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# *In vitro-In vivo* correlation (IVIVC) study of Gemifloxacin immediate release (IR) oral formulation

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## ABSTRACT

*The aim of this study was to establish a correlation between in vitro dissolution and in vivo absorption data of prepared immediate release Gemifloxacin tablets (Zagam) and compare with conventional tablets of Gemifloxacin (Factive). In vitro release data were obtained for test and reference tablets by using the USP apparatus II, 0.01N HCl of pH 2.0 at 50 rpm. A group of six healthy, male human subjects participated for in vivo study. Serial blood samples were collected at 0, 1, 2, 3, 4, 6, 8, 10, 12 and 24 hr. Gemifloxacin was measured by Ultra performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) method to establish in vitro-in vivo correlation while absorption profiles were derived using Wagner-Nelson equation. $f_2$ and $f_1$ were determined for the time intervals of 5, 10, 15, 20, 25, 30, 45 and 60 minutes and the obtained values were 97.97, 99.94, 95.87, 91.02, 99.05, 96.97, 86.80 and 100.00% for $f_2$ and 11.5, 9.4, 14.0, 12.5, 10.6, 0.5, 0.2 and 0.9% for $f_1$ at respective time intervals. The bioavailability of Gemifloxacin IR tablet containing 320 mg of Gemifloxacin mesylate and reference tablet was measured using pharmacokinetic parameters $C_{max}$, $T_{max}$, $t_{1/2}$ and AUC. Moreover, the value of correlation coefficients for % in vivo absorption versus % in vitro dissolution of the two products were calculated to be 0.9443 and 0.9208.*

**Keywords:** Gemifloxacin, *In vitro-In vivo* Correlation, Human plasma, Immediate release tablets.

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## INTRODUCTION

Correlations between *in vitro* and *in vivo* data (IVIVC) are often used during pharmaceutical development in order to reduce development time and optimize the formulation. A good correlation is a tool for predicting *in vivo* results based on *in vitro* data. IVIVC allows dosage

form optimization with the fewest possible trials in man, fixes dissolution acceptance criteria, and can be used as a surrogate for further bioequivalence studies; it is also recommended by regulatory authorities [1, 2]. The Center for Drug Evaluation and Research (CDER) at the Food and Drug Administration (FDA) released a guidance that set the information which should be provided to CDER to assure continuing product quality and performance characteristics of immediate-release oral solid dosage formulations for specific post-approval changes [3]. This is commonly called Scale-Up and Post Approval Changes for Immediate Release (SUPAC IR) that has the major intent to reduce the number of preapproval supplements required for manufacturing changes. According to SUPAC IR guidance, a manufacturer will frequently need to demonstrate that the dissolution profiles of the pre-change product and post-change product are "similar". SUPAC IR suggests that dissolution profiles may be compared by determining similarity and difference factor ($f_2$ *and* $f_1$ metric). SUPAC IR also states that if $f_2$ value lies between 50 to 100% suggests that the two dissolution profiles of test and reference formulations are similar [4, 5].

Gemifloxacin mesylate is a potent, novel fluoroquinolone agent with a broad spectrum of antibacterial activity and it is used to treat respiratory and urinary tract infections that are proven or strongly suspected to be caused by susceptible gram-positive and gram-negative bacteria [6]. Gemifloxacin mesylate is chemically (*R, S*)-7-[(4*Z*)-3-(amino methyl)-4-(methoxy imino)-1-pyrrolidinyl]-1cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-1, 8-aphthyridine-3-carboxylic acid and its empirical formula is C18H20FN5O4•CH4O3S with molecular weight 485.49 [7, 8]. Gemifloxacin is rapidly and almost completely absorbed after oral administration and showed excellent tissue penetration by absorbing two-thirds of those in plasma. Peak plasma concentrations are usually attained one to two hours after oral dosing [9, 10]. In this work, the behavior of Gemifloxacin has been studied through *in vitro* tests and used the current pharmacokinetic assessment to correlate with *in vivo* test significantly for the bioavailability of the drug.

## MATERIALS AND METHODS

### Tablet formulations and *in-vitro* study

Dissolution testing was performed for both formulations of 320 mg Gemifloxacin mesylate (Factive as reference product, batch number: 3044478) from Cornerstone Therapeutics Inc., Cary, N.C under the licensing authority of LG Life Sciences Ltd., Seoul, Korea and (Zagam® as test product, batch number: 022/044) from Orchid Healthcare Ltd., India. Tablet dissolution was assessed using standard USP 24 Apparatus II equipment. A stirring speed of 50 rpm was used to agitate the dissolution medium, which was kept at 37 ± 0.5ºC throughout and consisted of 0.01 N Hydrochloric acid. The drug concentration was determined by UV spectrophotometer (Varian Cary 50 CONC) at 342 nm at various time points 5, 10, 15, 20, 25, 30, 45 and 60 minutes, 10 mL of solution was withdrawn and replaced by equal amount of 0.01 N HCl solution. Then the solutions were filtered through Whatman No.41 filter paper.

### *In vivo* study in humans

Six healthy male subjects with a mean age of 25.3 ± 1.8 years (ranging from 23 to 27 years), a mean body weight of 65.3 ± 4.5 kg (ranging from 60 to 70 kg) and a mean height of 165.1 ± 5.8 cm (ranging from 160 to 171 cm) participated in this study. The volunteers were judged healthy on the basis of their previous medical history, physical examination and routine clinical laboratory tests.

None of the subjects used alcohol or tobacco. All subjects were free from other drugs 15 days before and during the study.

**Study design**

A single-centre, non-blind, two-period, open-label, single dose, randomized block design (RBD) (n=6) in which six volunteers received single treatment to evaluate the pharmacokinetic profile of both reference and test formulations of Gemifloxacin. The subjects were fasted overnight for approximately 10 hours prior to dosing and until 4 hours post dose during Period 1. Subjects were discharged after the completion of the 24-hour procedures and were instructed to return 36 hours post dose for a pharmacokinetic blood sample collection. During Period 2, subjects were dosed within 5 minutes after the completion of a standardized meal. Water was allowed ad libitum two hours post dose.

**Blood Analysis**

An indwelling venous catheter was inserted into a forearm vein, and venous blood samples were collected for pharmacokinetic measurements at predose (0 hour) and at 0.25, 0.5, 1, 2, 3, 4, 5, 6, 8, 12, 16 and 24 hours. The whole blood samples were centrifuged to separate the plasma within 30 min after sample collection at 4°C at approximately 3,000 rpm for at least 10 minutes. Until centrifugation, the samples were stored in ice bath, and then samples were stored immediately in a freezer at −20°C.

The plasma concentration of Gemifloxacin from the selected formulation and reference product were measured by validated UPLC-MS/MS method (Waters ACQUITY UPLC®-Ultra performance liquid chromatography, Milford Massachusetts, USA) was coupled to a tandem mass spectrometer with 2996 PDA detector and turbo electrospray ion source (4000 Qtrap, Applied Biosystems, Foster City, CA, USA) and was used with negative-ionization mode with the following source settings: the turbo ionspray interface was maintained at 530 °C with a zero air nebulization). In brief the analytical method involved a robotized solid phase extraction in the 96-well plate format (Oasis MCX 30 mg), followed by reversed phase liquid chromatography (isocratic mode, Purospher, RP18e, column dimension 150 x 4.6 mm, particle diameter 5µm, column temperature 35°C). Venlafaxine was used as an internal standard. The mobile phase consisted of pH 3.0 phosphate buffer, acetonitrile and methanol were mixed in the ratio of 75:17:8 and the flow rate was 1.2 mL/min with 50 µL volume of sample injection. Six quality control (QC) samples (in duplicate) at three concentration levels: one near the lower limit of quantification (QC1: 50 µg /mL), one in the mid-range (QC2: 100 µg /mL) and one near the upper limit of quantification (QC3: 500 µg/mL). The inaccuracy and imprecision of the data obtained was below 5.00% and 7.00% respectively. The ion transition was monitored as *m*/*z* 390.100 → 372.100. The analytical method in human plasma (EDTA) was shown to be linear from 10 to 5000 ng/mL. Concentrations were determined using the slope and the intercept of the calibration line obtained by least square regression using the appropriate weighing factor ($1/x^2$).

**Dissolution data analysis**

The *in vitro* dissolution data were analyzed by estimation of a similarity factor ($f_2$) and difference factor ($f1$) [4, 11] and parameterized by the sigmoid $E_{max}$ model. The dissolution profiles were compared using similarity factor ($f_2$) and difference factor ($f_1$), presented in the following equation:

$$f_2 = 50\log[\{1+1/n\sum_{t=1}^{n}(R_t - T_t)^2\}^{-0.5} x100] \quad \text{..... (1)}$$

$$f_1 = [\{\sum_{t=1}^{n}|R_t - T_t|/\sum_{t=1}^{n}R_t\}x100] \quad \text{..... (2)}$$

Where Rt and Tt are the percent drug dissolved at each time point for the reference and test products, respectively; n is the number of dissolution sample times and t is the time points for collecting dissolution samples. The mean dissolution time ($MDT_{in\ vitro}$), mean absorption time ($MAT_{in\ vivo}$) were also calculated both for test and reference formulations by using equations (4) and (5) [11, 12].

$$MDT_{invitro} = \sum_{t=1}^{n} t_{mid}\Delta M / \sum_{t=1}^{n}\Delta M \quad \text{..... (3)}$$

$$MAT_{invivo} = \sum_{t=1}^{n} t_{mid(invivo)}\Delta M_{(invivo)} / \sum_{t=1}^{n}\Delta M_{invivo} \quad \text{..... (4)}$$

Here, $t_{mid}$ is the time at midpoint between *i* and *i*-1, $\Delta M$ is the additional amount of drug dissolved between *i* and *i*-1, $\Delta M_{\text{in vivo}}$ is the additional amount of drug absorbed between *i* & i-1.

***In vivo* data analysis**

The pharmacokinetic parameters such as the highest Gemifloxacin concentration measured for a subject was the $C_{max}$, the time at which $C_{max}$ occurred was the $T_{max}$ and the area under the plasma concentration-time curve to 24 hr ($AUC_{0-t}$) was determined by the trapezoidal rule and the area under concentration-time curve extrapolated to infinity ($AUC_{0-\infty}$) was calculated by using the following formula:

$AUC_{0-\infty} = AUC_{0-t} + C_t/K_{el}$ ..... (5)

where $C_t$ is the last quantifiable plasma level [13].

The percent of drug absorbed was calculated by means of model dependent technique such as Wagner-Nelson procedure [14]. According to Wagner–Nelson equation,

$$A_t / A_0 = \frac{C_t + K_{el} * AUC_0^t}{K_{el} * AUC_0^\infty} \quad \text{..... (6)}$$

Here, $A_t/A_0$ denotes the fraction of drug absorbed at time t, $C_t$ is the plasma drug concentration at time t, $K_{el}$ is elimination rate constant, $AUC_{0-t}$ and $AUC_{0-\infty}$ are the area under the plasma concentration-time profile curve at time t and α respectively.

## RESULTS AND DISCUSSION

The dissolution results for individual tablet of both test and reference product is listed in Table 1 and presented graphically in Fig 1. From the graphical presentation it is observed that the dissolution pattern of test (Zagam) product is almost similar to that of reference (Factive) product. Similarity factors (*f2*) and difference factors (*f1*) for reference and test products are also presented in Table 1. Mean Gemifloxacin plasma concentrations through 24 hr for reference and test formulation are found almost similar. Mean area under the plasma concentration-time curve (AUC) for test product (Zagam® ) versus reference product (Factive) is given in the Fig 2 and indicates that reference formulation (Factive) has similar bioavailability ($F_{relative}$ = 1.38) to the test product (Zagam). Similar type of curve is obtained from percent drug absorbed versus time plot for both products (Fig 3). The values of mean dissolution time ($MDT_{in\ vitro}$) and mean absorption time ($MAT_{in\ vivo}$) are also presented in the Table 2 both for test and reference formulation. The chromatogram of Gemifloxacin standard along with internal standard is presented in Fig 4. The retention time of Gemifloxacin and internal standard (Venlafaxine) are 6.7 min and 9.4 min respectively. The blank sample is clean and no interfering peak is observed at the retention times of Gemifloxacin and there is no interference between the peaks of Gemifloxacin and internal standard.

**Table 1. Dissolution profile for test and reference products of Gemifloxacin 320 mg IR tablets**

| Percent released | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Test product | | | | | | | | |
| Sample | 5 min | 10 min | 15 min | 20 min | 25 min | 30 min | 45 min | 60 min |
| 1 | 11.6 | 30.05 | 43.2 | 67.7 | 91.6 | 99.6 | 99.2 | 100.9 |
| 2 | 11.4 | 32.5 | 44.6 | 68.9 | 90.5 | 98.7 | 98.8 | 100.3 |
| 3 | 10.5 | 31.5 | 43.8 | 67.2 | 91.1 | 99.5 | 99.4 | 100.2 |
| 4 | 9.9 | 30.4 | 44.2 | 69.1 | 92.3 | 100.4 | 99.5 | 101.9 |
| 5 | 10.4 | 30.8 | 42.5 | 67.5 | 91.8 | 99.4 | 99.6 | 101.4 |
| 6 | 11.7 | 31.04 | 43.9 | 68.5 | 92.4 | 98.7 | 98.6 | 100.8 |
| **Mean (%)** | **10.92** | **31.05** | **43.70** | **68.15** | **91.62** | **99.38** | **99.18** | **100.91** |
| ±SD | 0.75 | 0.87 | 0.75 | 0.79 | 0.73 | 0.64 | 0.40 | 0.66 |
| SE | 0.31 | 0.36 | 0.31 | 0.32 | 0.30 | 0.26 | 0.16 | 0.27 |
| **f2 (%)** | **97.97** | **99.94** | **95.87** | **91.02** | **99.05** | **96.97** | **86.8** | **100.0** |
| **f1 (%)** | **11.50** | **9.40** | **14.0** | **12.50** | **10.60** | **0.50** | **0.20** | **0.90** |
| Reference product | | | | | | | | |
| Sample | 5 min | 10 min | 15 min | 20 min | 25 min | 30 min | 45 min | 60 min |
| 1 | 12.8 | 27.42 | 37.82 | 60.4 | 83.2 | 98.7 | 99.4 | 100.6 |
| 2 | 11.6 | 28.16 | 40.05 | 59.9 | 81.6 | 98.2 | 98.8 | 99.6 |
| 3 | 12.3 | 27.96 | 41.23 | 60.1 | 83.4 | 99.4 | 99.4 | 100.2 |
| 4 | 12.9 | 28.52 | 36.94 | 60.8 | 82.8 | 99.7 | 99.7 | 100.4 |
| 5 | 11.2 | 30.02 | 36.62 | 60.5 | 82.7 | 98.4 | 99.6 | 99.7 |
| 6 | 13.2 | 28.22 | 37.25 | 61.8 | 83.4 | 99.2 | 99.6 | 99.8 |
| **Mean (%)** | **12.33** | **28.38** | **38.32** | **60.58** | **82.85** | **98.93** | **99.42** | **100.05** |
| ±SD | 0.79 | 0.88 | 1.88 | 0.67 | 0.68 | 0.59 | 0.33 | 0.41 |
| SE | 0.32 | 0.36 | 0.77 | 0.28 | 0.28 | 0.24 | 0.13 | 0.17 |

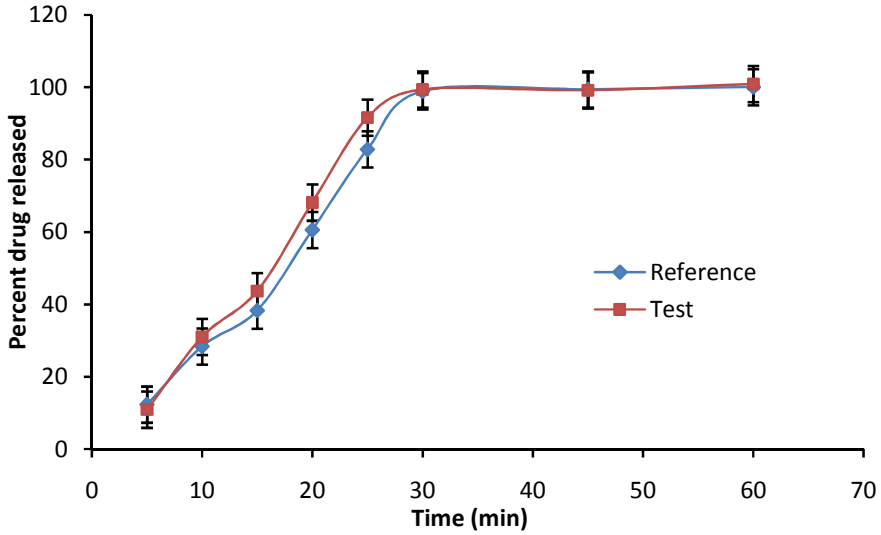


**Fig 1. Comparison of mean dissolution rate between test (Zagam) and reference (Factive) products**

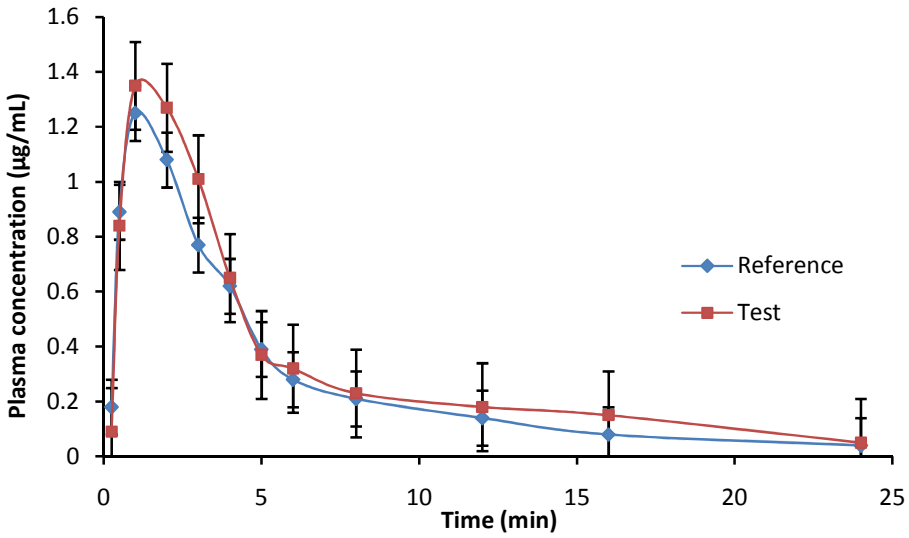


**Fig 2. Mean plasma concentrations of Gemifloxacin at different time intervals of test (Zagam) and reference (Factive) formulation**

## Evaluation of *in-vitro*/*in-vivo* relationship

A multiple level C correlation relates one or several pharmacokinetic parameters of interest ($C_{max}$, AUC, or any other suitable parameters) to the amount of drug dissolved at several time points of the dissolution profile. Multiple level C correlations is the highest category of correlation and represents a point-to-point (1:1) relationship and test products according to compendia dissolution method using USP apparatus II (paddle type) which has been discussed earlier and the results are presented in the Table 1. Percent of drug released and percent of drug absorbed which was calculated from the mean plasma drug concentrations, using Wagner-Nelson equation (Equation 6) for both reference and test products (Table 2).

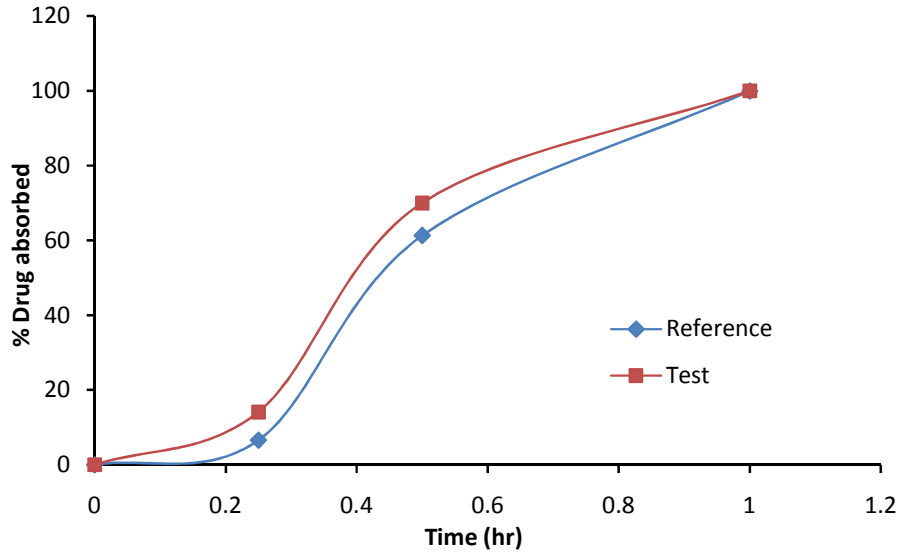


**Fig 3. Mean Wagner-Nelson plot for test (Zagam) and reference (Factive) products after administration of Gemifloxacin to six healthy male volunteers**

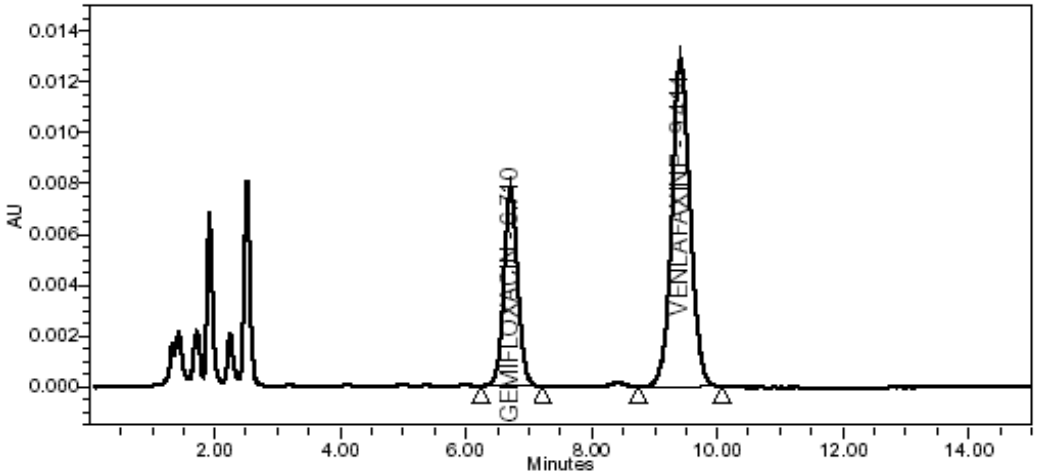


**Fig 4. UPLC chromatogram of Gemifloxacin standard along with internal standard Venlafaxine**

Then the values of percent of drug released are plotted against the percent of drug absorbed to find out the *in vitro/in vivo* relationship (Fig 5). Table 1 also describes the similarity factor ($f_2$) for 5, 10, 15, 20, 25, 30, 45 and 60 minutes and the obtained values are 97.97, 99.94, 95.87, 91.02, 99.05, 96.97, 86.80 and 100.00% respectively. As, similarity factors ($f_2$) are within the acceptable range of 50% to 100%, test formulation is similar to reference formulation [5]. Difference factors ($f_1$) are obtained 11.5, 9.4, 14.0, 12.5, 10.6, 0.5, 0.2 and 0.9% at the same time intervals. The values for $f_1$ are also within the acceptable range (less than 15%) [5].

From the Table 3, the mean absorption time (MAT) for test formulation is shorter due to low mean dissolution time (MDT) and opposite circumstance for reference formulation. Percent of drug absorbed from test product and reference product have followed similar pattern and it is

very close to each other in the present study (Fig 3). It is observed that there is a gradual increase in the percent of drug absorbed both for test and reference product with a rapid increase in the terminal phase (Fig 5). The most common pharmacokinetic parameters such as total area under the plasma concentration–time curve (AUC $_{0-\infty}$, peak plasma concentration ($C_{max}$), time to reach maximum plasma concentration ($T_{max}$) and the elimination half-life ($t_{1/2}$) are estimated from the plasma concentration-time profiles of two preparations (test and reference) for each volunteer is presented in Table 4 [16].

**Table 2. Mean percent of drug released and absorbed for both test and reference products**

| Time (hr) | Test product | | Reference product | |
|---|---|---|---|---|
| | % drug released | % drug absorbed | % drug released | % drug absorbed |
| 0.25 | 43.70 | 14.13 | 38.32 | 6.57 |
| 0.50 | 99.38 | 70.04 | 98.93 | 61.2 |
| 1.00 | 100.91 | 100.01 | 100.05 | 99.97 |

**Table 3. The values of $MDT_{in\ vitro}$ and $MAT_{in\ vivo}$, both for reference and test formulation**

| Formulation | $MDT_{in\ vitro}$ (min) | $MAT_{in\ vivo}$ (min) |
|---|---|---|
| Reference | 19.83 | 40.75 |
| Test | 17.20 | 35.69 |

*MDT = Mean dissolution time, MAT = Mean absorption time*

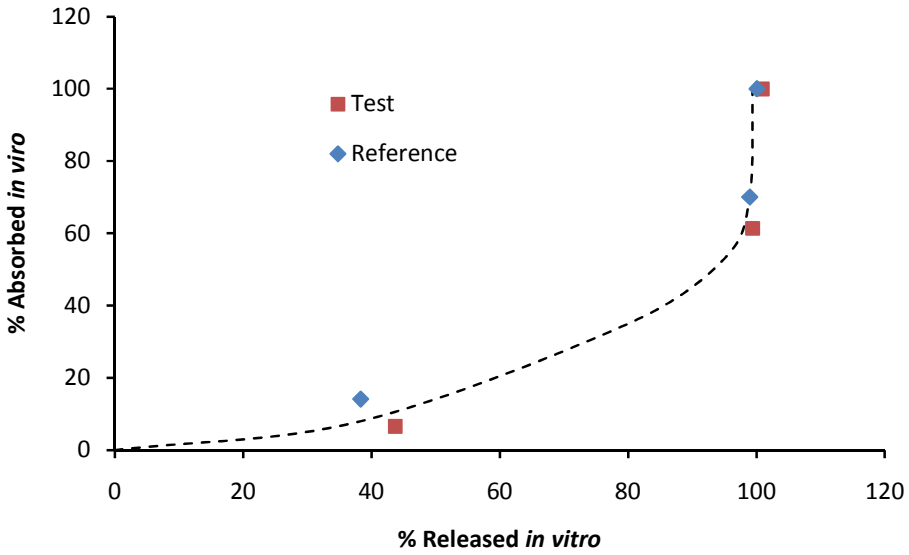


**Fig 5. Non linear multiple level C correlation (IVIVC) for both test and reference products**

From the multiple level C correlations, it is concluded that there is no linear correlation between percent of drug released and percent of drug absorbed for both the products. This can be attributed to the Gemifloxacin film coated tablet is an immediate release formulation, as dissolution is not a rate limiting step in IR products, the fraction of drug absorbed against the fraction of drug released. Since absorption cannot "keep up" with dissolution, a non linear relationship between the fractions of drug absorbed and the fractions of drug released is

obtained. Thus the *in vitro*/*in vivo* correlation is well established and justified for both test and reference formulations with multiple level C correlation.

**Table 4. Mean pharmacokinetic parameters after immediate release test and reference formulation**

| | Test product | | | | Reference product | | | |
|---|---|---|---|---|---|---|---|---|
| Subject | $C_{max}$ (µg/mL) | $T_{max}$ (hr) | AUC $_{0-\infty}$ (µg h/mL) | $T_{½}$ (hr) | $C_{max}$ (µg/mL) | $T_{max}$ (hr) | AUC $_{0-\infty}$ (µg h/mL) | $T_{½}$ (hr) |
| S1 | 1.61 | 1.00 | 8.08 | 7.3 | 1.58 | 1.30 | 7.69 | 7.5 |
| S2 | 1.78 | 1.00 | 8.51 | 7.7 | 1.29 | 1.20 | 7.52 | 7.2 |
| S3 | 1.49 | 1.00 | 6.83 | 7.2 | 1.23 | 1.00 | 6.39 | 6.7 |
| S4 | 1.24 | 1.40 | 8.00 | 6.9 | 1.19 | 1.30 | 5.61 | 7.8 |
| S5 | 1.53 | 1.00 | 6.02 | 7.4 | 1.44 | 1.00 | 6.11 | 7.1 |
| S6 | 1.75 | 1.30 | 8.06 | 7.6 | 1.12 | 1.00 | 7.65 | 7.4 |

## CONCLUSION

To summarize the results from the current study, a multiple level IVIVC is adequately demonstrating the *in vivo* plasma pharmacokinetic profiles of Gemifloxacin test product along with reference product is established based on the release properties obtained from the *in vitro* investigations and the pharmacokinetic properties obtained after administration of an immediate-release tablet. The IVIVC developed makes Gemifloxacin dissolution profiles more meaningful, as it allows for predicting their impact on the pharmacokinetics and for the replacement of bioequivalence studies in situations defined by the SUPAC-IR guideline. The benefit of this current study is to minimize the number of cost effective bioequivalence studies performed during the initial approval process, the scaling-up and post-approval changes. Therefore it can be concluded that two different products (test and reference) had little effect on the bioavailability of Gemifloxacin.

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