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IN-VITRO AND IN-VIVO CORRELATION OF IMMEDIATE RELEASE ACYCLOVIR TABLET USING WAGNER NELSON METHOD

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ABSTRACT

The purpose of this study was to establish In-Vitro and In-Vivo correlation of immediate release Acyclovir tablets of 800 mg. In vitro and in vivo studies are done on the test product as Acyclovir Tablet USP 800 mg (containing Acyclovir 800 mg) of Cadila Pharmaceuticals Ltd., India versus Zovirax® Tablet 800 mg (containing Acyclovir 800 mg) of GlaxoSmithKline, USA. In vivo studies are done in 36 healthy, adult, human subjects under fasting condition.In vitro dissolution study was done using USP apparatus II at 50 rpm in 0.1N HCL for 45 minutes. The *in vitro–in vivo* correlation of Acyclovir shows R-squared value 0.9794 in excel work sheet, which depicts a successful correlation between *in vitro* and *in vivo* Characteristic of the drug. In addition, $\%PE_{AUC}$ and $\%PE_{Cmax}$ was found to be –4.604 and -11.19 respectively for each formulation. The present study shows a good correlation between *in vivo* and *in vitro* PK profiles of the formulation used as the test drug in the study.

Keywords: Acyclovir 800 mg tablets, In Vitro Dissolution, In Vivo absorption, IVIVC.

INTRODUCTION

In vitro in vivo correlations play a key role in the drug development and optimization of formulation is an integral part of manufacturing and marketing which is certainly a time consuming and expensive process. *In vitro-in vivo* correlation (IVIVC) demonstrates the direct relationships between *in vitro* dissolution / release and *in vivo* absorption profiles. The in vitro property generally is the rate or amount of drug dissolution or release, while in vivo response is plasma drug concentration or amount of drug absorbed. [1]

The in vitro release data of a dosage form containing the active substance serve as characteristic in vitro property, while the in vivo performance is generally represented by the time course of the plasma concentration of the active substance. These In vitro & In vivo data are then treated scientifically to determine correlations. For oral dosage forms, the in vitro release is usually measured and considered as dissolution rate. The relationship between the in vitro and in vivo characteristics can be expressed mathematically by a linear or nonlinear correlation. However, the plasma concentration cannot be directly correlated to the in vitro release rate; it has to be converted to the in vivo release or absorption data, either by pharmacokinetic compartment model analysis or by linear system analysis. Different IVIVC model are used as a tool for formulation development and evaluation of immediate and extended release dosage forms for setting a dissolution specification and as a surrogate for bioequivalence testing. 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 (profiles). Before one considers relating in vitro results to in vivo, one has to establish as to how one will establish similarity or dissimilarity of in vivo response i.e. plasma drug concentration profiles.

As a result, considerable effort goes into their development and the main outcome is "the ability to predict, accurately and precisely, expected bioavailability characteristics for an extended release (ER) drug product from dissolution profile characteristics**.** [2,3] The methodology of establishing similarity or dissimilarity of plasma drug concentrations profile is known as bioequivalence testing. There are very well established guidances

and standards available for establishing bioequivalence between drug profiles and products [4, 5]. There are four levels of IVIVC that have been described in the FDA guidance, which include levels A, B, C, and multiple C [6,7].

The concept of correlation level is based upon the ability of the correlation to reflect the complete plasma drug level-time profile which will result from administration of the given dosage form. An IVIVC Level A correlates the entire in vitro and in vivo profiles has regulatory relevance. This level of correlation is the highest category of correlation and represents a point-to-point relationship between in vitro dissolution rate and in vivo input rate of the drug from the dosage form [8].

The objective of IVIVC evaluation is to estimate the magnitude of the error in predicting the in vivo bioavailability results from in vitro dissolution data. This objective should guide the choice and interpretation of evaluation methods. Any appropriate approach related to this objective may be used for evaluation of predictabilityPrediction errors are estimated for C_{max} and AUC to determine the validity of the correlation. Various approaches of are used to estimate the magnitude of the error in predicting the in vivo bioavailability results from in vitro dissolution data [8,9].

It can be calculated by Prediction error that is the error in prediction of in *vivo* property from *in vitro* property of drug product. Depending on the intended application of an IVIVC and the therapeutic index of the drug, evaluation of prediction error internally and/or externally may be appropriate. **[5]**

Acyclovir is a synthetic purine nucleoside analogue with *in vitro* and *in vivo* inhibitory activity against herpes simplex virus types 1 (HSV-1), 2 (HSV-2), and varicella-zoster virus (VZV). The inhibitory activity of Acyclovir is highly selective due to its affinity for the enzyme thymidine kinase (TK) encoded by HSV and VZV. This viral enzyme converts Acyclovir into Acyclovir monophosphate, a nucleotide analogue. The monophosphate is further converted into diphosphate by cellular guanylate kinase and into triphosphate by a number of cellular enzymes. *In vitro*, Acyclovir triphosphate stops replication of herpes viral DNA. The greater antiviral activity of Acyclovir against HSV compared to VZV is due to its more efficient phosphorylation by the viral TK **[10]**. Acyclovir pharmacokinetics has been extensively investigated during the various phases of clinical development. Most of the administered drug is eliminated from the body unchanged, via the kidneys by glomerular filtration and tubular secretion. After intravenous dosing of patients with normal renal function, 8 to 14% of the dose is recovered in the urine as the metabolite 9-carboxymethoxymethylguanine.

After oral administration, the bioavailability of Acyclovir was approximately 20%. The plasma elimination half life of acyclovir is 2.5 to 3.3 hr and protein binding is 9%-33% [11].

MATERIAL AND METHODS

Materials: All materials used for analysis were of analytical grade. The test product for *In Vivo* study used Acyclovir Tablet USP 800 mg of Cadila Pharmaceuticals Ltd., India reference product used was Zovirax® Tablet 800 mg of GlaxoSmithKline.

In vitro Evaluation:The dissolution of Acyclovir tablets was carried out using USP Type II Dissolution apparatus for 45 minutes. The dissolution media used was 900 ml 0.1 N HCL at 37ºC ± 0.5ºC and rotated at a speed of 50 rpm. Analysis of the withdrawn samples was carried out using UV spectrophotometer at the maximum 254 nm against 0.1 N Hydrochloric acid as a blank.

***Dissolution procedure*:** Parameters of the instrument were set as mentioned above and the medium was degas prior to use. 900 ml of Dissolution media was transferred into each of the six dissolution vessels and apparatus was operated as per requirement. One tablet was dropped into each of six different vessels & dissolution apparatus was started immediately. After 45 min solution was withdrawn, & fiiltered the solution through whatmann filter paper No.1; discard first 2-3 ml of the filtrate. One mL of the filtered sample was diluted to 100 ml with dissolution medium and mixed. (Use this solution as sample preparation)

***Procedure for analysis*:** Absorbance was measured for the standard preparation and sample preparation on a suitable spectrophotometer at the maximum 254nm against 0.1 N Hydrochloric acid as a blank. Percentage of Acyclovir dissolved in 45 minutes in individual tablets was calculated.

In vivo Absorption study: A randomized, open label, two-treatment, two-period, two-sequence, two-way crossover comparative bioavailability study of a single oral dose of Acyclovir Tablet USP 800 mg (containing Acyclovir 800 mg) of Cadila Pharmaceuticals Ltd., India versus Zovirax® Tablet 800 mg (containing Acyclovir 800 mg) of

GlaxoSmithKline, USA in 36) healthy, adult, human subjects under fasting condition.

***Screen Procedure*:** During screening procedure Demography data, standard physical examination with Vital signs, Clinical laboratory tests on blood and urine samples, Electrocardiogram (ECG) and Chest X-ray were done.

Study Design; A randomized, open-label, two-treatment, two-period, two-sequence, two-way crossover, comparative bioavailability study, during which subjects were administered a single dose of test or reference product under fasting condition with at least 7 days washout period between each administration.

***Sample Size*:** Minimum of 36 + (04 standby) healthy, adult, subjects was enrolled to allow dosing in both periods.

***Administration*:** Single a single oral dose of test (Acyclovir Tablet USP 800 mg) or reference product (Zovirax® Tablet 800 mg) products were administered along with 240 mL of drinking water after an overnight fasting of at least 10 hours in each Period.

***Bio-analysis of Plasma sample*:** Samples were analyzed for the quantification of Acyclovir in plasma using Liquid Chromatography with Mass Spectrometry (LCMS) procedures. Data analysis was carried out using Win-Nonlin software to get the Cmax, AUC_{0-t}, $AUC_{0-\alpha}$ and kel.

***Statistical Analysis*:** Statistical analysis was performed on the pharmacokinetic parameters data obtain from subjects (Completing both the periods) using the SAS Statistical Software version 9.1.3 (SAS Institute INDIA Pvt. Ltd).

RESULTS AND DISCUSSION

***In Vitro dissolution*:** The mean percent dissolved is calculated on the basis of time and it showed that within the first 15 min, 91.0% of Acyclovir drug and within the first 20 min, 89 % of Zovirax had dissolved. It was observed that 96.0 % of acyclovir and Zovirax dissolved within 45 min.

Percent dissolved versus *in vitro* dissolution time (in min) when plotted generates a dissolution profile curve as shown below in table 1. The amount of drug dissolved over a period of time for test & reference formulation is given in the figure 1.

In vivo absorption: The fraction of drug absorbed was calculated by Wagner Nelson method using following equation.

$$\frac{(X_A)_T}{(X_A)_\infty} = \frac{CT + K\int_0^T Cdt}{K\int_0^\infty Cdt}$$

Above equation relates the cumulative amount of drug absorbed after a certain time to the amount of drug absorbed. The fraction of drug absorbed calculated using Wagner Nelson method for test & reference formulation is summarized in the Table 2.
The following figure 2 and figure 3 summarizes the fraction of drug absorbed of the Acyclovir molecule Test and reference respectively at given blood sampling time points for test drug. It shows that at 1 hr almost 90% of the drug was absorbed for both test & reference formulation.

Determination of intensity factor *:* Since the in vitro dissolution data was available only for one hour and hence a time scaling factor (i.e., intensity factor) was calculated using Ratio of time of 90 % absorbed and 90 % dissolved & Ratio of time of 50 % absorbed and 50 % dissolved.

In vivo observed data: With the help of time scaling factor the in vitro data was compared with In vivo data. Table 3 summarizes fraction of drug absorbed & percent drug absorbed vs. time data of Zovirax 800 mg tablets obtained using Wagner Nelson Method.

Development and evaluation of Level A IVIVC Model: The in vitro data was taken based on minutes and the in vivo absorption based on hours. It was apparent that these two processes occurred over different time scale. To make the time difference between in vitro and in vivo data, uniform, a time scaling factor (intensity factor) was calculated. The intensity factor, I, obtained was 6.66. Thus by using this factor we have converted the in vitro time points to hours in order to match with the in vivo time points. Time scaled normalization of in vitro and in vivo data with intensity factor is given in figure 4.

Correlation calculation: The correlation graph (Figure 5) was plotted as % absorbed verses % dissolved and there exists an extremely good correlation of formulation between *in vitro* and *in vivo* data.

Prediction Error: *IVIVC* model predictability was determined by the calculating percent Cmax and AUC prediction errors. Prediction error as %PE, AUC and %PE $_{Cmax}$ was found to be – 4.604 and -11.19 respectively for each formulation.

An *IVIVC* should be evaluated to demonstrate that predictability of *in vivo* performance of a drug product from its *in vitro* dissolution characteristics is maintained over a range of *in vitro* dissolution rates and manufacturing process.

If *in vitro* dissolution is shown to be independent of dissolution conditions such as PH, surfactants, osmotic pressure, agitation intensity, a set of dissolution data obtain from one formulation is correlated with that of *in vivo* absorption data.

To demonstrate a correlation, fraction absorbed *in vivo* should be plotted against fraction dissolved *in vitro*. If this relationship becomes linear with a slope of 1, then curves are super imposable, and there is a 1:1 relationship which is defined as point-to-point or Level A correlation.

Regression analysis was also performed on Excel spread sheet with the same *in vitro* and *in vivo* data. R-squared value obtained from graph was 0.9794.
According to FDA guidelines and expertise, statistic from Level A analysis is r, the correlation coefficient. It's square, i.e. R-squared, ranges from 0 to 1 and is a measure of strength of relationship between fractions absorbed against fraction dissolved. Often, results with sufficient large R-squared (e.g., greater than 0.9) yielded "a successful correlation".

The *in vitro–in vivo* correlation of Acyclovir shows R-squared value 0.9794 in excel work sheet, which depicts a successful correlation between *in vitro* and *in vivo* Characteristic of the drug.

In a linear correlation, *in vitro* dissolution and *in vivo* input curves may be directly super imposable or may be made to be super imposable by the use of appropriate scaling factor (time corrections). Time scaling factor should be the same for all formulations and different time scales for each formulation indicate absence of an *IVIVC.*

Percent prediction error (PE%) of 10% or less for Cmax and AUC establishes the predictability of the *IVIVC*. In addition, the PE% for each formulation should not exceed 15%. Here $\%PE_{AUC}$ and $\%PE_{Cmax}$ was found to be –4.604 and -11.19 respectively.

CONCLUSION

The present study shows a good correlation between *in vivo* and *in vitro* PK profiles of the formulation used as the test drug in the study. The *in vitro–in vivo* correlation of Acyclovir shows R-squared value 0.9794 in excel work sheet, which depicts a successful correlation between *in vitro* and *in vivo* Characteristic of the drug. In addition, %PE $_{AUC \text{ and}}$ %PE $_{Cmax}$ was found to be –4.604 and -11.19 respectively for each formulation. The concept of correlation level is based upon the ability of the correlation to reflect the complete plasma drug level-time profile which will result from administration of the given dosage form. An IVIVC Level A correlates the entire in vitro and in vivo profiles has regulatory relevance. This level of correlation is the highest category of correlation and represents a point-to-point relationship between in vitro dissolution rate and in vivo input rate of the drug from the dosage form.

TABLE 1: *IN VITRO* DISSOLUTION DATA OF ACYCLOVIR 800 MG TABLETS (TEST) AND ZOVIRAX 800 MG TABLETS (REFERENCE)

	0.1 N HCl	
Time (min)	**Acyclovir 800 mg (test) Tablets**	**Zovirax 800mg (Reference) tablets**
0	0	0
10	86	74
15	91	81
20	94	89
30	95	94
45	96	96

TABLE 2: FRACTION OF DRUG ABSORBED FOR TEST & REFERENCE

Fraction of Drug Absorbed		
Time (hr)	**Test**	**Reference**
0	0	0
0.333	0.208509	0.283505
0.667	0.600566	0.809927
1	0.902686	0.99656
1.333	1.086002	1.195828
1.667	1.187093	1.321714
2	1.13907	1.327762
2.5	1.173928	1.330732
3	1.257711	1.281141
4	1.137136	1.175827
5	1.067112	1.088926
6	0.956591	0.981582
8	0.886052	0.901321
10	0.851755	0.878418
12	0.835008	0.851137
14	0.841072	0.844374
16	0.852433	0.850673
24	0.876586	0.875232

TABLE 3: Fraction of Drug Absorbed

Time (hr)	Fraction drug absorbed	% Drug absorbed
0	0	0
0.333	0.283505	28.3505
0.667	0.809927	80.9927
1	0.99656	99.656
1.333	1.195828	119.5828
1.667	1.321714	132.1714
2	1.327762	132.7762

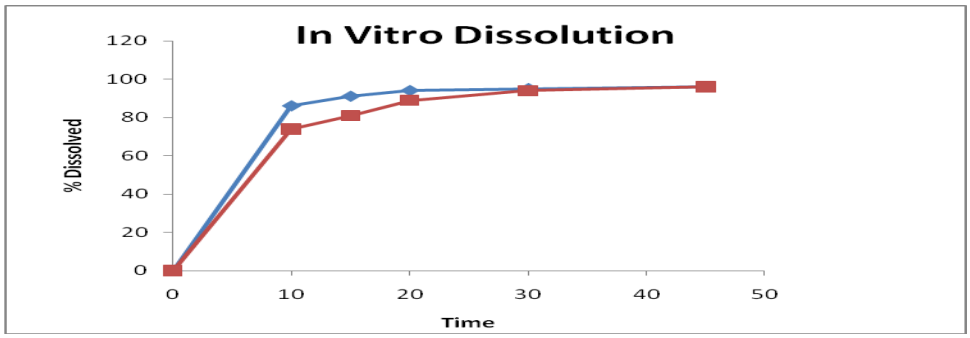

FIGURE 1: FRACTION OF DRUG DISSOLVED VS TIME GRAPH FOR ACYCLOVIR 800 MG TABLETS

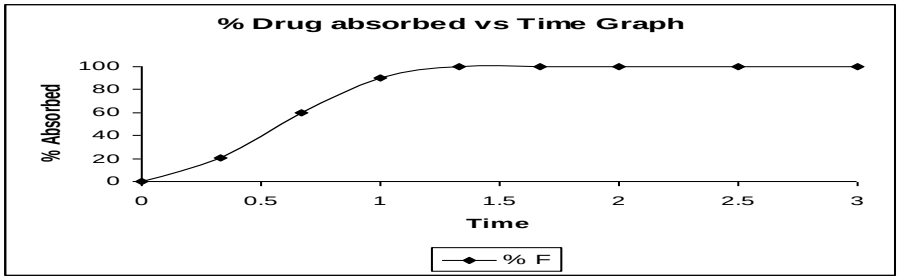

FIGURE 2: FRACTION OF DRUG ABSORBED VS TIME GRAPH FOR ACYCLOVIR 800 MG TABLETS (TEST)

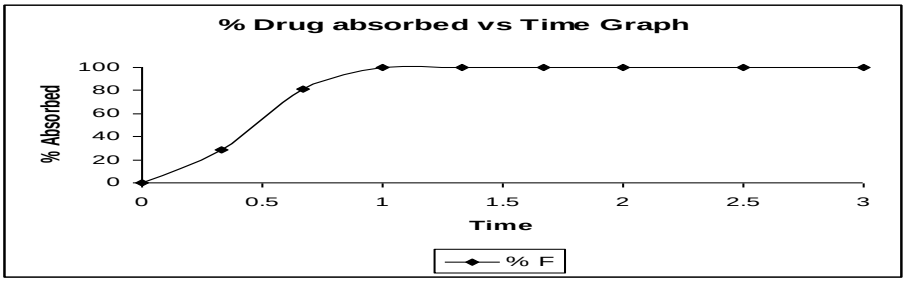

FIGURE 3: FRACTION OF DRUG ABSORBED VS TIME GRAPH FOR ZOVIRAX 800 MG TABLETS (REFERENCE)

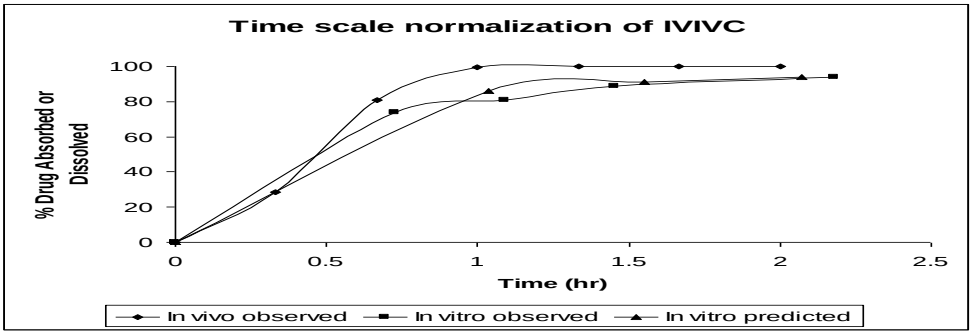

FIGURE 4: TIME SCALED NORMALIZATION OF *IN VITRO* AND *IN VIVO* DATA WITH INTENSITY FACTOR

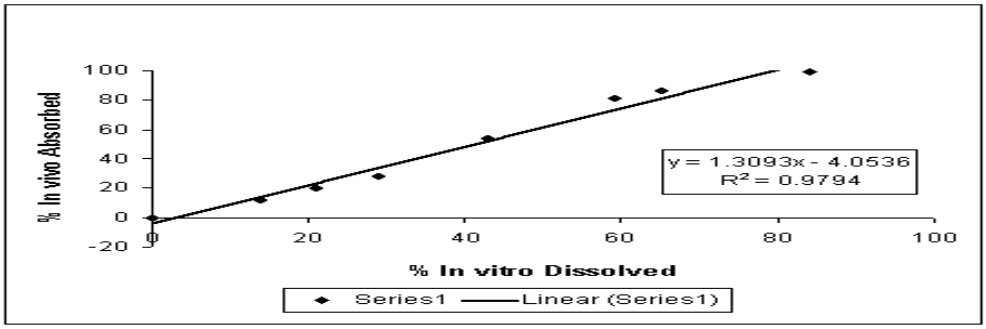

FIGURE 5: THE LINEAR REGRESSION PLOT OF % ABSORBED AND % DISSOLVED

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