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In Vitro-In Vivo Correlation Study of A newly Formulated Effervescent Ciprofloxacin Tablets With reference Tablets

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Abstract

Effervescent tablets of ciprofloxacin were developed to increase bioavailability. Tablets were prepared by direct compression and wet granulation. Tablets were evaluated for their physical characteristics, weight variation, hardness, friability, thickness, diameter, microbiological assay and drug release pattern over the dissolution medium comparing to conventional marketed brand Ciprobay as a reference, furthermore was proceed *in vivo* studies by measuring drug concentrations in plasma of rabbits by high performance liquid chromatography. The results showed that similarity between *in vitro in vivo* effects. Thus it was concluded that the effervescent ciprofloxacin containing sustained release properties was found improve bioavailability, patient compliance, minimize side effects and decrease microorganisms resistant.

Keywords: Ciprofloxacin, HPLC, *In vitro In vivo* correlation, Rabbits.

1. Introduction

In vitro in vivo correlations (IVIVC) play a key role in the drug development and optimization of formulation which is certainly a time consuming and expensive process. Formulation optimization requires alteration in formulation, composition, equipments, batch sizes and manufacturing process. If such types of one or more changes are applied to the formulation, the in vivo bioequivalence studies in human may required to be done to prove the similarity of the new formulation which will not only increase the burden of carrying out a number of bioequivalence studies but eventually increase the cost of the optimization process and ultimately marketing of the new formulation. To overcome these problems it is desirable to develop in vitro tests that reflect can bioavailability data. IVIVC can be used in the development of new pharmaceuticals to reduce the number of human studies during the formulation development. Thus, the main objective of an IVIVC is to serve as a surrogate for *in vivo* bioavailability and to support biowaivers[(1)].

1.1. Effervescent Tablets

Tablet formulations may be rendered effervescent for several reasons, including improvement of their disintegration characteristics, increase dissolution rate and thus enhance liberating the ciprofloxacin HCl beside together with sweetener, flavor and guar to mask the taste. [(2)]

Effervescent agents have been shown to be useful and advantageous for oral administration of drugs and have

been employed for use as taste masking agent for ciprofloxacin HCl (in ratio 1:2:3.4). [19] It comprise effervescent base, an orally administrable medicament, a taste masking generator of carbon dioxide, and optionally a taste bud desensitizing composition by other non active material such as sweeteners, flavoring agent, guar gum and filers. Thus all that contributes in success the formula. [2, 3,4]

1.2. Ciprofloxacin hydrochloride (HCl)

Quinolone antibacterial drugs have been in use since 1964, when nalidixic acid was released. Oxolinic and cinoxacin were introduced somewhat later. These drugs had fallen into disuse because of their limited antibacterial spectra, and resistance to them rapidly develops (17).The introduction of 6-fluoro and 7-(1-piperazinyl) group expanded the spectrum, increased potency and appears to have prevented the development of plasmid-mediated resistance in microorganisms (Figure 1 and 2).

The fluoroquinolones are bacteriostatic at low concentrations and bactericidal at high concentrations. They were used as alternative to chloramphenicol due to the high risk of chloramphenicol (e.g. bone marrow depression). They are now considered as drugs of choice for enteric fever. [5]

1.2.1. Mechanisms of Action

The fluoroquinolone drugs inhibit DNA gyrase (topoisomerase II), which results in abnormal linkage between opened DNA and the gyrase. Negative supercoiling (absent in mammalian nuclei) is impaired, so protein synthesis is prevented. [5]

Ciprofloxacin hydrochloride (effervescent tablets) (figure 1 and 2) . [5]

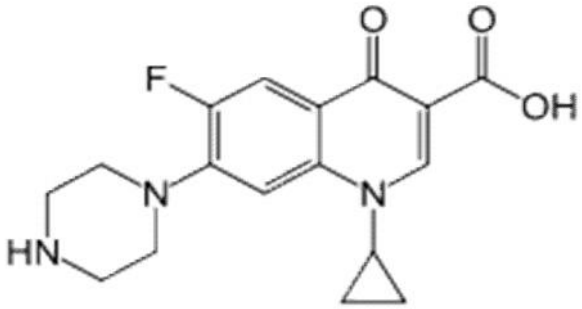

Figure 1. Structure of Ciprofloxacin

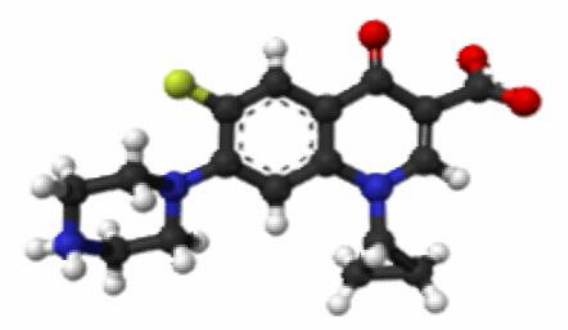

Figure 2. 3D Structure of Ciprofloxacin

2. Method

2.1. Formulation of Tablets:

Tablets were prepared by two methods. In the two Methods: The ratios of the effervescent ingradients were taken as (1:2:3.4) respectively for citric acid: tartaric acid: sodium bicarbonate according to the following equation.

Citric acid:

$$3NaHCO3+C6H8O7\cdot H2O \rightarrow 4H2O+3CO2+Na3C6H5O7 \quad (1)$$
$$3\times84 \qquad 210$$

Tartaric acid:

$$2NaHCO3+C4H6O6 \rightarrow 2H2O+2CO2+Na2C4H4O6 \quad (2)$$
$$2\times84 \qquad 150$$

From the above equations the ratio of effervescent ingredients used was (1:2:3.4) for the citric acid tartaric acid: sodium bicarbonate [41].

2.1.1. Wet Granulation:

The most widely used and most general method of tablet preparation is the Wet Granulation method. Its popularity is due to the greater probability that the granulation will meet all the physical requirements for the compression of good tablets. Its chief disadvantages are the number of separate steps involved as well as the time and labor necessary to carry out the procedure, specially on a large scale. The steps in the wet method are weighing, mixing, granulation, screening, drying, dry screening, lubrication and compression. The equipment involved depends on the quantity or size of the batch. The active ingredient, diluents, and disintegrant are mixed or blended well. [2] [69][70][71][72]

Specific amount of ciprofloxacin and saccharin were weighed and were divided into two pestles in equal amount and well mixed to each one of pestles effervescent base was added Citric and Tartaric acid in one and sodium bicarbonate in another one to avoid reaction then the binder combination (Guar and Poly vinyl pyrollodine) was added slowly after dissolving in a very small amount of water and then the mixture was blended continuously to make the paste, granulated using mesh (10), and then put in oven for drying for twenty hours. The mixture was passed through mesh (14) after drying using mesh (14). The micro crystalline cellulose when added before granulation used as disintegrant and after granulation as glident. Talc powder and magnesium stearate were added as lubricant and glident. Granules were compressed into two types one tablet 250 mg (0.25 gm active ingredient) by 20mm die and 125 mg (0.125 gm ingredient) by 13mm die as divided dose.[2][69][70][71][72]

2.1.2. Calculations:

Formula (1) (high binder concentration):

Guar: 1% & PVP: 4% (w/w)

Formula (2) (low binder concentration):

Guar: 0.005% & PVP: 2% (w/w)

- Guar with poly Vinyl pyrrolidone as a binder in different ratios for the two formulae.
- Saccharin was used from three to five time of active ingredient and the best one it was used in ratio five times to active ingredient.
- Saccharine itself can be used as a binder.
- Tablet weight in these two formulae 1600 mg and 2000 mg can be used in two tablets to be easy to carry, handle, stand packaging and transportation.
- Micro crystalline cellouse (Avicil) 5% is used as disintegrating agent and glidant and lubricant.
- Mg stearte and Talc powder combinations as lubricant and glidant.
- Vanillin was used as flavoring agent.

2.1.2. Direct Compression:

As its name implies, Direct Compression consists of compression of tablets directly from powdered material without modifying the physical nature of the material itself. Formerly, direct compression as the method of tablet manufacture was reserved for small group of crystalline chemicals having all the physical characteristics required for the formation of a good tablet. [2][69][70][71][72] Ciprofloxacin is mixed with lactose to improve compression characteristic then $NaHCO_3$ and saccharine sodium four times (active ingredient) were added to active ingredient and mixed well and named (A). In another mortar, specified amount of tartaric acid and citric acid were weighed accurately and named (B). Then (A) and (B) were mixed in third mortar and specified amount of banana and vanillin flavor was added and then the whole mixture was passed through a sieve for more mixing. One percent of guar is used in dry form for all formula, vanillin was added as flavor agent. The powder was put in an oven for drying and then tableting machine.[2][6][7][8][9]

2.2. Dissolution Test:

The dissolution test was undertaken using (USP apparatus1) (basket method) in six replicates (six tablets for each brand). The dissolution medium was 900ml 0.1NHCl which was maintained at 37±0.5 C^o. In all the experiments, 5ml of dissolution sample was withdrawn at 45 min and replaced with equal volume to maintain sink condition.

Samples were filtered and assayed by ultraviolet spectrophotometer at 277λ (nm) and compared to standard.

The concentration of each sample was determined from a calibration curve obtained from pure samples of ciprofloxacin according to the monograph. [8]

2.3. Microbiological Test:

Microbiological test was carried out for new formula in four isolated laboratory species to inhibit and ensure the effectiveness of the antibiotics. And those species are *Salmonella typhi, Salmonella paratyphi, Staphlococcus aureus* and *Escherechia coli* [15].

2.3.1. Ciprofloxacin Sensitivity Test using Disc diffusion Kirby-Bauer:

Sensitivity Test:

For each test and standard 1mg is taken and dissolved in 10 ml distilled water then 1ml was taken from it and dissolved in other10 ml distilled water.[10]

2.3.2. Antibiotic disc preparation:

Filter paper was cut into small disks of about 4 mm in diameter then it enclosed in a sealed container and sterilized in oven.

Halve number of the disks impregnated with ciprofloxacin test suspension and the others with standard suspension then the disks are dried in oven at $60C^0$ for 20 minutes (serial dilution was made to obtain concentration 10µg/ml as follow: 1mg was dissolve in 10ml and then 1ml was taken and dissolve in another 10 ml).

$$\text{Dilution factor} = \frac{R*V}{O}$$

Where:

R is required concentration,
V is required volume
O is origin concentration

Inoculums was prepared from each bacterium under test

- *Staphylococcus aureus*
- *E. coli*
- *Salmonella* species

Inoculums preparation is the most important step in any susceptibility test. Inocula are prepared directly by inoculating colonies grown overnight on an agar plate, into broth media. Then the numbers of bacteria tested was standardized using McFarland turbidity standards [10].

McFarland turbidity standards: The McFarland 0.5 standard is used, which contains 99.5 ml of 1% sulfuric acid and 0.5 ml of 1.175% barium chloride, this solution is dispensed into tubes comparable to those used for inoculums preparation.

The McFarland 0.5 standard provides turbidity comparable to that of a bacterial suspension containing 1.5×10^8CFU/ml [10].
Inoculation and incubation:

After preparation of standard inoculums suspension, a sterile cotton swab is dipped into the suspension, pressed to remove excess liquid, and then swabbed evenly across the surface of a Mueller Hinton agar plate (plates of 9mm are used). (Each inoculum suspension was inoculated into three media labeled test (T), standard (S) and control(C)).

- Within 15 minutes of inoculation, the individual ciprofloxacin disks (one disc per plate) are applied to the agar media with a forceps and gently pressed to ensure contact with the agar. (Koletar, 2000)
- The ciprofloxacin Test disks are applied in the plates labeled (T)
- The ciprofloxacin Standard disks are applied in the plates labeled(S). While other plate's labeled (c) without antibiotic disks were used to control growth.
- Within 15 minutes of disks placement, plates are inverted and placed in a 37 C^0 for 18 hours [10].
- After incubation the plates were examined, to make certain the test organisms has grown satisfactory, the diameter of each inhibition zone is measured using ruler or calipers.[10]
- Once zone measurements have been made, the millimeter reading for each brand and effervescent formula are compared with that specified in the interpretive tables of the NCCLS documents [10].

2.4. 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 (K3EDTA) 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[83].

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 [11-12-13-14-15-16-17-18-19] figure (3-1).

2.5. Protocol of the Study

Pharmacokinetics of Ciprofloxacin from the different generic formulations was studied after administration of an single oral dose in normal healthy male rabbits. The study was approved by the research and ethical committee in College of Pharmacy Taif University [20].

2.6. 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 [20-82-83-84].

2.7. Subjects

Twenty healthy white albino adult male rabbits participated in the study. 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.[80] 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[20].

2.8. 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.

2.9. 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 [14-20-17].

2.10. 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[20].

2.11 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[19]. 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 ($AUC_{0\rightarrow30}$) was calculated from measured data points from time of administration to time of last quantifiable concentration (C_{last}) by the linear trapezoidal rule. The area under the plasma concentration-time curve extrapolated to infinity ($AUC_{0\rightarrow\infty}$) was calculated according to the following formula:

$$AUC_{0\rightarrow\infty} = AUC_{0\rightarrow30} + C_{last} / [Ln (2) / T½]$$

Where, C_{last} is the last quantifiable concentration. The ratio $AUC_{0\rightarrow30}$ / $AUC0\rightarrow\infty$ as a percent, was determined as an indicator for the adequacy of sampling time. The elimination half-life (T½) was calculated as:

$$T_{½} = Ln (2) / (−b)$$

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 $AUC_{0\rightarrow t}$ and $AUC_{0\rightarrow\infty}$. 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 $AUC_{0\rightarrow30}$, $AUC_{0\rightarrow\infty}$ and C_{max}[19].

2.12. Statistical analysis

Statistical analyses were performed by the two-way analysis of variance (ANOVA) for crossover design at an alpha = 0.05 using the general linear modeling (GLM) procedure of the statistical analysis system (SAS) software (SAS Institute, Inc., Cary, NC, USA). The model contained the main effects of subject within sequence, period and formulations. Sequence effects were tested against the mean square term for animals within sequence. All other main effects were tested against the mean square error term. The pharmacokinetic parameters: $AUC_{0\rightarrow30}$, $AUC_{0\rightarrow\infty}$, C_{max}, T_{max}, K_{el} and $T_{½}$ were analyzed assuming multiplicative model. Drug concentrations at each sampling time point were also analyzed statistically using analysis of variance. Bioequivalence of the two formulations were assessed by calculating the 90% confidence intervals based on the ANOVA (parametric) of the mean Test/Reference ratios of $AUC_{0\rightarrow30}$, $AUC_{0\rightarrow\infty}$ and C_{max} using log-transformed data. In addition, bioequivalence between the two formulations was also assessed by Schuirmann's two one-sided t-tests procedure [17]. Ciprofloxacin is a drug with an intermediate to low intra-subject variability (ANOVA–CV of C_{max} and AUC ≤ 20%). The method of Hauschke et al. For sample size determination for bioequivalence assessment using multiplicative model was used [18].

3. Results and Discussion

3.1. *In vitro* Dissolution and *In Vivo* Correlation:

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. That is agree with Sakore *et al,*[21], Sirisuth[22] *et al.,* and . Qureshi *et al*[23] . An In-vitro in-vivo correlation (IVIVC) has been defined by the Food and Drug Administration (FDA) as "a predictive mathematical model describing the relationship between an in-vitro property of a dosage form and an in-vivo response [24-25]. Generally, the In vitro property is the rate or extent of drug dissolution or release while the In vivo response is the plasma drug concentration or amount of drug absorbed. Practically, the purpose of IVIVC is to use drug dissolution results

from two or more products to predict similarity or dissimilarity of expected plasma drug concentration (Figures (3-1) (3-2) (3-3) and (3-4).Thus correlation between the formulated tablets and reference product construes the high pharmacokinetics parameters figure (3-3) and figure (3-4). This agree with Galia *et al.* [26], Modi *et al.* [27], Amidon *et al.*[28], Dunne *et al.*[29],

Solubility, dissolution rate and intestinal permeability are the major biopharmaceutic factors that affect the rate and extent of absorption of an oral drug product. Particularly for (BCS 11) like ciprofloxacin. This agree with agree with Parrot *et al* [30], Djorjevic *et al.* [31]. Dressman *et al.* [32], Macheras *et al* [33].

The proposed Biopharmaceutics Classification System (BCS) is based on determining the underlining process that is controlling the drug absorption rate and extent, namely, drug solubility and intestinal membrane permeability. The goal of the (BCS) is to function as a tool for developing in vitro dissolution specifications like effervescent tablets that are predictive of their in vivo performance, like figure (3-3) when correlated to figures (3-4) there is a complying and similar results between in vitro and in vivo which agreement with Shan *et al*[34], and O'Hara *et al,*[35] that is strong correlation between enhancement in vitro properties with in vivo results like Shah *et al.*[36], Amidon *et al.*[37], and Hwang *et al,*[38]. The predicted bioavailability is compared with known bioavailability and % P.E is calculated. The prediction error for external validation should be below10% whereas prediction error between 10-20% indicates inconclusive predictability and need of further study using additional data set, so in this study the % of prediction for C_{max} and $AUC_{0-\alpha}$ was calculated as follows:

For Cmax

%Prediction error (P.E.)

$$= \frac{(C_{max}\ observed - C_{max}\ predicted)*100}{C_{max}\ observed}$$

For AUC:

%Prediction error (P.E.)

$$= \frac{(AUC\ observed - AUC\ predicted)*100}{AUC\ observed}$$

Interestingly %for both C_{max} and $AUC_{0-\alpha}$ is 4% and 4.2% this illustrate intimately correlation between in vitro in vivo in this research. This finding agree with. Uppoor *et al.* [39], Chilukuri *et al.*[40], Jaber *et al.*[41], and Jayaprakasam *et al,*[42].

In microbiological assay the effervescent formulated tablets give higher effects rather than reference tablets, Table (3-1) and table (3-2) similar result as concentration –time curve profiles figures (3-4) which reflect high concentration of new formulated drug in plasma rather than reference. This illustrate the good correlation between in vitro microbiological assays with in vivo, for the medicament distribution. Similar findings by Ahmed *et al.*[43],Cristina *et al.*[44].

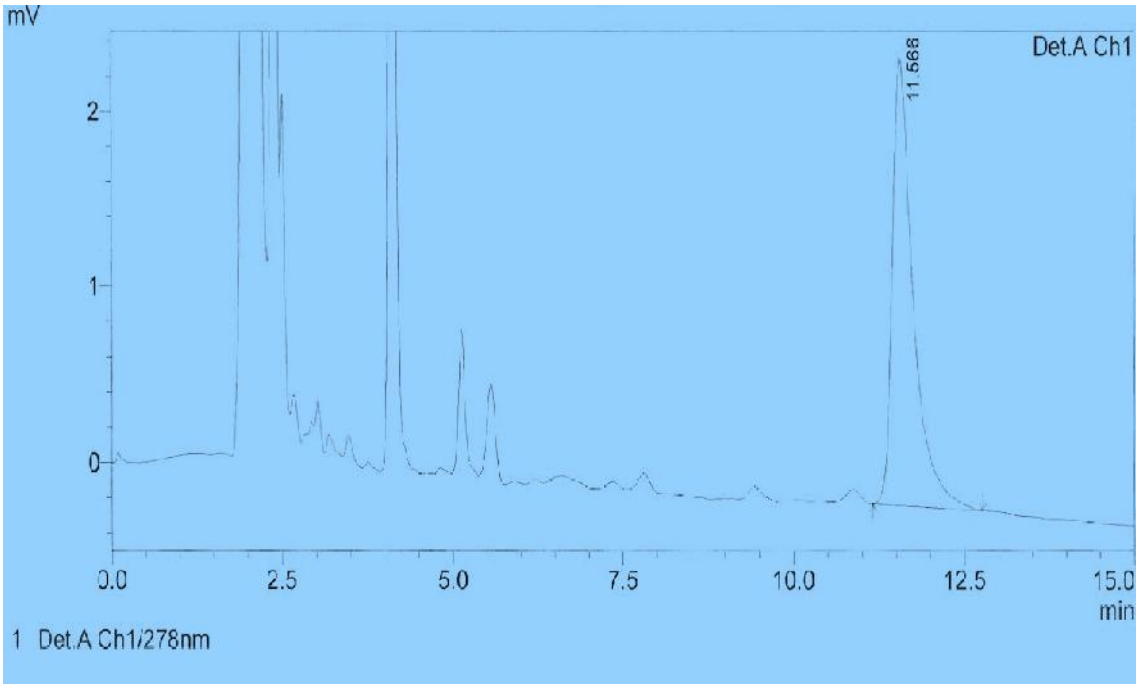

Figure (3-1) HPLC chromatogram showing rabbit plasma sample containing ciprofloxacin 1626.6 ng/ml of test tablets

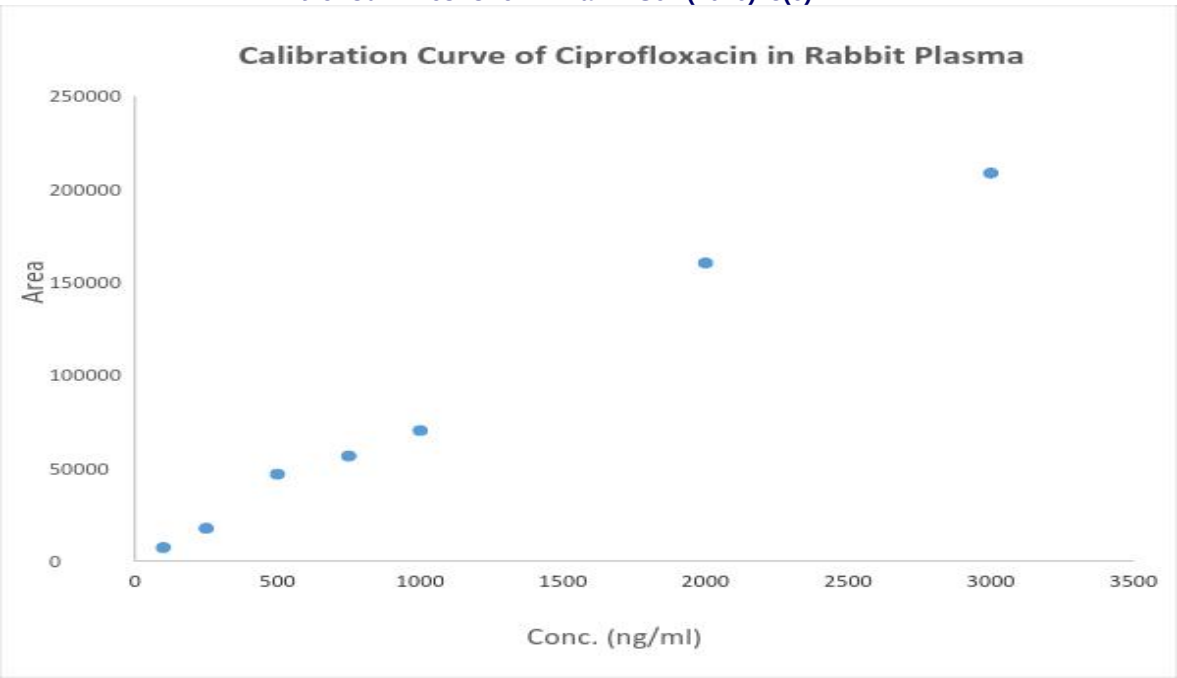

Fig (3-2) Calibration Curve of ciprofloxacin in healthy Adult Male Rabbit Plasma.

Table (3-1): Plasma Concentrations of Ciprofloxacin (ng/ml) after single oral administration of Ciprobay (Reference Product A) and Effervescent Ciprofloxacin (Test Product B) to Rabbit # 16

Sampling Frequency (hrs after administration)	Reference Product (A) ng/ml	Test Product (B) ng/ml
0.5	188.0	360.9
1.0	394.6	1003.6
1.5	712.9	801.7
3.0	390.3	420.7
4.0	212.7	260.5
8.0	155.0	167.1
12.0	112.3	140.8
24.0	0.0	11.0

Figure (3-3) Dissolution profile of New Effervescent Ciprofloxacin Formula (B) Compared to Reference Product Ciprobay (A).

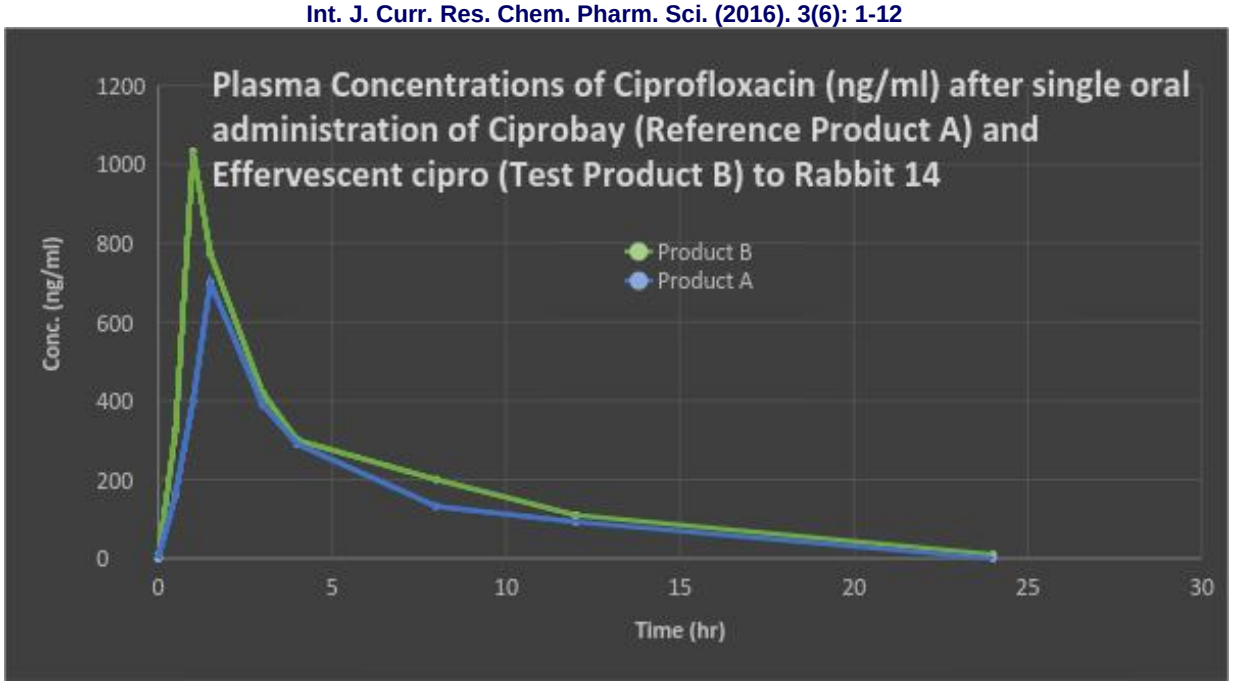

Figure (3-4): Plasma Concentrations of Ciprofloxacin (ng/ml) After Single Oral Administration of Ciprobay (Reference Product A) and Effervescent Ciprofloxacin (Test Product B) to Rabbit.

Table (3-2): Comparison between the Two Types of Effervescent With Reference Tablets on Different Species of Microorganisms

Tablets	Diameter (mm)			Surface Area (mm^2)		
	E. coli	***Staph aureus***	***Salmonella***	***E. coli***	***Staph aureus***	***Salmonella species***
Direct Compression	17	15	14.8	266.9	176.7	172
Wet Granulation	18	15.5	15.4	254.3	188.6	186.2
Reference	14.5	14	13.9	165	153.9	151.7

In two types of effervescent tablet (wet granulation method and direct compression method) the zone of inhibition is slightly larger in wet granulation method than in direct compression method this might be due to good distribution of active ingredient and both of them is higher than reference product(Ciprobay)[46].

4. Conclusion

- The pharmaceutical industry has been striving to find a ways to saving precious resources in relevance to the budgets and increasing cost of drug development. IVIVC is a tool applied in various areas and stages of drug development to find a place in the regulatory bodies around the world. IVIVC can serve as surrogate for in vivo bioavailability and to support biowaivers also allows setting the dissolution specification and methods. The substitute of expensive clinical trials with the use of IVIVC is perhaps the most important feature of IVIVC. From the regulatory point of view IVIVC can assist certain scale-up and post approval changes. IVIVC principles have been mostly applied to oral products, there exists a need to develop methodologies and standards for non-oral delivery systems, to develope more meaningful dissolution and permeation methods.
- Due to the high solubility of the effervescent drug, ciprofloxacin was formulated as effervescent tablets to become more effective, convenient, and easy to use and swallow. Premeasured dosage forms that are already in solution when ingested effervescent mixtures have been moderately popular.
- The study also managed to improve the palatability of the drug solution, via the utilization of saccharin sodium and vanillin flavor and using effervescent formula.
- 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.

- Formulating tablet by wet granulation method (when it is possible and applicable) is better than in direct compression method because of good distribution of active ingredient.
- The effervescent tablets may be very effective in medicine this clear from the microbiological sensitivity test and bioequivalence study which has been carried out against different bacteria.
- 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.
- 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 [45].
- *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.
- HPLC analysis is suitable method for analysis drugs in plasma due to it is precise values.

5. Recommendations:

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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