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Bioequivalence Study of A newly Formulated Effervescent Ciprofloxacin Tablets With reference Tablets in Rabbits

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Abstract

The pharmacokinetic parameters of ciprofloxacin were determined following oral administration of a single dose of 20mg/kg body weight for Ciprobay as reference and a new formulation of effervescent ciprofloxacin as test product in 20 normal healthy rabbits male. High performance liquid chromatographic method was employed for estimation of ciprofloxacin in plasma samples. A significant ($P<0.05$) increase in drug plasma concentration was recorded at 0.5, 1, 3. 4, 8, 12 hours sampling time, and highly significant ($P<0.01$) increase of drug concentrations was observed at 1.5 for test formula, except at 24 hour. A highly significant ($P<0.01$) increase in absorption half-life (25%), distribution rate constant (34.95) area under the curve $AUC_{0-\alpha}$ (38%) and peak plasma concentration (C_{max}) (42.30%) for effervescent tablets. Distribution half-life and, elimination half-life, and T_{max} decreased ($P<0.05$) significantly for effervescent tablets. MRT and lag time significantly ($P<0.05$) were found higher in rabbits which take test product. The results reflect that enhancement in dissolution rate by effervescent base with sustained release in new formula increase bioavailability.

Keywords: ciprofloxacin, rabbits, drug plasma, HPLC, MRT.

1. Introduction

There are many works published in the last two decades regarding HPLC methods for determination of ciprofloxacin in biological fluids of different species[(1-2)]. For a laboratory, to develop a method is sometimes a compromise between cost, time consuming and purpose of study. Some of the reported methods about ciprofloxacin quantification in blood plasma supposed expensive sample extractions by using switching devices [(3)], time and materials consuming extraction methods such as liquid–liquid extraction [(4,5,6,7)]or derivatization methods [(8)].

The number of articles about determination of ciprofloxacin in plasma after protein precipitation is great due to the relative simplicity of the sample treatment. In many works acetonitrile or methanol was used as precipitating agent [(11)]. The methods had good sensitivity, accuracy and precision, but internal standards were used, special devices or further sample treatment was applied to improve the results. Perchloric or trichloroacetic acid causes protein precipitation without significant dilution of the sample. Three of studied works [(10)] applied this kind of precipitation for quantification of ciprofloxacin in blood plasma and all used an internal standard. There are also a few articles in which ciprofloxacin metabolites are chromatographic determined in plasma, applying a more or less complicate extraction or chromatographic procedure or using special stationary phase [(9,10)].

Extending this work, the present study was carried out to calculate the pharmacokinetics parameters of newly formulated effervescent ciprofloxacin in experimentally healthy male rabbits[(12,13)]. Ciprofloxacin is a flouroquinolone derivative with outstanding antibacterial activity against gram-negative and some gram-positive

bacteria as well as on some Chlamydia and Mycoplasma, and many mycobacterium species. In animals quinolones especially ciprofloxacin exhibit favorable pharmacokinetic properties, their apparent volume of distribution suggested substantial tissue penetration. [14, 15].

2. Materials and Methods

2.1 Subjects

Twenty healthy white albino adult male rabbits participated in the study[22]. They are small mammals in the family of *Leporidae* of the order *Lagomorpha,* found in several parts of the world, being less aggressive as a good model for pharmacokinetics analysis, their habitats include meadows, wood, forest, and grass lands all the animals were maintained under similar conditions. The animals were fed with fresh green fodder and black gram in the morning and evening, while water was provided freely as much they required. The mean age (± SD) of the rabbits was 2.00 ± 0.40 years, with a range of 1.5 – 2.3 years, mean body weight was 3.5 ± 0.50 kg with a range of 3 - 4 kg. No rabbit had a history or evidence of any acute or chronic diseases or allergy to ciprofloxacin or any flouroquinolone antibiotics. The ethics committee of the College of Pharmacy and the Institutional Review Board (IRB) of Taif University, Taif, Saudi Arabia, approved the study protocol.

2.2. Protocol of the Study and Subjects

Pharmacokinetics of Ciprofloxacin from the different generic formulations was studied after administration of a single oral dose in twenty normal healthy male rabbits.Twenty healthy white albino adult male rabbits participated in the study[(2)]. They are small mammals in the family of *Leporidae* of the order *Lagomorpha,* found in several parts of the world, being less aggressive as a good model for pharmacokinetics analysis, their habitats include meadows, wood, forest, and grass lands all the animals were maintained under similar conditions. The animals were fed with fresh green fodder and black gram in the morning and evening, while water was provided freely as much they required.[(16)] The mean age (± SD) of the rabbits was 2.00 ± 0.40 years, with a range of 1.5 – 2.3 years, mean body weight was 3.5 ± 0.50 kg with a range of 3 - 4 kg. No rabbit had a history or evidence of any acute or chronic diseases or allergy to ciprofloxacin or any fluoroquinolone antibiotics. The study protocol was approved by the ethics committee of the College of Pharmacy and the Institutional Review Board (IRB) of Taif University, Taif, Saudi Arabia[(2,17)].

2.3. Identity of Study Medications

Test product (B) newly formulated ciprofloxacin effervescent tablets (250 mg ciprofloxacin/tablet); formulated in College of Pharmacy, Taif University, KSA, and the Reference product (A) Ciprobay® tablets (250 mg ciprofloxacin/tablet); Batch No. 285 manufactured by Bayer, Germany.

2.4. Drug Administration

Rabbits were randomly divided into two groups, A, B, crossover design. Tablets were crushed and mixed with carboxymethylcellulose (CMC) 1% w/v solution, ensuring that rabbits consumed all the dose. Drug was prepared in a solution form and was administered through the feeding tube orally. A single dose was given for each rabbit and was administered as a single dose of 20 mg/kg of body weight [(19-20-21-22)].

2.5. Collection and Handling of Blood Samples for Analysis

The administration of the two products to the rabbits was carried out by means of a two-way crossover design with a 1-week washout period. Rabbits were randomly divided into 2 equal groups and assigned to 1 of the 2 sequences of administration. In the morning of study day 1 of each study period and before drugs administration, a cannula was inserted into the rabbit's ear vein and remained there until the 24-hour blood sample was collected. The rabbits were returned the next day for the 24-hour blood samples. Each rabbit received a single oral dose of (20 mg/kg body weight) of either brand with 100 ml of water after overnight fast for at least 10 hours. Rabbits were allowed to eat a standard meal 4 hours after drug administration. The volume of blood taken for determination of ciprofloxacin in plasma was 2 ml per sample. The following blood samples for the analysis of ciprofloxacin in plasma were collected at (- 0.50 hour) and at, 0.50, 1.00, 1.50, 2.00, 3.00, 4.00, 8.00, 12.00, and 24.00 hours after drugs administration. The number of blood collections for drug analysis was 10 samples in each study period. Blood samples were collected, protected from light, into evacuated glass tubes containing heparin as an anticoagulant (heparinized vacutainers, Beckton and Dickinson, Rutherford, NJ, USA) through the indwelling cannula placed in the rabbit's ear veins, slightly shaken and immediately centrifuged at approximately 3500 r.p.m for 5 minutes. After centrifugation, plasma samples were transferred directly into two labeled 1.5 ml-plastic micro centrifuge tubes protected from light. These samples were immediately stored in a freezer at a nominal temperature of −80°C pending analysis. For each rabbit, the total amount of blood loss during the whole study did not exceed 30 ml[(18)].

2.6. Study Design

Bioequivalence evaluation is usually carried out in vivo by comparing the rate and extent of drug absorption of the test and reference formulations in healthy subjects.

In a standard in vivo bioequivalence study design, study subjects received test and reference products on separate occasions, in single dose, with random assignment to the two possible sequences of product administration. Samples of plasma were analyzed for drug concentrations, and pharmacokinetic parameters were obtained from the resulting concentration-time curves. These pharmacokinetic parameters were then analyzed statistically to determine if the test and reference products yielded comparable values. Standard statistical methodology based on the two one-sided T-tests procedure to determine whether average values for pharmacokinetic parameters measured after administration of the test and reference products are comparable. This procedure involves the calculation of a 90% confidence interval for the ratio between pharmacokinetic variable averages of the test and reference products. The limits of the observed confidence intervals were within the pre-determined range for the ratio of the product averages. The determination of the confidence interval range and the statistical level of significance were based on the parametric theory. Standard non-compartmental and compartmental procedure were employed for the analysis of pharmacokinetic data derived from in vivo bioequivalence studies. Analysis of variance (ANOVA) was performed on the pharmacokinetic parameters to assess the effect of variables (subjects, sequence, period and formulation) on the study outcome. On the basis of these considerations, a single-dose, two treatment, two-period, two-sequence crossover bioequivalence study on healthy normal rabbits was adopted as described in the study protocol [1.2.5].

2.7. Pharmacokinetic analysis

The pharmacokinetic parameters of ciprofloxacin were estimated using standard non-compartmental methods. The analysis procedure followed the scaled bioequivalence limits imposed by the FDA. All parameters were determined from the true (actual) sample collection times and assayed plasma concentrations at these times. The maximal plasma concentration (C_{max}) and the time to peak plasma concentration (T_{max}) of ciprofloxacin were taken directly from the measured data. The area under the plasma concentration-time curve ($AUC0 \rightarrow 30$) was calculated from measured data points from time of administration to time of last quantifiable concentration (Clast) by the linear trapezoidal rule. The area under the plasma concentration-time curve extrapolated to infinity ($AUC0 \rightarrow \infty$) was calculated according to the following formula:

$$AUC0 \rightarrow \infty = AUC0 \rightarrow 30 + Clast / [Ln (2) / T½]$$

Where, Clast is the last quantifiable concentration. The ratio $AUC0 \rightarrow 30$ / $AUC0 \rightarrow \infty$ as a percent, was determined as an indicator for the adequacy of sampling time. The elimination half-life (T½) was calculated as:

$$T½ = Ln (2) / (-b)$$

Where, b was obtained as the slope of the linear regression of the Ln-transformed plasma Concentrations versus time in the terminal period of the plasma curve. At least 3 non-zero plasma Concentration-time points were used in the calculation. The extent of absorption is determined by

$$AUC0 \rightarrow t \text{ and } AUC0 \rightarrow \infty.$$

The rate of absorption is determined by Cmax. For the parametric analysis of bioequivalence for Ln-transformed data, the acceptance boundaries were set at 80.00-125.00% for $AUC_{0 \rightarrow 30}$, $AUC_{0 \rightarrow \infty}$ and C_{max}.

2.8. Validation of Calibration Curve:

The specificity of the method was verified using six different plasma blanks obtained from healthy rabbits which did not take before ciprofloxacin. The anticoagulant (K_3EDTA) interference was also verified during this stage. In the lack of ciprofloxacin metabolites standards, the specificity of the proposed chromatographic conditions was also verified by monitoring the chromatographic behavior of blood plasma of one healthy rabbits after oral administration of 20 mg/kg body weight ciprofloxacin dose.

The linearity of the peak height against standard concentration was verified using least-squares linear regression in 5 different days. The calibration curves parameters were computed by the HSM D7000 software. Distribution of the residuals (% difference of the back-calculated concentration from the nominal concentration) was investigated. The calibration model was accepted, if the residuals were within ±20% at the lower limit of quantification and within ±15% at all other calibration levels and at least 2/3 of the standards meat this criterion.

To establish the lower limit of quantification in a single validation batch five replicates of QC sample with 0.0412 µg/ml ciprofloxacin were analyzed. On each of 5 different days, a single QC sample (0.0412 µg/ml) was analyzed against daily calibration (inter-day assay).

The intra- and inter-day precision (CV%) and accuracy (bias%) of the assay procedure were determined by the analysis of five samples at each lower, medium and higher QC concentration in the same day and one sample at each QC concentration in 5 different days, respectively. The absolute recoveries at each concentration were measured by comparing the response of the pre-treated plasma standards (QC) with the response of standards diluted with water in

the same proportion as the pre-treated standards. On-instrument stability of ciprofloxacin in extract was verified at one level of concentration (0.5152 µg/ml) by performing the experiment five times during 10 h of storage at room temperature, looking for the change of signal height. The long-term stability of ciprofloxacin in rabbit plasma was verified at three levels of concentration (0.0429, 1.288, 2.576 µg/ml ciprofloxacin in plasma) by performing the experiment after 7, 15, 26 and 40 days of storage at −80 °C. The freeze-thaw stability was also verified at two levels of concentrations, lower and higher, after three freeze-thaw cycles. The aliquot was separated for injecting into the HPLC system (Agilent 1100 series with vacume, Quaternary pump, Autosampler 10v, Thermostated column compartment, Variable wave length detector) and a column (Zorbex Eclipse x DB C18 Analytical 4.6 x 150mm - 5µ)[4,5,6,7,8,9].

3. Results and Discussion

3.1 Bioequivalence study:

In the current study, the bioequivalence (or rate and extent of absorption) of a single dose of effervescent ciprofloxacin (250 mg /tablet), as a test formulation, and of A ciprobay Pharma, Germany (Tarivid® 250 mg/tablet), as the reference one, were compared under fasting conditions. Bioequivalence of the two products was assessed based on the plasma concentration data obtained following their administration to 20 healthy adult rabbit in a balanced single center, open-label, randomized, single-dose study with two-way crossover design, to compare the bioequivalence of ciprofloxacin tablets between two products Figure (3). This is similar to Sajed *et al.*[17], Sahar *et al*.[2]. Animal models are being used for experimental studies in various branches of medical sciences, because certain of the research areas obviously cannot be done on human beings for practical and ethical reasons and for resemblance to human this agree with *Hirayama et al.*[18], and Willy *et al*[19]. The use of animals in experimental research must be based on scientific, ethical, and legal principles .When research is performed on the appropriate animals, the information obtained should approximate what can be expected in human beings this agreed with Ferreira *et al.,*[20], Petroianu *et al*.,[21] and Jun *et al*[22]. Animal models are being used for experimental studies in various branches of medical sciences, because certain of the research areas obviously cannot be done on human beings for practical and ethical reasons. The use of animals in experimental research must be based on scientific, ethical, and legal principles with similarity to human beings, this agree with Sajed *et al.,*[17]. When research is performed on the appropriate animals, the information obtained should approximate what can be expected in human beings. Rabbits are one of the animals used as research models approved by ethical committees [23].

Rabbits are one of the animals used as research models approved by ethical committees and give more realty to human beings especially in pharmacokinetics of BCS class II like ciprofloxacin which agreed with Green L C *et al.*[24], Sajed *et al.*[17], Sahar *et al*.[2], Bashir S *et al.*[25], Hanan *et al.,*[26]. They are small mammals in the family of *Leporidae* of the order *Lagomorpha*, *Oryctolagus cuniculus* found in several parts of the world, being less aggressive as a good model[27].Their habitats include meadows, wood, forest, and grass lands. In animals quinolones especially ciprofloxacin exhibit favorable pharmacokinetics properties, this agreed with (Abd El-Aty *et al.*,[23] and Albarellos *et al.,*[16] their apparent volume of distribution suggested substantial tissue penetration. This study was performed to investigate the bioequivalence of two brands of ciprofloxacin, namely, newly formulated effervescent ciprofloxacin HCl 250 mg/tablet (formulated in College of Pharmacy, Taif University, KSA, as a generic test product 'B') relative to Ciprobay® 250 mg/tablet (from Bayer, Germany, as a reference product 'A') after a single dose oral administration of 20 mg /kg body weight to 20 healthy adult male rabbits under fasting conditions .this a complied to Sajed *et al.,*[17]. The bio-analysis of plasma samples was accomplished by a HPLC method for the determination of plasma ciprofloxacin concentrations.

3. 2. Pharmacokinetic Analysis

Pharmacokinetic analysis was performed by non-compartmental approach along with compartmental approach as well, using two compartment model, by a software pharmacolysis® this agreed with Ahmad *et al.,*[28]. Pharmacokinetic parameters, determined by standard non-compartmental methods and analysis of variance (ANOVA) statistics were calculated using statistical analysis system (SAS) software. The parametric 90% Confidence intervals (CIs) of the least squares mean test/reference ratios were found to be within the confidence limits of 80.00-125.00% for $AUC_{0\rightarrow 30}$, $AUC_{0\rightarrow \infty}$ and C_{max}, *i.e.* 89.10% to 103.95%, 91.49% to 102.30%, and 91.71% to 105.30%, respectively. This single-dose study demonstrated that the test product (B) was found more bioavailable to the reference product (A) following an oral dose of 20 mg/kg body weight, as per predetermined regulatory criteria for bioequivalence, in the 20 fasting healthy rabbits tables(1-20)and figures (1-20) similar finding by Hespe *et al.,*[29], Eichman, *et al.,*[30]. Therefore, the new formula was considered to be more bioequivalent than the reference product due to enhancement in the physicochemical properties and decrease dissolution rate time that is agreed with Shweta *et al.,*[31], honey et al[32] Bhagavan *et al.,*[33], Chowhan *et al.,*[34].

3.3. Statistical Analysis:

All values are expressed as the mean ±of standard deviation (SD) of twenty animals. The pharmacokinetic parameters obtained for the drug (A) and the drug (B),after a single oral dose of 20/kg body weight were compared using a paired t test, considering a probability of ($p<0.05$) to be significant.

3.4. Ciprofloxacin Plasma Concentration-Time Profile:

Plasma concentration of rabbits following oral administration of ciprofloxacin solution (20/kg body weight) for rabbits that were administered reference drug Ciprobay (A) and test product effervescent ciprofloxacin drug (B) was measured from the blood samples by High Pressure Liquid Chromatography (HPLC).The mean plasma concentrations against time are obtained from calibration curve figure (3), table (4.2) and then are plotted in figure (4.1) and presented along with Statistical analysis in Table (2) with calibration curve figure (2).

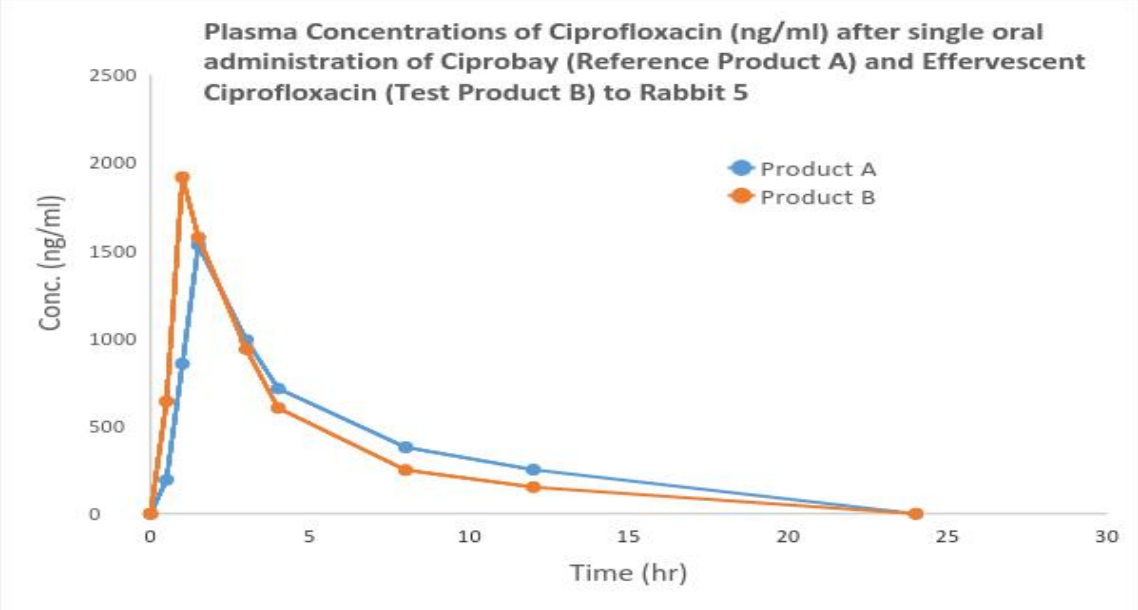

Figure (1): Plasma Concentrations of Ciprofloxacin (ng/ml) After Single Oral Administration of Ciprobay (Reference Product A) and Effervescent Ciprofloxacin (Test Product B) to Healthy Male Rabbit.

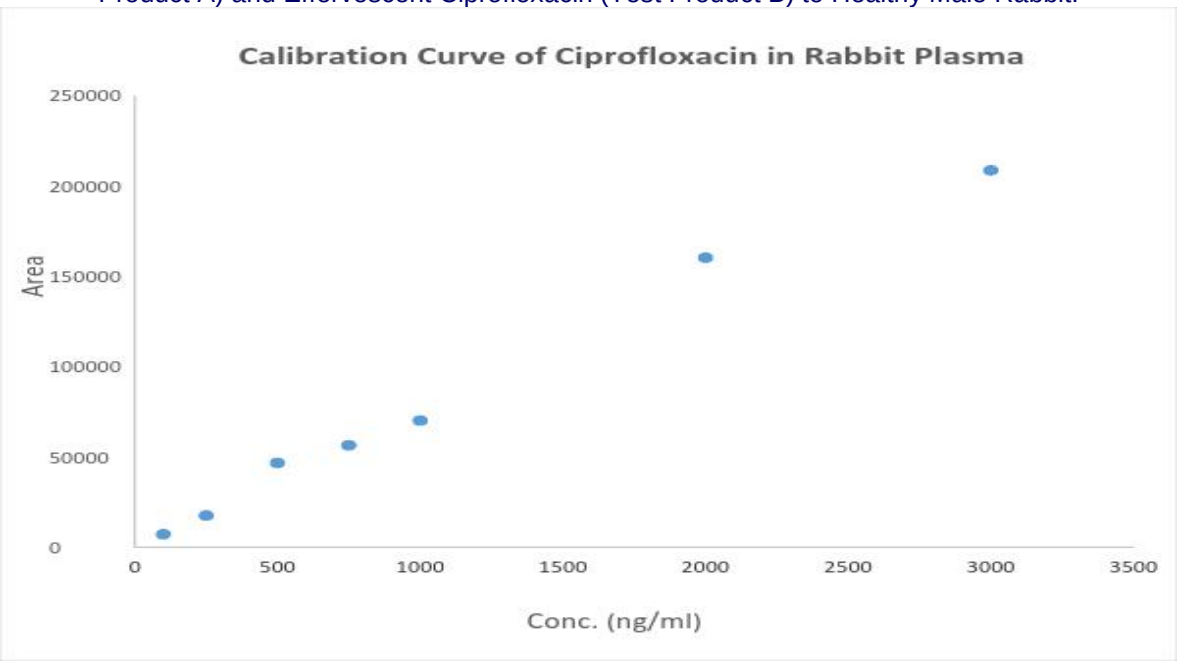

Fig (2) Calibration Curve of ciprofloxacin in healthy Adult Male Rabbit Plasma.

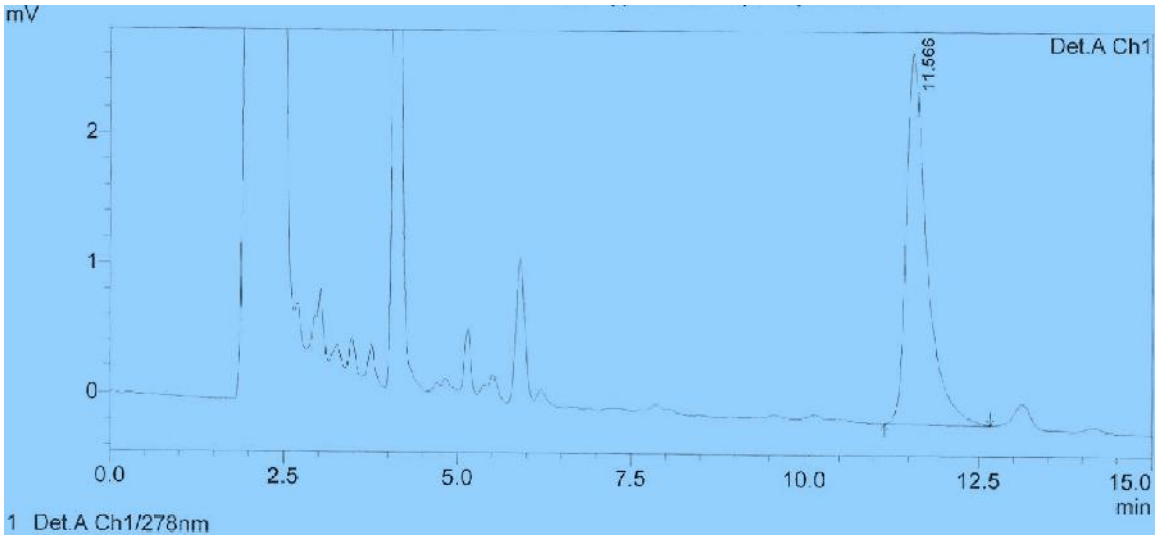

Figure (3) Chromatogram after Single Dose 20mg/kg body weight for the test ciprofloxacin tablets.

A highly significant ($p<0.05$) increase in drug plasma concentration was recorded at 0.5 and 1.5 hours sampling time, and highly significant ($p<0.01$) increase of drug concentrations was observed at 0.5,3,8,12 hours for effervescent ciprofloxacin drug (B) rather than reference drug (A). Increased plasma levels of the effervescent ciprofloxacin may be attributed to highly dissolution rate of effervescent tablets which enhance the dissolution rate and bioavailability of drugs that is agree with Jiao *et al*., [36], Eichman *et al.,*[35], Hespe *et al.,*[29], Kareem *et al.,*[37], Lynn *et al.,*[38].The other possible reason might be the faster absorption rate and sustained release effect in the new effervescent formula that is agree with Ahmed *et al.,*[39], Sarat *et al.,*[40],that resulted in increased drug concentration in plasma similar to Flower et al[41]. These findings are in accordance with the previous studies of drug plasma concentration in effervescent base Wesley *et al.,*[42] , Sonar *et al.,*[43] and Ali *et al.,*[44].

On the other hand, sampling at 24.0 did not reveal any difference of plasma level between reference ciprobay (A) and test formulated effervescent ciprofloxacin (B) this result was similar to Adnan et al[45], Sarathchandiran *et al.,*[46], Sajed *et al.,*[17], Sahar *et al.,*[2], Baumgartner et al[47] that drug is totally cleared from the body, especially ciprofloxacin is highly soluble and highly permeable drugs (BCS class 111) this agree with Chen et al[48]. The maximum plasma concentration of 0.701 ±.02 µg/ml and 0.873±.03 µg/ml Table-1) were attained in Rabbits administered drug (A) and (B) respectively, after 1.5 hour. This agree with Sajed *et al.,*[17], Sahar *et al.,*[2],Bashir et al[25], Edyta *et al.,*[21].

Table (1) Plasma level Vs Time {Mean (n=12) ± SEM} of Ciprofloxacin Tablets in Reference (A) and Test Effervescent Following Oral Administration of A Single Dose of 20mg/kg Body Weight.

Time (hours)	Group		Ratio	% age Difference	Paired t-test
	Reference A	Test B			
0.5	0.659± .03	0.680± .01	0.97	+3.23	**
1.0	0.687+ .02	0.742± .01	0.93	+7.40	**
1.5	0.701+ .02	0.873± .03	0.89	+10.55	***
3.0	0.407+ .02	0.443± .01	0.92	+7.90	**
4.0	0.281+ .02	0.305± .02	0.96	+4.41	**
8.0	0.180± .01	0.243± .02	0.74	+25.51	**
12.0	0.133± .00	0.200± .02	0.92	+8.40	**
24.0	0.50 ± .00	0.50± .03	1.00	0.00	ns

ns = non-significant difference ** = significant difference
*** = highly significant difference + = increase

3.5. Pharmacokinetics of Ciprofloxacin (A and B) in Rabbits:

Pharmacokinetic parameters as presented in (Table-2) showed a highly significant ($p<0.01$) increase in absorption half-life (25%) distribution rate constant (34.95%), elimination rate constant (37.5%), area under the curve AUC $_{(0-t)}$ and peak plasma concentration (C_{max}) (43.30%) for drug (B) (effervescent ciprofloxacin) in rabbits compared to drug (A) (Ciprobay).The higher plasma concentrations recorded for drug (B) in rabbits in comparing to drug (A) (Fig 1) and other pharmacokinetics parameters attributed due to enhancement the dissolution rate of the formulated ciprofloxacin as an effervescent tablets, this agree with Thakkar *et al*[49], which the higher bioavailability was recorded for drug effervescent potassium calcium citrate tablets over tablets formulated as convential calcium citrate[49].

Table(2) Plasma levels Vs Time [Mean(n=12)± SEM] of A Reference Ciprofloxacin Ciprobay(A) and Test effervescent Ciprofloxacin(B) in Normal Healthy male Rabbit Following Oral Administration of A Single Dose of 20 mg/Kg Body Weight.

parameters	A	B	Ratio	% difference	Paired t-test
Absorption Rate Constant Ka {hr^{-1}}	3.66±0.31	4.31±0.18	1.17	-15.08	***
Absorption Half Life {hr}	0.2±0.01	0.16±0.01	0.8	+25.00	***
Distribution Rate Constant {Hr^{-1}}	1.03±0.10	1.39±0.11	0.74	+34.95	***
Distribution Half-Life {hr}	0.73±0.06	0.55±0.06	1.32	-24.65	**
Elemination Rate Constant Ke {hr^{-1}}	0.08±.00	0.11±0.01	0.72	+37.5	***
Elemination Half Life{hr}	8.66±0.47	6.77±0.36	1.27	-21.82	***
AUC $_{(0-t)}$ (hr.mg.$^{-1}$)	3.46±0.06	3.86±0.07	0.89	+11.56	***
AUC $_{(0-inf)}$ (hr.mg.$^{-1}$)	5.13±0.16	5.78±0.15	0.88	+0.97	***
MRT(area) {hr}	4.16±0.03	4.33±0.03	0.96	+4.08	**
C $_{(max)}$ {$mg.1^{-1}$}	0.26±0.01	0.37±0.02	0.70	+42.30	***
$T_{(max)}$	1.04±0.06	0.90±0.03	0.91	+9.47	**
Lag time {hr}	0.39±0.07	0.29±0.04	0.74	+34.48	**

** = significant difference (P<0.05) *** = significant difference (P<0.01)
- = decrease + = increase

Distribution half-life and elimination half-life decreased ($p<0.05$) significantly for effervescent tablets. These results are comparable with observation made by Waterbird *et al* [50].

MRT and lag time significantly ($p<0.05$) were found higher in effervescent tablets (B) rather than reference drug Ciprobay (A).MRT provides a quantitative estimation of persistence time of drug in the body due to the uses of guar gum which prolong the effect as sustained release formula this agreement with Ahmed *et al*[39].Like half-life MRT is the function of both distribution and elimination. Which is in agreement with Sajed *et al.,*[17],

The results in table (2) demonstrate that the higher values of pharmacokinetics parameters AUC_{0-30}, AUC_{0-t}, $AUC_{0-\alpha}$, mean plasma concentration, C_{max}, for test product rather than reference this indicate that it possesses higher bioavailability this as the result of improvement the physicochemical properties by effervescent base which is in agreement with Patel *et al*[51].

Area under curve ($AUC_{0-\alpha}$) (Table 2) show significant ($p<0.05$) was found higher in effervescent tablets rather than reference Ciprobay this due to enhancement the dissolution rate with sustained release in new formula[39],this agreement with Patel *et al,*[51].Which finding that improvement the physicochemical properties especially dissolution rate increase bioavailability. Similar finding by Rao *et al.*[52], Wu *et al.,*[53]

3.6. Bioavailability:

The relative bioavailability of the a newly formulated effervescent ciprofloxacin tablets was calculated as shown in Table (3). The higher bioavailability value indicate that improvement of physicochemical properties lead the formulated tablets to be superior the reference tablets. Similar finding by Sahar *et al*., [2], and Abd El-Aty *et al*[23].

Table (3): Relative Bioavailability of The A New FormulatedEffervescent Tablets Compared To The Reference.

Bioavailability	Reference Drug (A)	Formulated Tablets
%	100	141

4. Conclusion

- Rabbits are one of the animals used as research models approved by ethical committees and give more realty to human beings especially in pharmacokinetics of BCS class II like ciprofloxacin
- Animal models are being used for experimental studies in various branches of medical sciences, because certain of the research areas obviously cannot be done on human beings for practical and ethical reasons and for resemblance to human.
- Formulation into effervescent tablet is suitable for larger dose size which has difficulty in production of a convention tablets due to the difficulty in swallowing, besides enhancing solubility and masking the taste.
- HPLC analysis is suitable method for analysis drugs in plasma due to it is precise values.
- *In Vitro–In Vivo* Correlation (IVIVC*)* plays a key role in pharmaceutical development of dosage forms. This tool hastens the drug development process and leads to improve the product quality. It is an integral part of the immediate release as well as modified release dosage forms development process.

Recommendation

1-The monitoring and quality control testing of medicines in pharmacies randomly to ensure the good storage conditions might ensure drug's effectiveness.
2- The effervescent formula is needed and sometimes it is a must to enhance solubility, palatability of certain drugs.
3- Wet granulation method (when it is applicable) is better than the direct compression method; this might lead to good distribution of active ingredient.
4- Effervescent tablet from ciprofloxacin might reduce the microbial resistance, increase effectiveness, and increase patient compliance, and the effervescent tablets need well tight container.
5- The microbiological sensitivity test can be used as an indicative for variations of drugs activities in different formulae.
6- Rabbits is good animal model for doing many researches, which give good indicator similarity for human being.
7-The correlation can be made between dissolution rate and microbiological sensitivity test of effervescent tablets as an indication for its effectiveness in vivo studies.

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