

Establishing Prospective IVIVC for Generic Pharmaceuticals: Methodologies Assessment

Sumon Chakraborty*, Kavan Pandya and Deepika Aggarwal

Centre of Excellence-Biostudies, Integrated Product Development Organization, Dr. Reddy's Laboratories, Hyderabad, Andhra Pradesh, India

Abstract: The present study was aimed to establish prospective IVIVC method for generic products using example of two different drug formulations (aprepitant capsules, immediate release and donepezil tablets, sustained release). The *in vitro* dissolution of these formulations was examined by using USP-II apparatus and different range of dissolution media. The dissolution profile was matched with the deconvoluted profile of drugs obtained from literature data to select biorelevant dissolution media. An IVIVC was established by using the mean fraction dissolved (FD) and mean fraction absorbed (FA) and used to simulate the plasma profile of these formulations by convolution from optimized dissolution media. The *in vivo* drug disposition was studied in an open label, balanced, randomized, single dose, two way crossover study in healthy subjects. Predicted PK parameters were compared with observed parameters. A positive correlation was seen between the FD and FA for both formulations with $r^2=0.989$ for aprepitant and $r^2=0.995$ for donepezil. The percent prediction error for both C_{max} and AUC_t were $\leq 15\%$ while predicting the plasma concentration time profile for human bioequivalence studies for these formulations. Results supports use of prospective method in establishing IVIVC while predicting *in vivo* pharmacokinetic profile for bioequivalence studies for generic product development.

Keywords: Convolution, deconvolution, aprepitant, donepezil, IVIVC.

INTRODUCTION

In vitro-in vivo correlation (IVIVC) is a predictive mathematical model describing the relationship between an *in vitro* property and a relevant *in vivo* response of a drug. Dissolution testing of dosage forms has extensively been used for quality control, ensuring batch to batch similarity, formulation discrimination etc. [1, 2]. Such dissolution testing does not assure bioequivalence (BE). However *in vitro* dissolution in biorelevant media has been used for setting up IVIVC and *in vitro* bioequivalence for certain class of drugs [3, 4]. The IVIVC has its own significance in product designing, changing or scaling up, setting up dissolution specification with respect to *in vivo* behavior of formulation [5-7].

For a generic product to come into existence, bioequivalence needs to be proven against the innovator. The prime strategy of generic formulation development is to deliver a product having similar *in vivo* performance (plasma profile) as that of innovator product. In order to achieve the sameness, formulator needs to know the influence of various formulation changes on *in vivo* outcome of the formulation. *In vivo* performance can be predicted from dissolution testing (*in vitro*) using *In vitro in vivo* correlation (IVIVC). IVIVC has mainly two elements: convolution and deconvolution.

The convolution approach assures direct correlation of dissolution data and pharmacokinetic parameters (C_{max} , T_{max}). Predicting plasma concentration time course in a single step using convolution method is relatively simple when compared to the two stage methodology in deconvolution model (Fig. 1) [8]. Classical Level A IVIVC is not possible always due to the extensive data requirement (three formulations and IV/IR data) [6], which would not be completely available during developmental phase of drug product. A recent article by Gillespie also reiterated that convolution IVIVC models afford certain advantages over the deconvolution models [9].

The use of different type of IVIVC definitely affects strategic decision in the generic pharmaceutical development. For a generic company, establishing complete IVIVC using deconvolution approach would be expensive, complex and time consuming. On the other hand, prospective IVIVC would be much easier and efficient in predicting bioequivalence metrics from reference and to test *in vitro* dissolutions.

Thus, the objective of this article is to exemplify the significance of prospective IVIVC to its application during generic product development especially during strategy development of the product. We have performed BE for two different generic pharmaceuticals (aprepitant and donepezil) and have correlated the observed results with the predicted results from convolution method.

MATERIAL AND METHODS

Dosage Forms

Two different formulations, one containing 125 mg of aprepitant and other having 23 mg of donepezil were used in

*Address correspondence to this author at the Centre of Excellence-Biostudies, Integrated Product Development Organization, Dr. Reddy's Laboratories, Bachupally, Hyderabad, Andhra Pradesh, India; Tel: +91 404334647; Fax: +91 404346285; E-mail: sumonc@drreddys.com

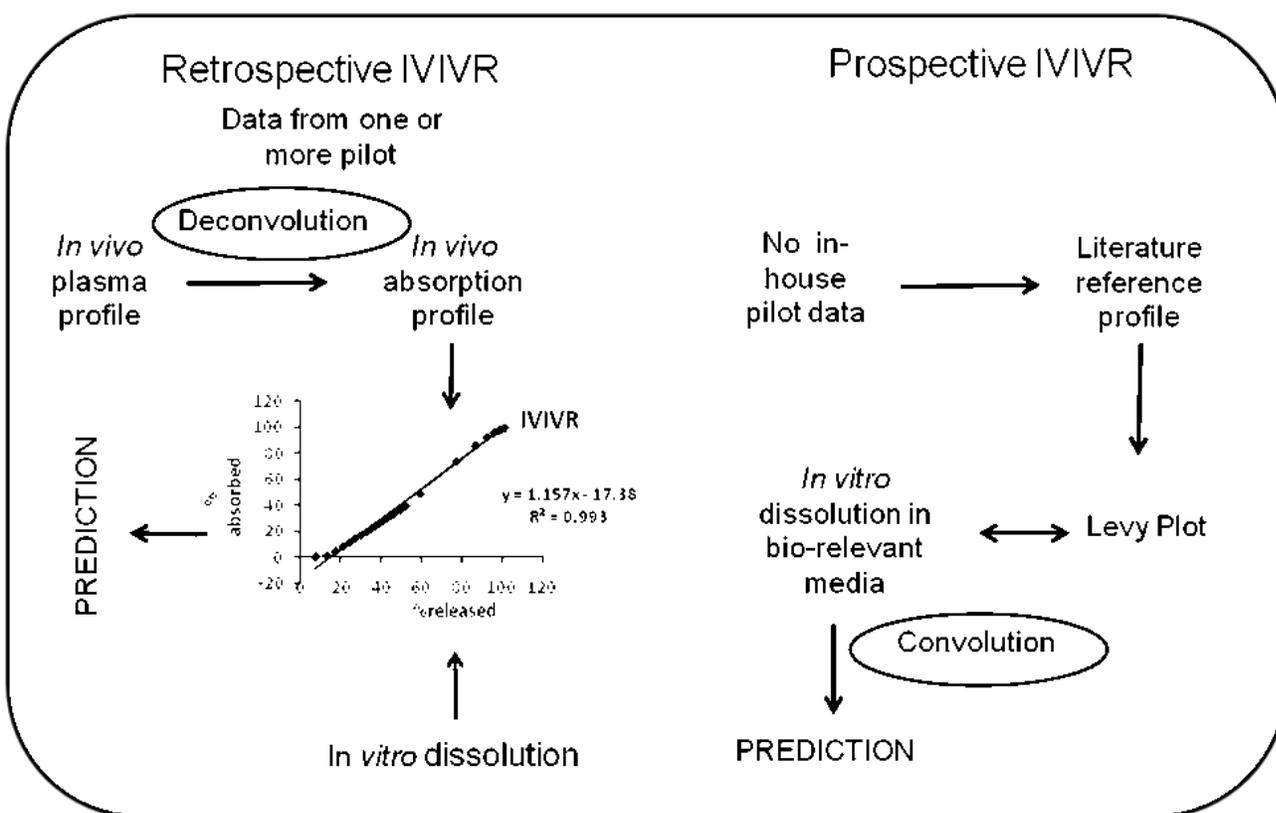


Fig. (1). Schematic representation of retrospective and prospective IVIVC approaches for predicting pharmacokinetic parameters prior to human bioequivalence studies for generic products.

this study. The first formulation was an immediate release (IR) aprepitant capsule containing (w/w) 30% aprepitant, 6% hydroxypropyl cellulose, 0.1% SLS, 30% sucrose, 15% MCC (18.9% hard gelatin capsule shell weight). The technology used for the formulation of aprepitant capsules was drug nanoparticles coated over MCC beads (Wet milling of drug dispersion to achieve particle size diameter of less than 400 nm). Hydroxypropyl cellulose, aprepitant and SLS were added in the purified water and stirred for 20 minutes, and the suspension was milled using bead mill until getting desired particle size. Then the drug suspension was added in the sucrose solution. Drug loading was carried out using the above prepared suspension by bottom spray over celphere (MCC). Drug loaded pellets were lubricated with SLS for 5 minutes and filled in a capsule filling machine [Dr. Reddy's Laboratory, Batch # F015]. The reference for aprepitant capsules used in the study was commercially available as EMEND capsules [Merck and Co., INC, USA, Batch # 193027]. The other formulation was a sustained release donepezil tablet containing (w/w) 12% donepezil, 38% lactose, 21% Eudragit L 100 55, 23% HPMC K100 LV CR, 2.7% each of low grade HPC and HPC (EXF) and 0.6% magnesium stearate [Dr. Reddy's Laboratory, Batch # EC 10261]. The reference for donepezil tablets was commercially available as Aricept tablets [Eisai Inc., Batch # 004837].

In Vivo Testing

Only healthy subjects having normal health conditions determined by medical history and physical examination

were selected for the study. The age of the subjects included in bioequivalence study (aprepitant/donepezil) was between 18-45 years and weighing at least 50 Kg (52-70 Kg) for both BE. Selected routine clinical laboratory measurements were performed during screening. Informed consent was obtained from subjects after explaining the purpose and the nature of the study. Subjects were instructed to abstain from taking any medication for one week prior to and during the study period. Also, it was ensured that the subjects had not consumed any alcohol and had not smoked for 48 h before drug administration and throughout period of the sample collection.

Aprepitant: An open label, balanced, randomized, single dose, two way crossover study was conducted in 18 healthy subjects to compare the bioequivalence of test product [Dr. Reddy's Laboratory 125 mg aprepitant capsules] and reference product [Emend, Merck and CO, INC, USA]. All the study protocols were reviewed and approved by Independent review boards, IRB. The study was conducted under fasting conditions with a washout period of 10 days. The drug was administered in fasting subjects (~10 h overnight fasting) with 240 mL of water. Blood samples were obtained from each subject before dosing and at 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 11.0, 12.0, 13.0, 14.0, 16.0, 18.0, 20.0, 24.0, 48.0 and 72.0 h post-dose. Plasma was isolated from the whole blood and stored at -20 °C until analysis. The concentration of Aprepitant was quantitated at Dr. Reddy's Laboratories Ltd., India using LC-MS / MS method, which is validated according to the international guidelines.

Donepezil: This was a randomized, single dose two way crossover, bioequivalence study of two formulation of donepezil tablet [Test: Dr. Reddy's Laboratory 23 mg tablet and Reference: Aricept manufactured by Eisai Inc.]. This study was conducted in normal, healthy, adult human subjects under fasting conditions with a washout period of 28 days. All the study protocols were reviewed and approved by Independent review boards [IRB]. Both test and reference drugs were administered in fasting subjects (~10 h overnight fasting) with 240 mL of water. Blood samples were collected at 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 11.0, 12.0, 13.0, 14.0, 16.0, 18.0, 20.0, 24.0, 48.0 and 72.0 h post-dose. Plasma was isolated by centrifugation and analyzed for concentration of donepezil at Dr. Reddy's Laboratories Ltd., India using LC-MS / MS method, which is validated according to the international guidelines.

Pharmacokinetic Parameters

Mean plasma concentration time profiles for each of the formulation were constructed using WINNONLIN version 5.2 software. Maximum plasma concentration (C_{max}) was determined by direct inspection of the mean plasma concentration time profile. The time at which C_{max} occurred was the T_{max} . AUC from time 0 to t (AUC_t) was determined by trapezoidal method. The area under the plasma concentration versus time curve from time zero to infinity was calculated using following formula, $AUC_{inf} = AUC_{0-t} + C_t/\lambda_z$, C_t is the last measurable concentration and λ_z is the terminal elimination rate constant. The elimination rate constant was determined for each of the molecule by linear regression of the linear portion of the curve [ln (concentration versus time curve)].

Dissolution Testing

Aprepitant: *In vitro* dissolution studies were performed using USP-II (paddle apparatus) at 50 rpm in the following bio relevant dissolution media: (a) Water with 0.5% SLS, (b) 6.5 pH FaSSIF and (c) 6.5 pH FaSSIF where sodium taurocholate (NaTc) and lecithin replaced by 0.25 % SLS. Twelve capsules were used in the dissolution experiment. Each capsule was dipped in the above mentioned media (500 ml for first two media and 900 ml for the final media) at 37°C. Concentrations of aprepitant were measured by UV spectrophotometry at 0, 5, 10, 15, 20, 30, 45, 60, 90 and 120 minutes in fasted state simulated intestinal fluid (FaSSIF) and 0, 5, 10, 15, 20, 30, 45, 60 minutes in Water with 0.5% SLS. Cumulative amount release of aprepitant at these time points was calculated.

Donepezil: USP-II (paddle) apparatus at 50 rpm was used for *in vitro* dissolution testing with 900 ml of following bio relevant dissolution media: (a) 2.1 SGF without SLS, (b) 0.1 N HCl, (c) 4.5 pH sodium phosphate buffer, (d) 5.5 pH sodium phosphate buffer and at 25 rpm in 900 ml of 0.1 HCl (2 h) followed by 6.8 pH phosphate buffer. For dissolution experiment, 12 tablets were used and each tablet was dipped into the above mentioned media at 37 °C. The concentration of donepezil was measured by UV spectrophotometry at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12 and 14 h. Cumulative amount release of donepezil at this time points was calculated.

IVIVC

The IVIVC was developed using the mean *in vitro* dissolution data (percentage dissolve) and mean *in vivo* dissolution data (percentage absorb *in vivo*). Fraction of drug absorbed to the systemic circulation was obtained by deconvoluting the plasma profile by numerical deconvolution method [6, 10]. In this method, time course of the drug input is estimated by using a mathematical model based on convolution integral.

$$C_t = \int_0^t C\delta(t-u) r_{abs} u (du)$$

From above equation, r_{abs} is absorption rate time course, C_δ represents the concentration time profile resulting from an instantaneous absorption of a unit amount of drug which is typically from bolus or infusion intravenous injection or reference oral solution data (in this case intravenous infusion), C_t represents the plasma concentration versus time profiles of the tested formulations and u is the variable of integration.

At each time point, mean FD (Fraction dissolved) was plotted versus mean FA (fraction absorbed) in a levy plot. The slope, intercept and correlation coefficient describing the relationship between mean FD and mean FA were determined using linear regression.

Aprepitant: In general, FaSSIF media is used to assess the *in vitro* dissolution of poorly soluble drug which is correlated with *in vivo* performance. However amount of sodium taurocholate and lecithin may be insufficient for certain poorly soluble drugs such as aprepitant and hence there is need to develop a simplified version of FaSSIF with common surfactants used in dissolution keeping other ingredients same. Aprepitant follows one compartment pharmacokinetic model, so from the literature plasma profile [11] pharmacokinetic profile of 125 mg of aprepitant was deconvoluted to get *in vivo* dissolution profile. 2 mg intravenous infusion (for 4 hours) of aprepitant was used to calculate the unit impulse response (UIR) [11]. *In vitro* dissolution profiles were generated in (FaSSIF) and FaSSIF where sodium taurocholate (NaTc) and lecithin were replaced by 0.1 %, 0.25 % and 0.5 % SLS. All the dissolution media were similar to FaSSIF in terms of osmolarity and buffer capacity. Dissolution rate and extent were very slow in FaSSIF so NaTc and lecithin were replaced by SLS. Levy plots were used to optimize the media and from these media results, PK parameters were computed by convolution technique and were validated in human bioequivalence study of test formulation versus reference formulation (emend) in healthy human subjects. Ratios of test and reference formulation for C_{max} and AUC were computed and compared with predicted ratios.

Donepezil: Donepezil follows two compartment pharmacokinetic models, so from in-house data pharmacokinetic profile of reference formulation [12] was deconvoluted to get *in vivo* dissolution profile. In house IR data of 10 mg were used as UIR for the SR product. Deconvoluted profile was compared with the *in vitro* dissolution profile in different dissolution media and the particular dissolution profile was selected which is super imposable to the doconvoluted profile for further

convolution in order to get the predicted plasma profile for 23 mg SR donepezil.

Prediction error: The prediction error (% PE) calculation was used to access quantitatively, how accurate a given model can predict a pharmacokinetic parameter for a drug. The % PE of both C_{max} and AUC was calculated using the same formula. In this formula, the observed value is subtracted from the predicted values in the model. This resulting value is then divided by the observed value. The value is then converted to a percentage to by multiplying by 100.

$$\%PE C_{max} = [(C_{max\ obs} - (C_{max\ pred})) / C_{max\ obs}] * 100$$

$$\% PE AUC = [(AUC)_{obs} - (AUC)_{pred}] / (AUC)_{obs} * 100$$

RESULTS AND DISCUSSION

Aprepitant

The mean values of C_{max}, AUC_t and T_{max} after oral administration of the immediate release EMEND capsules extracted form literature sources were 1539 ng/mL and 19455 ng/mL for 1h and 4 h respectively. These values were taken from the SBOA of aprepitant approved by US FDA [13].

The mean *in vitro* dissolution profile for aprepitant IR capsule (EMEND, 125 mg) along with the deconvoluted profile is shown in Fig. (2). Dissolution profile in 0.25% SLS in FaSSIF is similar and super imposable on the deconvoluted profile of aprepitant.

The IVIVC was established using *in vitro* dissolution data and *in vivo* dissolution data (Deconvoluted profile). Linear regression analysis in the levy plot showed (Fig. 3)

that a statistically significant relationship ($r^2 = 0.989$) existed between the FD and FA for aprepitant reference formulation in 0.25% SLS with FaSSIF media.

So this media was further explored as bio relevant media. Based on the dissolution profile obtained in 0.25% SLS with FaSSIF media, pharmacokinetic parameters were predicted for test and reference formulation by convolution method and test to reference ratio was calculated for C_{max} and AUC_t

The comparison of predicted and observed PK parameters, T/R ratio and the % PE for test and reference formulations have been tabulated in Table 1. From these results, it is evident that the prospective convolution using the optimized biorelevant media was able to successfully predict the *in vivo* PK parameters with a minimal (<15 %) prediction error. Hence, for poorly soluble drug like aprepitant, there is a need of critical evaluation in selecting bio relevant media with appropriate modification which will further help to achieve IVIVC.

Donepezil

The mean values of C_{max}, AUC_t and T_{max} for sustained release Aricept tablets were 32.63ng/mL and 1561.46ng/mL for 1h and 6.15 h, respectively [12].

The mean *in vitro* dissolution profile for donepezil SR tablet (Aricept) along with the deconvoluted profile is shown in Fig. (4). From the *in vitro* dissolution profile of Aricept, 0.1 N HCl followed by 6.8 PB was able to closely describe the *in vivo* absorption profile and thus it was used for IVIVC for donepezil.

The IVIVC was established using *in vitro* dissolution data and *in vivo* dissolution data (Deconvoluted profile).

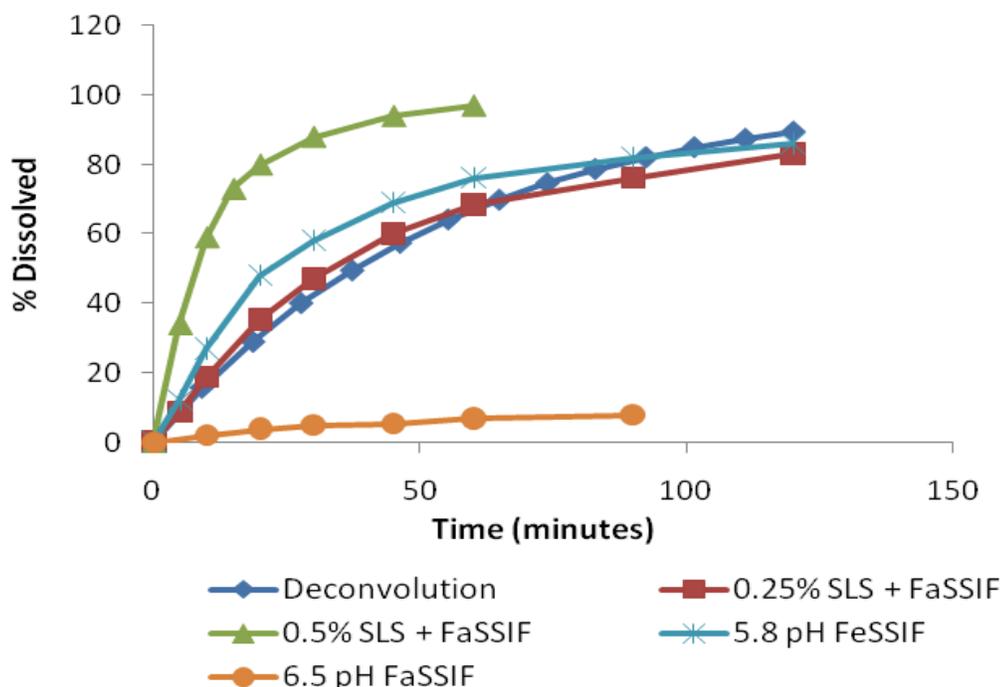
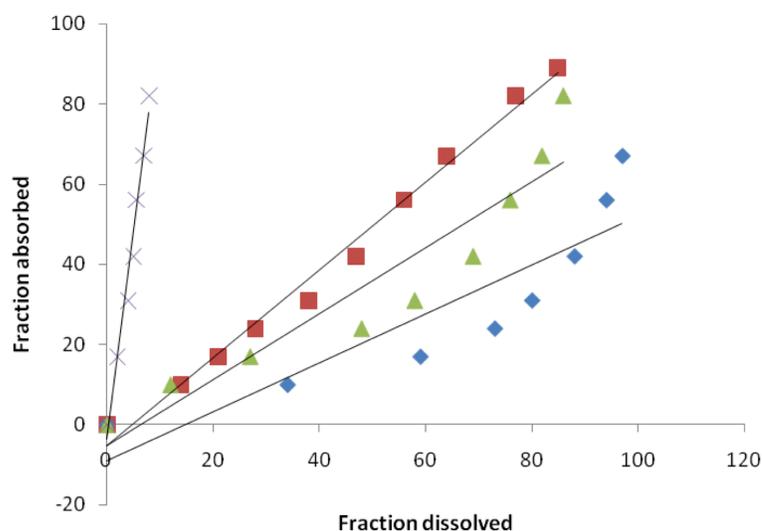


Fig. (2). *In vitro* dissolution profile along with the deconvoluted profile of EMEND in USP-II (paddle apparatus) at 50 rpm speed. Each time point represents the mean fraction dissolved of twelve capsules of EMEND (aprepitant, 125 mg).



	Dissolution media	Linear trend line equation	r ² value
■	0.25 % SLS+ FaSSIF	y = 1.097x - 5.395	R ² = 0.989
◆	0.5 % SLS+ FaSSIF	y = 0.610x - 9.193	R ² = 0.798
▲	5.8 pH FeSSIF	y = 0.822x - 5.276	R ² = 0.886
×	6.5 pH FaSSIF	y = 10.18x - 3.679	R ² = 0.977

Fig. (3). Relationship between the mean fraction absorbed *in vivo* and the mean fraction dissolved *in vitro* for EMEND. Mean FD and mean FA were determined for the reference formulation and used to establish the IVIVC. The line represents the linear regression of the data.

Table 1. Prediction of Pharmacokinetic Parameters (C_{max} and AUC_t) for Reference and Test Formulation of Aprepitant IR Capsule (125 mg) by Numerical Convolution Method Using Winnonlin Version 5.2 IVIVC Toolkit as an Simulation Software and the Corresponding Prediction Error

Formulation	C _{max} Observed (ng/mL)	C _{max} Predicted (ng/mL)	% PE	AUC _t Observed (ng/mL)	AUC _t Predicted (ng/mL)	% PE
Reference	1735	1539	11%	41828	46757	11%
Test	1378	1275	7%	35368	39336	11%
T/R	79.42%	84.40%		84.50%	85.00%	

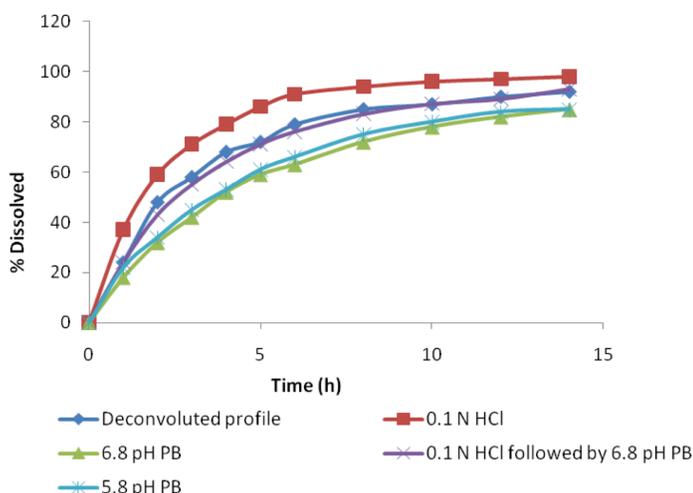
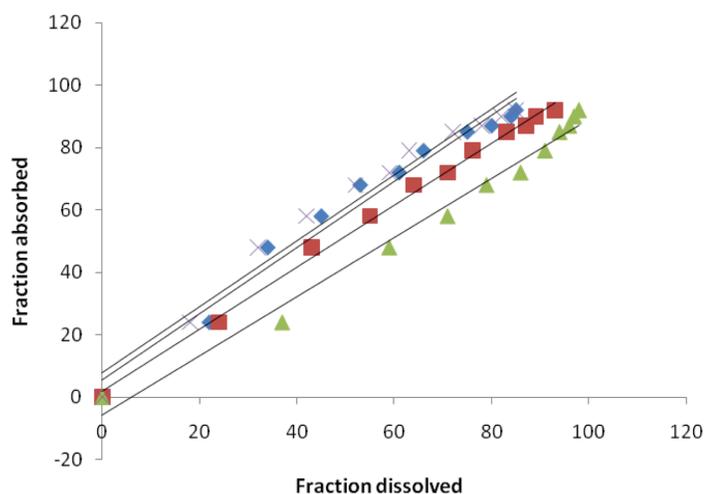


Fig. (4). *In vitro* dissolution profile along with the deconvoluted profile of Aricept in USP-II (paddle apparatus) at 50 rpm speed. Each time point represents the mean of fraction dissolved of twelve tablets.



Dissolution media	Linear trend line equation	r^2 value
▲ 0.1 N HCl	$y = 0.949x - 5.825$	0.983
× 6.8 pH Phosphate buffer	$y = 1.056x + 7.918$	0.970
■ 0.1 HCl followed by 6.8 pH phosphate buffer	$y = 0.997x + 1.806$	0.995
◆ 5.5 pH phosphate buffer	$y = 1.059x + 5.659$	0.975

Fig. (5). Relationship between the mean fraction absorbed *in vivo* and the mean fraction dissolved *in vitro* for Aricept. Mean FD and mean FA were determined for the reference formulation and used to establish the IVIVC. The line represents the linear regression of the data.

Table 2. Prediction of Pharmacokinetic Parameters (C_{max} and AUC_t) for Reference and Test Formulation of Donepezil SR Tablet (23 mg) by Numerical Convolution Method Using Winnonlin Version 5.2 IVIVC Toolkit as an Simulation Software and the Corresponding Prediction Error

Formulation	C_{max} Observed (ng/mL)	C_{max} Predicted (ng/mL)	% PE	AUC_t Observed (ng/mL)	AUC_t Predicted (ng/mL)	% PE
Reference	28.19	29	2.87	1543.18	1601.82	3.75
Test	25.8	27.9	8.1	1501.81	1541.78	2.7
T/R	91.52 %	96.21 %		97.32	96.25	

Linear regression analysis in the levy plot showed (Fig. 5) a statistically significant relationship ($r^2 = 0.995$) between the FD and FA for donepezil reference formulation in 0.1 N HCl followed by 6.8 phosphate buffer media.

So this media was further explored as bio relevant media. Based on the dissolution profile obtained in 0.1 N HCl followed by 6.8 phosphate buffer, pharmacokinetics parameters (C_{max} and AUC_t) were predicted for both formulations by convolution method and test to reference ratio was calculated for C_{max} and AUC_t . Table 2 shows the comparison of predicted and observed ratios along with the % PE.

From the above results, it is clear that using prospective convolution in an optimized biorelevant media we can closely predict the *in vivo* PK parameters with a low prediction error.

CONCLUSION

This study clearly advocates the benefit of prospective IVIVC method using an optimized biorelevant media for predicting PK parameters for human bioequivalence for a generic product. The *in vivo* C_{max} and AUC_t were predicted

by calculating F_a from the IVIVC. In both examples (aprepitant and donepezil) *in vivo* plasma profiles were closely predicted (PE% <15%). This further fortifies the fact that the *in vitro* dissolution of drugs in biorelevant media was similar to *in vivo* absorption profile of both drugs. This study provides an easy prospective convolution approach for predicting and establishing IVIVC for generic product development.

CONFLICT OF INTEREST

All the authors are employees of Dr. Reddy's laboratory and have no competing interest to declare.

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