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Development & validation of level A: In-vitro and in-vivo correlation for ofloxacin using a systematic approach

Nidhi Makadiya, Neha Padwal & Arti Swami*

Department of Pharmaceutical Sciences, School of Health Sciences & Technology,
Dr. Vishwanath Karad MIT World Peace University, Pune, Maharashtra, India

*E mail: arti.swami@mitwpu.edu.in

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The key objective of the current research is to establish an *IVIVC* (in vitro and in vivo correlation) model for ofloxacin tablet formulations. The USP apparatus II is used to determine the in vitro drug release characteristics of drug, rpm ranging from 50-150, in a pH-varying medium. The in vitro dissolution testing is found to be applicable for calculating the in vivo drug concentration. Three different formulations are used for the study. The in vivo data is referred from the study aimed to assess bioequivalence of ofloxacin tablets. Plasma concentration is converted to cumulative percentages of drug absorbed using the Wagner-Nelson equation. The percentage in vitro cumulative drug dissolved, intercept, and slope are used to calculate the in vivo drug release. The cumulative drug release is plotted against the percent cumulative drug absorbed and the level A *IVIVC* model is developed using regression technique.

Keywords: Bioequivalence, Dissolution, *In vitro-in vivo* correlation, *IVIVC*, Ofloxacin

Introduction

As the generic market is increasing, need of bioequivalence study increases which involves human and animal studies. Bioequivalence studies are required throughout drug development process, scale-up and post approval changes, submissions of generic product applications. Establishing bioequivalence is expensive and time-consuming process. So, developing alternative methods for establishing bioequivalence is of prime importance. *IVIVC* stands for in-vitro in-vivo correlation for predicting the in vivo drug release through in-vitro studies is a mathematical tool. *IVIVC* model can serve as a surrogate for human studies. It can act as a simple, fast, and inexpensive alternative for bioequivalence studies. It can also be used as a tool to check batch-to-batch variations. The in vitro properties (release of drug) are correlated with in vivo properties (AUC, plasma drug concentration). FDA has already come up with *IVIVC* guidelines for the oral formulation but still they are not completely defined, so there is need to carry out the studies which can support the regulatory guidelines[1-4].

Establishing *IVIVC* model is a two-step process which includes developing and then validating the model. Level A *IVIVC* model's development is usually established in the first stage using a two-stage procedure. The numerical deconvolution approach is used to estimate the observed proportion of drug absorbed in the first phase. The obtained amounts of drug absorbed along with drug dissolution are used for developing the *IVIVC* model. The observed fraction of drug dissolved is used to calculate the expected measure of drug absorption based on the *IVIVC* model. The convolution approach is then used to convolve the expected proportion of drug absorbed to the predicted plasma concentrations. Determining the percentage of predicted errors (%PE) will be the second stage of the *IVIVC* model's predictability evaluation. Assessing the %PE either internally or externally may also prove beneficial[5-7].

No. of *IVIVC* studies have been reported till date which elaborates about the application of IVIVC as an alternate to bioequivalence studies for oral dosage forms which include *IVIVC* of Immediate release levofloxacin tablet[8], extended-release matrix formulation of propranolol hydrochloride[9], differently dissolving ketorolac tablets[10], immediate release ibuprofen tablet[11], divalproex sodium III tablets[12], amorphous solid dispersion immediate-release suvorexant tablets[13]. It has been reported in the literature that BCS Class III drugs are best candidate drugs for IVIVC study as these are highly permeable

and poorly soluble drugs. Therefore, the in vivo dissolution is the main factor influencing oral bioavailability of these compounds. Hence ofloxacin being the BCS Class II drug is an ideal candidate for *IVIVC* study. The present manuscript describe about establishment of Level A *IVIVC* model for ofloxacin tablets.

Ofloxacin is a quinolone/fluoroquinolone antibiotic which acts by blocking DNA replication by binding to DNA gyrase enzyme, which allows the untwisting required to replicate one DNA double helix into two. Depending of the type of infection, dose of ofloxacin ranges from 200-800 mg divided in 2-3 times per day. Level A association is most likely when a class I drug candidate is designed as an extended-release product, in which the release profile regulates the rate of absorption and the drug's solubility and permeability are site independent. Hence, ofloxacin was a best candidate to formulate as extended-release formulation[14,15].

Experimental Section

Bile salt, glycerol monooleate, sodium oleate, sodium chloride, sodium hydroxide were procured from Research-Lab Fines Chem; lecithin, malecic acid was from Analab-fine Chemicals; pepsin was from Oxford Laboratory; sodium acetate from Bhortek Chemicals; sodium dihydrogen phosphate was from Vishal. Chem; Hydrochloric acid and Dichloromethane from Molychem. All the above mentioned chemicals and materials were of analytical reagent grade.

Development of the UV method for Ofloxacin Analysis

The sample solution was prepared by using 20 tablets from which the equivalent quantity was weighed. Stock solution was diluted to make the final solution of concentration of 10 µg/mL. As per ICH guidelines[16] linearity was established using calibration curve of ofloxacin. The linearity was obtained after plotting of calibration curve from six different aliquots of standard solutions, prepared at concentrations of 2-12 µg/mL. The intra-day and inter-day precision was studied at three concentration levels viz. 2, 6 and 8 µg/mL and the results were estimated as per ICH guidelines. Recovery is estimated using formulation and standard solution at 80%, 100% and 120% recovery level. The analysis was carried out in triplicate and % recovery was calculated[16].

Dissolution Studies

The *in vitro* studies of three formulations Zanocin 200 (Sun Pharmaceuticals Industries Ltd), Zanocin 400 (Sun Pharmaceuticals Industries Ltd) and Oquin 200(Nepal Pharmaceutical laboratories) were carried out using the dissolution test apparatus. The drug release was estimated with USP apparatus type-II, i.e. paddle apparatus. The dissolution was carried out using multiple dissolution media and the change in RPM was carried out in the range of 50 to 150 RPM to obtain the dissolution condition in which the best *in vivo* predictability can be achieved. The sample of 5ml was withdrawn at appropriate time interval 1, 2, 3, 4, 6, 8, 12, 16, and 24. After sample collection 5 mL of buffer is injected in every vessel to maintain the dissolution volume[10].

In vivo Data

The *IVIVC* was developed using the reported plasma concentration data which was extracted from the previously published literature [17].

In vitro Data Analysis

The graph of cumulative percentage against time points is plotted for each tablet of ofloxacin for dissolution analysis. The similarity factor was calculated using dissolution study data. The similarity between the curves is indicated by f_2 value which should be more than 50 and less than 100.

In vivo Data Analysis

Wagner-Nelson Equation was employed for conversion into cumulative percentages of drug absorbed from the plasma concentration data. This equation is widely used in pharmacokinetics to estimate the cumulative fraction of drug absorbed over time[17,18].

Model Evaluation

The internal and external predictabilities was used to evaluate *IVIVC* model. To define the *IVIVC* model internal predictability involved the initial data. The in vitro dissolution data of two formulations (Zanocin 400 and Zanocin 200) was used to calculate the predicted plasma concentration profiles of these formulations. External predictability was assessed by introducing an additional formulation (Oquin 200) with a moderate release rate. The projected plasma concentration profile of the formulation was computed using the dissolution data of the formulations.

The intercept, slope, and "% *in vitro* cumulative dissolved (t)" data were used to calculate "predicted % in vivo cumulative input (t)" data. The predicted in vivo input rates (R_{input}) were obtained from;

$$R_{input} = \frac{[\%\ in\ vivo\ input(t_2) - \%\ in\ vivo\ input(t_1)]}{t_2 - t_1} \times dose$$

The predicted plasma concentration-time profiles were calculated using following equation;

$$Cp(t) = \sum_{t=1}^{m} [{}^{R_j}/_{\lambda}\{e^{-\lambda(t-\tau 2)} - e^{-\lambda(t-\tau 2)}\}]$$

The absolute % prediction error (%PE) value for C_{max} and $AUC_{0-\infty}$ was estimated from following formula;

$$\%PE = \frac{P_{obs} - P_{pred}}{P_{obs}}$$

The validity of the *IVIVC* model is determined by the mean value of absolute %PE not exceeding 10% and the %PE for each formulation not exceeding 15%[1].

Results and Discussion

Spectrophotometric Method Development and Validation

The UV spectrophotometric technique was devised to estimate the amount of ofloxacin in the formulations at a wavelength of 292 nm as depicted in UV spectrum shown in Fig. 1.

Linearity

Within the concentration range of 2-12 µg/mL, the method was found to be linear as depicted in Fig. 2. This means that as the concentration of ofloxacin increases, the measured absorbance values also increase proportionally. The regression equation obtained from the linearity studies was;

y = 0.0765x - 0.0436

The equation enables the estimation of the concentration of ofloxacin based on the measured absorbance values obtained from the spectro-photometric analysis.

Recovery

Using the standard addition method, the recovery analysis was carried out at three levels 80%, 100% and 120%. Recovery values for the drug ofloxacin were found to be in the range 98 to 102% as depicted in Table 1. As the % RSD value was found to be less than 2 the proposed method was reported to be accurate.

Precision

The inter day and intraday precision was performed at three concentrations and the % RSD was within the limits as depicted in Table 2.

LOD & LOQ

The LOQ and LOD values were estimated as per ICH guideline and wherein the LOQ was 0.455 µg/mL and LOD was 0.15 µg/mL.

Robustness

There was no significant difference observed and as the %RSD was less than 2, the developed method proves to be robust Table 3.

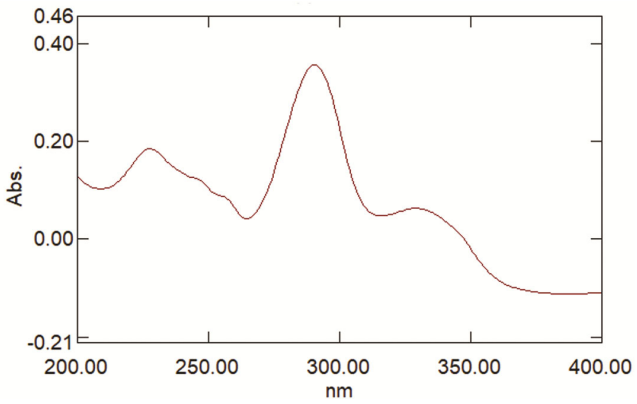

Fig. 1 — UV spectrum of ofloxacin

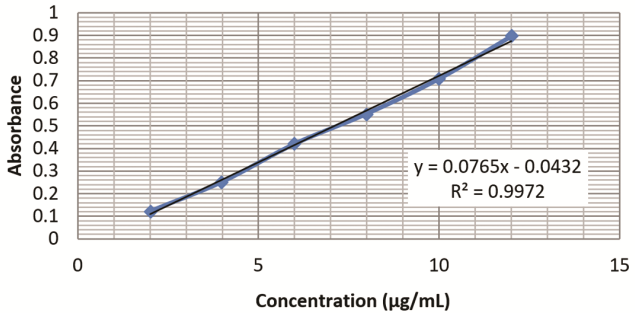

Fig. 2 — Calibration curve of ofloxacin

Table 1 — Recovery data for ofloxacin

Recovery Level	% Recovery	% RSD
80%	99.12%	1.36
100%	98.15%	0.87
120%	98.62%	0.45

Table 2 — Precision study data for ofloxacin

Description/ concentration level	Inter day Precision (%RSD)	Intraday Precision (%RSD)
1	0.565	0.124
2	0.045	0.423
3	0.184	0.552

Table 3 — Robustness data of ofloxacin

Parameters	SD	%RSD
Wavelength(± 1 nm)		
293	0.004	0.578
292	0.001	0.181
291	0.002	0.382
Manufacturers		
Manufacturer1	0.001	0.181
Manufacturer2	0.001	0.091
Equipment		
Schimadzu UV 1900i	0.001	0.361
Lab India UV 3200	0.001	0.235

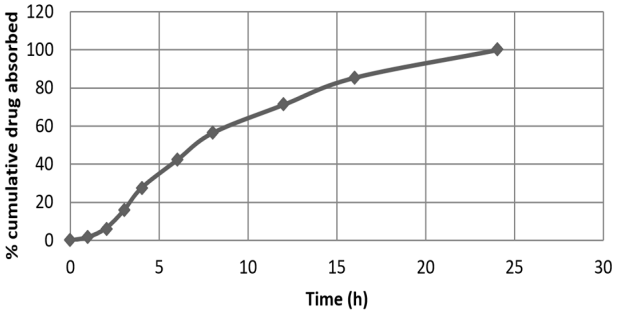

Fig. 3 — % Cumulative drug absorbed- Zanocin 400

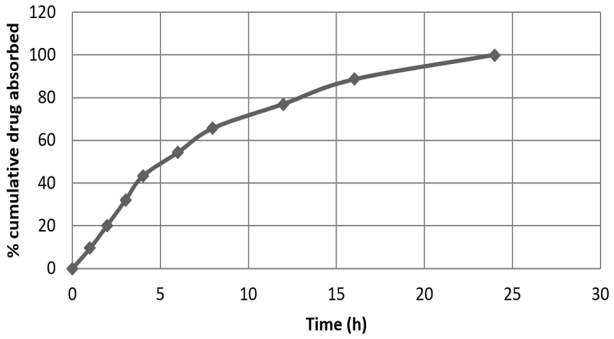

Fig. 4 — % Cumulative drug absorbed- Zanocin 200

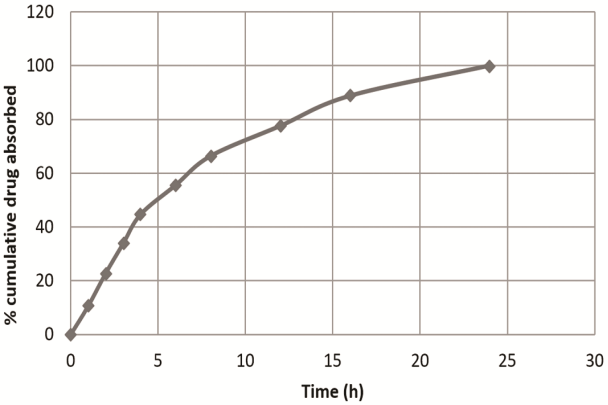

Fig. 5 — %Cumulative drug absorbed- Oquin 200

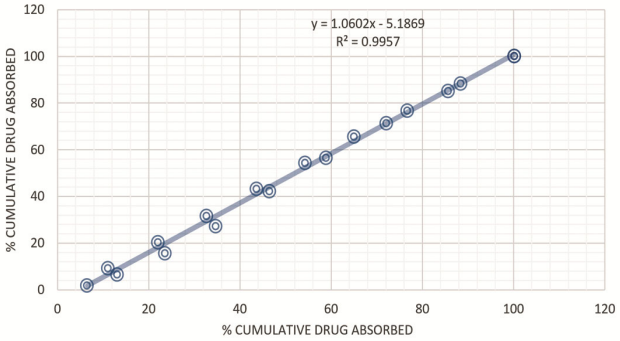

Fig. 6 — *IVIVC* Model FESSGF pH 5 and RPM 100

In vitro Dissolution Studies

As discussed, the complete dissolution studies were obtained in the 10 dissolution conditions and by opting change in RPM. The maximum drug release was obtained in FESSGF pH 5.0 with 100 RPM paddle speed. The similarity factor (f_2) value of Zanocin 400 and Zanocin 200 which are used for internal predictability was 42.73. This means the curves of these two formulations are not similar and the formulations shows different release rates. Another formulation Oquin 200 was introduced for external predictability. The f_2 value of Oquin 200/Zanocin 200 and Oquin 200/Zanocin 400 was found to be 83.21 and 41.66, respectively, indicating different release from the formulations.

In vivo data

The Wagner Nelson model was used to convert the plasma concentration to the cumulative % of drug absorbed. Fig. 3 represents the absorption profile of Zanocin 400, Fig. 4 represents absorption profile of Zanocin 200, and Fig. 5 represents absorption profile of Oquin 200.

IVIVC Model

Zanocin 400 and 200 mg tablets were used to develop *IVIVC* model. Fig. 6 represents the *IVIVC* Model of dissolution condition FESSGF pH 5 and RPM 100 at which optimum drug release was observed among all dissolution conditions.

Predicted Plasma Concentration & Prediction Error

The predicted plasma concentration (Cp) was calculated by developing the polynomial equation of the % cumulative drug absorbed and the plasma concentration. The % prediction error was calculated for the AUC and C_{max} for the 10 dissolution conditions, the prediction error of dissolution condition with optimum release is depicted in Table 4.

Table 4 — Percent prediction error associated with AUC and C_{max}

Formulations	C_{max}	AUC
Internal predictability		
Zanocin 200	8.75	1.80
Zanocin 400	0	18.35
Average absolute %PE	4.38	10.08
External predictability		
Oquin 200	14	8.60

Conclusion

Strong nonlinear level A correlation for the formulation of ofloxacin, which meets the requirements for both internal and external predictability was found. This *IVIVC* can be used to forecast the absorption performance of ofloxacin products with varying release rates and as a substitute for bioequivalence studies in the event of future site, process, or scale-up changes. The dissolution data used to determine bio relevant media are included in this study. Correlating dissolution requirements with equivalent plasma concentrations (the upper and lower limit) will be made easier with the use of this data. The correlation can be utilised as an alternative for clinical studies because the average percent prediction error is less than 15%, indicating that the correlation is predictive.

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