

## FEASIBILITY BIOWAIVER EXTENSION OF IMMEDIATE RELEASE ORAL ACYCLOVIR 800 MG TABLET FORMULATIONS: A BCS CLASS III DRUG

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### ABSTRACT

This article describes the preparation and characterization (*in vitro* and *in vivo*) of three immediate release Acyclovir 800mg tablets (T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub>) formulations and reference Zovirax® Tablets to allow a waiver of *in vivo* bioequivalence testing for approval and assessed for feasibility of bioequivalence extension of a BCS Class III Drug recommendations such as therapeutic use, therapeutic index, pharmacokinetic properties, reported bioequivalence and possibility of excipients interactions. A good linear correlation (R<sup>2</sup> = 0.9760, 0.9525, 0.9568 and 0.9973 for T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> formulations and Zovirax® Tablets) was obtained between the percent cumulative drug released (*in vitro*) and the percent cumulative drug absorbed (*in vivo*) data of these formulations at specific time points to develop level A *in vitro-in vivo* correlation. The *in vitro-in vivo* correlation (IVIVC) analysis demonstrated that the T<sub>1</sub> formulation exhibited dissolution rate-limited absorption compare to other formulations and found closer to the reference Zovirax® tablets. Hence, the extension of the bioequivalence concept to BCS Class III drugs of acyclovir IR tablets seems to be feasible and appropriate.

**Keywords:** Acyclovir IR Tablet, IVIVC, BCS Class III, Biowaiver

### INTRODUCTION

Food and Drug Administration Authority (FDA)<sup>[1]</sup> has developed a regular guidance for both immediate- and modified-release dosage forms to reduce the requirement of bioavailability studies as part of the formulation design and optimization. Increased development of immediate release dosage forms necessitates investigating the broader aspects of *in vitro-in vivo* correlation (IVIVC). Biopharmaceutical Classification System (BCS) represents a criterion for the classification of drugs on the basis of their solubility and permeability. In principle, BCS Class III (high soluble and low permeable) active pharmaceutical ingredients (APIs) have been identified to be eligible for the BCS-based bioequivalence approach. The BCS Class III drug was converted into an immediate release multi-unit dosage form in which the release profile controls the rate of absorption, and the solubility and permeability of the drug was site independent and an IVIVC is expected. The BCS Class III compounds, dissolution conditions may be set as those performed with Class I drugs as they are also poorly soluble, and consideration of food-drug interactions has to be concerned as for the BCS Class I cases. In this continuity, present article describes the waiving *in vivo* bioequivalence testing for the approval of new and/or reformulated of acyclovir immediate release solid oral dosage forms. A bioequivalence implies that bioequivalence assessment studies is waived for marketing authorizations by Health Authorities for a new tablet formulation of an existing immediate release (IR) dosage form and the product is considered bioequivalent to its reference product, without carrying out a bioequivalence study. The scientific basis for waiving request for acyclovir 800 mg IR tablets has been developed according to BCS Class III<sup>[2-6]</sup>.

Literature data was assessed from PubMed<sup>[5]</sup>, Micromedex<sup>[6]</sup> databases and although the International pharmaceutical abstracts.<sup>[7-11]</sup> Keywords used for searching, in various combinations were: acyclovir, Biopharmaceutical Classification System (BCS), bioequivalence, permeability, solubility, dissolution, *In vitro-in vivo* correlation (IVIVC).

#### General Characteristics

The INN and WHO chemical name for acyclovir is 2-amino-1, 9-dihydro-9-[(2-hydroxyethoxy)methyl]-6H-purine-6-one, or 9-[(2-hydroxyethoxy)methyl]-guanine. Its molecular weight is 225.21 g/mol.

#### Therapeutic Indication

The acyclovir is an effective agent in the therapy of herpesvirus infections in both immuno-competent and immuno-compromised

patients.<sup>[12-14]</sup> Acyclovir is the first line option for treatment and prophylaxis of herpes simplex virus (HSV)<sup>[15-18]</sup> and varicella zoster virus (VZV) infections.<sup>[18-22]</sup>

#### Therapeutic Index and Toxicity

Oral dosages of 600 to 1600 mg/day, 600 to 2000 mg/day and 600 to 3200 mg/day are given as prophylaxis against VZV, Epstein-barr virus (EBV) and CMV immunonon-compromised patients. The daily defined dose, either orally or parenterally is 4000 mg. Oral administration up to doses 4800 mg/day is usually well tolerated. Neurotoxicity (coma, confusion, delirium, encephalopathy, hallucinations, psychosis seizures, or tremor) may be seen with high doses in patients with compromised renal function<sup>[2-4]</sup>. Neurologic adverse reactions usually occur within 1-2 days of achieving maximum acyclovir concentrations at the time of the toxic effects appear.<sup>[23]</sup> Diarrhea, nausea and vomiting, and elevated serum creatinine levels may be observed in conjunction with plasma levels over 20 mcg/mL, but recede when the dose is reduced.<sup>[22]</sup> Nephrotoxicity and neurotoxicity are rarely reported with oral acyclovir therapy, but can occur more frequently with intravenous administration, especially with high plasma acyclovir concentrations and in patients with renal dysfunction.<sup>[22-26]</sup>

#### Physicochemical properties

Acyclovir is commonly used as the free acid form in solid dosage forms, where as the sodium salt is used in parenteral dosage form<sup>[10-11]</sup>. Valacyclovir, the L-valyl ester of acyclovir, has been used orally to increase its bioavailability.<sup>[26-29]</sup> Several dipeptide ester prodrugs are being tested to assess their usefulness in therapeutics. Acyclovir is normally present in a hydrated form consisting of three acyclovir molecules to two molecules of water, corresponding to a theoretical water content of about 5%, but dose and solubility are normally expressed in units of anhydrous acyclovir.<sup>[8, 31]</sup> A stable anhydrous form can be obtained by drying hydrated acyclovir at temperature above 150 °C<sup>[31]</sup>. Although only slight and insignificant differences in solubility values exist between these two forms, the anhydrous form of the acyclovir possesses poorer dissolution properties than the hydrated form<sup>[31]</sup>. Acyclovir is described as "slightly soluble in water" in different pharmacopoeias<sup>[8-9, 31]</sup>. The partition coefficient (log *P*) in n-octanol at 22°C is -1.57.<sup>[32]</sup> Acyclovir is an ampholyte with both weak acid and basic groups. The pK<sub>a</sub> values for acyclovir are 2.16 and 9.04 at 37 °C.<sup>[31]</sup>

#### Pharmacokinetic properties

Absorption of oral acyclovir across the small intestine appears to be passive and is incomplete, resulting in 15-30 % bioavailability and

mean peak plasma concentrations 1.5 to 2.5 hours post dose.<sup>[33-35]</sup> The pharmacokinetic disposition of the drug is not affected by dose, duration or frequency of administration. Plasma protein binding occurs in a range of 9 to 33%, irrespective of plasma concentrations. Acyclovir appears to be distributed to a wide range of tissues and fluids in human after oral and intravenous administration. The elimination half-life of acyclovir after intravenous administration is 2 to 3 hours.<sup>[37-38]</sup> The main metabolite of acyclovir, 9-carboxy-methoxymethyl guanine, is pharmacologically inactive. The main route of elimination of acyclovir is via renal excretion, with 45 to 79% of an intravenous dose recovered unchanged in the urine, which decreases with reduced creatinine clearance. The renal impairment affects the plasma concentrations, extent of metabolism and rate of elimination of the drug.<sup>[38-39]</sup>

## MATERIALS AND METHODS

### Excipients and/or Manufacturing Variations

The ingredients used in the test formulations were micro-crystalline cellulose (Avicel® PH101), sodium starch glycolate, FD&C Blue No. 2, purified talc and magnesium stearate. The reference product was 800-mg Zovirax® Tablet. The ingredients used in the Zovirax® formulation (according to the information provided by the company) were: micro-crystalline cellulose, sodium starch glycolate, FD&C Blue No. 2, providone and magnesium stearate. The T<sub>1</sub> (fast), T<sub>2</sub> (medium) and T<sub>3</sub> (slow) 800 mg released tablet formulations are prepared with different mixture of methacrylate copolymer (a retarding agent) were chosen as the promising formulations for comparative bioequivalence study in humans at 1:1, 1:2 and 1:3 ratio respectively.

### Physicochemical evaluation of tablet formulations

The three different tablet formulations were evaluated physically with respect to their weight variation, hardness, friability and thickness using suitable instruments.<sup>[40]</sup> *In vitro* dissolution study of various tablet formulation was conducted using United State Pharmacopoeia (USP) XXIV apparatus II (rotating paddle, six replicates, Pharma test, Germany) and 5.0 mL samples were drawn at pre-determined time intervals (0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10 and 12 hours) after filtration through millipore filters followed by UV spectrophotometric analysis.<sup>[41]</sup> Solubility investigation was conducted at 0.1 N HCl (pH 2.2), pH 1.2 in SGF (simulated gastric fluid without enzymes), phosphate buffer pH 4.5, pH 6.8 in SIF (simulated Intestinal fluid without pancreatine) and deionized water (pH approximately 5.5), respectively at stirring speed 50 rpm were also varied to study their influence on dissolution behavior.<sup>[8]</sup>

### Data analysis of dissolution profiles

The acyclovir release profiles obtained from three different acyclovir tablets formulation in various dissolution media were compared in accordance with the model independent approach using a difference factor ( $f_1$ ) and a similarity factor ( $f_2$ ) as shown in Equation 1 and 2, respectively.<sup>[42]</sup>

$$f_1 = \left\{ \frac{\sum_{t=1}^n (R_t - T_t)}{\sum_{t=1}^n R_t} \times 100 \right\} \quad (1)$$

$$f_2 = 50 \log \left[ 1 + \frac{1}{n} \sum_{t=1}^n \left( \frac{R_t - T_t}{R_t} \right)^2 \right]^{-0.5} \times 100 \quad (2)$$

Where n was the number of time point.  $R_t$  and  $T_t$  are the percent dissolved at each time point of the Zovirax® and the test batch at time t. Generally,  $f_1$  values up to 15 (0-15) and  $f_2$  values between 50 and 100 ensure that two dissolution profiles are similar.

### Formulation dissolution Study

Dissolution of the tablets was studied using two methods. The first was the USP II method, i.e. the paddle apparatus, operated at 50

rpm, with medium volume 900 mL, in 0.1 N HCl (pH 2.2), pH 1.2 in SGF, pH 4.5 phosphate buffer and pH 6.8 in SIF, in order to evaluate the dissolution behavior of pure drug substance without the effect of inactive ingredients at  $37 \pm 5$  °C. UV absorbance was measured at 260 nm. For each dissolution profile, one tablet was added to the medium and samples of the medium were drawn at 5, 10, 15, 20, 30 and 45 min (n = 12 per product). The second dissolution method was according to BP98. It was identical to the first method, except water was used instead of hydrochloric acid 0.1 N as the dissolution medium.

### Comparative Bioavailability Studies

The study was designed as open-labeled, balanced, randomized, four-treatment, four-Sequence, four period, single dose, crossover bioavailability study with 7 days washout period in fasting conditions.<sup>[43]</sup> Twenty four healthy, male, adult, non-smoker Indian human subjects weights (55-74 Kg) and having no clinical and biological abnormality were selected after screening through haemodynamic, haematological and urinalytic evaluation and divided into four groups (A, B, C and D), each consisting of 12 subjects. The alcohol breath test, and urine drug of abuse test were performed on the day of admission of each period of the study. In first sampling, group A, B and C received T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> tablet formulations, respectively, and group D received Zovirax® 800 mg IR Tablet, GlaxoSmithKline, UK were dosed as per randomization schedule with 240 mL of water. All subjects were fasted overnight at least 10 hour before dosing in fasting conditions. The subjects were not allowed to ingest water 1 hour before dosing and until 2 hours of the doses were given. The subjects were allowed to ingest water *ad libitum* after 2 hours of doses, and identical, nutritionally balanced meals were provided to all subjects during the remainder of the study period. After drug administration, 5 mL blood samples were drawn through an indwelling intravenous cannula at a pre-dose and at 0.33, 0.67, 1.0, 1.25, 1.50, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0, 10.0, 12.0, 16.0 and 24 h followed by centrifugation immediately at 4000 rpm for 10 minutes. Plasma was separated and stored at -20 °C until analysis.<sup>[37-38, 43]</sup>

### Chromatographic conditions

The analytical method used for determination of drug concentrations in *in-vitro* plasma samples was a validated LC/MS/MS liquid chromatography method with Mass spectrometer detection mode.<sup>[35-36]</sup> The assay method involves a solid phase extraction and chromatographic separation on a Hypersil GOLD C<sub>18</sub>, 4.6 x 50 mm, 5µm (Thermo Electron Corporation) column using a gradient detection. To 500 µL of human plasma, 20 µL internal standard working solution (1.00 ng/mL in diluents water: methanol, 50:50 v/v) were added. The samples were vortexed for 15 seconds and stored in a refrigerator for about 10 minutes. Then the samples were centrifuged at 10000 rpm and 10°C for 10 minutes. The clear upper layer was transferred into vials and an aliquot injected into the LC/MS/MS system. The column used for separation from endogenous plasma components was Hypersil GOLD C<sub>18</sub>, 4.6 x 50 mm, 5µm (Thermo Electron Corporation) and the mobile phase was 50 volumes of Milli Q-water and 50 volumes of methanol.<sup>[35-36]</sup>

### Pharmacokinetics and Statistical Analysis

Pharmacokinetic parameters were calculated by non-compartmental analysis using WinNonlin pharmacokinetic software (Pharsight Corporation, USA).<sup>[37-38, 43]</sup> Maximal plasma concentrations ( $C_{max}$ ) and time to reach the peak concentrations ( $T_{max}$ ) were obtained directly by the visual inspection of each subject's plasma concentrations-time profile. The slope of the terminal log-linear portion of the concentrations-time profile was determined by least-square regression analysis (from the data of the last 4-6 points of each plasma concentration-time curve) and used as the elimination rate constant ( $K_{el}$ ). The elimination half-life was obtained from the formulation  $t_{1/2} = \ln(2)/K_{el}$ . The AUC<sub>0-t</sub> from time zero to the last quantifiable point ( $C_t$ ) was calculated using the trapezoidal rule and the extrapolated AUC from  $C_t$  to infinity (AUC<sub>0-∞</sub>) was to be determined at  $C_t/K_{el}$ . The area under the plasma concentration-time from 0 to infinity (AUC<sub>0-∞</sub>) was calculated as the sum of the AUC<sub>0-t</sub> plus the ration of the last measurable concentration to the elimination rate constant.<sup>[37-38, 43]</sup>

### Invitro - Invivo correlation (IVIVC) analysis

For the develop level A correlation was to estimate the *in vivo* absorption or dissolution time course using deconvolution techniques such as Wagner-Nelson procedure for each formulation and subject. Wagner-Nelson method was model dependent in which former used for a one compartment model.<sup>[44-48]</sup>

Calculation of fraction of drug absorbed (in vivo) by using Wagner Nelson method was carried on excel worksheet which was shown in Equation 3.

$$\text{Fraction of Drug Absorbed at time "t" (F}_a\text{)} = \frac{C(t) + K_e \times \int_{t_0}^{t_0+t} C dt}{K_e \times \int_{t_0}^{t_0+\infty} C dt} \quad (3)$$

Where,  $K_e$  = Elimination rate constant of the drug.

$\int_{t_0}^{t_0+t} C dt$  = Area under the curve of the plasma concentration versus time profile of drug, for time period between  $t = 0$  to  $t = t$ .

$\int_{t_0}^{t_0+\infty} C dt$  = Area under the curve of the plasma concentration versus time profile of drug, for time period between  $t = 0$  to  $t = \infty$ .

### Computational simulation using PK-Sim®

The computer program based on physiologically based pharmacokinetics (PBPK) model, PK-Sim® was applied for prediction of acyclovir absorption patterns from Zovirax® tablets as well as those from three different acyclovir tablet formulations. Drug physicochemical parameters including acid dissociation constant ( $K_a$ ), lipophilicity, plasma protein binding, molecular weight, aqueous solubility, hepatic clearance, renal clearance as well as drug release data *in vitro* were set for simulations. Predicted results presented as plasma drug concentration-time profiles and pharmacokinetic parameters and experimental results were then compared and evaluated.

### Internal and external predictability of a level A IVIVC model

*In vivo* properties of a drug can be predicted from its relevant initial *in vitro* dissolution performance by evaluating predictive mathematical IVIVC model, known as internal predictability. Following approach, based on  $C_{max}$  and AUC, was used to evaluate the error in internal predictability as shown in Equation 4.<sup>[47-52]</sup> The predictability of a level A IVIVC model was tested by calculating percent prediction error (%PE):

$$\text{Prediction error (\%PE)} = \frac{(\text{Observed} - \text{Predicted})}{\text{Observed}} \times 100 \quad (4)$$

In this study the observed and predicted values of  $C_{max}$  and AUC were used. A level A IVIVC model has acceptable predictability if the average percent prediction errors for the formulation series are less than 10% (FDA guidance, 1997; EMEA, 2000). In addition, the percent prediction error for each formulation should not exceed 15%. Validity of IVIVC models was tested internally with data used to define the IVIVC, or externally with data that was not used for model development. External predictability has tighter limits for percent prediction error (10%) and it was recommended especially for narrow therapeutic index drugs.<sup>[47-52]</sup>

### Biowaiver and Dose proportionality in immediate release oral dosage forms

For the biowaiver of several strengths of the active substance a bioequivalence study investigating only one strength has been acceptable. However the choice of the strength used should be justified on analytical, pharmacokinetic and safety grounds.<sup>[48-50]</sup> If a new strength (within the approved dose range) was applied for on the basis of an already approved medicinal product and all of the stated conditions hold then a bioequivalence study was not necessary.

## RESULTS AND DISCUSSION

### Physical characterization of Acyclovir Formulations

Physical properties of three different formulations containing various amount of acyclovir, including tablet hardness, tablet weight, recovered drug content and content uniformity were found satisfactory. Flowability of T<sub>1</sub> formulation was considered as the best among the three formulations and the results indicating more consistent hardness values. However, tablet weight values obtained and acyclovir content uniformity in all three tablet formulations were consistent and within the acceptance range. The designed tablets showed low weight variation ( $\pm 3.0\%$ ).<sup>[2-4]</sup>

### Solubility

The solubility classification of a drug in the BCS<sup>[48-50]</sup> was based on the API as highest dose strength available in an immediate release product. Aqueous drug solubility of API (highest strength) in the GI tract physiological pH range, i.e., pH 1.0-7.5 at 37°C described as pH-solubility profile was desired for setting solubility class based on the BCS, in particular for ionizable compounds. A drug substance was considered as highly soluble when the highest dose strength was soluble in 250ml or less of aqueous media over the aforementioned pH range; otherwise, it was ranked poorly soluble. The dose/solubility (D/S) ratio of less than or equal to 250 ml indicates high solubility calculated based on the highest dose available commercially at each administration and the minimum solubility value in aqueous media.<sup>[55-56]</sup> The API of 800 mg strength does not meet the dose/solubility ratio criterion of below 250 ml.<sup>[57-58]</sup> For acyclovir, the minimum solubility value required for D:S ratio of 250 ml was 3.5 mg/ml, while the lowest aqueous solubility determined in the pH range 1.0-7.8 at 37°C was reported as 2.3 mg/ml. In this study, the acyclovir concentrations values evaluated upto 24 hours were higher than 3.5 mg/ml, while the lowest aqueous solubility determined in the pH range 1-7.8 at 37°C was found as 2.4 mg/ml affirming that acyclovir solubility was more in acidic medium pH conditions as anticipated in the upper GI tract.<sup>[57-58]</sup>

### Permeability

According to BCS, a drug showing high solubility and high permeability was considered as class-I drug. Absorption of acyclovir from GI tract was variable and incomplete. Oral bioavailability was almost 10-30% and peak plasma concentrations usually are attained within 1.5-2.75 hours after oral administration. Food does not appear to affect GI absorption. Acyclovir was widely distributed into body tissues and fluids including the brain, kidney, saliva, lung, liver, muscle, spleen, uterus, vaginal mucosa, CSF, herptic vascular fluid, and semen. About 9-33% was bound to plasma protein. Metabolized partially to 9-carboxymethoxymethyl-guanine; also converted intracellularly in cells infected with herpesviruses to acyclovir triphosphate, the pharmacologically active form of the drug. Excreted principally in urine as unchanged drug. Adults with normal renal function: initial serum half-life averages 0.34 hour and terminal half-life averages 2.1-3.5 hours. Renal impairment may reduce clearance.<sup>[54-57]</sup>

### Dissolution behavior of Acyclovir Test formulations

The comparison of dissolution profiles obtained from three formulations of acyclovir tablets formulations and the innovator, Zovirax® in different media i.e., deionized water (pH roughly 5.5), 0.1 N HCl (pH 2.2), pH 1.2 in SGF, pH 4.5 phosphate buffer and pH 6.8 in SIF respectively. The selected dissolution media represent the human physiological GI tract conditions in the fasted state. Since it was dissolved rapidly and completely within 15 minutes, for Zovirax®, dissolution testing was determined for 45 minutes. (Figure 1 and 2)<sup>[54-55, 58]</sup> The result shows that all acyclovir formulations dissolved quicker in both 0.1 N HCL and SGF medium, but T<sub>1</sub> formulation which more than or equal to 85% of drug substance is released within 30 minutes<sup>[2-4]</sup>. This may be possibly because of the component of micro-crystalline cellulose (Avicel® PH101). The result shows that, acyclovir tablet formulations were conceived to cover release characteristics ranging from "rapid enough to facilitate absorption" through to "slow enough to retard or even possibly

reduce absorption". The results of this study clearly revealed that a dissolution specification for higher strength of acyclovir, a BCS Class III compound, of 85% drug release in 15 minutes under BCS-conform conditions would result in comparable pharmacokinetic parameters, indicating bioequivalency of these products and permeability-limited absorption. Although some studies have demonstrated that oral absorption of BCS Class III drugs are affected by excipients which alter GI motility and absorption, the excipients used in this study were all used in amounts approved by the USFDA.<sup>[64]</sup>

The dissolution test results demonstrated that different Acyclovir tablet formulations containing various amounts of methacrylate copolymer held different release kinetics compared to each other as anticipated. The significance of the differences in dissolution profiles

obtained was affirmed by the 'Model Independent Approach', one of the dissolution profile comparison methods recommended by the USFDA.<sup>[42]</sup> This comparison approach composes of the similarity factors ( $f_2$ ) and the difference factors ( $f_1$ ). According to difference factor ( $f_1$ ) and similarity factor ( $f_2$ ), the release profiles of following pairs of formulations were different from each other:  $T_2$  versus Zovirax® and  $T_3$  versus Zovirax® as their  $f_1 > 15.00$  and  $f_2 < 50.00$ . While  $T_1$  versus Zovirax® has  $f_1 < 15.00$  and  $f_2 > 50.00$  which indicates the mutual similarity of the compared release profiles but to a very less extent. The dissolution results exhibited that the release profiles of each tablet formulation acquired were similar across the different dissolution media indicating their robustness of release characteristics in the different conditions extant in the upper GI tract milieu. The similarity of dissolution patterns obtained can be construed by the physicochemical properties of acyclovir itself.<sup>[55]</sup>

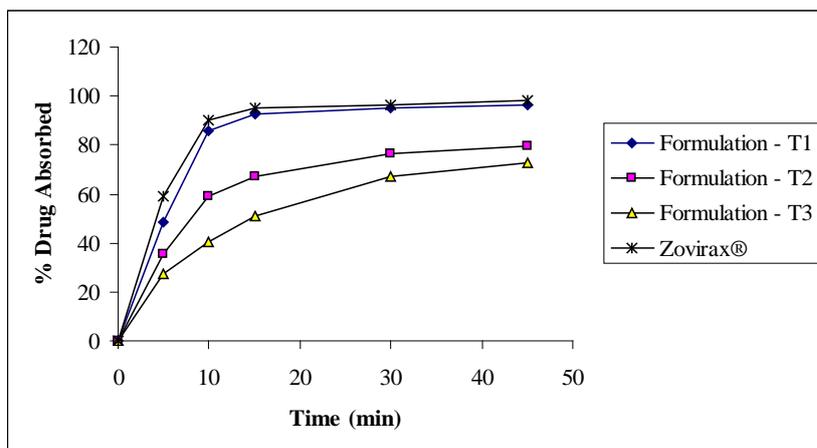


Fig. 1: Release profiles of Acyclovir formulations against Zovirax® in 0.1N HCL medium.

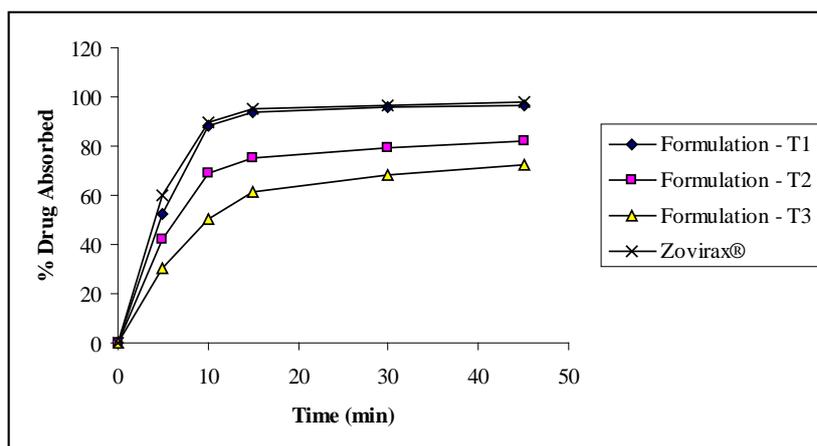


Fig. 2: Release profiles of Acyclovir formulations against Zovirax® in SGF medium

Based on model independent approach, utilizing similarity factor,  $f_2$ , and difference factor,  $f_1$ , the dissolution profiles obtained were significantly different from each other, whereas,  $T_1$ - tablet formulation of acyclovir showed the borderline and inconclusive release patterns in accordance with the BCS criteria. The results of the pharmacokinetic study with acyclovir tablet formulations revealed that, the  $T_1$ - tablet formulation of acyclovir and Zovirax® 800 mg IR tablets, the innovator product provided comparable plasma drug concentration-time profiles. The results clearly demonstrated that the release rate first becomes limiting to the overall absorption rate when the product releases more than 85% of acyclovir formulation ( $T_1$ ) in 15 minutes. Employing computational simulation programs coupled with *in vitro*

dissolution tests for predicting of acyclovir absorption using PK-Sim® programs showed relatively good correlation compared with *in vivo* results.

Based on these results, it can be concluded that the  $T_1$ -tablet formulation exhibited dissolution characteristics that have borderline similarity to those of Zovirax®. According to the FDA and CPMP criteria<sup>[3-4, 42]</sup>, the release characteristic of  $T_1$ -tablet formulation must also be considered borderline, since just about 85% of acyclovir was dissolved within 15 minutes. For  $T_2$  and  $T_3$  tablet formulation, the  $f_1$  and  $f_2$  analysis showed clear differences in dissolution from Zovirax® and it goes without saying that both failed to meet the BCS criterion of 85% dissolution in 15 minutes.<sup>[3-4, 42]</sup>

**Comparative Bioavailability Studies**

The three acyclovir 800 mg tablet formulations were administered to healthy Indian male subjects on separate occasions using Zovirax® IR 800 mg tablet as references for evaluating relative and absolute bioavailability, respectively. In total, twenty four subjects completed the comparative BA study. Plasma acyclovir concentration was determined by validated LC/MS/MS method

involves a solid phase extraction technique. Assay performance was validated and conducted according to the US FDA guideline for biological method validation.<sup>[59-60]</sup> The corresponding pharmacokinetic parameters from one-compartmental analysis of the data of all four formulations were summarized in Table I. <sup>[56]</sup> With the bioavailability criteria, food did not significantly affect the  $C_{max}$  or  $AUC_{0-t}$  or  $AUC_{0-\infty}$  under fasting and non-fasting conditions respectively.<sup>[23,25]</sup>

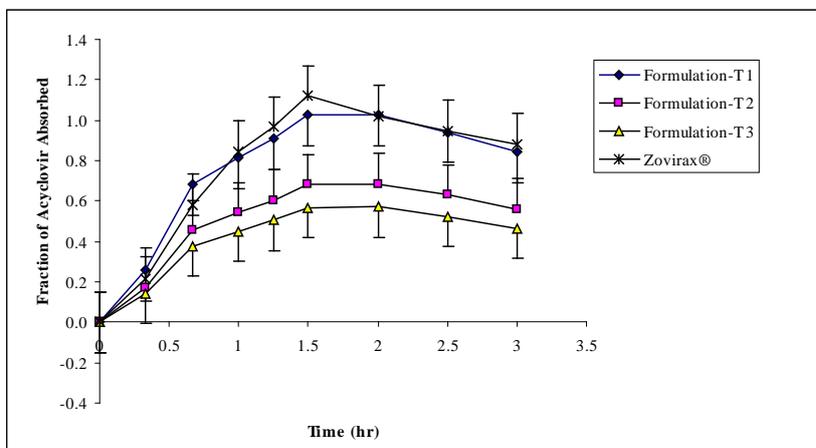
**Table 1: Mean ± SD Pharmacokinetic parameter of Acyclovir formulations and Zovirax® Tablets**

Parameters	Test (T <sub>1</sub> )	Test (T <sub>2</sub> )	Test (T <sub>3</sub> )	Zovirax®
$C_{max}$ (ng/mL)	1161.56 ± 150.38	876.90 ± 46.71	772.08 ± 34.55	1181.167 ± 47.02
$AUC_{0-t}$ (ng.hr/mL)	5278.82 ± 236.73	3973.08 ± 210.41	3611.60 ± 178.79	5713.64 ± 195.98
$AUC_{0-\infty}$ (ng.hr/mL)	5515.93 ± 242.81	4207.18 ± 215.36	3859.90 ± 183.11	5930.23 ± 201.73
$T_{max}$ (hr)	1.85 ± 0.25	1.21 ± 0.09	1.08 ± 0.06	1.91 ± 0.18
MRT (hr)	5.94 ± 0.09	4.10 ± 0.09	3.78 ± 0.05	5.69 ± 0.09

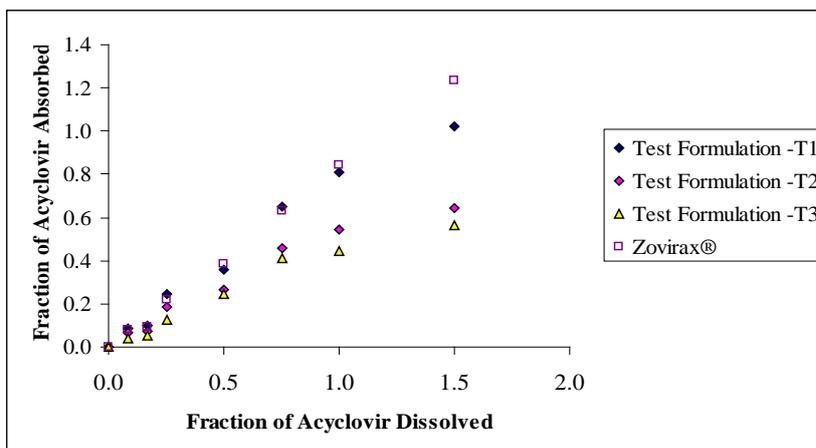
**In vitro-in vivo correlation analysis**

In order to attain the relative contribution of dissolution to IR acyclovir products' overall absorption kinetics, IVIVC analysis correlating fraction of drug absorbed ( $F_a$ ) and fraction of drug dissolved ( $F_d$ ). First, acyclovir fraction absorbed profiles correlating acyclovir cumulative input against time, which estimate the rate at which the drug reaches the systemic circulation, were established. <sup>[44, 46-48]</sup> The  $F_a$  values were calculated using numerical deconvolution approach.<sup>[44]</sup> To associate the fraction absorbed-time profiles and acyclovir release

curves, the percentage values of  $F_a$  and  $F_d$  were correlated on the same time basis. A good correlation between the dissolution and pharmacokinetic data was observed. A high value of determination coefficient ( $R^2 = 0.9760, 0.9525, 0.9568$  and  $0.9973$  for  $T_1, T_2, T_3$  formulations and Zovirax® respectively) suggested good correlation between in vitro and in vivo profiles (Figure 3 and 4). This correlation shows that dissolution profile can be utilized as a predictive tool for in vivo data. Figure 3 shows a faster test formulations dissolution rate than its absorption rate. <sup>[46-48]</sup> It elaborates that gastric emptying is a rate controlling factor in the absorption of acyclovir from tablet formulations. <sup>[46-47]</sup>



**Fig. 3: Fraction of Acyclovir absorbed in Test formulations and Zovirax® Tablets**



**Fig. 4: Plot of mean fraction Acyclovir dissolved and mean fraction Acyclovir absorbed of Test formulations and Zovirax® Tablets**

### Computational Simulation of Acyclovir Absorption

*In silico* prediction of acyclovir absorption was conducted. The results were compared with those attained experimentally for acyclovir plasma profile simulation using PK-Sim® program. First, physicochemical properties of acyclovir and human's physiologic parameters including demographic data were collected and applied to the program. The program then interpreted the data and resulted in plasma profiles. The comparison of computational

simulated and experimentally observed plasma acyclovir concentration-time profiles obtained from Zovirax® tablets and acyclovir tablet formulations administrations. The prediction of different acyclovir tablet formulations fates *in vivo* are in very good agreement with the experimental values. For T<sub>1</sub> tablet formulation of acyclovir 800 mg, although significant differences between simulated and observed values were shown, the program could speculate tendency of the product in humans effectively (Figure 5).

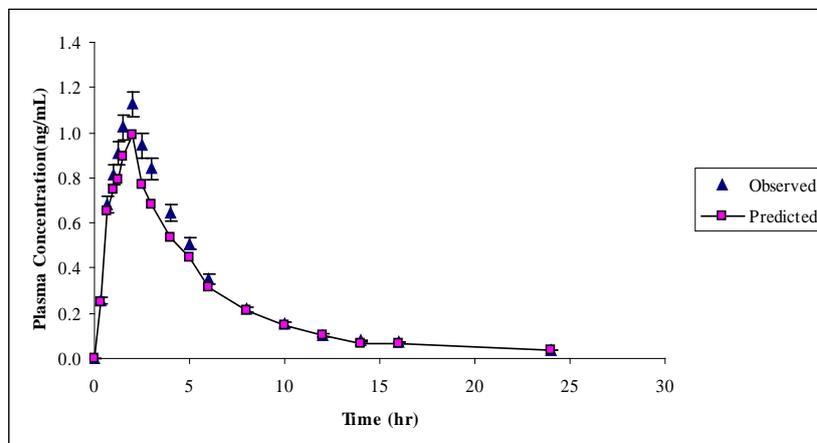


Fig. 5: PK Sim® predicted and observed plasma acyclovir concentration-time profile of Acyclovir T<sub>1</sub>-Formulation

### Validation of Results

Validity of IVIVC models was tested internally with data used to define the IVIVC. The prediction error was 7.68%, 8.14%, 9.26% and 8.37% for C<sub>max</sub> and 9.33%, 7.09%, 6.99% and 8.54% for AUC for the T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and Zovirax® formulations and found to be within the limits (<15%). The average prediction error for the formulation series were 8.36% and 7.995 for C<sub>max</sub> and AUC and found to be within the limits (<10%) (FDA guidance, 1997; EMEA, 2000)

### BCS Classification

Acyclovir drug is a BCS class III as per disposition characteristics of the API as estimate for its data of solubility and permeability. [2,4, 61] Kasim et al. [62] also classified acyclovir as BCS Class III as per API, but their classification was based on correlations of partition coefficients with permeability, a method not supported by HHS-FDA and other regulatory authorities due to limited predictability. It should be noted that the cut-off for "highly permeable" varies with regulatory authority. The FDA sets a limit for the fraction of dose absorbed of not less than 90%, the EMEA requires "highly permeability" but does not define a limit for the fraction of dose absorbed and the WHO requires not less than 85% fraction of dose absorbed. The 90% fraction of dose of test formulation (T<sub>1</sub>) of acyclovir 800 mg tablets was absorbed and meets the criteria of FDA and WHO guidance extended the possibility of biowaiver approval of Class III APIs under certain conditions. Therefore, acyclovir 800 mg tablet is a candidate for biowaiver according to the FDA and WHO guidance. [2-4] Food does not appear to affect GI absorption. [23,25]

### Surrogate techniques for In vivo Bioequivalence Testing

Based on the *in vitro* dissolution test results, it can then be anticipated that the acyclovir tablets evaluated will possess alike attributes in their dissolution *in vivo*. They were also expected not to be sensitive to the GI tract environment in the fasted state across both inter- and intra-subject variations. Taking into consideration the biowaiver criteria indicated by the FDA and the CPMP for the BCS Class I drug products [2,4, 61], by analogy to BCS Class III drug products, Zovirax® tablets and T<sub>1</sub>-tablet formulation would contribute similar plasma drug concentration-time profiles. Both were rapidly dissolved, inferred

bioequivalency of these two acyclovir products, while the other tablet formulations may not result in the same fashion. In principal, bioequivalence could also be caused by a difference in GI absorption, resulting from differences in composition between the test formulation and the Zovirax® product with respect to the excipients. Moreover, it can be further stipulated that slower dissolution patterns of acyclovir tablets beyond the 85% release within 15 minutes criteria as found with T<sub>2</sub> and T<sub>3</sub> tablet formulation would result in significantly different clinical effects reflected by differences in plasma drug concentration-time profiles that would lie outside the BE determination limit [61]. In other words, T<sub>1</sub>- tablet formulations, dissolution of drug products is expected to become a rate-controlling step instead of drug permeability and linear IVIVC correlating drug release and drug absorption is possible to be achieved. Furthermore, a wide variety of excipients has been used to formulate acyclovir IR drug products, having a Medicinal Authorization (MA) in a number of countries, suggesting that the fraction absorbed is not crucially influenced by these excipients. [2-4] The comparative bioavailability study between T<sub>1</sub> tablet formulation and T<sub>1</sub> tablet formulation (solution form) was found to be within limits (<10%) which is additional support for surrogate for *In vivo* bioequivalence study and food does not appear to affect GI absorption.

However, it is the ultimate objective of this study to elucidate the rather restrict criteria whether they can be applied and further set to be more flexible for highly soluble drugs, particularly for BCS Class III drug products. As a consequence, these tablets were then evaluated *in vivo* in human subjects in the subsequent comparative BA study. Further more, bioequivalence of acyclovir formulations was reported neither *in vivo* nor *in vitro* and is unlikely to occur for this insoluble API. Hence, the stricter dissolution methodology for biowaiver of BCS Class III drugs according to FDA and WHO guidance [2-4] over the pH range of 1.2–6.8, should be capable of detecting poor quality of formulations.

### Risks of bioequivalence caused by excipients and/or manufacturing

The report of bioequivalent drug product has been published in a literature. [62] The risk of bioequivalence caused by an excipients

interaction is further reduced if the test product contains only excipients present in drug products having MA in an ICH or associated country. Patient risks associated with bioinequivalence of Acyclovir IR 800 mg dosage forms can lead to decreased antiviral efficacy.

### Risks of bioinequivalence in patients

Acyclovir is a broad therapeutic index. Oral administration of acyclovir is usually well tolerated, and oral overdosing does not provoked serious adverse effects. Oral acyclovir has no life-threatening indication, and its therapeutic range seems wise enough to open the possibility for a biowaiver. <sup>[62-64]</sup>

### CONCLUSIONS

In the current study, Acyclovir tablet formulations were conceived to cover release characteristics ranging from "rapid enough to facilitate absorption" through to "slow enough to retard or even possibly reduce absorption". The results of this study clearly revealed that a dissolution specification for higher strength of acyclovir, a BCS Class III compound, of 85% drug release in 15 minutes under BCS-conform conditions would result in comparable pharmacokinetic parameters, indicating bioequivalency of these products and permeability-limited absorption. Although some studies have demonstrated that oral absorption of BCS Class III drugs were affected by excipients which alter GI motility and absorption, the excipients used in this study were all used in amounts approved by the USFDA <sup>[64]</sup>. Based on model independent approach, utilizing similarity factor,  $f_2$ , and difference factor,  $f_1$ , the dissolution profiles obtained were significantly different from each other, whereas, T1- tablet formulation of acyclovir showed the borderline and inconclusive release patterns in accordance with the BCS criteria. The results of the pharmacokinetic study with acyclovir tablet formulations revealed that, the T1- tablet formulation of acyclovir and Zovirax® 800 mg IR tablets, the innovator product provided comparable plasma drug concentration-time profiles. The results clearly demonstrated that the release rate first becomes limiting to the overall absorption rate when the product releases more than 85% of acyclovir formulation ( $T_1$ ) in 15 minutes. Employing computational simulation programs coupled with *in vitro* dissolution tests for predicting of acyclovir absorption using PK-Sim® programs showed relatively good correlation compared with *in vivo* results.

Conclusively, these results suggest that the current biowaiver criteria for product dissolution would be sufficient to guarantee bioavailability of orally administered formulations of acyclovir and would not pose a risk in terms of an incorrect biowaiver decision and its consequences for the patient. By analogy, it is likely that the biowaiver concept can also be applied to other BCS Class III drugs with a similar intestinal absorption pattern, provided that any influence of excipients and/or the manufacturing process on the permeability can be excluded. Further, computational simulation of *in vivo* behaviors together with *in vitro* dissolution of acyclovir products probably play an important role in determination of drug fates in humans once crucial parameters were adjusted as the inputs. The extension of the biowaiver concept to immediate release drug products containing acyclovir thus seems to be feasible and appropriate, assuming that a prudent selection of excipients is made. Still, similar studies with further BCS Class III drugs were needed to assess whether biowaivers should be generally applied to this class of compounds.

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