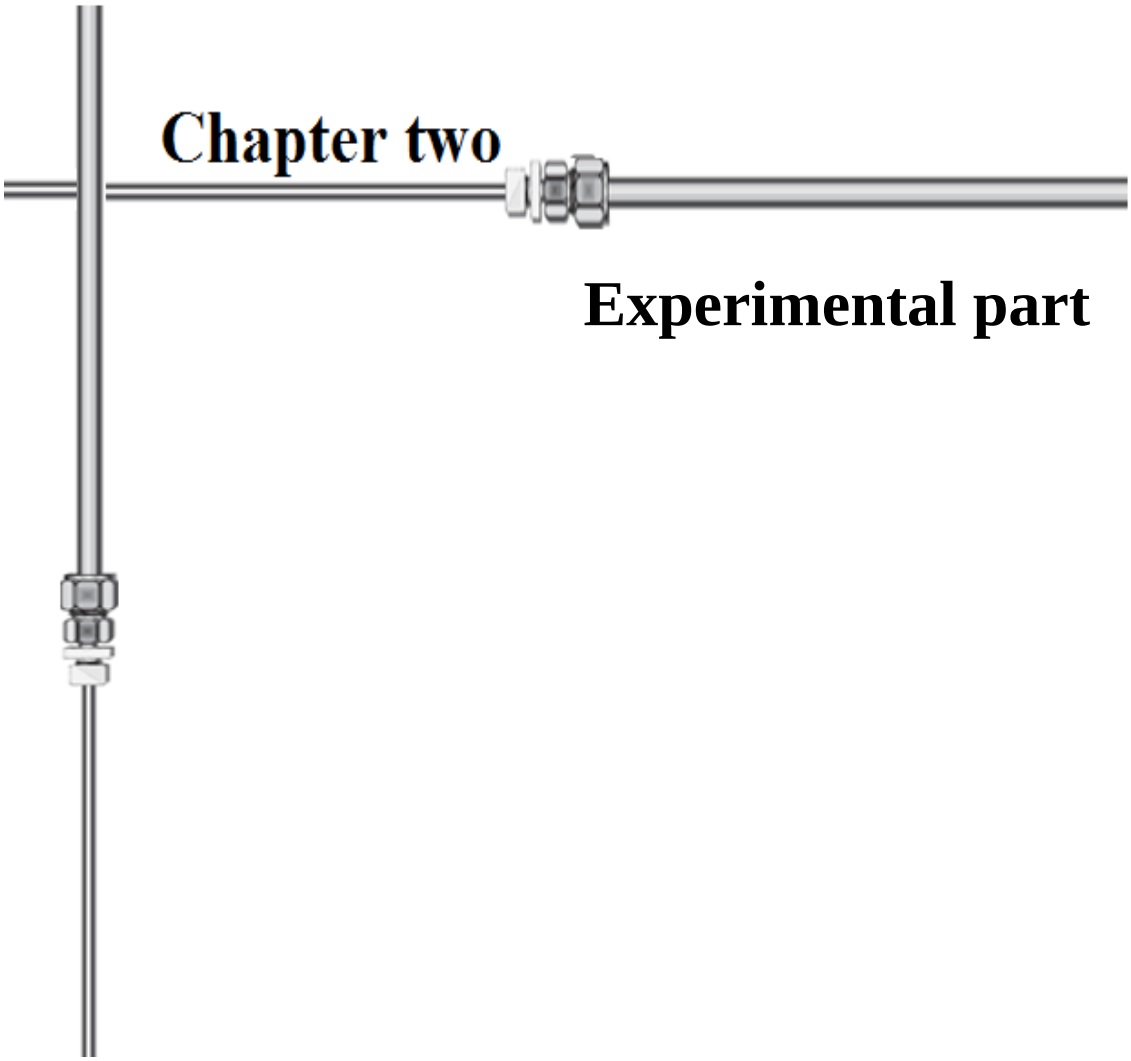
women) by HPLC method. The pharmacokinetics parameters; AUC_{0-36h}, T_{max} and C_{max}, there were no significant difference for two formulations, but observed significant differences in the pharmacokinetic between the genders. These differences probably are due to less metabolism of females when compared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

1. 5. The aim of the 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.



Chapter two

Experimental part

2. Experimental part

2. 1. Chemicals

All the chemicals used in this study are of the highest available assay as tabulated below Table (2. 1).

Table (2. 1): Characteristics of chemicals used.

Chemicals	Chemical formula	M.wt ($g.mol^{-1}$)	Assay	Supplied from
Standard ampicillin	$C_{16}H_{19}N_3O_4S$	349.405	99.99%	SDI, Iraq
Phosphoric acid	H_3PO_4	97.994	30 %	BDH
Acetonitrile (ACN)	C_2H_3N	41.052	HPLC grade	BDH
Potassium hydrogen phosphate	K_2HPO_4	174.200	99.9%	BDH
Methanol	CH_3OH	32.040	HPLC grade	BDH

2. 2. Apparatus and instruments used and their suppliers

1. HPLC, Shimadzu LC-20 A, gradient and isocratic system, (Koyota-Japan).
2. Sensitive Balance, Sartorius, 4 digitals, (Germany).
3. Ultra-sonic bath, Karl Kolb, (Germany).
4. Oven, Binder Hotline (Germany).
5. Special boxes, Runlab Labware (China).
6. Centrifuge, Hermle Laborti, 6000rpm, (japan).
7. pH-meter: Hanna, HI 98150 PH/ORP-meter.

2. 3. Experimental

2. 3. 1. Preparation of standard ampicillin

Stock solution of ampicillin (standard 1000 µg.ml^{-1}) was prepared by dissolving 1g of standard ampicillin [pure powder in this study was obtained from SDI-Iraq] in deionized water, the solution was transferred into 1000 ml volumetric flask and diluted using deionized water to mark, to obtain 1000 µg.ml^{-1}. This solution was used to prepare standard calibration solution (0.02 – 15 µg.ml $^{-1}$) by serial dilution method.

2. 3. 2. Volunteers selection

This study includes 20 healthy volunteers (11 males and 9 females), with average of age 35 ±8.3 years, mean weight of 74±9.1 kg and mean height of 173.1± 10.6 cm, from Ministry of Science and Technology (Iraq), as tabulated in Table (3. 7) with their consents. All volunteers were healthy and no foods random or medication taken after ingestion of ampicillin drug.

2. 3. 3. Subjects exclusion criteria

- The subjects who have a history of ; alcoholism, allergic to ampicillin, acute or chronic diseases (such as renal diseases, hepatic diseases, cardiac problems and diabetic).
- Blood samples that contained (RBC) breaker.

2. 3. 4. Blood samples collection

Single dose of ampicillin 500 mg capsule was administered orally with 250 ml of water to healthy volunteers. (3 – 4) ml blood samples were collected veinously (0.5, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0) hr, intervals after the oral administration, in polyethylene test tubes. The samples were centrifuged at 2400 rpm for 10 min and the sera was kept frozen until analysis.

2. 3. 5. Volunteers monitoring

No sides effects on healthy volunteers throughout the study interval (8 hr after drug administration) were noticed by physician.

2. 3. 6. Sample preparation

0.2 ml of sera was spiked with 0.2 ml of mobile phase (ACN). After stirring for 5 min the mixture was centrifuged at 2400 rpm for 10 min[87]. Supernatant layer was separated and 50 µl of sample were injected into HPLC system (Figure 2. 1),operate under the optimum separation conditions.

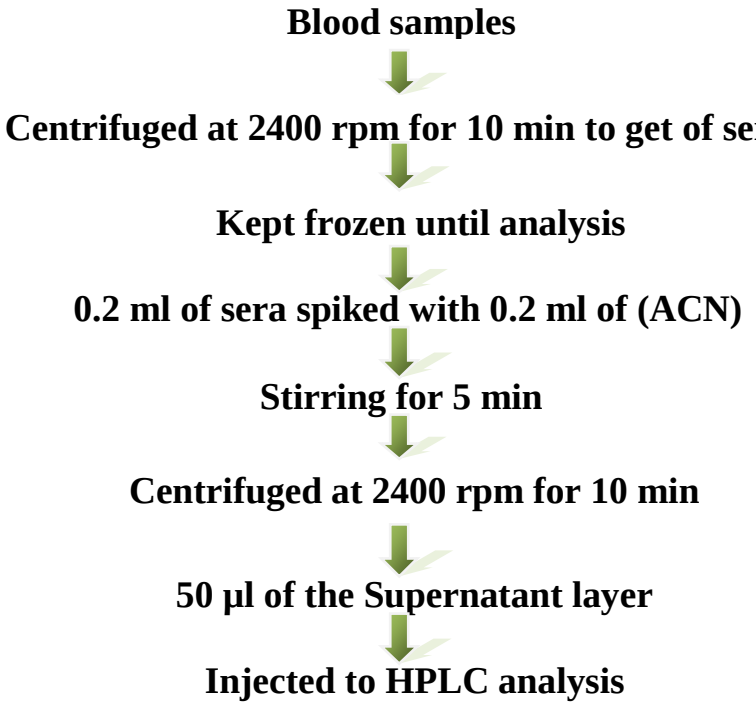


Figure (2. 1): Schematic figure steps preparation of sample

2. 4. HPLC instruments and equipments

2. 4. 1. Pumping system

A chromatographic system consists of binary pumps as solvent delivery model LC-20 A, from Shimadzu Corporation. Each pump has a single small plunger reciprocating pump of constant discharging and quick suctioning tube which delivers the mobile phase to the chromatographic column at a flow rate from (0.1 - 10) $ml.min^{-1}$; 1.0 $ml.min^{-1}$ was used in our work.

2. 4. 2. Solvent reservoir

Round - bottomed flasks of 500 ml capacity were used as reservoirs. Stainless steel filter 0.2 μm was fitted at the end of polytetrafluoroethylene (PTFE) tubes (4.35 × 6.35 mm I.D) to transfer the mobile phase from the reservoir to the pump to avoid any possibility of pump damage as a result of presence of any foreign particles.

2. 4. 3. Solvent degassing

In this study, solvent degassing was done by ultrasonic bath, because might be several gases soluble in the organic solvent especially when it pumped under high pressure. Gas bubbles that might be formed will interfere with separation process, steady baseline and the shape of the peak. So, degassing of solvent is important step to remove all dissolved gasses to increase the sensitivity and to reduce the baseline noise[96].

2. 4. 4. Connecting tubes

The pump to injector the system was connected by stainless steel tubing (1.6 x 0.8 cm I.D). To minimize sample diffusion (i.e. band spreading). The connecting tube between the injector and the column were stainless steel (2.6 x 0.3cm I.D), as short as possible.

2. 4. 5. Sample injection system

The sample was entered into the column by using a Rheodyne injector model 7125, where syringe loading sample injector fitted 50 µl injection loop.

2. 4. 6. Columns fast analytical

Phenomenex column, C-18, (50 × 4.6 mm I.D), 3 µm particle size, was used in this study.

2. 4. 7. Column oven (Thermostatic oven)

The column temperature (25 °C) is maintained by using columns oven model (TO – LC -20 A).

2. 4. 8. Detectors

A Shimadzu SPD-20 A ultraviolet –visible variable detector (190-800 nm) with (8 µl) cell volume, was used throughout this study at 254 nm.

2. 4. 9. System control unit and Computer

The calculation of retention time, peak area, peak height, concentration and plot chromatogram were done by Shimadzu – PC unit. The main parts component of HPLC instruments can be described in Figure (2. 2) and (2. 3).

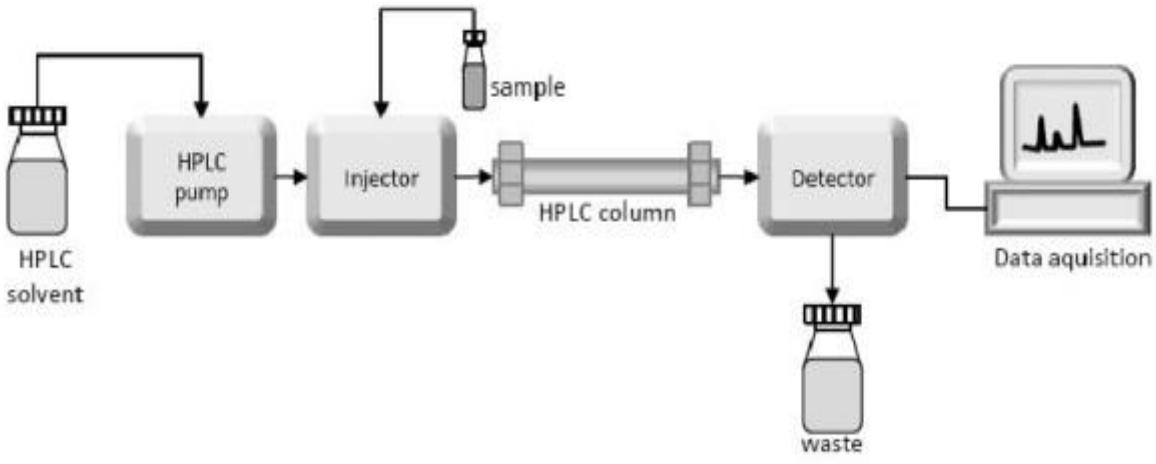
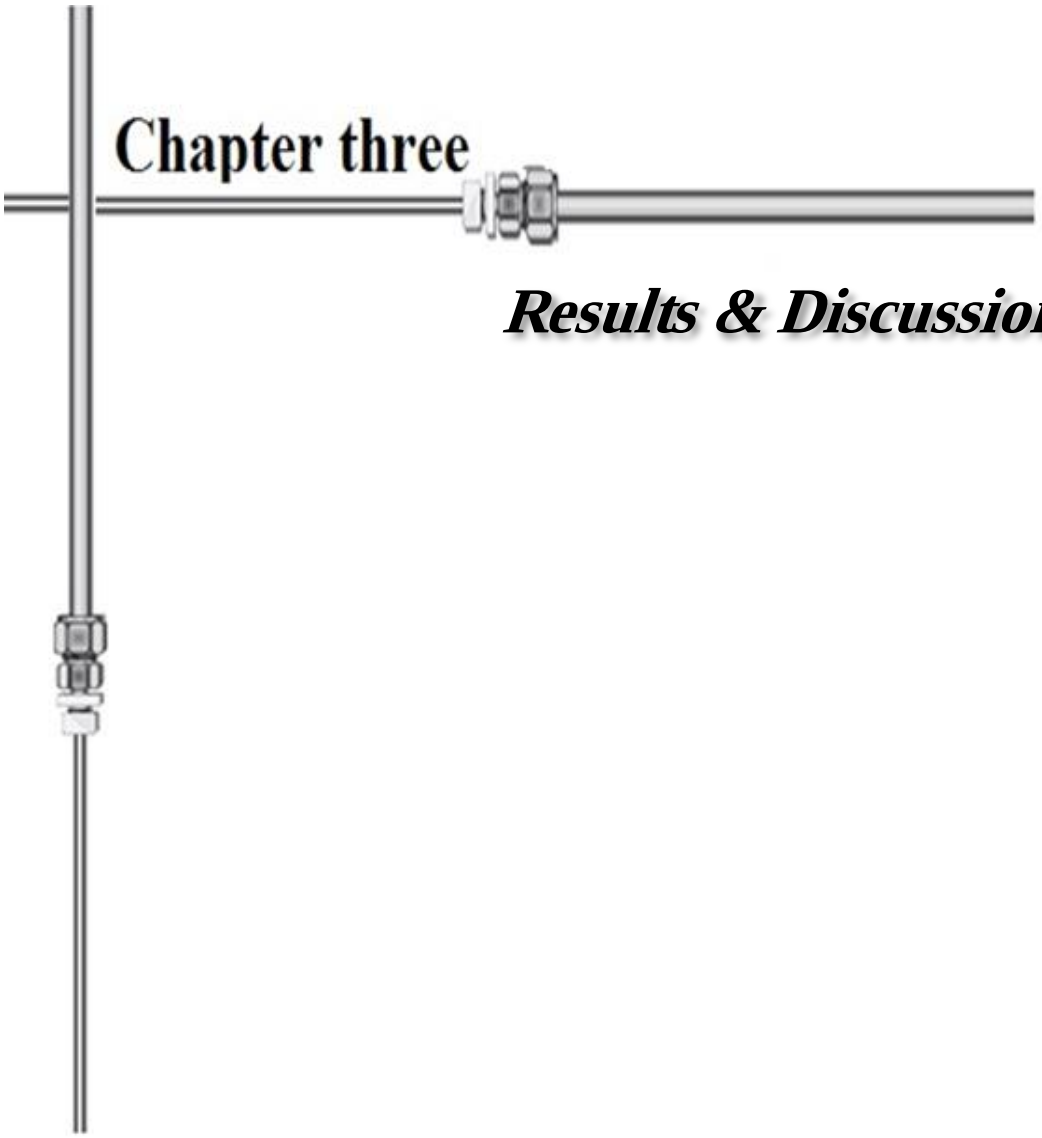


Figure (2. 2): HPLC system diagram.

Figure (2. 3): HPLC (LC-20 A) device picture.

2. 5. Statistical analysis

The results are expressed as mean ± SD. The data are statistically evaluated depending by ANOVA one way model analysis on SPSS software, ($P<0.05$) consider significantly different and ($p>0.05$) consider non significantly different, where P is (significance level)



Chapter three

Results & Discussion

3. Results and discussions

3. 1. Optimization of experimental condition

The most common technique for clinical pharmaceutical compounds separation is HPLC [26] . However, to get the highest efficient separation and acceptable retention time by optimization conditions of HPLC [64].

The optimization of separation is principally directed by the following goals:

- Give better separation and shorter retention time;
- Results in low cost separation (economic effort and highest throughput)[98].

3. 1. 1. Column

The selected column in this work was Phenomenex column C-18, (50 × 4.6 mm I.D), 3 μm particle size, for separation an ampicillin, this column gave high resolution, more efficient chromatographic separation as well as flexibility to rapidly separation with a wide range of antibiotics, because the high surface area of this column.

3. 1. 2. Flow rate

The mobile phase flow rate is very important for HPLC system to fix chromatographic quality and analysis time [99]. Therefore, the appropriate flow rate represent a clear of the interested peak without interference with other constituents of sera sample when separated onto HPLC column at optimum separation conditions. Thus, best flow rate with acceptable separation for ampicillin in sera is 1 ml.min.$^{-1}$ as shown in Table (3. 1).

Table (3. 1): Optimization flow rate for determination standard ampicillin.

Flow rate (ml.min.$^{-1}$)	**Retention time (min.)**
0.4	3.60
0.6	3.51
0.8	3.42
1.0	**3.307**
1.2	3.22

3. 1. 3. pH of mobile phase

Optimum pH control usually results in mobile phase containing buffer that resist any change when the sample is introduced throw the HPLC system. Slight variations in media of mobile phase can have dramatic effects by using K_2HPO_4 buffer (pH = 5) gave better peak shape.

3. 1. 4. Ratio of mobile phase of organic modifier

Deionized water and acetonitrile (ACN) in different percentage of organic modifier of the mobile phase (0 – 30 %) with in increment of 5 % were studied to get the optimum separations conditions as illustrated in Table(3. 2). The mobile phase ratio gave the best separation with low retention time and therefore, without sera components interference at 20% ACN.

Table (3. 2): Retention time of ampicillin at different percentage of ACN

% ACN	Retention time (min.)
0	10.0
5	8.0
10	6.0
15	5.0
20	**3.3**
25	2.1
30	1.8

3. 2. Optimum separation conditions

Table (3. 3) illustrates the optimum conditions which were applied for the separation and determination of ampicillin using RP-HPLC.

Table (3. 3): Applied optimum conditions of ampicillin separation

Instrumental conditions	**Values**
Injected sample volume	50 µl
Column	Phenomenex [C-18, (50 × 4.6 mm I.D) and 3 µm particle size]
Mobile phase	Deionized water (buffer phosphate pH=5, phosphoric acid)and ACN as ratio(80: 20 v/v)
Flow rate	1.0 $ml.min^{-1}$
Column temperature	25 °C
Wavelength maximum	254 nm [100]

Figure (3. 1) shows a typical chromatogram of standard ampicillin using optimum conditions. The mechanism of ampicillin separation by RP - HPLC via adsorption chromatography used for biochemical separation; is based on distribution of ampicillin between mobile phase and stationary phase, after the determination of chromatographic conditions, ampicillin was retained in stationary phase (non-polar) at retention time 3.307 min.

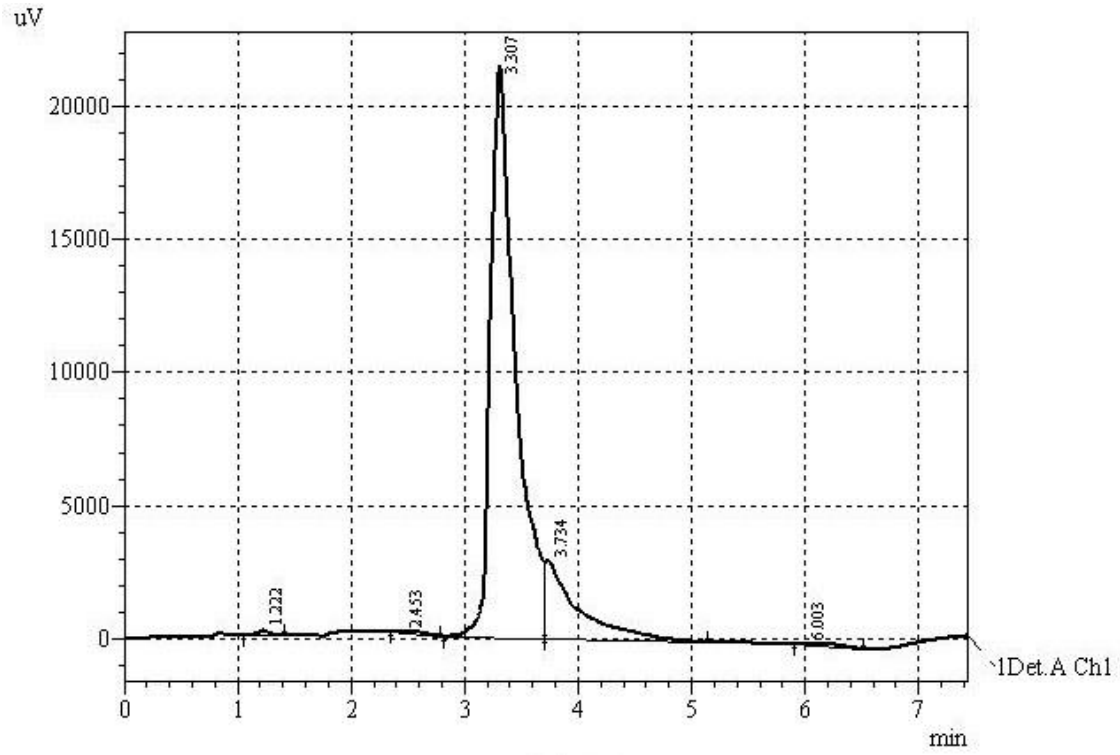


Figure (3. 1): HPLC chromatogram of standard ampicillin(0.2 µg.ml^{-1}).

3. 2. 1. Calibration graph and linearity

After selecting the optimum working conditions as previously described, a series of aqueous standard solutions of ampicillin were prepared. The peak area measurements are obtained as a function of concentration of ampicillin as shown in Table (3. 4).

Table (3. 4): Peak height measurements of standard solutions of ampicillin.

Concentration (µg.ml^{-1})	**Area (µv. sec)**
0.1	10680
0.3	33330
0.8	81005
1.0	106877
5.0	491168
8.0	801221
10.0	993452

The calibration curve of standard ampicillin Figure (3. 2); illustrates the linear relationship between standard concentrations and peak area, the regression equation; (y = 99171 x + 2662.27) and correlation coefficient R^2 = 0.9999.

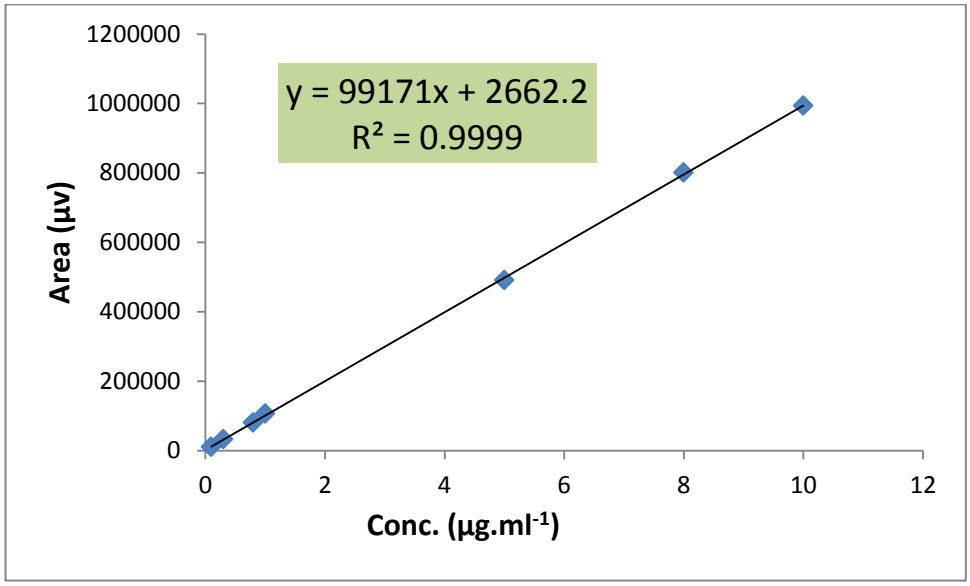


Figure (3. 2): Calibration graph of standard ampicillin

3. 2. 2. Detection limit

The chromatogram of 0.02 µg.ml^{-1} which is the lowest standard ampicillin concentration can be evaluated, at lowest concentrations that the HPLC chromatogram disappeared that the sensitivity of HPLC method was enough to measure all concentrations required through this study as shown in Figure (3. 3).

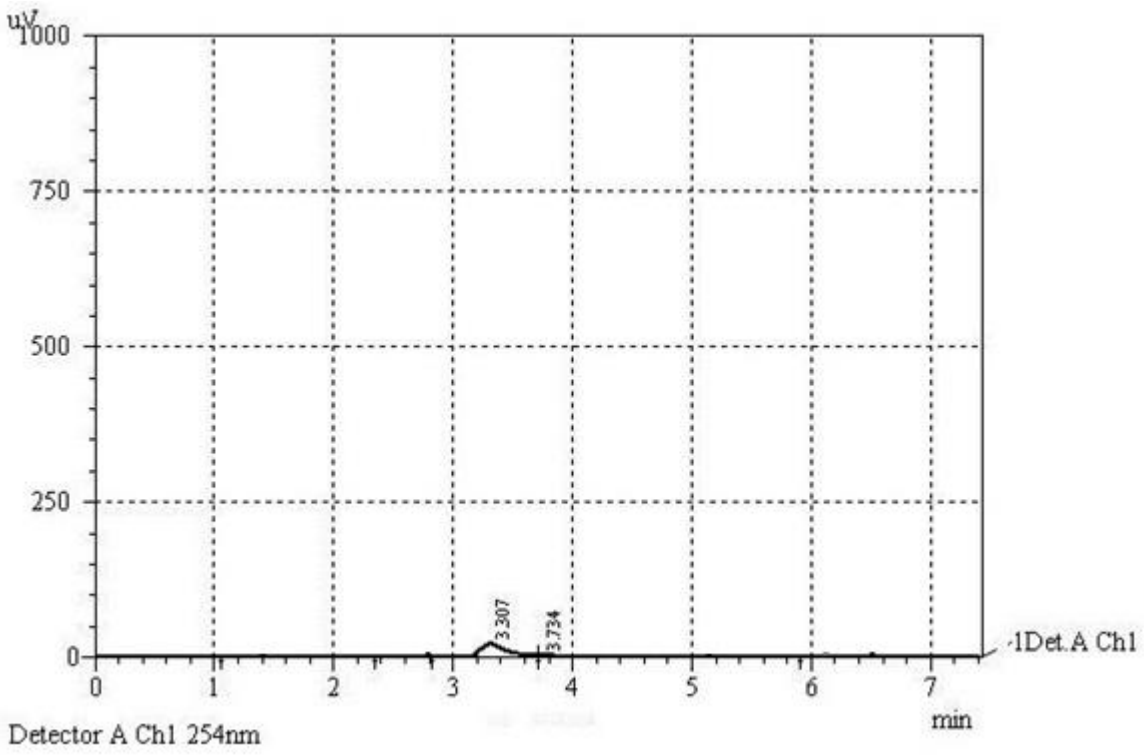


Figure (3. 3): HPLC chromatogram of 0.02 µg.ml^{-1} standard of ampicillin.

3. 2. 3. Statistical constants

Table (3. 5) illustrates the statistical data obtained from the linear equation of calibration curve of standard ampicillin. The results reveal that the limit of detection (LOD) and limit of quantification (LOQ) are 0.02 and 0.066 µg.ml^{-1} respectively, with R.S.D. value 0.250.

Table (3. 5): Statistical calculations for calibration graph of ampicillin.

Statistical factors	Value
Linear equation	y = 99171x + 2662.2
Slope **(m)** or sensitivity	99171
Intercept	2662.2
Correlation coefficient (**R^2**)	0.9999
Percentage linearity (**R^2 %**)	99.99
Correlation coefficient **(r)**	0.99995
Standard Error of intercept **(SE)**	2477.77

Standard Deviation of intercept **(SD)**	6555.56 (SD=SE × $\sqrt{N}$)
Relative Standard Deviation **(RSD%)(n=4)** for 5.0 µg.ml^{-1}	0.250
(LOD) in µg.ml^{-1} calculated by gradual dilution	0.02
(LOQ) in µg.ml^{-1}	0.066 (LOQ = 3.3 × LOD)
Linearity range in µg.ml^{-1}	0.1 -10
Calculated (**t**) values $\mathbf{t}_{cal.} = \frac{/r/\sqrt{n-2}}{\sqrt{1-r^2}}$	223.60 >>> 1.895

3. 2. 4. Reproducibility assay

In Figure (3. 4) represents the chromatogram of a blank sera of healthy volunteer, which is not subjected to ampicillin, and shows no peak retained at 3.307 min.

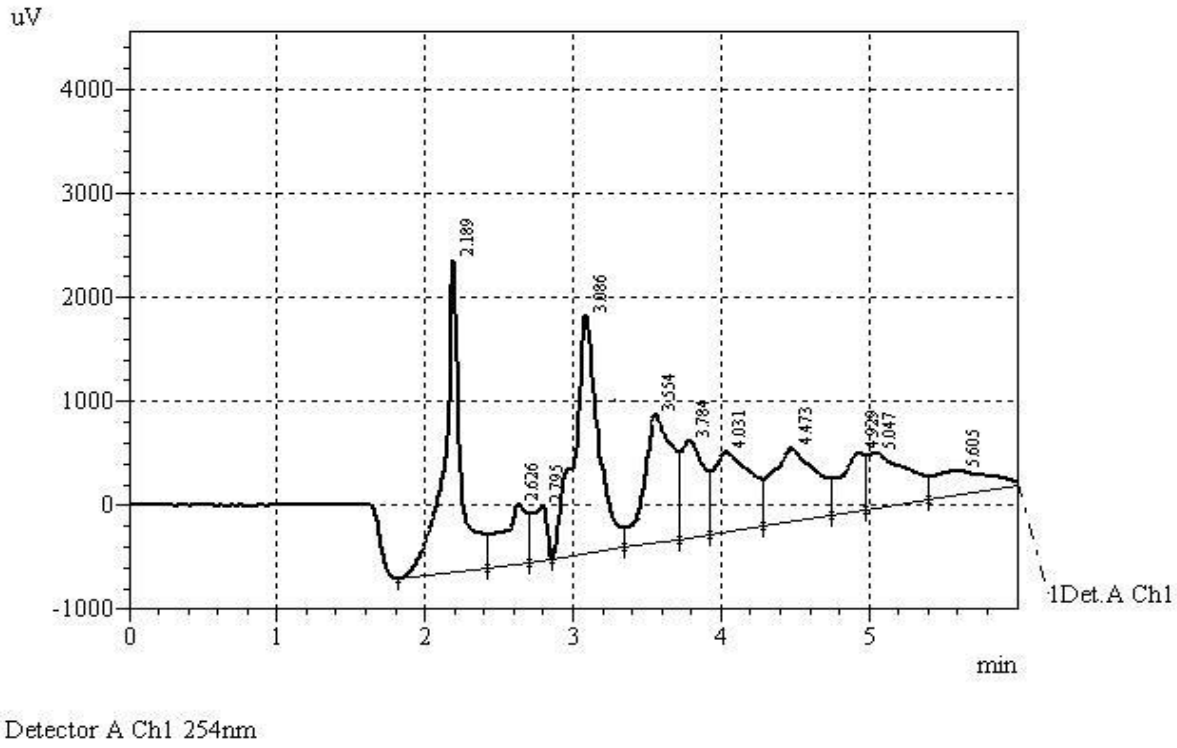


Figure (3. 4): HPLC chromatogram of a blank sera for healthy volunteer.

The chromatogram of ampicillin in sera predicated a new clear peak with retention time of 3.34 min as shown in Figure (3. 5).

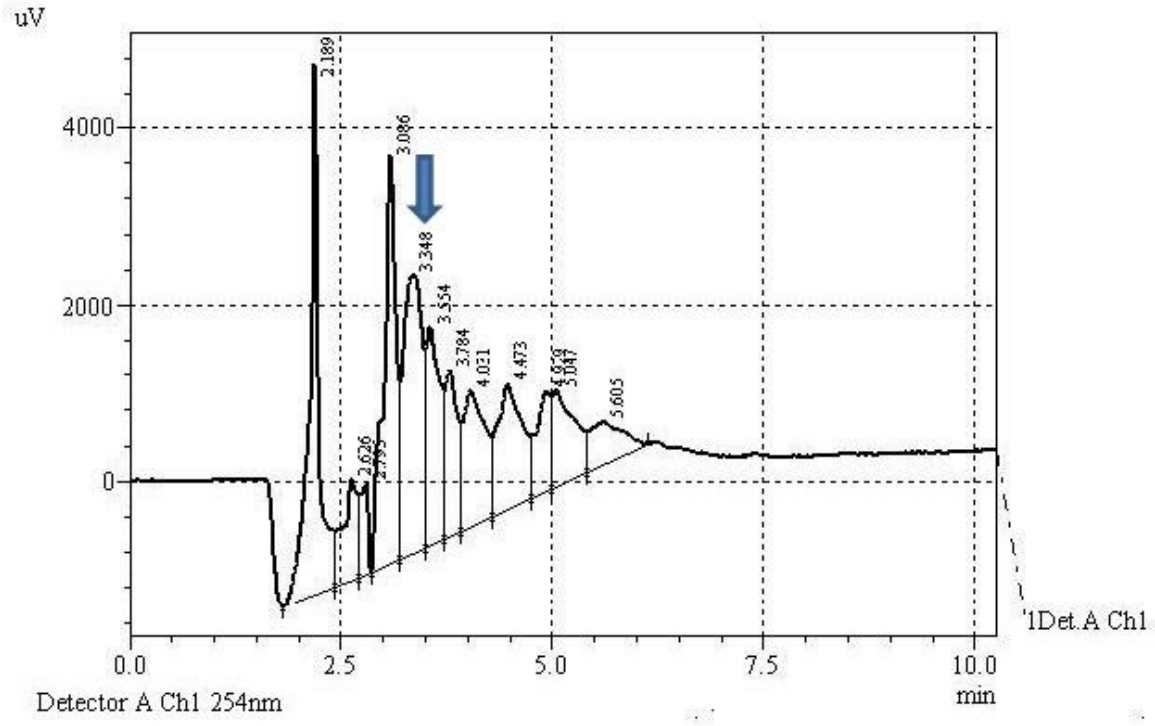


Figure (3. 5): HPLC chromatogram of ampicillin in sera.

For assay reproducibility (Rep.); the method of sample retention time, compared with standard retention time is calculated according to following equation;

Rep. = (retention of sample / retention of standard) × 100

Rep. = (3.348 / 3.307) × 100

= 101.24 %

3. 3. Recovery of spiked method

In order to confirm the reliability and stability for ampicillin determination in sera by HPLC; various concentrations (0.19 – 15 µg.ml^{-1}) were prepared from stock standard of 15 µg.ml^{-1} ampicillin by serial dilution method and spiked each concentration in sera. The results of recovery studies are shown in Table (3. 6), therefore the developed HPLC method can be used for the routine analysis of ampicillin in sera.

Table (3. 6): Recovery of spiked ampicillin in sera (n=3).

Added standard (amp.) µg.ml^{-1}	**Spiked (amp.) found in sera µg.ml^{-1}**	**Recovery %**	**E %**	**RSD %**
0.19	0.182	95.73	4.21	1.43
0.39	0.374	95.87	4.10	1.50
0.78	0.75	96.15	3.85	1.10
1.57	1.52	96.81	3.18	1.32
3.12	3.01	96.47	3.53	1.22
6.25	6.12	97.92	2.08	1.17
12.50	12.25	98.00	2.00	1.35
15.00	14.82	98.80	1.20	1.45
Mean	**4.88**	**96.97**	**3.02**	**1.32**
SD	**5.72**	**1.13**	**1.12**	**0.14**
Accuracy	**4.88±5.72**	**96.97±5.72**	**3.02±5.72**	**1.32±5.72**

3. 4. Volunteer overview

20 healthy volunteers (11males and 9 females) were selected, with average age 35.11 ± 8.3 years, an average weighing 74.10 ± 9.1 kg and average height 173.16±10.6 cm, with their consents. To assay the bioavailability of ampicillin, the characteristics population of healthy volunteers both females and males were studied. These characteristics were including the (age, gender, height and weight), of healthy volunteers with absence illness problems. As tabulated in Table (3. 7).

Table (3. 7): Characteristics of the volunteers (subjects).

subject	gender	Age (year)	Height (cm)	Weight (kg)
1	M	25	181	80
2	M	41	172	75
3	M	28	181	77
4	M	31	169	82
5	M	39	172	72
6	M	52	185	82
7	M	27	182	89
8	M	36	186	78
9	M	28	169	86
10	M	35	192	84
11	M	43	189	77
12	F	32	162	62
13	F	42	162	67
14	F	41	170	71
15	F	30	158	79
16	F	29	155	65
17	F	21	172	61

18	F	37	180	75
19	F	41	167	65
20	F	50	167	55
	µ±SD (Total)	**35.11±8.38**	**173.16±10.62**	**74.10±9.15**
	RSD%	**24**	**6**	**12**
	µ±SD (Male)	**35±8.27**	**179.81±8.13**	**80.18±5.02**
	µ±SD (Female)	**35.89±8.72**	**165.89±7.64**	**66.67±7.38**

3. 5. Pharmacokinetic analyses assessment

The individual and [mean ± standard deviation (µ ± SD)] of sera ampicillin concentrations at various times after single oral administration of 500 mg ampicillin of 20 volunteers are established and tabulated in Table (3.8) and Figures (3. 6 and 3. 7). The results revealed that the ampicillin concentrations in sera of both females and males volunteers are changed with times and indicate that the absorption take place at (0.5 - 1 hr) and elimination at (2 - 8 hr) of ampicillin in vivo. The highest absorption is occurred at (1hr) by the small intestine, that means a high activity and rapidly of the ampicillin in sera, this process considered as ampicillin distribution in sera is very important to determine the therapeutic effect[101]. When the concentration began minimizing, the ampicillin started to eliminate by kidney via liver. Upon taking the next dose, concentration-time curve is periodically changed.

The results in Table (3. 8) and Figures (3. 6 and 3. 7) show, that at (0.5hr, absorption stage) the lowest and highest ampicillin concentrations in sera are (5.04 and 6.91) $\mu g.ml^{-1}$ for males volunteers No. (3 and 5) respectively. While for females, the lowest and highest concentrations of ampicillin-sera are (4.59 and 6.54) $\mu g.ml^{-1}$ for volunteers No. (20 and 12)

respectively. This indicate that there is a clear deference in ampicillin concentrations between healthy for both gender.

At state of the highest absorption of ampicillin (at 1 hr), where the lowest and highest concentrations are (6.74 and 8.01) $\mu g.ml^{-1}$ for males volunteers No. (5 and 6) respectively. For females, the lowest and highest concentrations are (5.67 and 7.88) $\mu g.ml^{-1}$ for volunteers No. (17 and 19) respectively, this meaning a clear difference in ampicillin concentrations between the two healthy genders as a result of its distribution in sera of males more than females.

The highest average of elimination stage for healthy males started after about (2 hr) with (3.84 ± 0.34) $\mu g.ml^{-1}$, while the lowest elimination state is occur at (8 hr) with average concentration of (0.18 ± 0.03) $\mu g.ml^{-1}$. The highest average of elimination stage for healthy females began about 2 hr with (3.98 ± 0.29) $\mu g.ml^{-1}$, while the lowest elimination state is occur at 8 hr with average concentration of (0.17 ± 0.03) $\mu g.ml^{-1}$. This indicate that there is slightly clear difference in ampicillin concentrations between healthy males and females at elimination stage.

Table (3. 8): Variation of ampicillin concentration (µg.ml^{-1}) with time.

No.	Subj.	Time (hr.) after single oral administration						
		0.5	**1.0**	**2.0**	**3.0**	**4.0**	**6.0**	**8.0**
1	M	5.23	7.24	4.32	2.04	1.98	0.64	0.174
2	M	5.88	7.94	4.02	2.39	1.74	0.72	0.211
3	M	5.04	7.61	3.55	2.33	1.63	0.67	0.200
4	M	6.02	7.11	3.71	2.87	1.65	0.58	0.187
5	M	6.91	6.74	4.11	2.33	1.98	0.77	0.127
6	M	5.67	8.01	3.12	3.21	1.68	0.66	0.101
7	M	6.22	7.23	3.94	2.49	1.8	0.55	0.211
8	M	6.24	6.77	4.08	2.88	1.80	0.60	0.176
9	M	5.64	7.58	3.87	3.51	1.88	0.67	0.199
10	M	6.12	6.97	4.02	2.48	2.27	0.55	0.192
11	M	6.37	7.91	3.49	2.84	1.66	0.51	0.184
12	F	6.54	7.54	3.98	2.54	1.64	0.58	0.198
13	F	5.67	6.94	4.08	2.91	1.66	0.54	0.176
14	F	5.41	7.09	3.87	3.01	2.01	0.65	0.111
15	F	5.62	6.79	4.44	2.88	1.79	0.59	0.165
16	F	5.18	6.04	3.49	2.67	1.77	0.59	0.165
17	F	4.99	5.67	3.64	3.04	1.95	0.637	0.138
18	F	6.00	7.44	3.94	2.67	2.01	0.53	0.198
19	F	5.41	7.88	4.27	2.33	1.79	0.68	0.199
20	F	4.59	6.45	4.14	2.66	1.57	0.55	0.188
	µ±SD	**5.74± 0.58**	**7.15 ±0.63**	**3.90± 0.32**	**2.70± 0.35**	**1.81± 0.17**	**0.61± 0.07**	**0.17± 0.03**
	RSD%	**10**	**9**	**8**	**13**	**9**	**11**	**18**
	Max.	**6.91**	**8.01**	**4.44**	**3.51**	**2.27**	**0.76**	**0.21**
	Min.	**4.59**	**5.67**	**3.12**	**2.04**	**1.57**	**0.51**	**0.10**

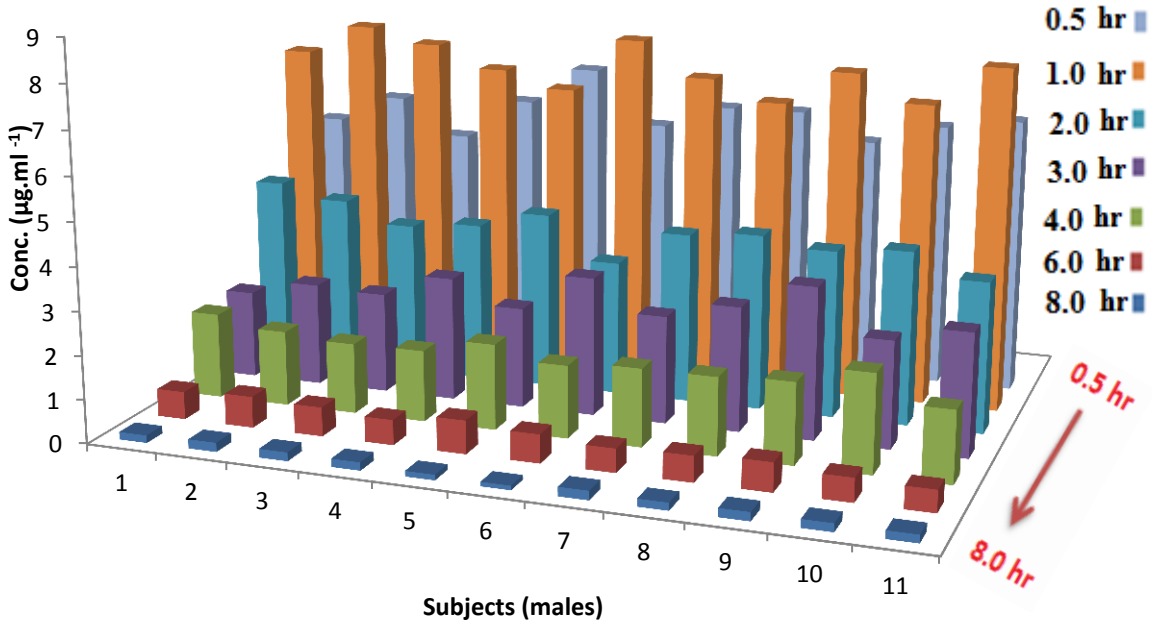


Figure (3. 6): Ampicillin concentrations in the sera of eleven of healthy male individuals for periods 0.5-8 hr.

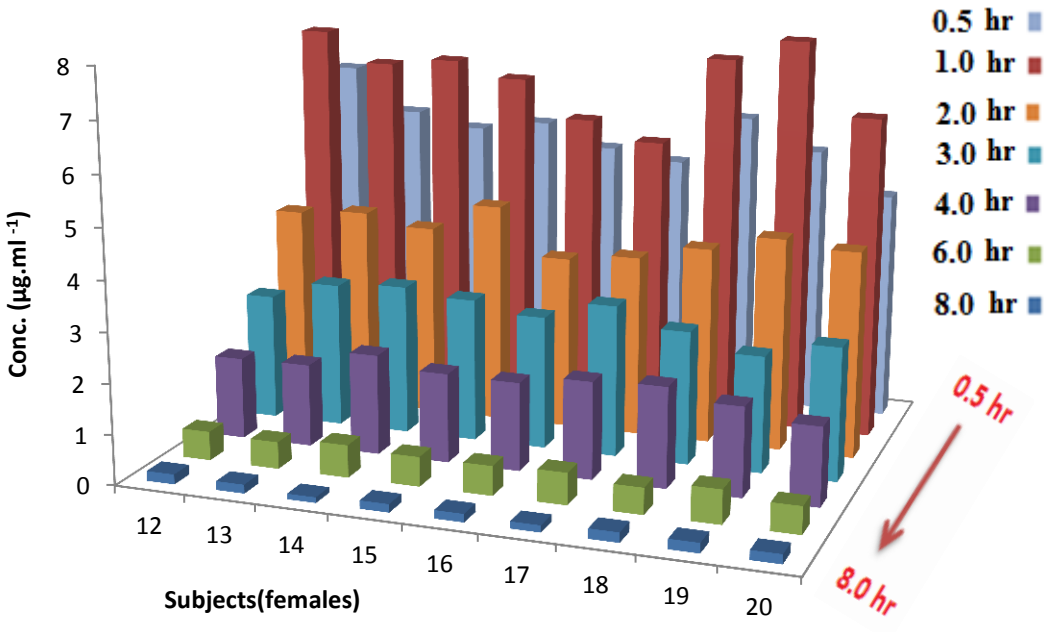


Figure (3. 7): Ampicillin concentrations in the sera of nine healthy female individual for the period 0.5 - 8 hr.

3. 6. Average ampicillin concentrations in sera

The study of finding the average ampicillin (500 mg) concentrations is accomplished after single oral administration for males (n = 11) and females (n=9), within the periods (0.5, 1, 2, 3, 4, 6 and 8 hr.).

The results show that after (0.5 hr) are (5.49 ± 0.57 and 5.94 ± 0.53) $\mu g.ml^{-1}$, and after (1 hr) are (6.87±0.72 and 7.37 ± 0.46) $\mu g.ml^{-1}$ for females and males respectively. The absorption and distribution of the drug were different i.e. the average ampicillin concentrations in the sera of males was found to be more than females, on the other found, the results in periods [(2, 3)(4, 6 and 8 hr)] of elimination stage, show that there are a slight significant difference in the average concentrations for both genders. All these significant differences may be due to the body's nature, activity of gastric enzymes, pH, cytochromes, body fat composition, body mass, lowest blood volume, transporter proteins and hormonal system for both genders[102]. The Table (3. 9) and Figure (3. 8) depict the results.

Total average ampicillin concentrations in sera for both males and females (n = 20) after administration of a single dose, way found to be (5.72± 0.32) $\mu g.ml^{-1}$ at the absorption stage (0.5hr),maximum concentration at (1 hr.) is (7.15 ± 0.31) $\mu g.ml^{-1}$ of sera for metabolism stage and the minimum concentration at (8 hr.) (0.18 ± 0.01) $\mu g.ml^{-1}$ which is the lowest ampicillin concentration in vivo volunteers, as shown in Table (3. 9) and Figure (3. 9).

Table (3. 9): Average ampicillin concentrations ($\mu g.ml^{-1}$) in sera with times for total volunteers, females and males.

Hour	µ±SD males n=11	µ±SD females n=9	µ±SD males & females n=20
0	0	0	0
0.5	5.94±0.53	5.49±0.57	5.72±0.32
1.0	7.37±0.46	6.87±0.72	7.15±0.31

2.0	3.84±0.34	3.98±0.30	3.91±0.10
3.0	2.67±0.43	2.74±0.24	2.71±0.04
4.0	1.82±19	1.79±0.17	1.81±0.02
6.0	0.63±0.08	0.59±0.05	0.61±0.03
8.0	0.18±0.03	0.17±0.03	0.18±0.01

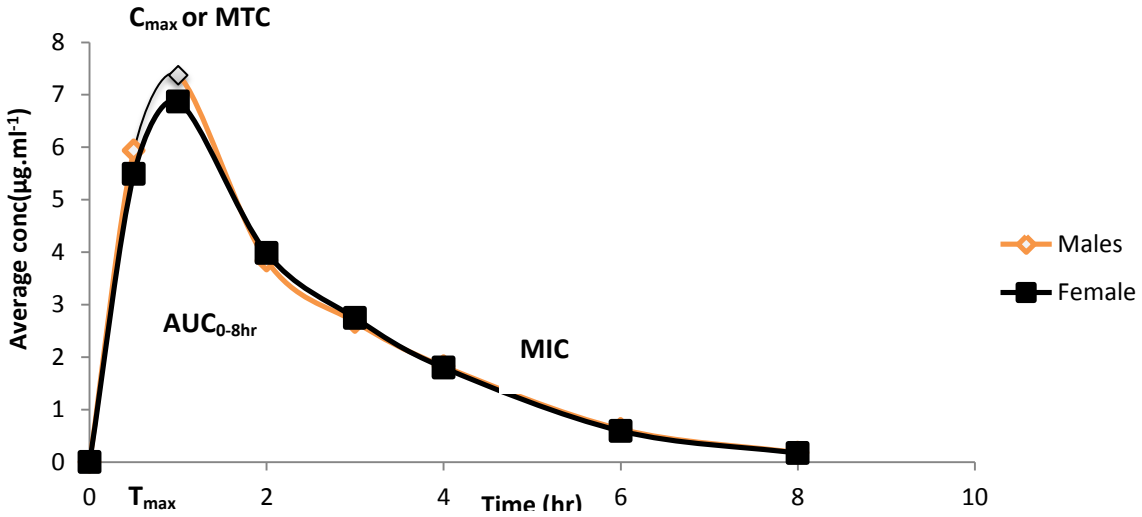


Figure (3. 8): Average ampicillin concentrations (µg. ml^{-1}) – time (hr) plot, after single oral administration of 500 mg ampicillin for both gender.

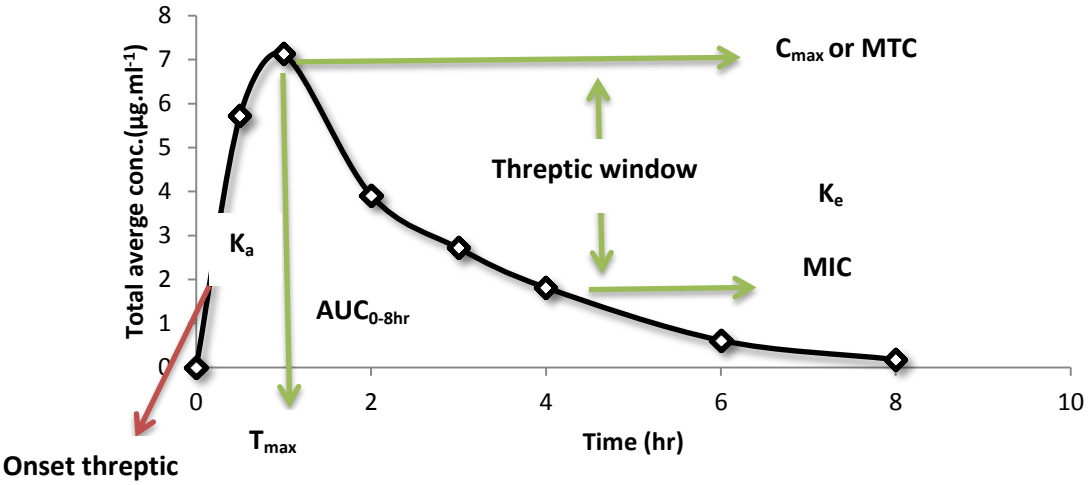


Figure (3. 9): Total average sera concentration (µg.ml^{-1}) – time (hr) plot.

The elimination stage, after maximum absorption of ampicillin, at (1 hr.), which a decrease through the time – concentration curve (especially from 2-8 hr.), shows a slightly difference between males and females volunteers.

The overall operation reveals that the elimination stage is nearly the same for both genders, as shown in figure (3. 10).

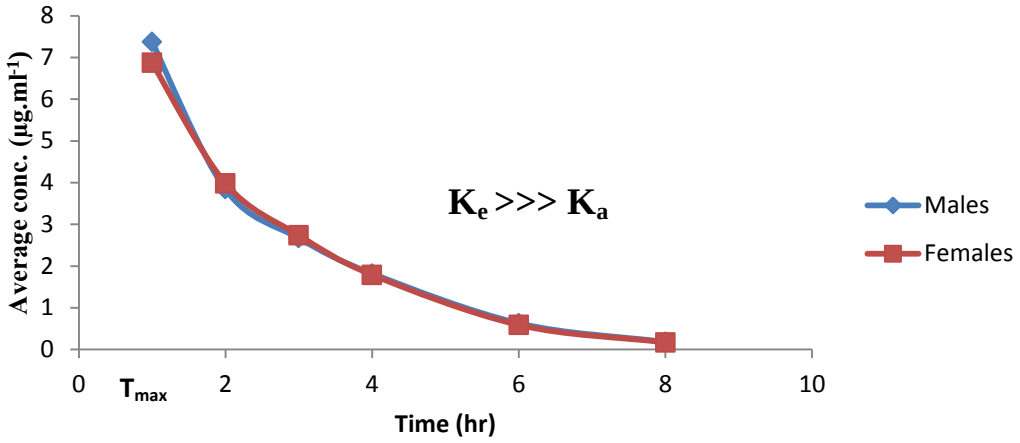


Figure (3. 10): Average ampicillin for both gender for elimination state.

3. 7. Volunteers personal characteristics effects on average ampicillin concertation in sera

The extent of oral administration absorption of ampicillin may varied among volunteers. In this study, some main personal characteristics of volunteers are taken into account and their effect on bioavailability ampicillin in sera; the age, gender, height and body weight. Age factor was significantly effective on ampicillin concentration (i.e. absorption and distribution) in sera of young ages; males(27-31) years and females (21-32) years, more than older ages; males (35-52) years and females (37-50) years, as shown in Figures (3. 11 and 3. 12) and Table (3. 10) on the slight contrast effect of gender for absorption stage (0.5 hr and 1 hr) and other characters such as weight, age and height, there is no significant effect on content of ampicillin in sera, depending on the ANOVA one way model analysis carried out with SPSS software, as shown in Table (3. 11).

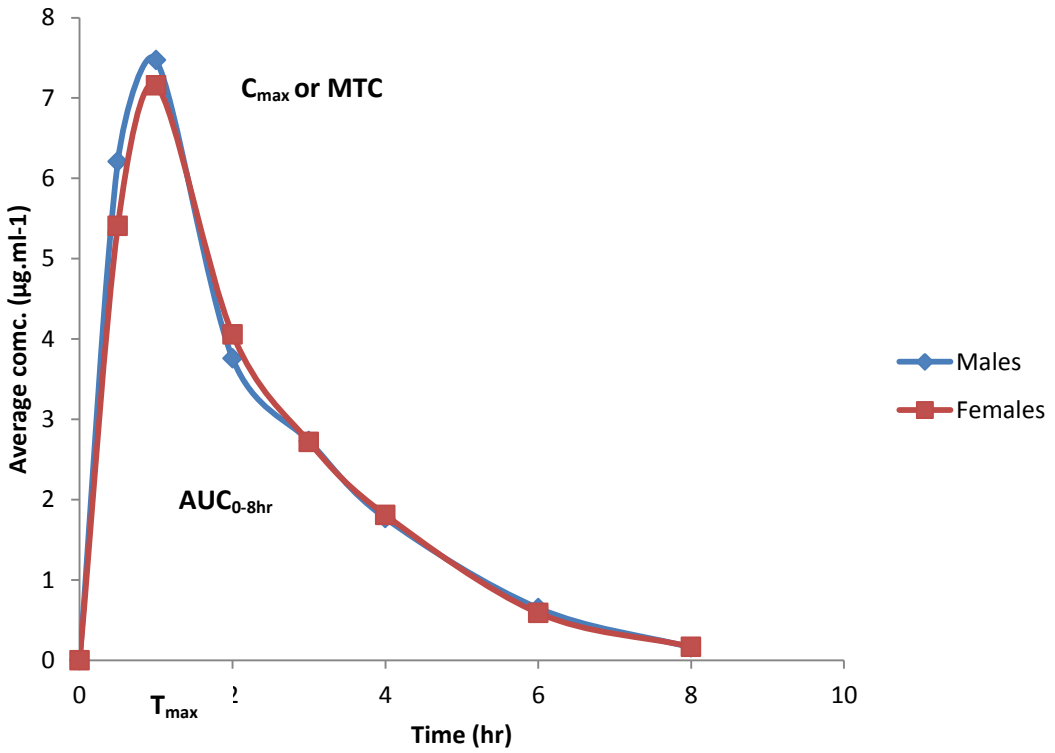


Figure (3. 11): Average ampicillin concentration with times (0.5 - 8 hr)plot of healthy volunteers for ages (males 35-52 year) and (females 37-50 year).

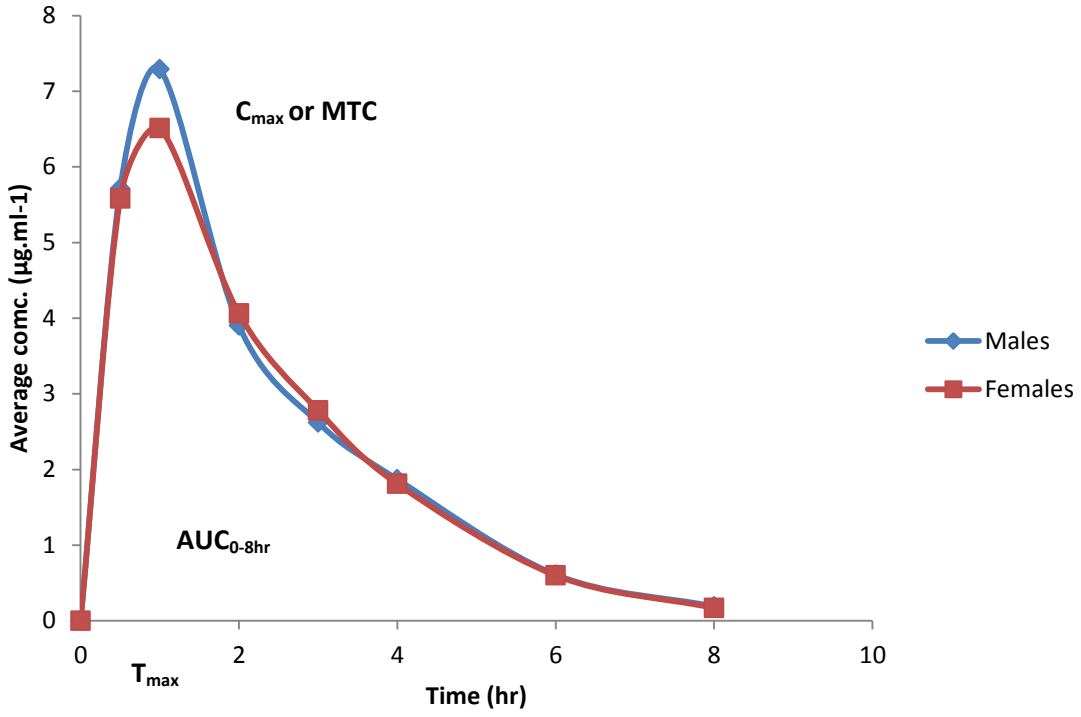


Figure (3. 12): Average ampicillin concentration with times (0.5 - 8 hr)plot of healthy volunteers for ages (males 27-31 year) and (females 21-32 year).

Table (3. 10): Pharmacokinetic parameters for older ages and young ages.

Pharmacokinetic parameters	Older ages		Young ages	
	Males (35-52) year	Females (37-50)year	Males (27-31) year	Females (21-32) year
C_{max} (µg.ml^{-1})	7.39	7.16	7.354	6.51
T_{max} (hr)	1.0	1.0	1.0	1.0
AUC_{0-8hr} (µg.ml^{-1}.hr)	19.35	18.92	18.9767	18.39
k_e (hr^{-1})	0.52	0.53	0.50	0.53
$T_{1/2}$ (hr)	1.33	1.30	1.38	1.31
K_a (hr^{-1})	1.71	1.69	1.76	1.69

Table (3. 11): ANOVA one way results for ampicillin concentration with times.

Hour	P value at 95% confidence interval			
	Gender	Height	Age	Weight
0.5	0.08	0.23	0.57	0.554
1.0	0.07	0.53	0.24	0.225
2.0	0.33	0.33	0.92	0.50
3.0	0.65	0.19	0.82	0.55
4.0	0.76	0.16	0.57	0.98
6.0	0.27	0.57	0.63	0.48
8.0	0.62	0.86	0.28	0.60

3. 8. Pharmacokinetic analyses

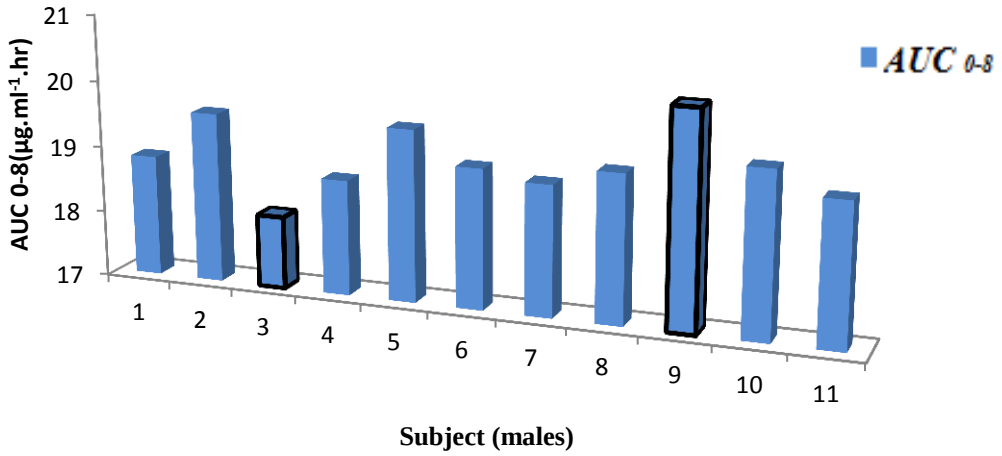
Pharmacokinetic parameters of individual subject and mean subjects following a single oral dose of 500 mg of ampicillin for all healthy volunteers, are shown in Table (3. 12) and (3. 13) ;

3. 8. 1. Area under curve concentration (AUC_{0-8hr})

It is derived from drug concentration and time, (0 - 8 hr). The properties of each effect height, gender, age and weight, were observed depending on ANOVA one way model analysis, there is no significant change of AUC_{0-8} ($p>0.05$) for all healthy volunteers, as shown in Table (3. 14). The average values AUC_{0-8} for males (19.18 ±0.56) $\mu g.ml^{-1}.hr$ more than females (18.68±0.89) $\mu g.ml^{-1}.hr$, as shown in Table (3. 13) , that may be the body mass index in males highest than females.

In this study, the highest value for AUC_{0-8hr} of male volunteer is for No.(9) (20.24 $\mu g.ml^{-1}$. hr) and lowest value of male volunteer is No. (3) (18.09 $\mu g.ml^{-1}.hr$). The results reveal that the highest value AUC_{0-8} for female volunteer No. (18, 19) with (19.46 $\mu g.ml^{-1}$. hr) and lowest value of female volunteer No. (16) is (17.28 $\mu g.ml^{-1}$. hr), as shown in Figures (3. 13 and 3. 14) and Table (3. 12) . AUC_{0-8} observation indicates significant change (nearly by one unit) between males and females. In general, the average AUC_{0-8} of ampicillin in healthy volunteers is 18.96±0.75$\mu g.ml^{-1}.hr$, as availability of ampicillin in sera for both genders of all healthy volunteers 0-8 hr.

The possibilities of the decreased and increased values AUC_{0-8hr} could be due to the possibility of saturation protein binding leading to increase or decrease of clearance (into urine) depending of enzymes responsible for drug metabolism [105].



Figure(3. 13): $AUC_{0\text{-}8hr}$ for healthy males volunteers.

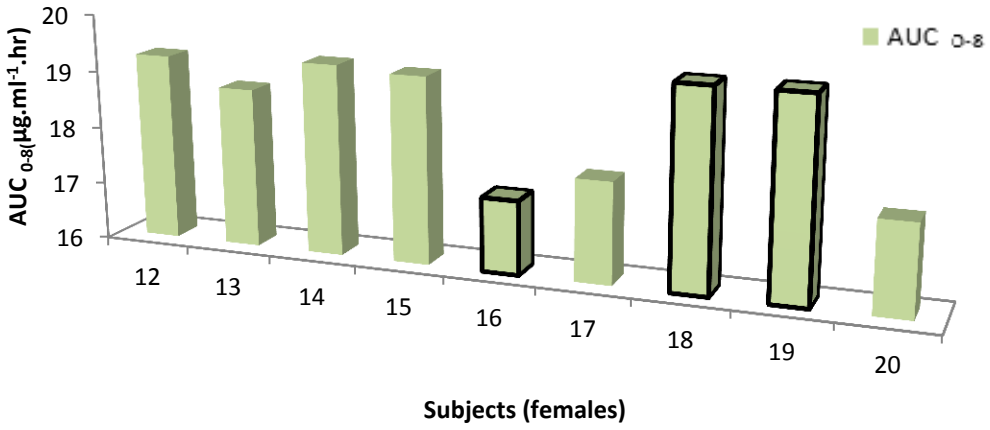


Figure (3. 14): $AUC_{0\text{-}8hr}$ for healthy females volunteers.

3. 8. 2. Maximum concentration (C_{max})

All pharmacokinetics studies reveal that the pharmacological effects of drugs depend upon the concentration of the drug in the body [106].

In this study, the highest value for C_{max} of male volunteer is for No. (6) 8.01 µg.ml^{-1} and the lowest value for male volunteer is for No. (5) 6.74 µg.ml^{-1}. The highest value of C_{max} for female volunteer No. (19) 7.88 µg.ml^{-1} and the lowest value is for No. (17) 5.67 µg.ml^{-1}, as shown in Figures (3. 15), (3. 16) and Table (3. 12) . This indicates that there is with significant change in C_{max} value by nearly one unit between males and females. However, average ampicillin concentration in sera found to be 7.37±0.46 µg.ml^{-1} for males and 6.87±0.72 µg.ml^{-1} for females as illustrate in Table (3. 13) . The values of the ampicillin C_{max} values in sera males is grantor than females because the males have more body mass index values and their hormone system characteristics differ from that for women, thus, metabolisms processes in males are highest than females, and the therapeutic efficacy in men more than women [107].

In general, the average C_{max} of ampicillin in healthy volunteers is 7.15±0.63 µg.ml^{-1} due to availability of ampicillin in sera of all healthy volunteers at (0-8 hr). The properties of each effect of genders and ages for C_{max}, shows a clear significant effect of ampicillin in sera as shown in Figures (3. 8 and 3. 12) . While, heights and weights, have no significant effect on ampicillin in sera, depending on ANOVA one way analysis at ($p>0.05$) as shown in Table (3. 14).

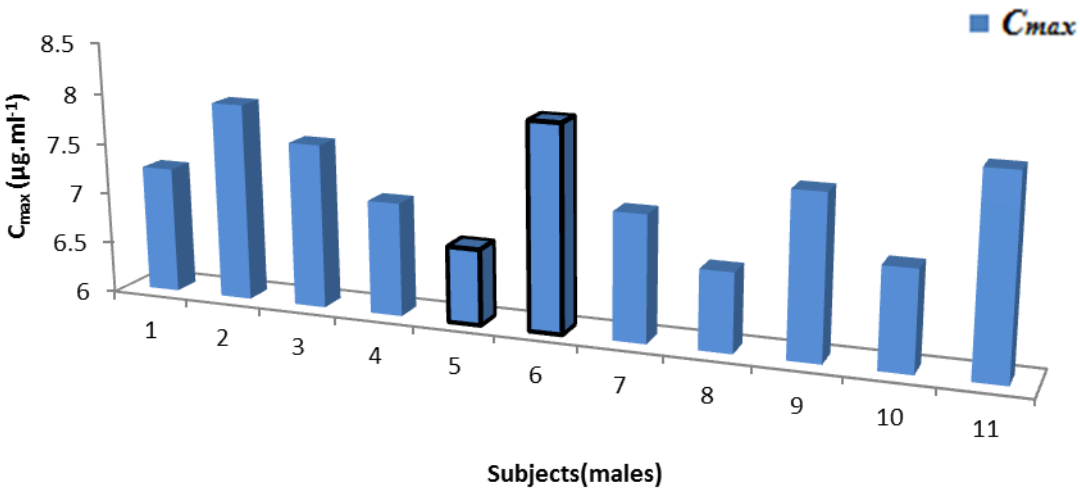


Figure (3. 15): C_{max} for healthy males volunteers.

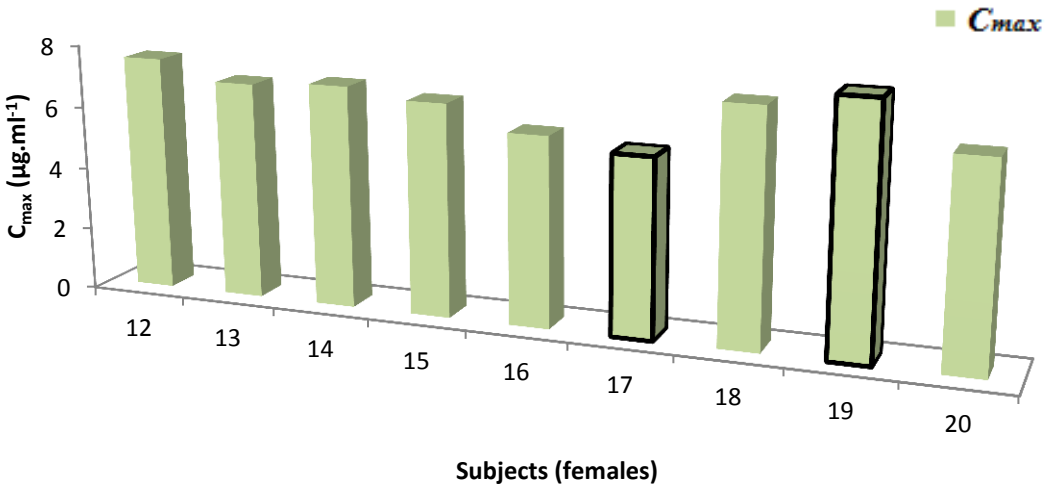


Figure (3. 16): C_{max} for healthy females volunteers.

3. 8. 3. Maximum time (T_{max})

In this study, T_{max} after single oral administration of ampicillin for all volunteers is 1 hr as shown in Table (3. 12) . This means that the highest concentration arrived of the 1.0 hr, and the extent of absorption and elimination processes are equal at this time. However, reaching the highest concentration is important to get rabidly absorb and highest effectiveness of ampicillin in short order to eliminate disease-causing bacteria [108]. The effect of the gender, weight, height and age of healthy volunteers on T_{max}, showed that there is no significant effect of ampicillin concentration in sera depending on ANOVA one way analysis of the resultant ($p > 0.05$), as shown in Table (3. 14).

3. 8. 4. Elimination rate constant (k_e)

k_e is a first order rate constant that describes elimination of ampicillin from the body. In this study, the highest value of k_e of male volunteer is for No. (6) 0.58 hr^{-1}, and lowest value is for No. (3) 0.47 hr^{-1}. The highest value for k_e of female volunteer is No. (14) 0.59 hr^{-1} and lowest value is for No. (19) 0.49 hr^{-1}, as shown in Figures (3. 17 and 3. 18) and Table (3. 12) . Average values of K_e are 0.52±0.03 and 0.53±0.03 hr^{-1} for males and females respectively, as shown in Table (3. 13) . These results indicate that there is no significant difference between males and females i.e. nearly similar elimination of ampicillin in both genders. Moreover there are no significant in k_e values due to effect of height, age, gender and weight for all healthy volunteers depending on the analysis of results ANOVA one way at ($p>0.05$), as shown in Table (3. 14). In general, the average value of k_e ampicillin of healthy volunteers is 0.52±0.03 hr^{-1} as the amount of ampicillin elimination in sera of all healthy volunteers for 0-8 hr.

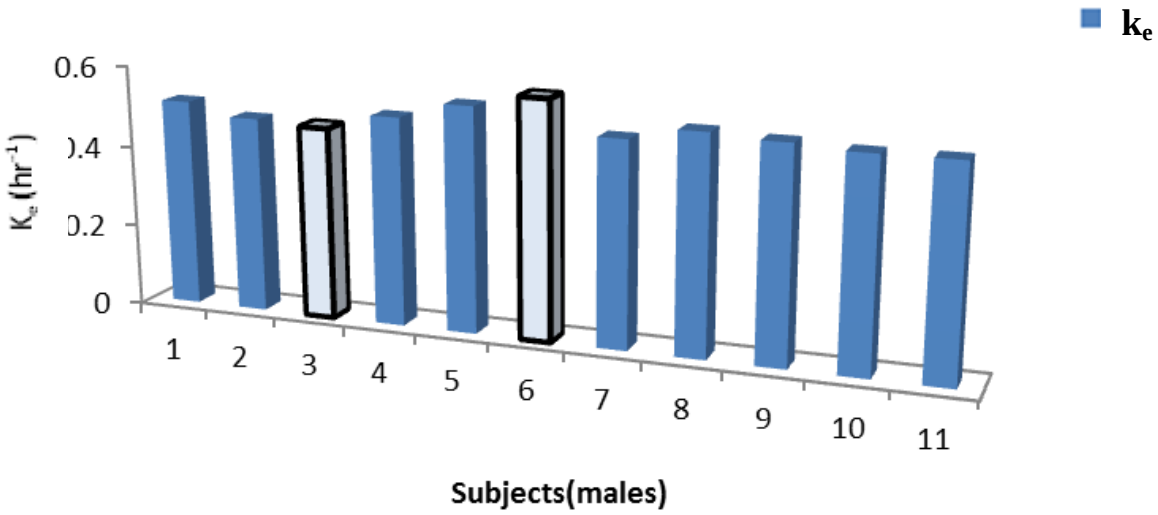


Figure (3. 17): K_e for healthy males volunteers.

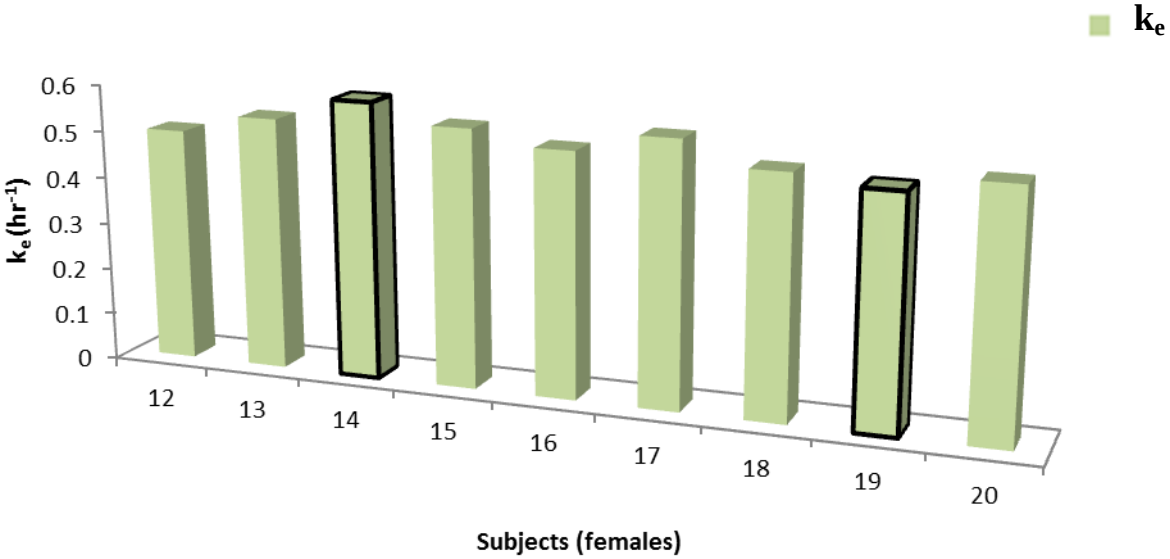


Figure (3. 18): K_e for healthy females volunteers.

3. 8. 5. Half-Life ($T_{1/2}$)

$T_{1/2}$ is the time required for the initial ampicillin concentration to be halved. In this study, the highest value $T_{1/2}$ of male volunteer is for No. (3) 1.47 hr and lowest value is for No. (6) 1.19 hr. Female volunteer No. (19) with highest $T_{1/2}$ 1.41 hr, while No. (14) has highest $T_{1/2}$ 1.17 hr, as shown in Figures (3.19 and 3. 20) and Table (3. 12) . Average values of $T_{1/2}$ are (1.35±0.08 and 1.31±0.08 hr) for males and females respectively, as shown in Table (3.13). So $T_{1/2}$ of ampicillin, nearly similar for both genders, also there is no significant effect of height, age, gender and weight for $T_{1/2}$ for all healthy volunteers depending on the ANOVA one way ($p>0.05$), as shown in Table (3. 14). In general the average value of $T_{1/2}$ ampicillin of healthy volunteers is 1.33±0.08 hr.

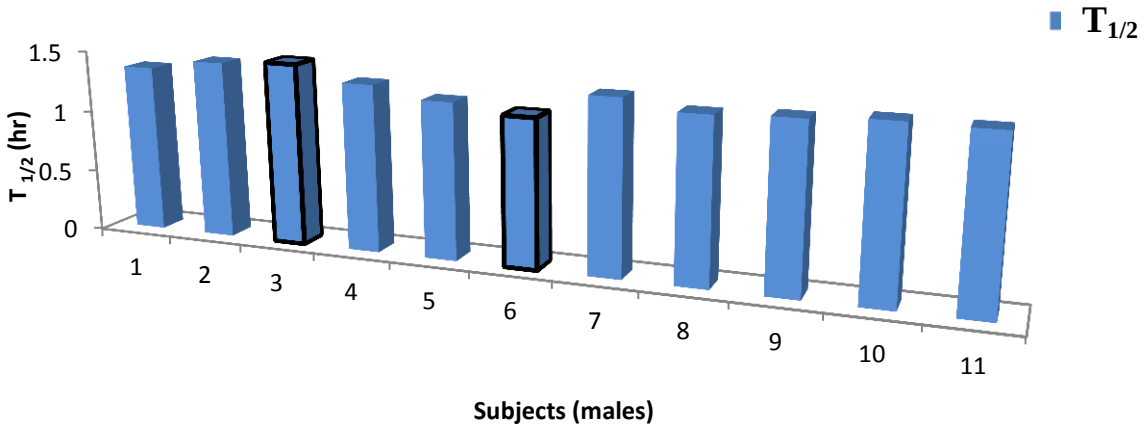


Figure (3. 19): $T_{1/2}$ for healthy males volunteers.

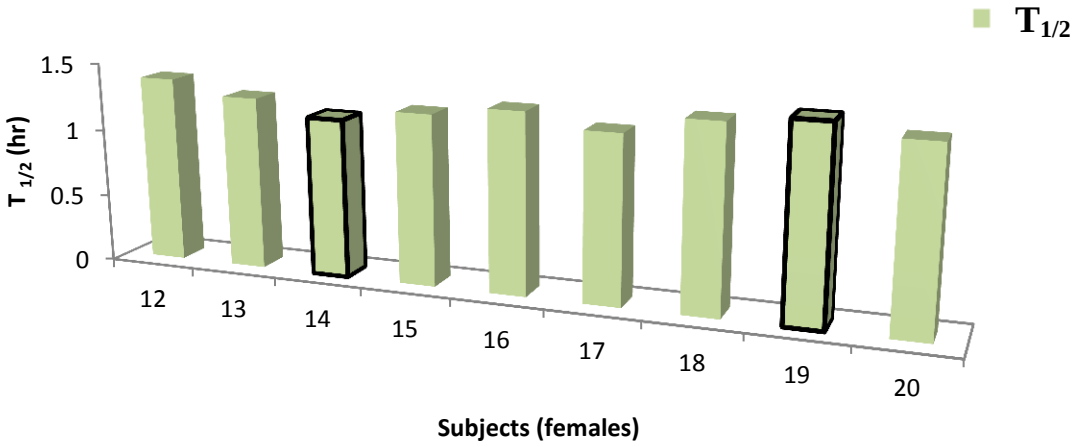


Figure (3. 20): $T_{1/2}$ for healthy females volunteers.

3. 8. 6. Absorption rate constant (k_a)

k_a describes fraction the absorption of ampicillin in the human body. From graphic method, at 0.5 hr, the absorption rate of ampicillin is greater than the rate of ampicillin elimination ($k_a > k_e$). Height peak-drug concentration of 1 hr, the rate of ampicillin absorption equal rate of elimination $k_a = k_e$. Elimination phase of (2-8 hr.), ampicillin absorption appeared zero ($k_e >>> k_a$).

In this study, the highest value k_a of male volunteer is for No. (6) 1.88 hr^{-1} and lowest value of male volunteer is for No. (5) 1.65 hr^{-1}. The highest value for k_a of female volunteer is for No. (19) 1.78 hr^{-1} and lowest value of female volunteer is for No. (14) 1.57 hr^{-1}**,** as shown in Figures (3. 21and 3. 22) and Table (3. 12) . Average values of k_a (1.69±0.06 and 1.75±0.07 hr^{-1}) for females and males respectively as shown in Table (3. 13). There is nearly significant effect between males and females because change mass bodies mass index and harmonic system for both ganders. Moreover, there is no significant effect of height, age and weight for k_a for all healthy volunteers depending on the ANOVA one way ($p > 0.05$), as shown in Table (3. 14). In general, the average value k_a of healthy volunteers is 1.73±0.07 $hr.^{-1}$

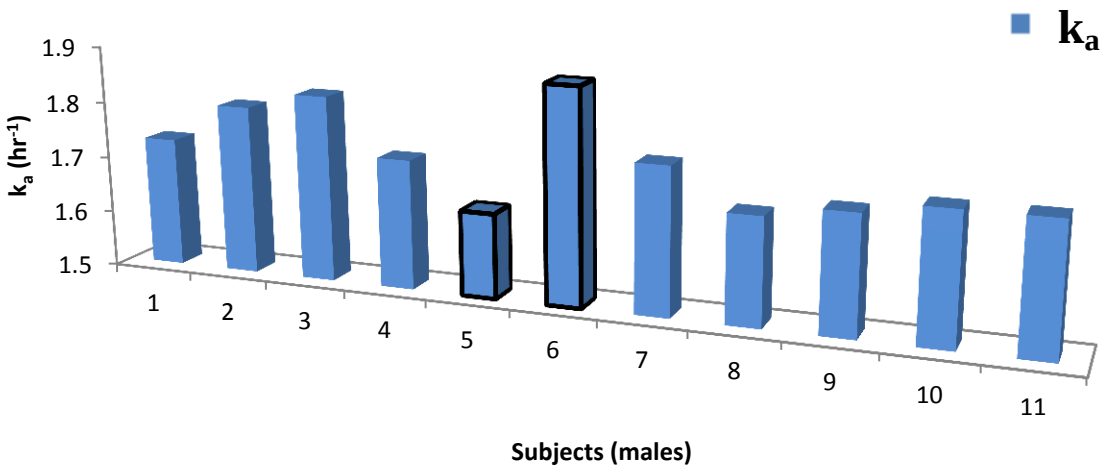


Figure (3. 21): K_a for healthy males volunteers.

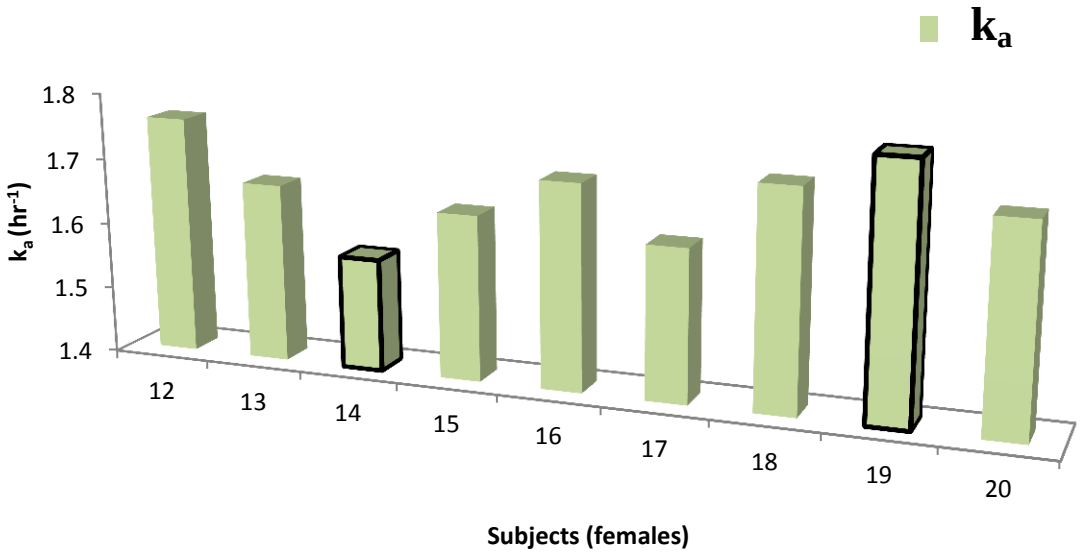


Figure (3. 22): K_a for healthy females volunteers.

Table (3. 12) : Volunteers pharmacokinetic parameters after a single oral dose of ampicillin(500 mg) to 20 healthy volunteers.

No.	Subj.	AUC_{0-8} ($\mu g.ml^{-1}.hr$)	k_a (hr^{-1})	k_e (hr^{-1})	$T_{1/2}$ (hr)	C_{max} ($\mu g.ml^{-1}$)	T_{max} (hr)
1	M	18.83	1.73	0.51	1.36	7.24	1.0
2	M	19.57	1.80	0.48	1.44	7.94	1.0
3	M	18.09	1.83	0.47	1.47	7.61	1.0
4	M	18.74	1.73	0.51	1.35	7.11	1.0
5	M	19.59	1.65	0.55	1.26	6.74	1.0
6	M	19.11	1.88	0.58	1.19	8.01	1.0
7	M	18.97	1.76	0.50	1.39	7.23	1.0
8	M	19.23	1.69	0.53	1.31	6.77	1.0
9	M	20.24	1.71	0.52	1.33	7.58	1.0
10	M	19.48	1.73	0.51	1.36	6.97	1.0
11	M	19.14	1.73	0.51	1.35	7.91	1.0
12	F	19.26	1.76	0.50	1.38	7.54	1.0
13	F	18.78	1.67	0.54	1.28	6.94	1.0
14	F	19.33	1.57	0.59	1.17	7.09	1.0
15	F	19.25	1.65	0.55	1.26	6.79	1.0
16	F	17.28	1.71	0.52	1.33	6.04	1.0
17	F	17.76	1.63	0.56	1.23	5.67	1.0
18	F	19.46	1.73	0.51	1.36	7.44	1.0
19	F	19.46	1.78	0.49	1.41	7.88	1.0
20	F	17.58	1.71	0.52	1.33	6.45	1.0
	μ±SD	**18.96±0.75**	**1.73±0.07**	**0.52±0.03**	**1.33±0.08**	**7.15±0.63**	**1**
	RSD%	**4**	**4**	**5**	**6**	**9**	**0**
	Max.	**20.24**	**1.88**	**0.59**	**1.47**	**8.01**	**1**
	Min.	**17.28**	**1.57**	**0.47**	**1.17**	**5.67**	**1**

Table (3. 13): Volunteers pharmacokinetic parameters average after a single oral dose of ampicillin(500 mg), for both males (n=11) and females (n=9).

parameters	μ±SD (males)	RSD% males	μ±SD (females)	RSD% females
AUC_{0-8}(μg.ml^{-1}.hr)	19.18 ±0.51	3	18.68 ±0.89	4
C_{max} (μg.ml^{-1})	7.37±0.46	6	6.87±0.72	10
T_{max} (hr)	1.0	0	1.0	0
K_e (hr^{-1})	0.52±0.03	6	0.53±0.03	5
$T_{1/2}$ (hr)	1.35±0.08	6	1.31±0.08	6
K_a (hr^{-1})	1.75±0.07	4	1.69±0.06	4

Table (3. 14): ANOVA one way results for pharmacokinetic parameters.

Pharmacokinetic parameters	P value at 95% confidence interval			
	Gender	Height	Age	Weight
AUC_{0-8hr}	0.33	0.91	0.80	0.840
C_{max}	0.07	0.91	0.20	0.776
T_{max}	0	0	0	0
K_e	0.28	0.54	0.98	0.59
$T_{1/2}$	0.25	0.62	0.99	0.61
K_a	0.06	0.50	0.96	0.59

3. 9. Conclusions

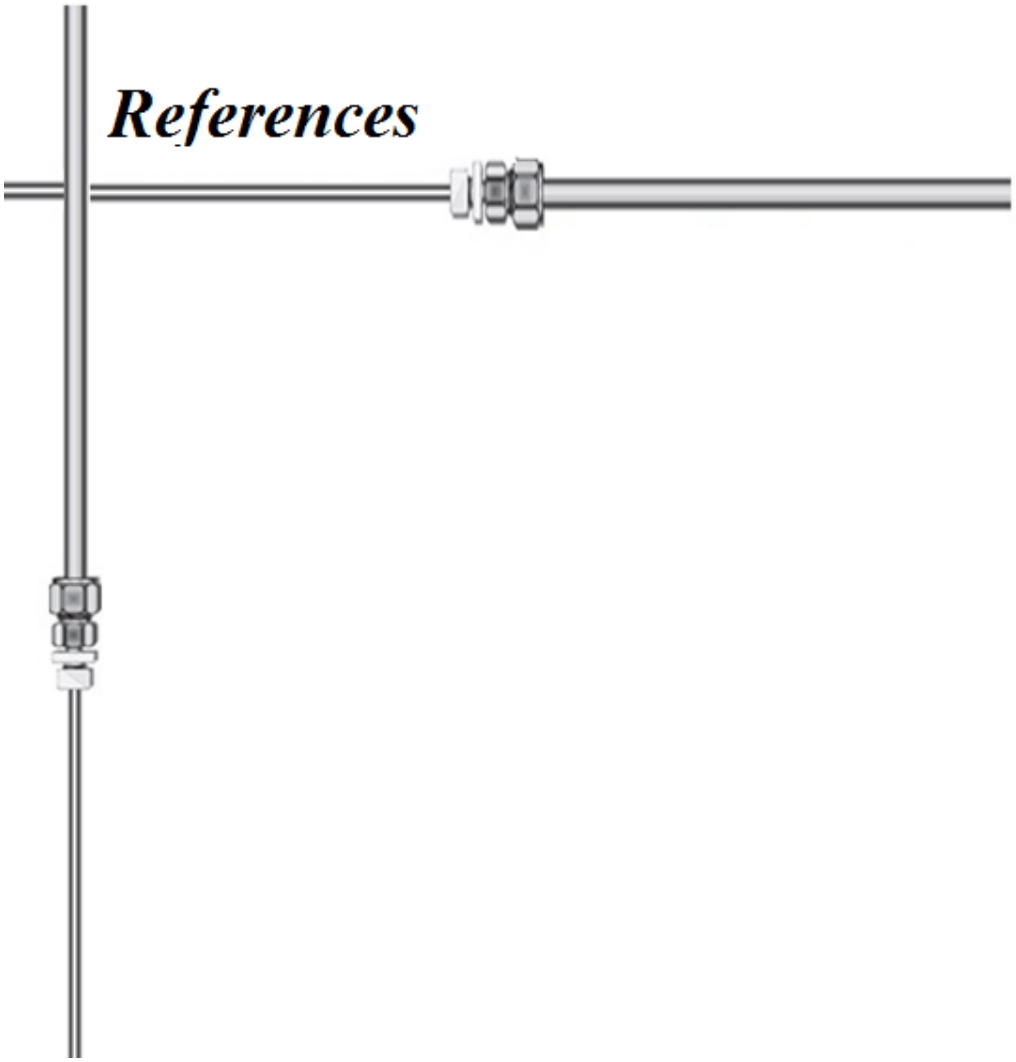
The following conclusions can be drawn as the following:

1. The importance and sensitivity of the RP-HPLC method using fast liquid chromatography to determination of ampicillin and might be used for other β – lactam antibiotics, in estimating concentration of antibiotic in sera for various concentrations and different times (hr).
2. The importance of this study is to estimate the bioavailability of ampicillin in sera of difference age, gender, weight and height of Iraqi healthy volunteers.
3. Study the pharmacokinetic parameters, the rate of absorption and the rate of elimination of drug through the period of various intervals time, to estimate the maximum absorption time, the last time and the drug elimination to the required dose.
4. Ampicillin absorption rate in males almost slightly highest than absorbed in females, but the elimination rate is nearly equal for both genders.
5. Characteristics of volunteers effect on concentration of ampicillin, The results observe a significant effect of both gender and age as well as there is no significant influence to other characteristics at absorption in sera.
6. Rapid absorption of ampicillin in the body.
7. The results this study of pharmacokinetic parameters match perfectly with the results of the program PKMP (version 1.01) from PHARMA CONSULTING COMPANY.

3. 10. Recommendations

After aching the aim of the study, a set of recommendations are drawn below:

1. The progress in developing RP- HPLC, offers a fast and accredited method to follow up drug in sera at different times, consequently, offer accurate method for bioavailability and bioequivalence studies for essential drugs.
2. Quality control of drugs, also can be performed using this method to avoid drugs fake which common in drug industry.
3. HPLC method offer a suitable non expensive method for drug bioequivalence compared with complicated and more expensive (HPLC- Mass) or other high expensive method.
4. This accurate method gives experience to support local drug industry.



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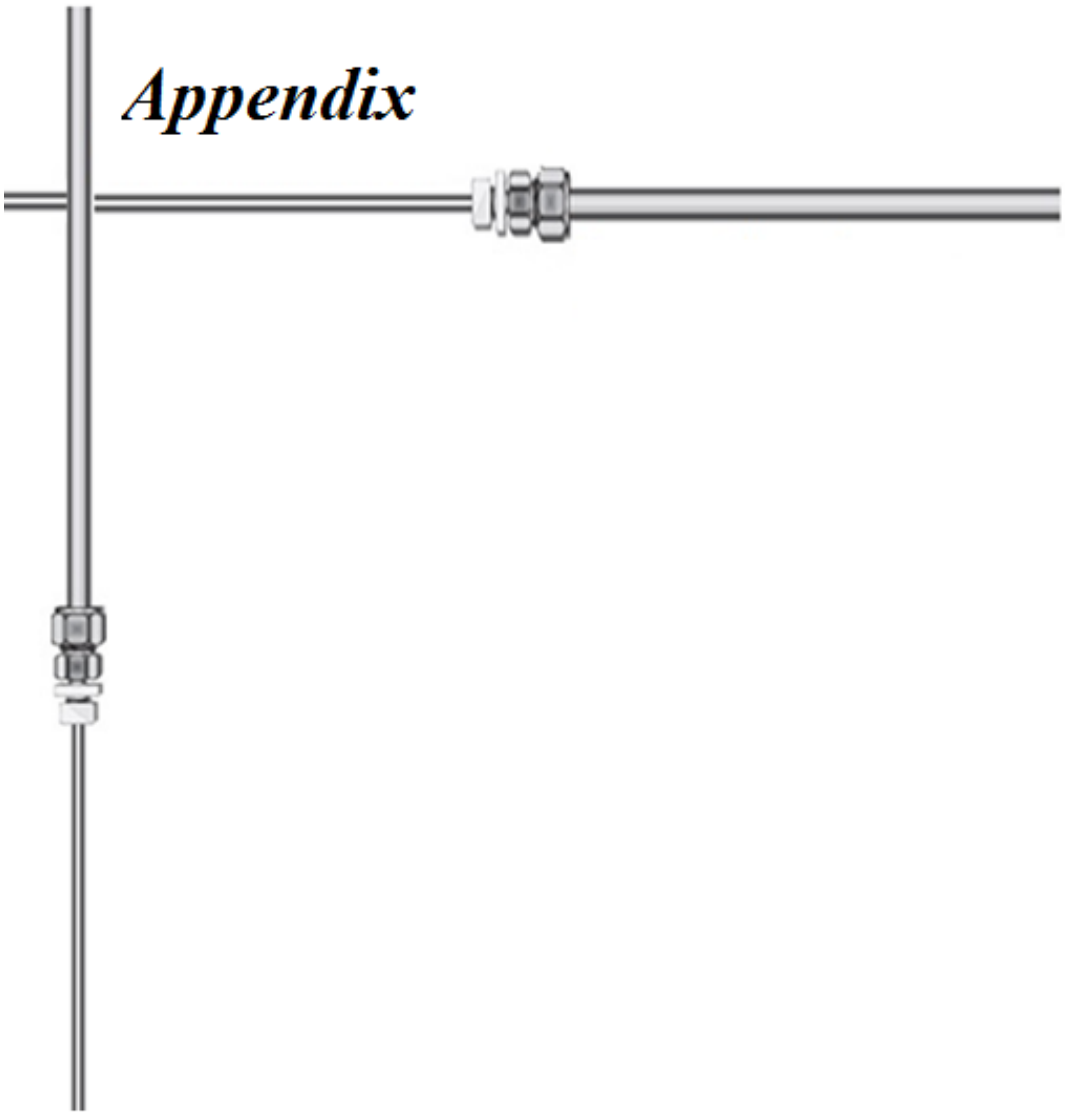
Appendix



Pharmacokinetic analyses

Pharmacokinetic parameters of individual subject and mean subjects following a single oral dose of 500 mg of ampicillin for all healthy volunteers [103];

- Area under curve concentration (AUC)

It reflects the absorbed actual amount of ampicillin in sera after administration of a 500 mg of the drug and is expressed in (µg.ml^{-1}. hr) and calculated by the trapezoidal rule: "the area under the curve can be divided into number of trapezoids as per the sampling times, the total area of each trapezoid is to be calculated by using its formula and summation all the areas added together to get the AUC."[104]. However for calculating AUC:

$$\mathbf{AUC}_{\mathbf{0\text{-}t\ hr}} = \sum_{i=0}^{n} \frac{Cn+Cn-1}{2} \ (t_n - t_{n-1}) \quad \ldots\ldots\ldots (3.1)$$

n = 1, 2, 3, 5, 6, 7.

C (µg.ml^{-1}) = Sera concentration of ampicillin at times

t (hr.) = 0, 0.5, 1, 2, 3, 4, 6, 8 hr

$$\mathbf{AUC}_{\mathbf{0\text{-}8hr}} = \frac{C(0.5)+C(0)}{2} \times (0.5 - 0) + \frac{C(1)+C(0.5)}{2} \times (1\text{-}0.5) + \frac{C(2)+C(1)}{2} \times (2\text{-}1) + \frac{C(3)+C(2)}{2} \times (3\text{-}2) + \frac{C(4)+C(3)}{2} \times (4\text{-}3) + \frac{C(6)+C(4)}{2} \times (6\text{-}4) + \frac{C(8)+C(6)}{2} \times (8\text{-}6).$$

- Maximum concentration (C_{max})

The highest observed concentration in a concentration-time profile to be the highest point of y- axis (graphic method), that represents higher concentration of a therapeutic ampicillin.

The unit used to measure C_{max} is (µg.ml^{-1}).

- Maximum time (T_{max})

It is the time after administration of ampicillin when the maximum sera concentration is reached; where the rate of absorption equals the rate of elimination. It can be calculated directly from the concentration versus time curve (graphic method) of single dose. The unit used to measure T_{max} is hr.

- Elimination rate constant (k_e)

It is the rate at which drug is cleared from the body assuming first-order elimination. The unit used to measure k_e is (hr. $^{-1}$). The calculation of k_e can be done as following :

First method: indirectly from a plot of concentration with time data, to perform this calculation, the concentration-time data must be plotted with a linear x-axis and a logarithmic y-axis. This is commonly referred to as a "semi-log" plot. Starting with the final data point and moving backwards, at least 3-5 data points (elimination phase) fit to a linear regression.

The slope of the line is = - k_e / 2.303

(the slope will be negative, but k_e is a positive value).

Second method: directly calculate (absolute value/k_e/) as an exponential function.

$$\mathbf{(Cp = Cp^0 . e^{-ke.t})} \text{ ..... } \mathbf{(3.2)}$$

Cp = Concentration profile, Cp^0= Initial concentration profile, t = time and k_e = elimination rate constant.

k_e in this study, was calculated in both methods and the results are identical for both ways.

- Half-Life ($T_{1/2}$):

The elimination half-life $T_{1/2}$ is the period of time required for the concentration or amount of ampicillin in the body to be reduced to exactly

one-half of a given concentration or amount. The unit used to measure $T_{1/2}$ is (hr), can be calculated by equation:

$T_{1/2} = 0.693 / k_e$ ……. (3. 3)

- Absorption rate constant (k_a):

The rate at which a drug enters the body after dose administration is called the absorption rate, represented the fractional rate of drug absorption from the site of administration into the systemic circulation. The assessment of the k_a depends on the value of both T_{max} and k_e for single dose:

$$T_{max} = \ln\left[\frac{k_a}{k_e}\right] \cdot \frac{1}{(k_a - k_e)} \quad \text{.....(3 .5)}$$

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الملخّـــص

الأمبيسلين هو مضاد حيوي من مركبات مجموعة البيتا لاكتام والذي يستخدم بشكل واسع في علاج الأمراض المعدية. تمت دراسة التوافر الحيوي بعد تناول جرعة واحدة عن طريق الفم من الأمبيسلين (500 mg) لعشرين شخصا من المتطوعين العراقيين الاصحاء لكلا الجنسين ذوات اعمار واوزان واطوال مختلفة مع موافقاتهم .حيث تم تقدير تراكيز الأمبيسلين في مصل دم المتطوعين بأوقات مختلفة كروماتوغرافيا السائل عالية الاداء (HPLC) ذات معامل ارتباط 0.9999 وزمن احتجاز الأمبيسلين 3.307min باستخدام العمود السريع (50×4.6 mm I.D, 3 µm) والحساسية العالية($0.02 \mu g.ml^{-1}$), ويمكن تطبيقها في تقدير الدواء في امصال الدم لدراسة الحركيات الدوائية. اذ كان امتصاص الأمبيسلّين سريع بعد اخذ الجرعة (زمن اعلى امتصاص الساعة الاولى) وبدون أي تأثيرات سلبية للدواء على المتطوعين خلال فترة الدراسة. وكان معدل امتصاص الأمبيسلين في الذكور أعلى قليلا مما في الإناث، ولكن معدل طرح الدواء تقريبا متساوي لكلا الجنسين وذلك بالاعتماد على الثوابت الصيدلانية التالية: المساحة تحت منحني التركيز AUC_{0-8} (0.51± 19.18 , 18.68 ±0.89) $\mu g.ml^{-1}.hr$ واعلى تركيز ملاحظ C_{max} (7.37±0.46, 6.87±0.72) $\mu g.ml^{-1}$ومعدل ثابت طرح الدواء(0.03±0.52, 0.03±0.53) hr^{-1} وعمر منتصف حياة الدواء البيولوجي $T_{1/2}$(0.08±1.35, 0.08±1.31)hr وثابت معدل الامتصاصk_a (0.07±1.75 , 0.06±1.69) hr^{-1} للذكور والاناث على التوالي.

وتم حساب الثوابت الحركيات لجميع المتطوعين الاصحاء :

C_{max} 0.63±7.15 $\mu g.ml^{-1}$, T_{max} 1hr, $T_{1/2}$, AUC_{0-8} , k_a and k_e 0.08±1.33 hr, 0.75±18.96 $\mu g. ml^{-1}$ hr, 0.07±1.73 hr^{-1} and 0.03±0.52 hr^{-1}.

تمت دراسة خصائص لجميع المتطوعين الاصحاء ولم يلاحظ هناك اي تأثير واضح بالنسبة الى الوزن والطول ولكن هناك تأثير طفيف بالنسبة الى الجنس والعمر في مرحلة امتصاص الأمبيسلين.

وزارة التعليم العالي والبحث العلمي

جامعـــــــة ديـــــــالى

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

بأشراف

أ.م. د. احمد مهدي سعيد

أ. د. فاضل محسن عبد

تشرين الأول 2017 م

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