ABSTRACT

Cephalexin is commercially available in the form of capsules and tablets containing 250mg or 500mg for oral administration. Cephalexin is presently used as the most common antibiotics. Twenty healthy human volunteers were characterized respected to their pharmacokinetic and bioavailability of two formulations of Cephalexin from two sources of industrial companies after a single dose administration was given orally. A developed procedure is described for determination the concentration levels of Cephalexin in human plasma of healthy volunteers using Reversed phase high performance liquid chromatography (Rp-HPLC) with ODS-C18-DB column at low wave length of UV-visible detection "254nm". An efficient drug extraction procedure was used for the separation of cephalexin after simple extraction with cold methanol. The pharmacokinetic of Cephalexin capsule "500mg" orally administrated treatment through 6 hours has been examined. The Cephalexin was eluted for "12.0 minutes" at flow Rate "1.0 ml/min." and Temperature equal to 298°K .The retention time of Cephalexin was observed at 4.9 minutes. The mean absolute recovery of Cephalexin in blood plasma of all healthy volunteers were 98.9% at 1.0 ppm, 99.0% at 5.0ppm, 93.5% at 10.0ppm, 101.8% at 15.0ppm,105.5% at 20ppm, 100.1% at 25 ppm, 100.0% at 30 ppm, 98.3% at 35 ppm, 95.5% at 40 ppm, 97.9% at 45 ppm and 103% at 50ppm respectively. The assay showed excellent relationships between the area under the curve ratios and drug concentration levels (P>0.005) .Oral Cephalexin administration in twenty healthy volunteers gave maximum concentration "T_max" peak plasma at two hours and decline through sixhours. Treatment with Iraqi formulation Cephalexin capsule produced higher area under the curve "AUC" and maximum concentration "C_max" of Cephalexin than Indian formulation.
KEY WORD: Cephalexin, Bioequivalence, Rp-HPLC, ODS -DB column.

INTRODUCTION
Cephalexin is an antibiotic useful for the treatment of a number of bacterial infections including: middle ear infections, strep throat, bone and joint infections, pneumonia, skin infections, and urinary tract infections. It may be used to prevent bacterial endocarditis. Cephalexin is taken by oral and is active against gram positive and some gram negative bacteria \cite{1}. It is not effective against methicillin-resistant Staphylococcus aureus. It may be used in those who have mild or moderate allergies to penicillin but is not recommended is those with severe allergies. It has no effect against viral infections \cite{2}.

It is in the class of first generation Cephalosporin and has similar activity to other agents within this group including the intravenous agent Cefazoline \cite{3}.

Cephalexin is a semisynthetic Cephalosporin antibiotic intended for oral administration. It is (7-(D-\(\alpha\)-Amino-\(\alpha\)-phenylacetam-ido)-3-methyl-3-cephem-4-carboxylicacidmonohydrate). Cephalexin has the molecular formula \(\text{C}_{16}\text{H}_{17}\text{N}_{3}\text{O}_{4}\text{S.H}_{2}\text{O}\) and the molecular weight is 365.41. Cephalexin has the following structural formula

![Cephalexin Structural Formula](image)

The nucleus of Cephalexin is related to that of other Cephalosporin antibiotics. The compound is a zwitterion. The isoelectric point of Cephalexin in water is approximately 4.5 to 5. The crystalline form of Cephalexin which is available is a monohydrate. It is a white crystalline solid having a bitter taste. Solubility in water is low at room temperature; 1 or 2 mg/mL may be dissolved readily, but higher concentrations are obtained with increasing difficulty \cite{4, 5}.

Cephalexin was different from penicillin in the structure of the bicyclic ring system and has a D-phenyl-glyceyl group as substituent at the 7-amino position and an un-substituted methyl group at the 3-position \cite{6, 7}.
Cephalexin is extensively used as antimicrobial agent in the treatment of Urinary Tract Infections, otitis media, skin and soft tissue infections and upper respiratory tract infection. While its use has largely been superseded by further generations of cephalosporin, it nevertheless remains an important antibiotic in the world. Cephalexin clinical efficacy, bioequivalence, pharmacokinetic profile and influence of co-administration of certain drugs were described in several reports [8-14]. In addition to its routine use, studies showed that it is effective in the treatment of Urinary Tract Infections during pregnancy and is safe to the fetus and the mother [9] and Urinary Tract Infections caused by Escherichia coli [10]. The efficacy of different doses of oral Cephalexin in the treatment of uncomplicated skin infections was described in comparison with topical preparations and other oral Cephalexin [11-13]. A study found that Cephalexin dosed twice daily or three times daily appear equivalent in bacteriologic and clinical cure of Group [14-18].

Bioequivalence studies of different brands of Cephalexin have been assessed in urine and plasma data from experimental animals and humans [19-23]. The comparative bioavailability of the two formulations was evaluated based in statistical comparisons of relevant pharmacokinetic parameters, obtained from data of drug concentrations in blood [24]. A randomized crossover study to investigate the influence of Ranitidine or Omeprazole on the pharmacokinetics and pharmacodynamics of Cephalexin monohydrate showed that there were no significant pharmacokinetic interactions between Cephalexin and different antibiotic drugs [25].

For several analytical procedures are available in the literature for the analysis of Cephalexin. These methods are spectrophotometry [26-31], high performance liquid chromatography [32-36], polarography [37] and titrimetric analysis [38].

Although Cephalexin has been studied in terms of therapeutic activity and commercialized, there is only one official pharmacopoeias monograph on its quantification by HPLC [39-40]. Recently, there are four published methods for the analysis of Cephalexin in powder for injection: microbiological assay [41], high performance liquid chromatography [42] and spectrophotometry [43-44]. Therefore, the use of Rp-HPLC procedures for determination of Cephalexin in plasma, serum and urine has been reported [45-47]. Few HPLC methods also have been reported for quantification of Cephalexin [48-51]. Some spectrophotometric and colorimetric methods also have been reported [52-53]. However till now, no stability indicating method for estimation of Cephalexin has been reported. Reversed phase high performance
liquid chromatography (Rp-HPLC) is preferred over alternative methods because it does not necessarily require extraction or volatilization for antibiotics, and is widely used for microbiological assays \cite{34} because of its precision and accuracy. A literature search indicates that HPLC has been used to analyze Cephalexin and in combination with other drugs. However, there have been few stability studies of Cephalexin analysis using HPLC techniques.

Regulations for registration of products from the national and international industries are relaxed with respect to bioequivalence studies. Treatment failure with antibiotics is always attributed to their irrational use and/or resistance of microorganisms. The question remains, whether this is true or the failure is merely a bioequivalence problem. Therefore, it is important to investigate the disposition and bioavailability of antibiotic brands, especially those manufactured nationally to ensure their bioequivalence to innovators products.

The paper presents the favored approach of clinical studies involved in qualitative and quantitative assay of pharmacokinetics and pharmacodynamics of two Cephalexin formulations, firstly, Iraqi formulation of capsule containing 500mg Cephalexin compared with Indian formulation capsule containing 500mg Cephalexin. After evaluation of the various condition of the (Rp-HPLC) assay, a suitable and simple assay for the determination of Cephalexin in human plasma of healthy volunteers was developed using isocratic (Rp-HPLC) for subsequent study of bioequivalence of two brands Cephalexin formulations.

**EXPERIMENTAL**

**MATERIALS AND METHODS**

**Subject:** Twenty healthy male Iraqi volunteers from the same ethnic group were recruited for the study through local advertisement. Their average age and weight were 28.0 ± 4.0 years (range 25 – 45 years) and 68.5± 9.5 Kg (range 70 – 95 Kg), respectively. All volunteers were asked to start fasting at midnight before the orally administration of Cephalexin capsule (500mg) single dose.

**Chemicals and drugs:** All chemicals used in this study were highest analytical grade purchased from commercial sources and used without any further purification. The deionized distilled-water was used for all preparation. Methanol and Acetonitrile (Absolute CH$_3$OH & ACN for HPLC grade) were purchased from (FLUKA).Cephalexin monohydrate white crystalline powder was purchased from (Zhengzhou Sigma Chemical Co. Ltd.).Cephalexin
monohydrate capsules (500mg) from two sources one from Iraq (SDI, Iraq) and the other from India (Ajanta Pharm. Limited" APKEF", India). Potassium di-hydrogen phosphate (KH$_2$PO$_4$), Di-potassium hydrogen phosphate (K$_2$HPO$_4$) and Phosphoric acid were purchased from (BDH, England). Sepelco-ODS-C$_{18}$-DB column (250 X 4.6mm I.D.) was purchased from (Sepelco, United Kingdom).

**Preparation of Standard Solutions:** Standard stock solution of Powder equivalent to 10 mg of Cephalexin monohydrate was accurately measured and transferred into 100 ml volumetric flasks, containing 50 ml of diluent of methanol : water (20:80) and ultrasonicated for 20 minutes; the volume was made up and mixed well. Solutions were filtered by a 0.2 μm filter to remove particulate matter, if any. The filtered solutions were properly diluted for analysis. The stock standard solution of Cephalexin (100ppm) was prepared freshly every month. The standard solutions of Cephalexin were prepared in serial concentrations (1.0, 5.0, 10.0, 15.0, 20.0, 25.0, 35.0, 40.0, 45.0 and 50.0 ppm) using the same diluents. The applied standard solutions were protected from light and stored at 25°C. Calibration standard curve was performed to achieve the concentration interval of standard solutions.( Figure-1).

![Figure 1: Linearity of different concentration levels of Cephalexin monohydrate using HPLC technique on ODS-DB column](image)

**Preparation of Sample solutions:** Blood sample (7-8 ml) were drawn from vein by syringes in to heparinized blood tubes, then transferred immediately into polypropylene tubes and centrifuged within 5 min. at 500G for 15 min. 100µl of 5% per-chloric acid was added for each one milliliter of plasma. Cephalexin (100 μl) was added as an internal standard. Cephalexin was extracted from human plasma samples by deproteinization using precipitation process. A 500µl aliquot form each plasma sample was transferred to a 5.0ml polypropylene tube. One milliliter of cold methanol was added. After slightly vortex mixing,
the tubes were centrifuged for 15min. at 500G. A 100μl aliquot of the supernatant was transferred to the injection vials and 50μl were injected into chromatographic system. All samples from volunteers were analyzed on the same day in order to avoid inter-assay variation. Plasma solutions were protected from the light and stored in a deep freezer at (203° K).

**HPLC Instrumentation:** This study was performed on Shimadzu instruments model LC-6A HPLC system. The unit was operated in the isocratic model using solvent reservoirs fitted with 0.22 μm stainlesssteel filter at the end of polytrifluoroethylene(PTFE) tubes, transferring the mobile phase from reservoirs to the pump, the system also involved an injector with 50μL sample loop model ( Reseadyre 7125 ), Column in type of ODS-C_{18}-DB "250 X 4.6mm I.D.",Thermostatic oven model CTO-6A Shimadzu, UV-visible detector model "SPD" and chromatopac unit model R4-6A Shimadzu.

**HPLC Operation Condition:** The analytical procedure for determination of Cephalexin in plasma was modified from the methods of Arshad Anjum et al.\(^{[49]}\). The developed procedure of HPLC for analysis of Cephalexin used the following estimation condition. The mobile-phase was phosphate buffer conc. 10mM: methanol(85:15) (v/v), pH buffer equal to 4.5, column temperature 298° K, flow rate equal to (1.0 ml/min.) and UV-visible detection at 254nm. The HPLC method was validated for linearity, specification, accuracy, precision and stability. Reasonable retention times were achieved for Cephalexin (4.9 minutes) .The typical chromatograms of standard solution and blood plasma samples of Cephalexin are shown in fig-2 and fig-3 respectively.

**Pharmacokinetics and statistical analysis:** Pharmacokinetic parameters, expressed as mean ± SD (coefficient of variation %CV), of test of two formulations of Cephalexin were tested statistically. Bioequivalence measures were determined by calculating and comparing the ratios and differences of the maximum plasma concentration levels (C_{max}), the maximum time consuming (T_{max}), and The area under the curve (AUC) of test formulations. Differences in values were considered statistically significant if p < 0.05. The decision rule was used to evaluate bioequivalence results and the test formulations are declared bioequivalence if the 90% CIs for ratios of mean C_{max} and AUC are within the United States Food and Drug Administration (FDA) and it will be acceptable interval of values. The observation of (C_{(max)}) and (T_{(max)}) were obtained from drug concentration versus time curves. The area under the curve ”AUC” of the Cephalexin concentration levels versus time
from 15.0 minute to five hours were estimated from "figure-4"

RESULTS

Figure 2: Typical chromatogram of HPLC analysis of Cephalxin standard solution on ODS-DB column.

Figure 3: Typical chromatogram of HPLC analysis of plasma Cephalxin on ODS-DB column.

Figure 4: Pharmacokinetics of Iraqi and Indian formulations of Cephalxin in blood plasma of healthy volunteers.
RESULTS
The isocratic reverse-phase HPLC technique described and used here for estimation of drug provides the appropriated sensitivity, specificity and high sample accuracy for bioavailability and pharmacokinetic studies. Fig.2 shows the retention time of Cephalexin standard solution that under described chromatographic condition. The retention time of Cephalexin was 4.9 minutes. The optimal chromatogram of analysis was given an ideal shape, symmetrical, and good resolution of peak. Fig.-3 shows the typical chromatogram of Cephalexin blood plasma sample of healthy volunteers which was appeared no endogenous Interfering peaks at the retention time of interest compound. The mean absolute recovery of Cephalexin in blood plasma were 98.9% at 1.0 ppm, 99.0% at 5.0 ppm, 93.5% at 10.0 ppm, 101.8% at 15.0 ppm, 105.5% at 20 ppm, 100.1% at 25 ppm, 100.0% at 30 ppm, 98.3% at 35 ppm, 95.5% at 40 ppm, 97.9% at 45 ppm and 103% at 50 ppm respectively. The calibration curve was linear with regression coefficient $R^2 = 0.995$ (Table-1). The analytical precision and accuracy values was obtained from assays of ten quality control (4.95, 9.35, 15.27, 21.09, 25.03,30.0, 34.40, 38.20, 44.04 and 51.50ppm) are shown in table-1. The accuracy were 99.0%, 93.5%, 101.8%,105.5%, 100.1%, 100.0%, 98.3%, 95.5%, 97.9% and 103% respectively. Andthere is not significant degradation of Cephalexin was observed during the period of storage.

Table 1: The linearity, precision and accuracy of blood plasma Amoxicillinsamples.

<table>
<thead>
<tr>
<th>Spiked concentration (ppm)</th>
<th>1.0</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>25</th>
<th>30</th>
<th>35</th>
<th>40</th>
<th>45</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recovered Average conc. (ppm)</td>
<td>0.9</td>
<td>4.9</td>
<td>9.4</td>
<td>15.3</td>
<td>21.1</td>
<td>25.0</td>
<td>30</td>
<td>34.4</td>
<td>38.2</td>
<td>44.0</td>
<td>51.5</td>
</tr>
<tr>
<td>Slope</td>
<td>74.344</td>
<td>R$^2$</td>
<td>P.V</td>
<td>0.995</td>
<td>0.005</td>
<td></td>
<td></td>
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</tbody>
</table>

Table 2 : The pharmacokinetic parameters“ Cmax , T max and AUC” for the Indian and Iraqi formulations of Cephalexin .

<table>
<thead>
<tr>
<th>Pharmacokinetic parameters</th>
<th>Indian formulation</th>
<th>Iraqi formulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (ppm)</td>
<td>28</td>
<td>39</td>
</tr>
<tr>
<td>Tmax (hours)</td>
<td>2.5</td>
<td>2.0</td>
</tr>
<tr>
<td>AUC</td>
<td>74.13</td>
<td>96.75</td>
</tr>
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</table>
DISCUSSION
The HPLC technique presented in this study decreases the lower limit of quantitation of Cephalexin monohydrate to about 0.1ppm. It was appeared that is more sensitive than many techniques for assay of Cephalexin monohydrate. The low limit of the estimation of the plasma concentration of Cephalexin monohydrate was sufficient to perform the pharmacokinetics study of drug. Cephalexin monohydrate plasma concentration levels were measured by several methods in combination with UV-visible detection. The lowest plasma concentration levels of Cephalexin monohydrate was obtained by UV-visible detection which was 0.5 ppm but the process was consumed long time that was 30min. [45-47]. Arshad Anjum et al were described a procedure for determination of Cephalexin in plasma [49]. Other complicated procedures for extraction and estimation of plasma concentration levels of Cephalexin has been also reported [48-51]. Nevertheless, these procedures were increased the cost of analysis. In order to improve the sensitivity, a column Rp- HPLC with post column derivatization has been used [51,52]. Where the low limit of quantitation was 0.5ppm, this procedure was more complicated due to the more step of post column derivatization and their retention time that will be longer than 10 min. which is compared with our procedure that the retention time of Cephalexin peak was 4.9 min. and the full time of process not greater than 10 min. . Also these procedures cannot be used in pharmacokinetics studies in human where a large number of samples were analyzed. The pharmacokinetics study was done in five hours and the results indicate that the Iraqi formulation has higher bioavailability compared to the Indian formulation depending on the area under the curve AUC and C_{max}. Our technique was evaluated and produced the best results in terms of selectivity and sensitivity consideration the fact that the present technique involves a shorter running time and a simple sample preparation process. The dose administered was the normal dose employed for soft tissue infections. It was well tolerated by the volunteers, no adverse effects were observed during or after the study period. Fig -4 shows the mean (± SEM) plasma concentrations of cephalexin observed following the administration of the two capsule formulations. Plasma levels of cephalexin were comparable and slightly significant difference from those previously reported in healthy volunteers following oral administration of equivalent doses [24-26]. Table-2 summarizes pharmacokinetic parameters of cephalexin following the administration of products Iraqi and Indian formulations. Cephalexin was rapidly and completely absorbed from the gastrointestinal tract with maximum plasma levels reached in about 2.0-2.5 hours for the two formulations. The mean values (± SD) of C_{max} were 28 ppm and 39 ppm for Indian and Iraqi formulations respectively. The extent of absorption appears to be nearly
similar in two formulations; AUC was 74.13 and 96.75 ppm. hour for Indian and Iraqi formulations respectively. Cephalexin was rapidly cleared off plasma; elimination was almost complete in 5 hours with elimination half lives of about one hour. Clearances and volumes of distribution of the two formulations were comparable among the volunteers. There were little statistically significant differences (P > 0.05), in all pharmacokinetic parameters of Cephalexin, were observed between the locally manufactured capsules "Iraqi formulation" and the import manufactured capsules "Indian formulation.

CONCLUSION

Our Rp-HPLC technique was employed here proved to be fast, simple, precise, specific, selective and sensitive enough to be used in clinical pharmacokinetic and bioavailability studies for Cephalexin monohydrate in plasma blood of human. The AUC and "C_{max}" of Iraqi formulation are higher than Indian formulation of Cephalexin monohydrate and the "T_{max}" of Iraqi formulation is 2.0 hours while the "T_{max}" of Indian formulation is 2.5 hours which are shown in table-2 and the relative bioavailability of Indian to Iraqi formulation was estimated from the curve in fig-4 is equal to 76.62%. Bioequivalence acceptable range of 80%–120% for all bioequivalence measurements, the two products can be considered bioequivalent. It can be concluded that since absorption profiles and disposition patterns of the Iraqi produced test formulation is highly comparable with the Indian product, they are expected to achieve the same levels and give the same clinical effect and can they be used interchangeably.

ACKNOWLEDGMENT

The author wish to express their gratitude to the volunteers and the technical staff at the Department of Medicinal Chemistry, Chemical Research Center, Ministry of Science & Technology for their caring out the experiments that was associated with preparation of samples and technical assistance.

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