International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.6, No.6, pp 1926-1932, Oct-Nov 2014

In Vitro and in Vivo Correlation Study of Levetiracetam Immediate Release Tablet using Wagner Nelson Method

S. Poongothai[1]*, R. Ilavarasan[2] and C.M. Karrunakaran[3]

[1]Bharath University, Selaiyur, Chennai-600 073, India
[2]Captain Srinivasa Murthi Drug Research Institute for Ayurvedha and Siddha, Arumbakkam, Chennai-600 106, India.
[3]SMK Fomra Institute of Technology, Chennai - 603 103, India

***Corres.author: poongothai.sun@gmail.com, Mobile: 09444648584**
Fax: +91-44-22293886

Abstract: The purpose of this study was to establish *in vitro-in vivo* correlation of immediate release Levetiracetam tablets of 1000 mg. *In vitro* and *in vivo* studies are done on the test product as Levetiracetam Tablet 1000 mg (containing Levetiracetam 1000 mg) of Orchid HealthCare Ltd., India versus Keppra® Tablet 1000 mg (containing Levetiracetam 1000 mg) of Teva Pharmaceuticals, USA. *In vivo* studies are done in 6 healthy, adult, human male subjects under fasting condition. In vitro dissolution study was done using USP apparatus paddle at 50 rpm in purified water for 30 minutes at 217nm. The *in vitro–in vivo* correlation of Levetiracetam 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, pharmacokinetic parameters like C_{max}, T_{max} and AUC was also measured using Wagner nelson method. 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: Levetiracetam 1000 mg tablets, *In vitro* Dissolution, *In vivo* absorption, IVIVC.

Introduction

In vitro-in vivo correlation 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* and *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 guidance 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 predictability and prediction 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].

Levetiracetam is a single enantiomer of (-)-(s) - ethyl-2-oxo-1-pyrrolidine acetamide. Levetiracetam is used in combination with other medications to treat certain types of seizures in people with epilepsy. Levetiracetam is in a class of medications called anticonvulsants and it works by decreasing abnormal excitement in the brain. Levetiracetam can prevent myoclonic jerks and generalized epileptiform activity in patients with photosensitive epilepsy whose mechanism of action is thought to involve binding of the synaptic vesicle protein SV2A, a protein involved in neurotransmitter vesicle exocytosis[10]. There is no monograph of this drug in any pharmacopoeia. Moreover, the literature presents few methods related to the quality control of Levetiracetam, mainly in its pharmaceutical dosage forms. The dissolution test has emerged as a valuable quality control tool to assess batch-to-batch product release performance and to assure the physiological availability of the drug. Its significance is based on the fact that for a drug to be absorbed and available on the systemic circulation, it must previously be solubilized.

Levetiracetam distributes well into saliva, with salivary levetiracetam concentrations being on average slightly higher than serum concentrations in patients receiving chronic levetiracetam therapy. Salivary levetiracetam concentrations correlate well with those in serum, which makes saliva an alternative sample to perform therapeutic drug monitoring. Levetiracetam has been determined in biological fluids by HPLC with mass spectrometry detection[11-13], gas chromatography with mass spectrometry detection[14], and impurity determination of levetiracetam using capillary electro chromatography[15]. The separation and quantitation of levetiracetam from other antiepileptic drugs was realized using microemulsion electrokinetic chromatography[16] and HPLC methods with ultraviolet detection[17-20].

Materials and Methods

Materials and *in-vitro* evaluation

All materials used for analysis were of analytical grade. The test product for i*n vitro* study used Levetiracetam Tablet 1000 mg of Orchid HealthCare Ltd., India and the reference product used was Keppra® Tablet 1000 mg of Teva Pharmaceuticals, USA.

Tablet dissolution was assessed using standard USP 24 Apparatus II equipment. A stirring speed of 50 rpm was used to agitate the 900 ml of the dissolution medium, which was kept at 37 ± 0.5ºC throughout and consisted of purified water. The drug concentration was determined by photodiode array detector and the wavelength was set at 217 nm using HPLC instrument at various time points 5, 10, 15, 20 and 30 minutes, 10 mL of solution was withdrawn and replaced by equal amount of purified water. Then the solutions were filtered through Whatman No.41 filter paper.

Procedure for analysis

Test sample and reference sample were analysed by using a Shimadzu Prominence HPLC system (Shimadzu, Kyoto, Japan) equipped with a SCL-10Avp system controller, LC-10 ADvp pump, DGU-14A degasser, CTO-10Avp column oven, SIL-10ADvp autosampler and a SPD-M10Avp photodiode array (PDA) detector. Detector was set at 217 nm and peak areas were integrated automatically by computer using a Shimadzu Class VP V 6.14 software program. The stationary phase was a Prontosil C18-EPS, column (150 mm x 4.6 mm i.d., with a particle size of 5 µm and pore size of 120 Å, Stuttgart, Germany by Bischoff Chromatography). A security guard holder was used to protect the analytical column. The Shimadzu Prominence HPLC system was operated isocratically at ambient temperature. The mobile phase was prepared by mixing 1.37 gms of potassium dihydrogen orthophosphate and 0.61 gms of sodium 1-heptane sulphonate in 1000 mL purified water (pH adjusted to 2.8 with orthophosphoric acid) and acetonitrile (90:10, v/v). The injection volume was 10 µl with the run time 8 minutes. The mobile phase was filtered using a 0.45 µm membrane filter (Milipore, Milford, MA) and degassed with helium. The mobile phase flow rate was 1.2 mL per minute. Elico pH analyzer (Model: Elico LII20) was used to determine the pH of all solutions. Percentage of Levetiracetam dissolved in 30 minutes in individual tablets was calculated.

***In vivo* absorption study**

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 Levetiracetam. 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.

Bio-analysis of plasma sample

Samples were analyzed for the quantification of Levetiracetam in plasma using Liquid Chromatography with Mass Spectrometry (LCMS) procedures. 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.

***In vivo* data analysis**

The pharmacokinetic parameters such as the highest Levetiracetam 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 also calculated.

Results and Discussion

***In vitro* dissolution**

The mean percent dissolved is calculated on the basis of time and it showed that within the first 10 min, 90.2% of Levetiracetam drug and within the first 10 minutes, 87.0 % of Keppra had dissolved. It was observed that 100 % of Levetiracetam and Keppra dissolved within 15 minutes.

Table 1. Dissolution profile for test and reference products of Levetiracetam 1000 mg IR tablets

Time (min)	Purified water	
	Test product (%)	Reference product (%)
0	0	0
5	60.8	52.0
10	90.2	87.0
15	100.6	100.4
20	101.2	101.0
30	102.4	102.2

Percent dissolved versus *in vitro* dissolution time (in min) when plotted generates a dissolution profile for test and reference product is shown in table 1. The amount of drug dissolved over a period of time for test and reference formulation is given in the figure 1 and the corresponding chromatogram of levetiracetam tablet and placebo obtained through the UPLC system is represented in figure 2 and figure 3.

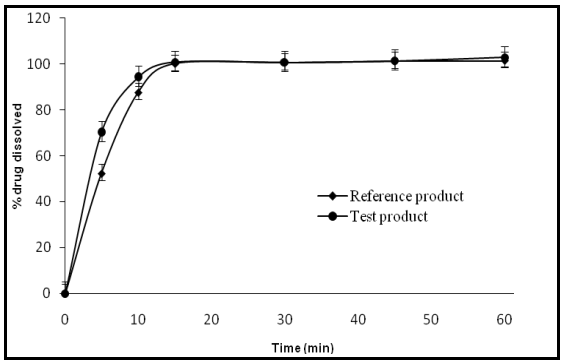

Figure 1: Fraction of drug dissolved vs. time graph for levetiracetam 1000 mg tablets

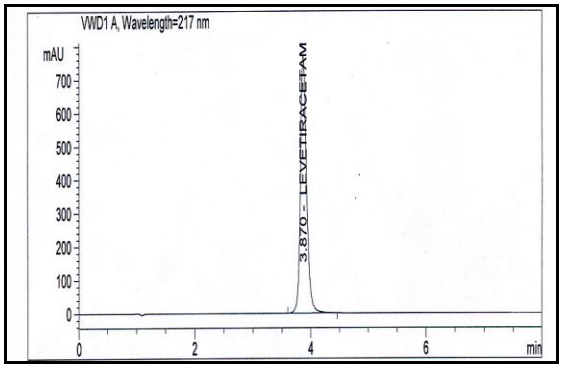

Figure 2. UPLC chromatogram of Levetiracetam detected at 217 nm

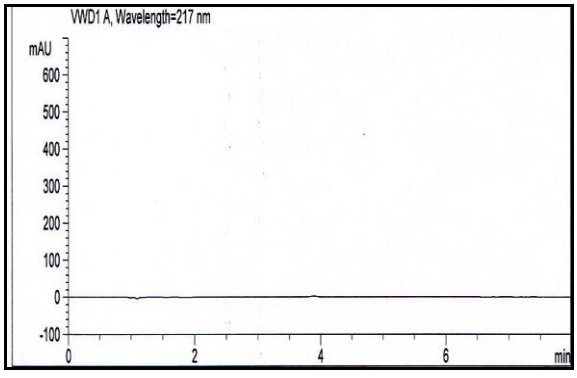

Figure 3. Chromatogram of placebo solution measured at 217 nm

***In vivo* absorption**

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

$$A_t / A_0 = \frac{C_t + K_{el} * AUC_0^t}{K_{el} * AUC_0^\infty}$$

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, $AUC_{0\text{-}t}$ and $AUC_{0\text{-}\infty}$ are the area under the plasma concentration-time profile curve at time t and α respectively.

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 and reference formulation shows that at 15 minutes almost 99% of the drug was absorbed for both test and 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 and Ratio of time of 50 % absorbed and 50 % dissolved.

***In vivo* observed data**

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

Time (hr)	Fraction of drug absorbed	Percent of drug absorbed
0	0	0
0.15	0.9920	99.20
0.35	1.0068	100.68
0.66	1.0234	102.34
1	1.1148	112.48
1.42	1.3266	132.66
2	1.3334	133.34

With the help of time scaling factor the *in vitro* data was compared with *in vivo* data. Table 2 summarizes fraction of drug absorbed and percent drug absorbed vs. time data of Keppra 1000 mg tablets obtained using Wagner Nelson method and the mean pharmacokinetic parameters after administration of immediate release test and reference formulation were represented in table 3.

Table 3. Mean pharmacokinetic parameters after administration of immediate release test and reference formulation

	Test product			Reference product		
Subject	C_{max} (µg/mL)	T_{max} (hr)	AUC (µg/h/mL)	C_{max} (µg/mL)	T_{max} (hr)	AUC (µg/h/mL)
S1	31.61	1.00	356.08	21.58	1.30	377.69
S2	46.78	1.00	408.51	41.29	1.20	407.52
S3	19.49	1.00	336.83	43.23	1.00	386.39
S4	28.24	1.20	416.00	21.19	1.30	415.61
S5	41.53	1.00	406.02	31.44	1.00	396.11
S6	33.75	1.30	376.06	42.12	1.00	387.65

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 7.12. Thus by using this factor we have converted the *in vitro* time points to hours in order to match with *in vivo* time points. Time scaled normalization curve of *in vitro* and *in vivo* data using Wagner Nelson method is given in figure 4.

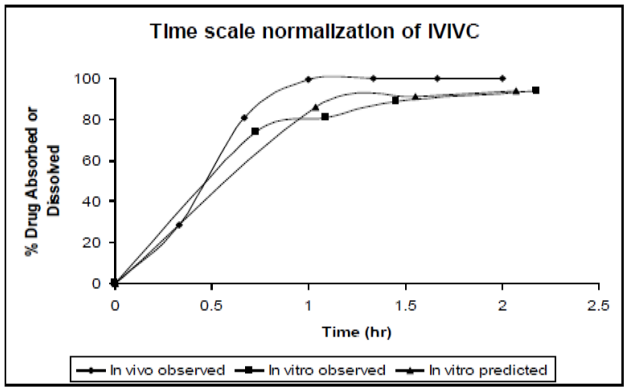

Figure 4. Mean Wagner-Nelson plot for test and reference (Keppra) products after administration to six healthy male volunteers

Correlation calculation

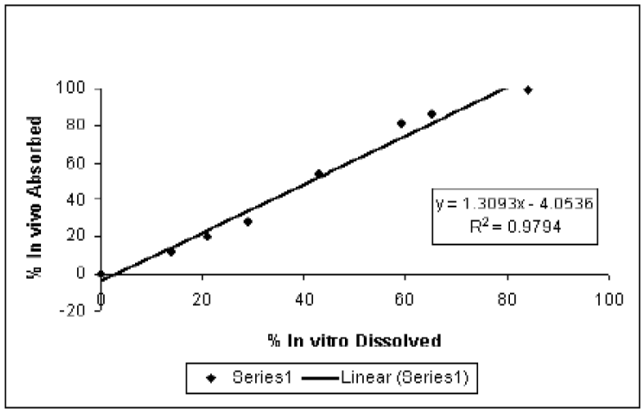

Figure 5: The linear regression plot of % absorbed and % dissolved

The correlation graph (Figure 5) was plotted as % absorbed vs. % dissolved and there exists an extremely good correlation of formulation between *in vitro* and *in vivo* data with R square value 0.9794.

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 Levetiracetam 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, AUC and Cmax were found to be 356.08 and 31.61 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. The IVIVC developed makes Levetiracetam 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.

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