ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Bioavailability of ampicillin 500 mg capsule on healthy Iraqi volunteers by HPLC.

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ABSTRACT

The bioavailability of ampicillin after a single dose (500 mg) orally administrated was investigated in 20 Iraqi healthy volunteers. Serum concentrations of ampicillin were determined at various times after administration by a sensitive and specific High Performance Liquid Chromatography (HPLC). The method is linear with R^2 value of (0.9994). Ampicillin was well absorbed rapidly after administration. The mean maximum concentration (C_{max}) was found to be 7.14 ± 0.62 µg/mL occurring at maximum time (T_{max}) of 1hr. The elimination half-life ($T_{1/2}$), area under the curve concentration (AUC_{0-8}), absorption rate constant (k_a), and elimination rate constant (k_e) values were found to be 1.34 ± 0.08 hr, 16.07 ± 0.61 µg. mL^{-1}. hr., 1.72 ± 0.06 hr^{-1} and 0.52 ± 0.03 hr^{-1}, respectively. The effect of volunteers characteristics on bioavailability ampicillin after administration 500 mg single dose were investigated.

Keywords: Ampicillin; Bioavailability; Pharmacokinetics; HPLC.

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ISSN: 0975-8585

INTRODUCTTION

Ampicillin is semi-synthetic to penicillin, it has a wide range spectrum, impervious to the action activity of the gastric. The ampicillin is bactericide, because it represses the biosynthesis of the cellular wall of the sensitive bacteria [1]. It is a common antibiotic that is effective against a wide assortment of Gram negative and Gram positive organisms [2]. The bioavailability of ampicillin in humans may be expanded to 88.7 % by association with sub-lactam or clavulanic acid [3]. A few analytical techniques have been described for assay of ampicillin in pharmaceutical formulations using spectrophotometric methods and in biological fluids of animals including colorimetric [4]. These techniques, although still applied routinely in numerous research laboratories, are considered time-consuming and of restricted application [5]. Chromatographic analysis, in specifically high performance liquid chromatography (HPLC), is the most utilized strategy these days for determination of ampicillin due to its specificity, sensitivity, efficiency and reproducibility[6]. There are a few detection conditions related with HPLC for the quantification of ampicillin including spectrophotometry (HPLC-UV), fluorescence, and mass spectrometry (HPLC-MS) [7]. In humans, ampicillin seems to be readily absorbed following oral administration of doses up to500 mg. Bioavailability becomes available at the site of drug action[8]. The aim of our study was to assess the bioavailability and pharmacokinetics assessment of ampicillin in 20 healthy Iraqi volunteers.

EXPERIMENTAL

Chemicals

Ampicillin standard as pure powder, were generous gift from Sammara Drug Industries (SDI) Iraq. Ampicillin capsule as administrate dose for healthy volunteers were obtained from local market. Methanol and Acetonitrile (HPLC-grade) was from BDH. Potassium hydrogen phosphate (K_2HPO_4) as buffer was from BDH.

Apparatus

HPLC (Shimadzu LC – 20 A, Japan), Sartorius balance (Germany), Ultra sonic bath (Karl Kolb, Germany), Shaking water bath (Taiwan) and oven (Memmert, Germany) were used through this study.

Study design and volunteers

The study protocol was approved by the Ministry of Science and Technology, Department of Materials Research (Baghdad- Iraq). The written informed consent was obtained from all volunteers prior to study enrolment. Eleven healthy males (mean age = 35 ± 8.3 years, mean body weights = 80.18 ± 5.01 Kg, mean height = 179.82 ± 8.13 cm) and nine healthy females (mean age = 35.25 ± 9.1 years, mean body weights = 66.67 ± 7.38 Kg, mean height = 165 ± 8.9 cm) participated in this study. No enrolled volunteers had any medical problems according to drug history. Healthy volunteers were don't taken medications (including over-the-counter) neither two weeks prior to nor during the study period and all nonsmokers. Volunteers were randomly assigned to receive orally single dose 500 mg of ampicillin along with 150 mL of water. Both volunteers were managed under supervision taking after an overnight fast of at least 8 hr and subjects continued to fast for no less than 2 hr before and after dosing.

Blood sampling

To determine the serum concentration of ampicillin, 3-5 mL of whole blood was drawn from each volunteer. The time-points at which blood was collected in each case were quickly in before (0 hr) and 0.5, 1.0, 2.0, 3.0, 4.0, 6.0 and 8.0 hr after administration of single oral dose in polyethylene test tubes. To obtain serum, the blood samples were then centrifuged at 4000 rpm for 15 min at room temperature, serum was separated just after sample collection and was frozen store for subsequent assessment.

Analytical assays

Serum samples were analyzed for ampicillin using a validated high-performance liquid chromatography (HPLC) method with UV detection at 254 nm [9]. Serum samples were defrosted at room temperature. Sample preparation was done by liquid phase extraction with 0.2 ml of a mixture of methanol

ISSN: 0975-8585

and (0.2 ml) of serum. Supernatant layer was separated pre-concentrated and 20 µl of sample were injected to HPLC analysis under the optimum separation conditions. Mobile phase consisting of a mixture of deionized water acidified with acetic acid (0.1 %) and Acetonitrile as ratio (80: 20 v/v) was delivered at a flow rate of 1.0 ml.min^{-1} with UV detection at 254 nm. The column was Phenomenex C-18 (50 × 4.6 mm I.D) and 3 µm particle size . Analysis was performed at room temperature (~25 °C) and the total run time was 10 min.

RESULTS AND DISCUSSIONS

Preparation of calibration graph and linearity study

For determining the linearity, a series of solutions with a different standard ampicillin concentration range of (0.02 – 15.0 µg/mL) were prepared by simple dilution of stock solutions. The calibration graphs were obtained by plotting the area under peak versus known concentrations in µg/ml.

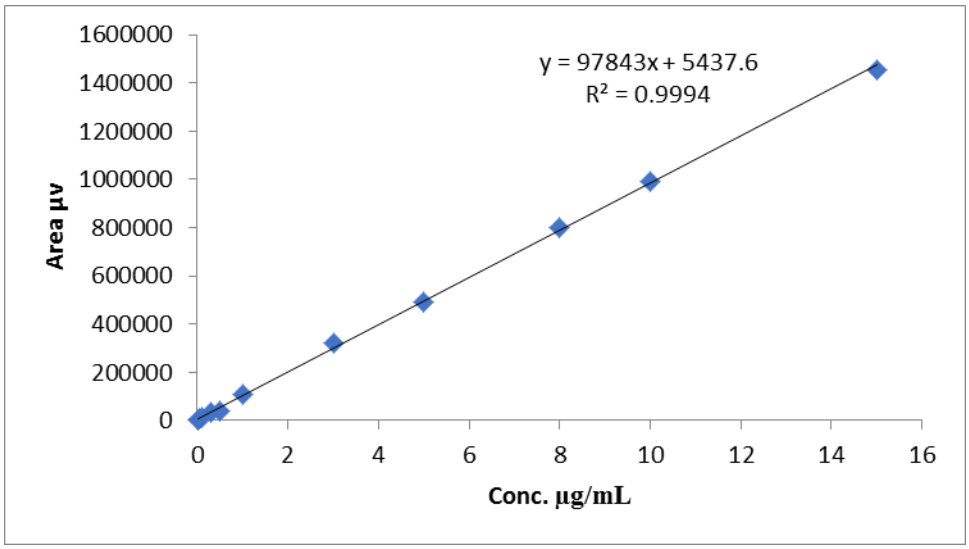

Figure 1: Calibration graph of ampicillin.

Figure (1), show the calibration graph plots of the ampicillin and the obtained results were tabulated in Table (1), which show that the values of t_{cal} are larger than t_{tab} values. The methods were linear with an R^2 of (0.9994) indicating that there is a strong correlation between the variation of concentration and response. Linearity was determined by the regression analysis.

Table 1: Statistical calculations for calibration graph of ampicillin.

Statistical factors	Value
Linear equation	y = 97843x + 5437.6
Slope **(m)**	97843
Intercept	5437.6
Correlation coefficient "**R^2**"	0.9994
Percentage linearity (R^2 %)	99.94
Correlation coefficient (r)	0.9997
Standard error of intercept	4870.18
Standard deviation of intercept	16152.56
Relative standard deviation "R.S.D."	0.250
Linearity range in µg/mL	0.02 -15.0
Limit of detection "LOD" µg/mL	0.02
Limit of quantification "LOQ" µg/mL	0.066
Calculated (t) values $t_{cal.} = \frac{/r/\sqrt{n-2}}{\sqrt{1-r^2}}$	122.46 >>> 2.26

ISSN: 0975-8585

Accuracy and precision of proposed method

Ampicillin was determined at eight different selected concentrations (0.19 - 15 µg/mL). The obtained results were shown in Table 2, which indicated that the proposed method for the determination of ampicillin using this method was quite satisfactory in reality with respect to the procedure and parameters calculated.

Table 2: Precision and accuracy for the determination of ampicillin in serum.

Ampicillin standard add µg/mL n=3	Ampicillin spiked serum n=3	Recovery %	RSD%
0.19	0.182	95.73	1.43
0.39	0.38	97.43	1.50
0.78	0.75	96.15	1.10
1.57	1.52	96.81	1.32
3.12	3.01	96.47	1.22
6.25	6.12	97.92	1.17
12.50	12.25	98.00	1.35
15.00	14.82	98.80	1.45
	Mean	97.16	
	SD	1.13	
	Accuracy	97.16 ±1.13	

Separation and determination of ampicillin

The optimum separation conditions obtained were applied to determined the ampicillin as standard and in serum. The method exhibited good specificity and selectivity which were assessed by the retention times of ampicillin standard and for ampicillin in subject as shown in the Figure 2 (A, B). The reproducibility of sample retention time compared with standard was calculated according to recovery 101.24 % .

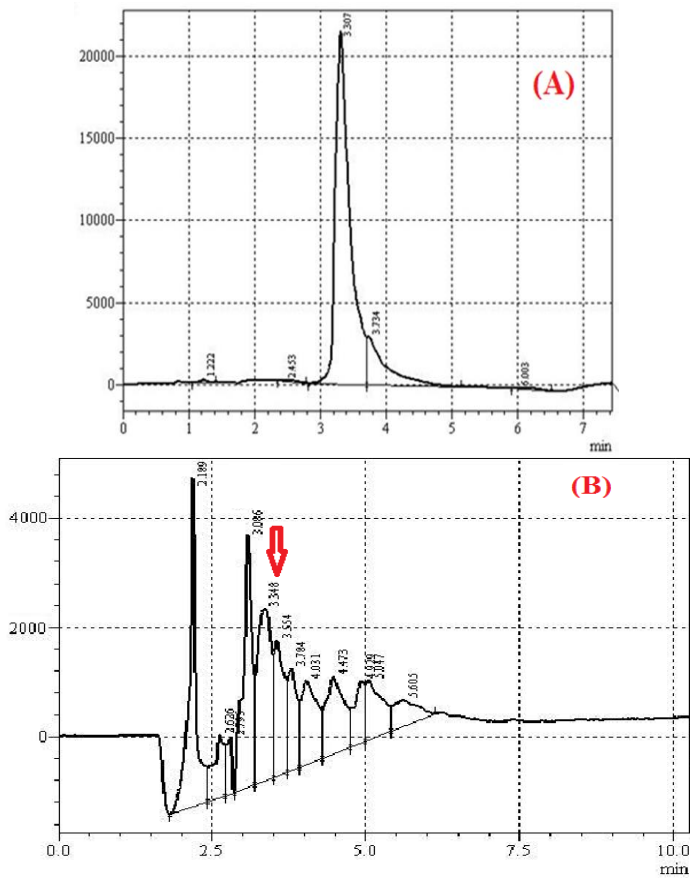

Figure 2 (A, B): Separation of ampicillin standard (A) and in subjects (B).

ISSN: 0975-8585

Pharmacokinetics and bioavailability

The mean serum concentration-time profile of ampicillin is shown in Figure (3) and relevant pharmacokinetic parameters have been listed in Table (3). The mean C_{max} was found to be 7.14 ± 0.62 µg/l occurring at T_{max} of 1 hr. The half-life ($T_{1/2}$), AUC_{0-8}, k_a and k_e values were found to be 1.34 ± 0.08 hr, 16.07 ± 0.61 µg. mL^{-1}. hr., 1.72 ± 0.06 hr^{-1} and 0.52 ± 0.03 hr^{-1} respectively.

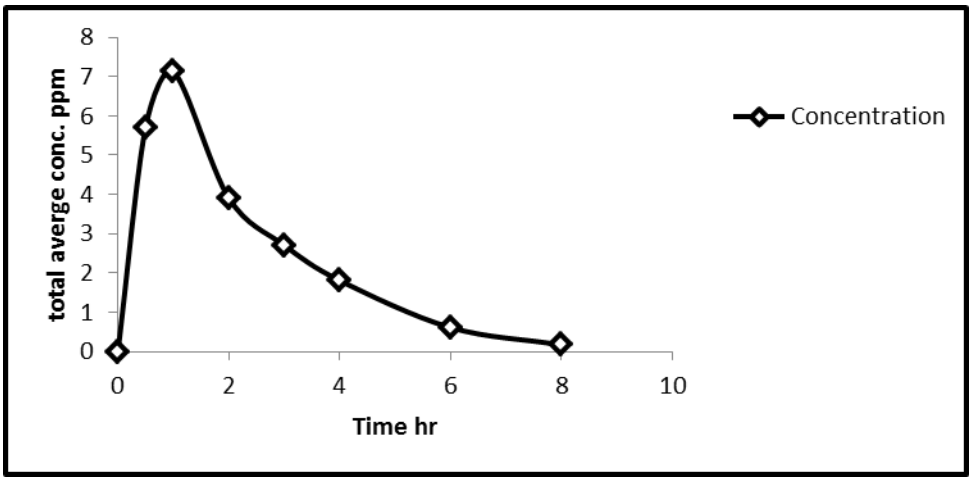

Figure 3: Mean ampicillin serum concentration versus time curve over (AUC) 8 hours in healthy iraqi volunteers (N=20).

Table 3: Pharmacokinetic parameter after administration of 500 mg single oral dose of ampicillin in healthy iraqi volunteers (N=20).

Pharmacokinetic parameter	Mean ± SD
AUC_{0-8} (µg. mL^{-1}. hr.)	18.93 ± 0.74
C_{max}(µg/mL)	7.14 ± 0.62
T_{max}(hr)	1 ± 0
K_a(hr^{-1})	1.72 ± 0.06
K_e (hr^{-1})	0.52 ± 0.03
$T_{1/2}$ (hr)	1.34 ± 0.08

According to the results obtained in the present study, that shown was rapidly and completely absorbed ampicillin after oral administration. In general the average AUC_{0-8} of ampicillin in healthy volunteers is (18.93 ± 0.74 µg. mL^{-1}.hr.), this indicates the amount of availability of ampicillin in serum of all healthy volunteers at 0-8 hr. The possibilities of decreased AUC could be due to the possibility of saturation of protein binding leading to increase in clearance and hence lower AUC. This is indicative of saturable mechanism, this time a process in the elimination of the compound and if the drug is metabolized, then it is quite possible that higher doses lead to saturation of the enzymes responsible for its metabolism[10]. If the drug is excreted through an active secretion (into urine or bile), that process might become saturated. Both will lead to an increase in AUC/Dose [11]**.** The results of C_{max} revealed that there is a significant change by nearly one unit between males and females. However, average ampicillin concentration in serum is 7.37 ± 0.46 µg/mL for males and 6.87 ± 0.72 µg/mL for females. The highest adsorption rate was occurred at the first hour for all volunteers in the adsorption process. Due to the value of adsorption rate constant, the process was occured rapidly. After one hour the ellimination process was takeplace gradualliy and slowly as clearly explain through the low value of ellimnation rate constant, untill reached the minmum concentration after eight hours as shown in Figure (4).

ISSN: 0975-8585

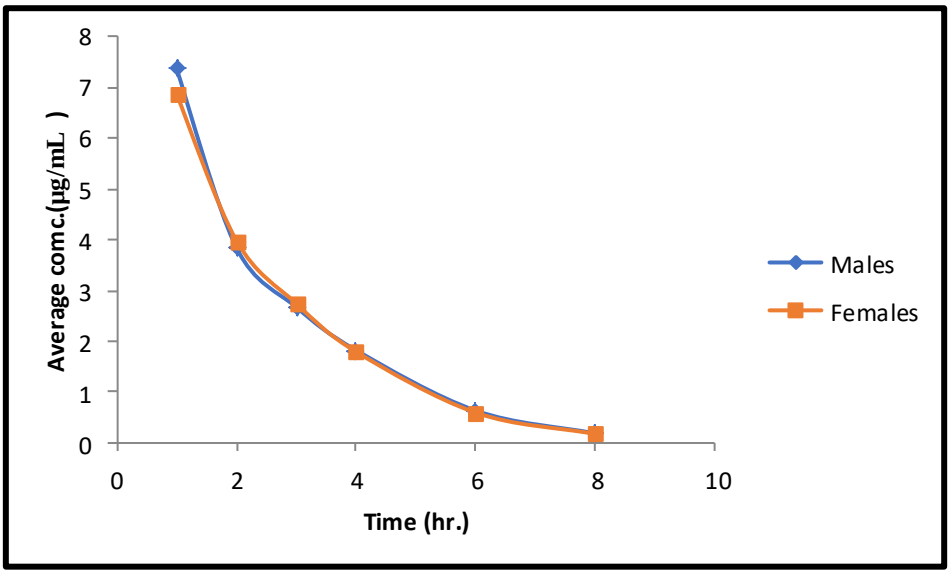

Figure (4): Average ampicillin for both males and females for elimination process.

Average values of $T_{1/2}$ are (1.35 ± 0.08 and 1.31 ± 0.08 hr.) for males and females respectively, that mean of $T_{1/2}$ of ampicillin ,nearly similar for both ganders . However, there was no significant effect of height, age, sex and weight for $T_{1/2}$ for all healthy volunteers. In general the average value of $T_{1/2}$ ampicillin of healthy volunteers is 1.33 ± 0.08 hr. This indicates the amount of halved of initial concentration ampicillin in serum of all healthy. The obtained results revealed that there is no significant difference in ampicillin bioavailability due to volunteers characteristics for height and weight , but there is a small significant effect on the bioavailability of ampicillin in serum by volunteers characteristics for age and gander depended on the results obtained from system anova (p >0.05) of program ssps as shown in Figure (5).

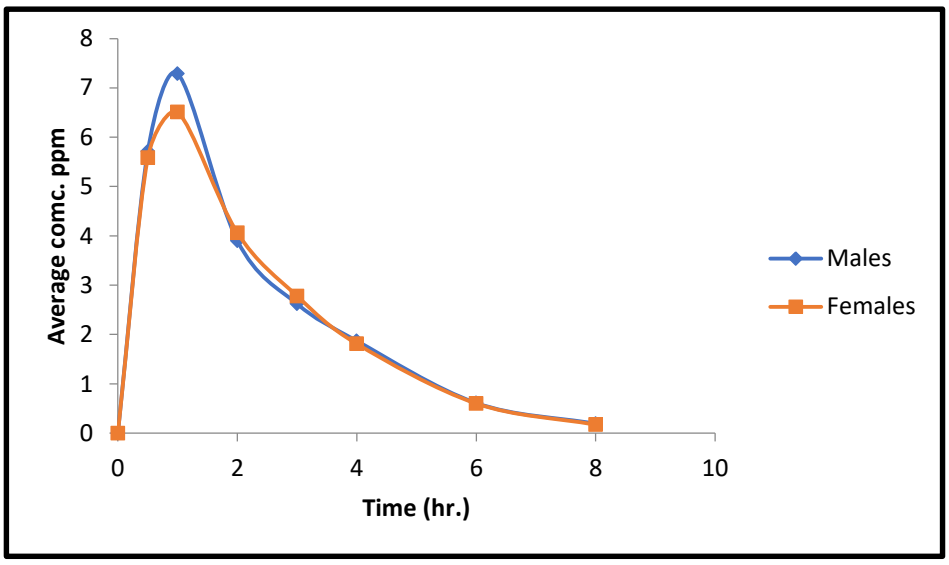

Figure (5): Average ampicillin concentration with times (0.5 - 8) hr. of healthy volunteers for ages (males 25-35 year) and (females 21-32 year).

CONCLUSIONS

The importance of this study is to estimation the bioavailability of ampicillin in serum of Iraqi healthy volunteers. Results of this study revealed that the ampicillin absorption rate in mail almost slightly higher than in females, while elimination is equal for both genders. There is a significant effect of age in absorption and

elimination process. The accuracy of method was validated by mean percentage recovery which was found to be in the acceptable range.

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