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RESEARCH PAPER

Use of *In Vitro–In Vivo* Correlation to Predict the Pharmacokinetics of Several Products Containing a BCS Class I Drug in Extended Release Matrices

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ABSTRACT

Purpose To determine if an IVIVC model can predict PK profiles of varying formulations of a BCS Class I drug that is a salt of a weak base.

Method An IVIVC model (Level A) was created by correlating deconvoluted *in vivo* absorption data obtained from oral administration of 50 mg, 100 mg, and 200 mg fast and slow extended release formulations with *in vitro* percent dissolved using residual regression analysis. The model was then used to predict the *in vivo* profile of five test products that varied in formulation characteristics.

Results The model passed internal validation for predicted Cmax and AUC. For external validation, *in vitro* data of five different test formulations was utilized. The model passed external validation for two test formulations that were different but belonging to the same release mechanism as that of the reference formulation. Three formulations failed external validation because they belonged to either a mixed or different release mechanism. The model and results were further confirmed using GatstroPlus™ simulation software.

Conclusions These observations indicate that an IVIVC model for a BCS class I drug may be applicable to varying formulations if the principle of the drug release is similar.

KEY WORDS BCS Class I drug · convolution · deconvolution · dissolution · IVIVC

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ABBREVIATIONS

AUC	area under the curve
BCS	biopharmaceutics classification system
C_{max}	maximum drug concentration observed in the blood plasma profile
FRA	fraction of drug absorbed into the body
FRD	fraction of drug dissolved during *in vitro* experimentation
IVIVC	*in vitro–in vivo* correlation
k_e	constant of elimination
MAPE	mean absolute percentage error
rpm	revolutions per minute
SUPAC-MR	scale up post approval changes modified release
V_d	volume of distribution
$\%PE_{AUC}$	percent error of AUC prediction
$\%PE_{Cmax}$	percent error of C_{max} prediction

INTRODUCTION

In vitro–in vivo correlation (IVIVC) has been defined by the United States Pharmacopeia (USP) Subcommittee on Biopharmaceutics as: "the establishment of a rational relationship between a biological property, or parameter derived from a biological property produced by a dosage form, and a physicochemical property or characteristic of the same dosage form" (1). The Food and Drug Administration defines IVIVC as "A predictive mathematical model describing the relationship between an *in vitro* property of an extended release dosage form (usually the rate or extent of drug dissolution or release) and a relevant *in vivo* response, *e.g.*, plasma drug concentration or amount of drug absorbed" (2). In most cases, the *in vitro* property is the rate or extent of drug dissolution or release while the *in vivo* response is the plasma drug concentration

or amount of drug absorbed. The parameter derived from the biological property is the AUC or C_{max}, while the physicochemical property is the *in vitro* dissolution profile. In the case of controlled release dosage forms, a greater emphasis is placed on the cumulative dissolution of a dosage form over time to indicate *in vivo* performance. Accordingly, the formulation-specific cumulative absorption-time profile is the most powerful indicator of *in vivo* performance in man (3). A linear relationship with slope of unity is preferred, signifying that the *in vitro* dissolution profile is representative of the extent of *in vivo* oral absorption that occurs.

Ideally IVIVC would replace *in vivo* testing It could serve as a surrogate for predicting *in vivo* bioavailability and in establishing bioequivalence, greatly improving time- and cost-efficiency for the pharmaceutical industry. In early formulation development, IVIVC can be used to optimize the formulations. During the later phases of development, IVIVC can aid in obtaining a biowaiver by utilizing the FDA SUPAC-MR guidance. The guidance provides the scientific basis for scale-up or minor post-approval changes without incurring the expense of costly bioequivalence clinical studies. IVIVCs are also utilized to support setting of meaningful dissolution specifications. In summary, IVIVCs can assist in the initial approval, scale-up and post-approval phases of a drug product (4).

In the establishment of an IVIVC, it is essential for drug release to be the rate-limiting process. Hence, IVIVC might be possible with controlled-release formulations of BCS Class 1 or BCS Class 2 drugs. IVIVCs are established by first conducting an *in vitro* dissolution test while consistently maintaining an identical system for all the formulations under investigation. The dissolution profiles are plots of the relationship between the cumulative percent of drug released *versus* time. Then the formulations are given to human subjects in a controlled study to obtain the plasma concentration-time profiles. Finally, the plasma concentration-time profiles are subjected to a numerical deconvolution in order to generate an *in vivo* dissolution profile that can be correlated with experimental *in vitro* dissolution data. Deconvolution is defined as the estimation of the time course of drug input (usually *in vivo* absorption or dissolution) using a mathematical model based on the convolution integral. Based on the knowledge of the pharmacokinetic system for a particular drug, the plasma concentration-time profile resulting from administration of an oral dosage form may be taken apart or deconvoluted to construct an absorption-time profile to represent the extent of *in vivo* oral absorption. Incorporation of deconvolution methods to simulate a worthwhile *in vivo* dissolution profile that follow either statistical moment analysis or model dependent approaches such as Loo-Riegelman and Wagner-Nelson require proficiency of the pharmacokinetics of the drug of interest as well as understanding of pharmacokinetic modeling (4). In the present study, the Wagner-Nelson method was utilized under the notion that this compound followed a one compartment pharmacokinetic model and the elimination constant, k_e was derived from human pharmacokinetic

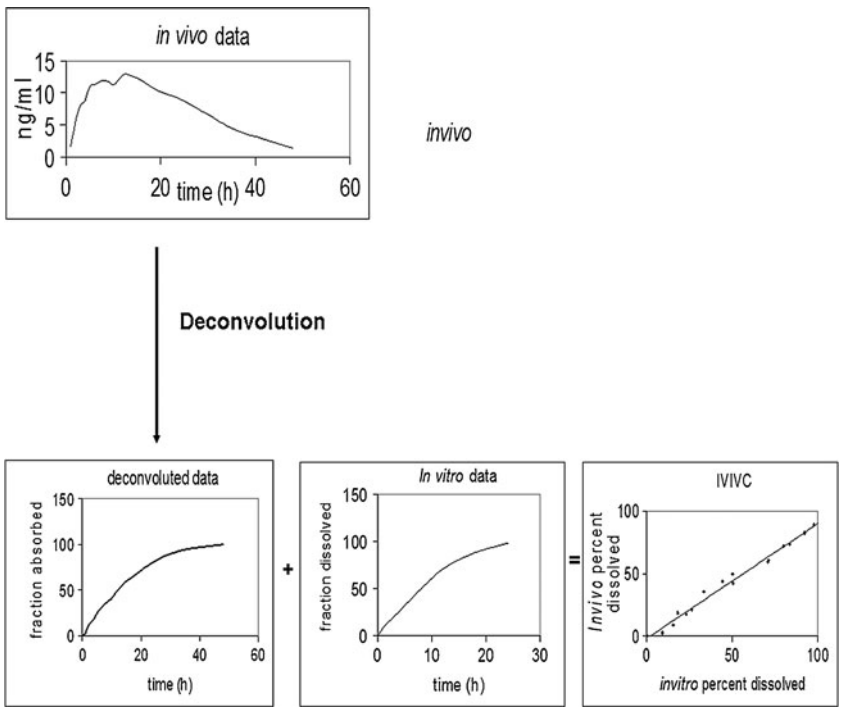

Fig. 1 Schematic representation of creation of IVIVC model using *in vivo* and *in vitro* data.

Table I Formulations Used for *In Vitro* and *In Vivo* Testing

Product	Strength of dosage for *in vitro* testing	Strength of dosage for *in vivo* testing
Reference extended release	25 mg, 100 mg[a], 200 mg	50 mg, 100 mg[a], 200 mg
Reference fast release	100 mg	100 mg
Test A	50 mg	50 mg
Test B	200 mg	200 mg
Test C	200 mg	200 mg
Test D	200 mg	200 mg
Test E	200 mg	200 mg

[a] Only used for external validation

data. The Wagner-Nelson relationship can be described by the equation (5):

$$F_{abs}(t) = (C(t) + k_e AUC_{0-t})/k_e AUC_{(0\rightarrow inf)}$$

After the deconvolution of the pharmacokinetic profile is performed to obtain the *in vivo* absorption time profile, it is visualized together with the *in vitro* dissolution profile to assess the degree of superimposition. Ideally, the two curves should superimpose each other with respect to rate and extent of drug release to guarantee a strong linear correlation. A strong correlation is indicative of a linear relationship between each time point in the dissolution profile to each individual point in the absorption time profile (Fig. 1).

MATERIALS AND METHODS

Dosage Form

All drug products used in this experiment were obtained from Bradley Drugs, a local pharmacy located in Bethesda, Maryland. The reference product used for this project was a BCS Class 1 weak base salt, formulated (as stated in the NDA submission) into fast and slow extended release oral tablet dosage forms. The fast dosage form (reference fast release) was formulated to contain 100 mg of the drug. The slow formulations (reference extended-release) contained 25 (*in vitro* only), 50 (*in vivo* only), and 200 mg, respectively (Table I). The test formulations A and C were manufactured (as stated in the ANDA submissions) by using a similar process as that of the reference product except that the release layer comprised of a different type of polymer. Additional formulations (B, D and E) with different release mechanisms were tested to challenge the model. The information pertaining to Test formulations B, D and E was also obtained from ANDA submissions. These drug products either had an extra coating of drug and polymer or had an instant release portion contained in the formulation. The composition of the various Reference and Test formulations presented in Table II. Dissolution testing was performed in-house on both the reference and from the five other test manufacturers. Data obtained through the *in vitro* dissolution of 25, 100, and 200 mg tablets from the reference manufacturer were used to create the IVIVC model.

Table II Description of the Formulations, Sources of Data and Name of Suppliers

	Beads	Bead coating	Excipient granules	Tablet coating	Source of *in vitro* data	Source
Reference extended release formulations	API, Silicon Dioxide	Ethylcellulose 10 cps, Hydroxypropyl Methylcellulose 6 cps,	Microcrystalline Cellulose	Hydroxypropyl Methylcellulose 6 cps,	Authors 25 mg Sponsors 100 mg 200 mg	Bradley Drugs Bethesda, MD
Reference fast release					Sponsors	Not available commercially
Test A	API, Silicon Dioxide	Microcrystalline Cellulose NF (PH200)	Magnesium Sterate NF	Opadry white YS-1-7003	Authors	Bradley Drugs Bethesda, MD
Test B	API, Silicon Dioxide	Microcrystalline Cellulose NF (PH200)	Sodium Stearyl Fumarate NF	Opadry white y-22-7719, Opadry clear Y-19-7483, Cannauba wax	Authors	Bradley Drugs Bethesda, MD
Test C	API, Silicon Dioxide	Ethylcellulose 10 cps, Hydroxypropyl Methylcellulose 6 cps	Microcrystalline Cellulose	Hydroxypropyl Methylcellulose 6 cps	Authors	Bradley Drugs Bethesda, MD
Test D	API, Opadry Clear YS 1R 7006	Ethylcellulose NF 20 cps	Triethyl citrate	Isopropyl Alcohol, Opadry white 03F28415, Purified water	Authors	Bradley Drugs Bethesda, MD
Test E	Polysorbate 80, API Hypromellose 2910, Sugar Spheres	Poloxamer 188, Cellulose acetate Butyrate	Microcrystalline Cellulose	Opadry White Ys-1-7003, Opadry Clear YS-1-7006	Authors	Bradley Drugs Bethesda, MD

Table III Physiochemical Properties of Drug Compound Used in Creating the Gastroplus IVIVC Model

Parameters	Values
Log P	1.9
Molecular weight	261.36 g
Ph off or reference solubility fully saturated solution	5.48
Concentration of fully saturated solution	16.9 mg/ml
Mean precipitation time	5 s
Diffusion. coefficient ($cm^2/s \times 10^5$)	0.74081
Drug particle density	1.2 g/ml
Particle size (diameter)	50 μm
Human jejunal permeability (Peff) ($cm/s \times 10^4$)	1.34

In Vivo Studies

The *in vivo* data used in creating the IVIVC was reference fast release formulation 100 mg, reference extended-release formulation 50 mg, and reference extended release formulation 200 mg. All of the *in vivo* data used to create the IVIVC model were generated by the sponsors. Randomized, single dose, two-way crossover comparative bioavailability studies in 24 to 36 healthy subjects with a washout period of at least 14 days under fasting condition were conducted. All subjects provided written informed consent and were evaluated, including medical history and a physical examination, prior to entering the study. Each sponsor took blood samples at pre-defined time points between 0 and 48 h following administration of the dose for pharmacokinetic analysis. The reference company and each of the test companies used differing numbers of subjects and differing time points to conduct their *in vivo* studies, and the resulting data was normalized for consistency. The plasma was isolated from whole blood by centrifugation at 4°C. All standards and samples were analyzed by using a validated high performance liquid chromatography (HPLC) method. Standard pharmacokinetic parameters (AUC, C_{max} and T_{max}) of the drug were determined.

In Vitro Studies

The FDA IVIVC guidance document suggests dissolution by USP I (basket) at 100 rpm, or USP 2 (paddle) at 50 or 75 rpm, though any dissolution method may be used. However, it specifies that all samples be analyzed using the same method (2). Dissolution testing was conducted using a Vankel 7000 USP 2 dissolution apparatus (Vankel Technology Group Cary, NC) at 50 rpm, integrated with a Varian 8000 semi automatic sampler (Varian Inc. Cary, NC). Samples taken through the autosampler were filtered using 0.45 µm PTFE Millipore filters (Millipore, Billerica, MA). The samples were collected at 1, 2, 4, 8, 12, 16, 20, 24 h time points. Experiments on test extended release formulations were conducted at 50 rpm only. The absorbance of the samples was measured at 230 nm using a Thermo Scientific Evolution 300 spectrophotometer (Thermo Scientific, Madison, WI). The dissolution medium was 500 ml of pH 6.8 phosphate buffer maintained at 37°C. The medium was prepared by dissolving monobasic sodium phosphate and sodium hydroxide in MilliQ™ (18 MΩ deionized) water. The pH was adjusted using sodium hydroxide. Additionally, dissolution of the reference product (25 mg dosage) was performed at 50 and 100 rpm to demonstrate that drug release from the reference products was primarily controlled by the formulation, and was not influenced by the agitation of the medium.

Deconvolution

The Wagner-Nelson deconvolution method was applied to the *in vivo* data to determine the fraction of the drug absorbed. It assumes a one compartment pharmokinetic model.

The Wagner-Nelson relationship can be described by the equation:

$$F_{abs}(t) = \left(C(t) + k_e AUC_{(0\rightarrow t)}\right) / k_e AUC_{(0\rightarrow inf)}$$

where C(t) is the concentration of drug in the plasma at time (t), k_e is the rate constant of elimination, AUC_{0-t} is the calculated area beneath the plasma concentration curve from time

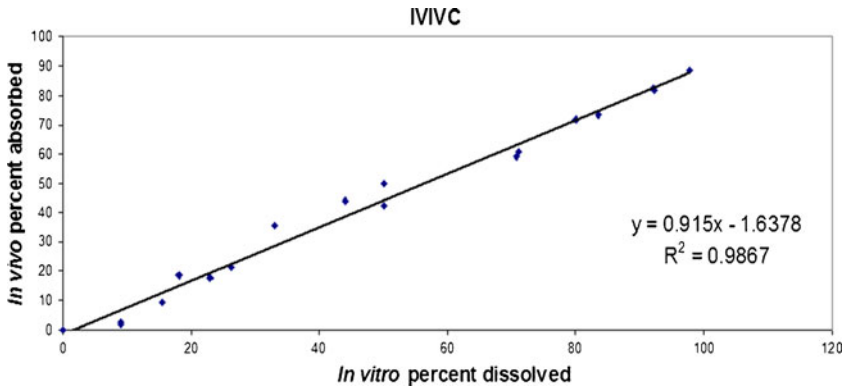

Fig. 2 IVIVC model created by numerical convolution using *in vivo* data from reference fast release (100 mg), and reference extended release formulations (50 and 200 mg).

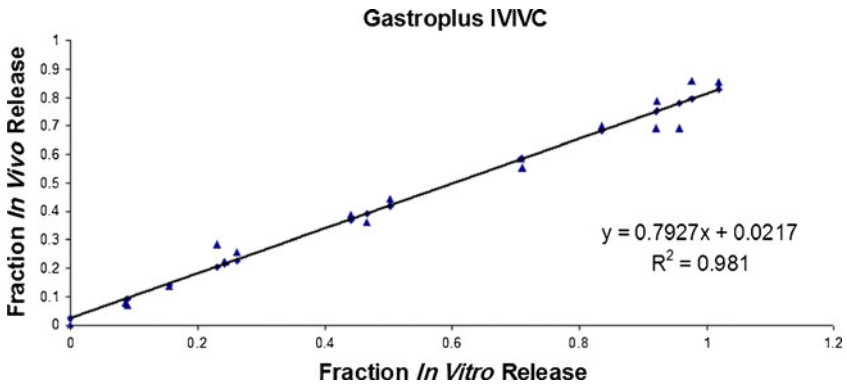

Fig. 3 IVIVC model created using GatstroPlus using reference fast release 100 mg, reference extended release 50 mg and 200 mg formulations.

zero to time (t), and $AUC_{(0\rightarrow inf)}$ is the calculated area beneath the plasma concentration curve from 0 to infinity.

The rate of elimination (k_e) was obtained by taking the negative slope of a time *vs*. Ln(concentration) plot for the final three time points of the plasma concentration of the immediate release dosage form. The $AUC_{(0 \rightarrow t)}$ was determined by dividing the plasma concentration at time (t) by k_e to determine the change from the previous AUC value. The delta AUC value is then added to $AUC_{(0 \rightarrow t)}$ at the previous time point value to determine the current $AUC_{(0 \rightarrow t)}$ value. The $AUC_{(0\rightarrow inf)}$ term was determined by dividing the plasma concentration at the last measured time point by the rate elimination constant, k_e, to determine delta AUC. The delta AUC is then added to the AUC value from the final time point to determine the $AUC_{(0\rightarrow inf)}$ value.

In Vitro-In Vivo Model (IVIVC) Model

To create the IVIVC model, in-house *in vitro* data from reference fast release (100 mg) and reference extended release (25 and 200 mg) were correlated with corresponding sponsor-submitted *in vivo* data for the reference fast release (100 mg) and reference extended release (50 and 200 mg), respectively. Note that limitations in availability of data necessitated scaling of the 25 mg *in vitro* data and correlating it with the 50 mg *in vivo* data. These products were chosen since the FDA guidance requires that an IVIVC model be created with products having at least two different release rates. Also the products had different dosage strengths. To create the IVIVC model, *in vivo* data was deconvoluted using the Wagner-Nelson method so it could be properly matched with *in vitro* data from the same time point. *In vitro* data for the 100 mg, and 200 mg product was obtained from the sponsor NDA submission. Due to the scarcity *of in vitro* data in the sponsor files for the 50 mg tablet, we collected our own dissolution data. The 50 mg tablets were not readily available to us so we used data gathered from the dissolution of a 25 mg tablet to match with the *in vivo* data of the 50 mg tablet. Comparison of the dissolution data available for 50 mg in the sponsor files and the data generated from the 25 mg tablet shows high similarity with an f_2 value of 79.21. The information gathered from all three of the data sets were pooled together, after which a best fit linear trendline was created for the data. The resulting model was then used in conjunction with numerical convolution to determine how accurately it could predict *in vivo* data.

Convolution

To convolute the *in vitro* dissolution data, a spreadsheet function was created that required an input of the predicted milligrams of drug dissolved at various time points. The convolution function consists of two parts, the rate function

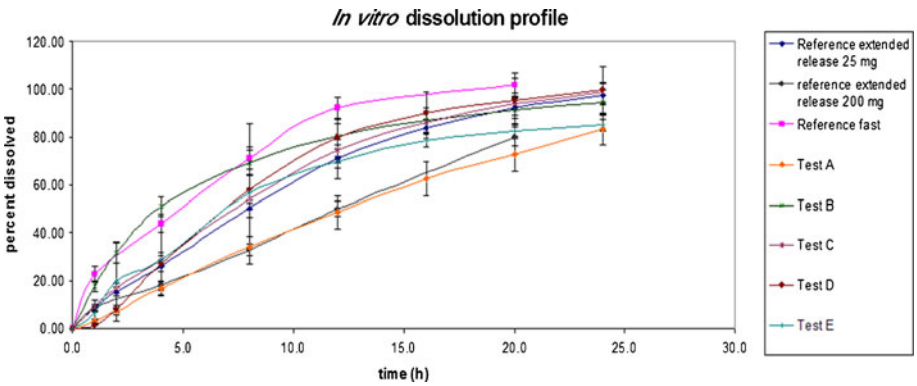

Fig. 4 *In vitro* dissolution profiles of reference fast release (100 mg), reference extended release (25 mg, and 200 mg) and test extended release drug products (25–200 mg).

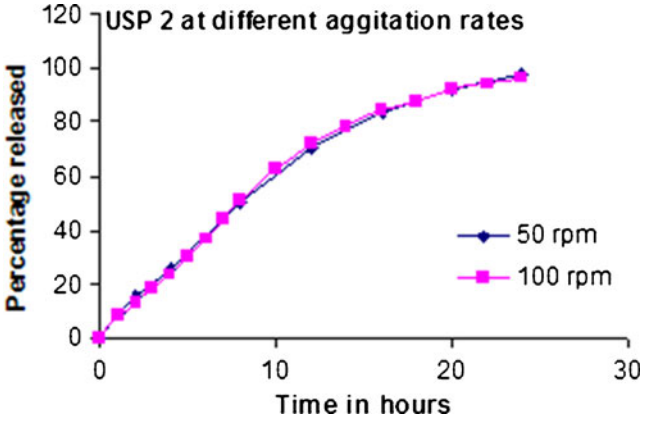

Fig. 5 Drug release study of reference extended release formulation (25 mg) in USP apparatus II at agitation rates of 50 rpm and 100 rpm.

f(t) and the unit impulse response function *g(t)*. Generally, the convolution of the two functions, *f(t)* and *g(t)* is expressed as $(f * g)(t) = \int_0^t f(\tau)g(t-\tau)d\tau$. For IVIVC, *g(t)* is a "unit impulse response" function, representing the plasma profile that would occur if 1 unit of drug were added to the plasma as a single bolus. The unit impulse response function, *g(t)*, is often expressed as $\frac{1}{V_d}e^{-k_e t}$ where V_d is the apparent volume of distribution and k_e is the elimination rate constant. Both must be determined prior to convolution. The function *f(t)* is the rate of predicted *in vivo* absorbance, expressed in this study in terms of mg/h. For this study, *in vivo* absorbance was predicted by applying the IVIVC model to *in vitro* dissolution data. The application of the IVIVC model allowed the prediction of the quantity absorbed (in milligrams) at each time point. Using this information the rate function, *f(t)*, was approximated numerically at each time point by $f(t_n) = \frac{1}{2}\left(\frac{A(t_n)-A(t_{n-1})}{t_n-t_{n-1}} + \frac{A(t_{n+1})-A(t_n)}{t_{n+1}-t_n}\right)$ where A(t_n) is the predicted quantity of the API absorbed at time t_n. Plasma concentration at each time point was then calculated by trapezoidal estimation of the convolution integral. Specifically, the predicted plasma concentration at time t_n is given by $C(t_n) = \sum_{i=1}^{n}\left[\frac{1}{2}\left(f(t_i)\cdot\frac{1}{V_d}\cdot e^{-k_e(t_n-t_i)} + f(t_{i-1})\cdot\frac{1}{V_d}\cdot e^{-k_e(t_n-t_{i-1})}\right)(t_i - t_{i-1})\right]$.

The rate and convolution calculations were carried out using Microsoft Office Excel® 2003 (Microsoft Corp., Redmond, WA).

Computer Simulation Program GatstroPlus™

In vitro data in combination with data relating to physiochemical, physiological, and pharmokinetic properties of the drug were used as input parameters in GatstroPlus™ version 7 (Simulation Plus Inc., Lancaster, CA) to accurately create an IVIVC model. The physiochemical data required to perform the prediction included molecular weight, pKa, dosage size, effective permeability, particle density, and solubility of the drug (Table III). GatstroPlus™ also uses physiological data, usually resulting in a more accurate prediction of the absorption profile of the drug. This data includes the time spent in various sections of the gastrointestinal tract, the pH in various sections, bile salt concentrations, volume, length, and radius of the various sections. GatstroPlus's™ default physiological values and calculated transit times were adjusted to match literature values for bioavailability, blood/plasma concentration ratio, and volume of compartment data, and colon transit time. After incorporating these values, the GatstroPlus™ model was created. The simulation program selected a two compartment model for pharmacokinetic predictions (6,7).

Prediction Error

The prediction error (%PE) calculation was used to quantitatively determine how well a given model can accurately predict a pharmacokinetic parameter of drug. The %PE of both C_{max} and AUC are calculated using the same formula. In this formula the observed value is subtracted from the

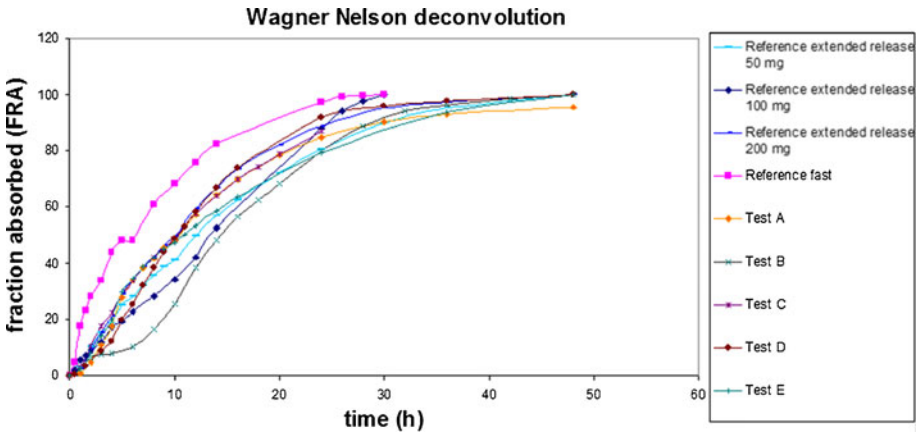

Fig. 6 Deconvolution of reference fast release (100 mg) up to 30 h., reference extended release (100 mg) up to 30 h, reference extended release (50 mg 200 mg) up to 48 h., Test product A (50) mg, Test products B–E (200 mg) all up to 48 h.

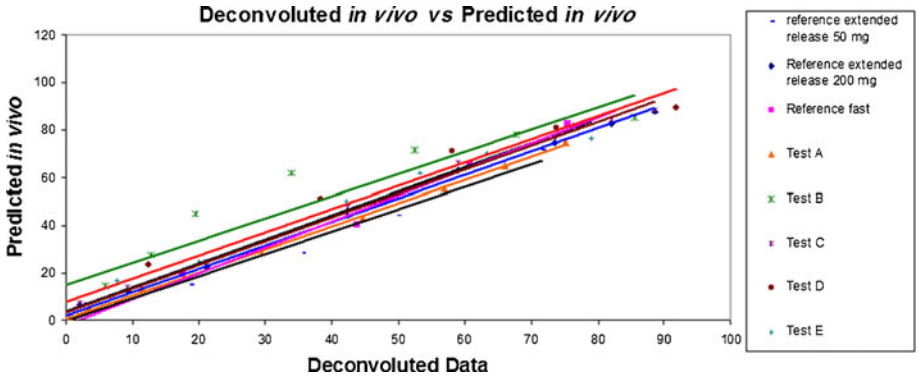

Fig. 7 Comparison of deconvoluted *in vivo* data to IVIVC model prediction. Reference fast release, reference extended release (50 mg, 200 mg) and Generics (50 mg and 200 mg).

value predicted 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.

$$\%PE_{AUC} = \left[\frac{AUC(obs) - AUC(pred)}{AUC(obs)}\right] * 100$$

$$\%PE_{c\max} = \left[\frac{C_{\max}(obs) - C_{\max}(pred)}{C_{\max}(obs)}\right] * 100$$

The mean absolute prediction error (MAPE) is used to determine the overall accuracy of the model in by looking at total accuracy of prediction with respect to multiple products. This is calculated by performing a summation of all the various individual %PE values that were calculated and then dividing by the total number of %PE values.

$$MAPE = \frac{100}{n} * \sum_{i=1}^{n} \left|\frac{(obs_i - pred_i)}{(obs_i)}\right|$$

RESULTS

A robust IVIVC was created using formulations of two different release rates, and doses 50 mg and of 200 mg at the slower release rate, and 100 mg at the faster release rate Separate IVIVC models were created using GatstroPlus™ software, and by using Excel® for conventional calculation.

Table IV Trend Line Equations and Correlation Coefficient Values of Deconvoluted Data *vs* Predicted Data from IVIVC Model of Innovator and Test Generic Drug Products

Drug	Linear trend line equation	R^2 value
Reference extended release (200 mg)	0.986*x + 2.1558	0.9967
Reference fast release	1.0942*x − 2.109	0.9915
Test A	0.975*x + .593	0.9982
Test B	0.933*x + 14.71	0.8942
Test C	0.9986*x + 3.616	0.9941
Test D	0.9808*x + 7.5967	0.9906
Test E	1.0066*x + 4.0281	0.9769

The two different models were created to determine if having different input parameters between the GatstroPlus™ and the conventional calculation model yielded dramatically distinct results. The conventional calculation model had a correlation coefficient (R^2) of 0.9867 (Fig. 2) indicating a strong linear relationship. The GatstroPlus™ model had a correlation coefficient (R^2) of 0.981 (Fig. 3).

The *in vitro* data used in the model was obtained by performing dissolution of the dosage forms using a common procedure that utilized USP Apparatus 2 (paddle) operated at an agitation rate of 50 rpm (Fig. 4). In order to determine whether the dissolution parameters affected the IVIVC model, the reference extended release tablets were tested at a paddle speed of 100 rpm. As seen in Fig. 5, the *in vitro* dissolution profile of the reference tablet at 50 and 100 rpm are largely the same. This was further reinforced by determining the similarity of the 50 and 100 rpm curve through an f_2 test, which yielded a value of 89.41 (8,9).

$$f_2 = 50 * \log\left[\frac{100}{\sqrt{1 + \frac{1}{n} * \sum_{i=1}^{n} (R_i - T_i)^2}}\right]$$

Wagner-Nelson deconvolution (as described in the Methods section) allows one to determine the fraction of the drug that has been absorbed in the body (FRA) at any time. The similarity of the various Wagner-Nelson profiles led us to believe that predicting the concentration profiles of these various drug would be possible (Fig. 6).

The accuracy of the IVIVC model was initially determined by predicting the deconvoluted *in vivo* data. Using the IVIVC model, predictions of the fraction of the drug absorbed into the body (FRA) could be made using the *in vitro* release data. The plot of FRA *vs*. predicted *in vivo* data yielded linear functions all with very high R^2 values (Fig. 7). A linear output was expected since it was a comparison of two sets of deconvoluted data. The model was best able to predict the deconvoluted profiles of test formulations A and

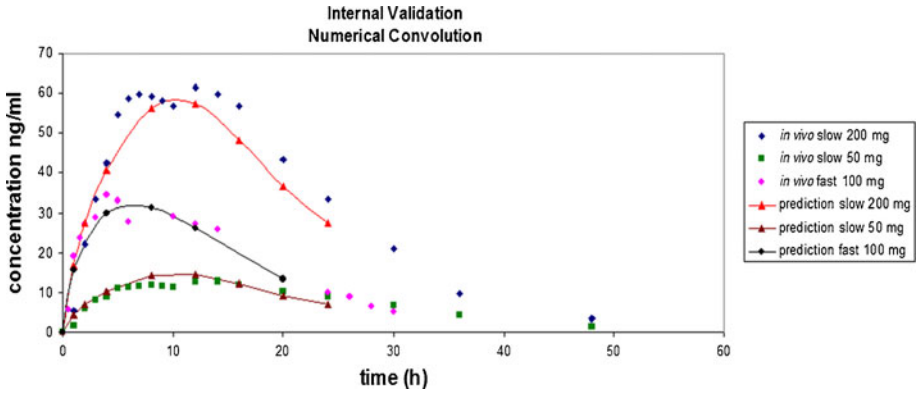

Fig. 8 Prediction of reference fast 100 mg, Reference slow extended release 50 mg and 200 mg. Predictions made up to 20 h for fast extended release, and 24 h for slow extended release using numerical convolution.

C meaning that model was able to best predict the pharmokinetic profiles of test formulations A and C (Table IV).

In accordance with FDA's IVIVC guidance, the model was validated both internally and externally for predictability, i.e., "the model's ability to describe *in vivo* bioavailability results from a test set of *in vitro* data (external predictability) as well as from the data that was used to develop the correlation (internal predictability)" (2). For internal predictability, IVIVC models that were created using Excel® and GatstroPlus™ were tested for their ability to predict AUC and C_{max} of the data sets that were used in the creation of the models. Comparisons of the conventional model created in Excel for AUC were done at 24 h (as opposed to AUC_{inf}) since the last *in vitro* dissolution time point was taken at 24 h. The IVIVC model that was created using GatstroPlus™ was able to make AUC predictions for the entire *in vivo* time course of 48 h.

The FDA IVIVC guidance indicates that the mean absolute percent prediction error for the IVIVC should be less than 10% for both C_{max} and AUC and that the percent prediction error should not be greater than 15% for any one formulation. The mean absolute prediction error (MAPE) for the AUC was approximately 7.09% and the mean absolute prediction error for C_{max} was 8.58% (Fig. 8). Since both of these values remain below the 10% threshold and no PE is above 15%, this model passes internal validation (Table V).

The IVIVC model was further tested by examining its predictive capability using GatstroPlus™. This simulation software allows the user to make predictions by utilizing multiple variables such as drug solubility, drug pKa, and GI transit time. The predictions made by GatstroPlus™ reinforce those made through numerical convolution (Fig. 9). The MAPE calculated for AUC by using GatstroPlus™ was 4.59% for the AUC and 2.54% for C_{max}. The improved (i.e., lower) MAPE for the GastroPlus™ model *vs.* the Excel model may have been because additional data points beyond 24 h were utilized in the predictions.

The model was able to pass external validation by accurately predicting AUC and C_{max} of two out of the five test formulations under consideration (Table VI). The results for test formulation C were most accurate with both AUC and C_{max} predictions being within 10% of the actual value, thereby conclusively passing external validation. However, only the predicted AUC of test formulation A was within 10% of the actual value, while the C_{max} was outside the 10% threshold but was within 20% (Fig. 10). Further investigation is needed to conclusively predict the C_{max} of test formulation A.

External validation through GastroPlus showed a broader range of predictability than the conventional model, accurately predicting three products (reference 100 mg, test formulation A, and test formulation C) (Fig. 11). The

Table V Internal Validation Statistics of Pharmacokinetic Parameters (C_{max} and AUC) from Actual Experiments

Drug	AUC actual ng/ml*h	AUC predicted ng/ml*h	Percent error	Cmax actual ng/ml	Cmax predicted ng/ml	Percent error
Reference fast release 100 mg	504.23[a]	469.9	−6.81%	34.37	31.96	−7.01%
	607[b]	603	−.596%	34.37	35.14	2.15%
Reference extended release 200 mg	1129.32[a]	1027.86	−8.98%	61.35	57.28	−6.63%
	1446[b]	1297	−10.26%	61.35	57.03	−7.11%
Reference extended release 50 mg	243.52[a]	256.96	5.52%	12.77	14.32	12.13%
	353[b]	343	−2.85%	12.77	13.14	2.63%

[a] Predicted by numerical convolution, AUC calculated at 20 h (fast), 24 h (200 mg) and 24 h (50 mg)

[b] Predicted through Gastroplus® model, AUC calculated at 30 h (fast), 48 h (200 mg) and 48 h (50 mg)

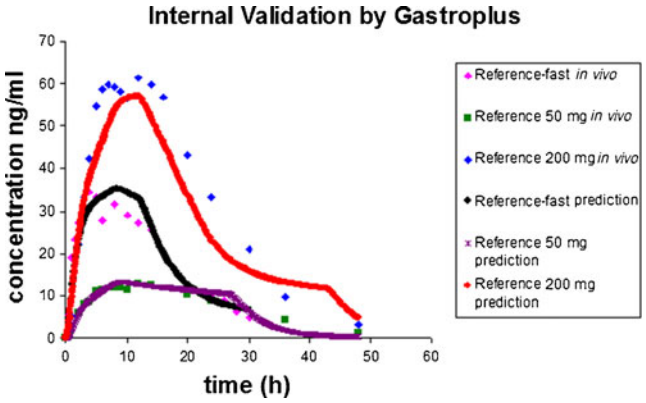

Fig. 9 Internal validation using Gastroplus®. Predicted PK profiles of reference fast (100 mg), and reference slow extended release dosages (50 and 200 mg).

calculated MAPE for these three products was 10.07% for AUC and 8.28% for C_{max}. However, unlike internal validation, some GastroPlus predictions were less accurate than predictions made through numerical convolution. Specifically both AUC and C_{max} for Test C and C_{max} of Test A were better predicted through numerical convolution.

DISCUSSION

An *in vitro–in vivo* correlation (IVIVC) model is developed during the early phase of drug development in order to predict the release rates of various formulations in order to facilitate the selection of the optimum formulation without having to perform additional bioequivalence studies. In the later phases of drug development, IVIVC can be beneficial in justifying minor SUPAC changes. It is also used to support biowaiver applications for lower strengths. However, the IVIVC model has an inherent limitation as it is generally considered as specific to a formulation. This hypothesis was tested and further challenged to determine if the model could be applied to a specific release mechanism even if the formulations and manufacturing processes were different. If true, it was postulated that one would be able to predict the pharmacokinetic profiles of different formulations such as those manufactured by using different processes or by utilizing different release-controlling excipients. One may also be able to predict *in vivo* plasma profiles for test formulations on the basis of the IVIVC model developed for the reference formulations. Such efforts could enable faster development time and lower costs if bioequivalence studies can be minimized for certain low risk products. The aim of the current work is to explore such possibilities and promote scientific discussion.

A thorough screening of existing databases was performed in order to choose a model drug and extended release formulation for which FDA approved test alternatives were available. The chosen model drug was a BCS Class 1 drug that was coated on granules/beads and then sealed by a release rate controlling polymeric coating. The beads/granules were compressed to form the extended release tablets. Five test alternatives were available for the reference drug formulation.

An IVIVC model (Level A) was successfully obtained by utilizing in-house data of slow- and fast-release tablets of the reference formulation. The model was created in two steps. In the first step, the *in vivo* data obtained from oral administration of 50 mg (slow release), 100 mg (fast release), and 200 mg (slow)

Table VI External Validation Statistics of Pharmacokinetic Parameters (C_{max} and AUC)

Drug	AUC observed ng/ml*h	AUC predicted ng/ml*h	Error	Cmax observed ng/ml	Cmax predicted ng/ml	Error
Reference fast release 100 mg	382.18[a]	513	25.64%	20.76	28.64	37.95%
	485[b]	413	−14.86	20.76	21.39	3.03%
Test A	223.42[a]	206.95	−7.37%	12.89	10.79	−16.29%
	341[b]	323	−5.21%	12.89	10.41	−18.46%
Test B	899.18[a]	1062.26	18.13%	51.11	72.36	41.57%
	1247[b]	965	−22.62%	51.11	26.44	−48.26%
Test C	1129.32[a]	1048.42	−7.16%	61.35	59.45	−3.09%
	1446[b]	1299	−10.15%	61.35	59.28	−3.37%
Test D	2931.31[a]	1060.54	−63.82%	171.54	68.22	−60.23%
	3703[b]	1293	−65.07%	171.54	68.23	−60.22%
Test E	1468.97[a]	932.93	−36.49%	89.21	58.27	−34.68%
	1377[b]	1019	−31.57%	89.21	61	−31.62%

[a] Predicted by numerical convolution, all AUC values calculated at 24 h time point

[b] Predicted through Gastroplus®, Innovator AUC predicted at 30 h time point, Generics A–E AUC predicted at 48 h time point

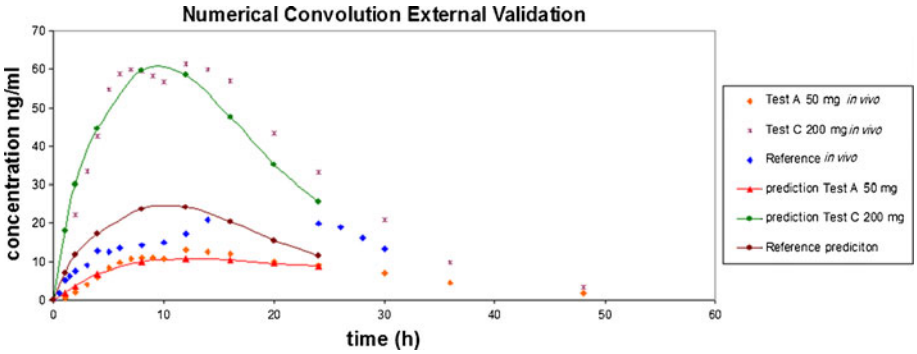

Fig. 10 Prediction of Test A 50 mg, reference 100 mg, and Test C 200 mg (slow extended release) up to 24 h using numerical convolution.

extended release formulations were deconvoluted to obtain cumulative percent *in vivo* drug absorbed (FRA). In the second step, the *in vitro* percent dissolved (FRD) was correlated with FRA using residual regression analysis. The accuracy of the model was examined through internal validation. This involved calculating the %PE for AUC and C_{max} and then calculating the respective MAPE of each. The model passed internal validation, with 5 of 6 metrics having %PE below 10% (Table V). The mean prediction error was 7.09% for AUC and 8.58% for C_{max} except for C_{max} of the reference extended release 50 mg formulation which had a %PE of 12.13%. The positive internal validation indicates that the model is useful amongst various formulations and dosages (10).

External validation was performed in the same manner as internal validation and showed an ability to predict two out of the five test formulations. Experiments were also conducted at an increased rate of 100 rpm to determine if paddle speed influenced dissolution rate. The *in vitro* dissolution profile remained largely similar. Models created using data from the experiment at 100 rpm (data not shown) tended to be more accurate in predicting C_{max} values (11). It appears that the increased number of time points taken during the 100 rpm experiment allows for the creation of a more accurate rate function, *f(t)* (the derivative of FRA in mg with respect to time), which led to a more accurate determination of C_{max} (12).

The model showed a poor ability to predict Test products B, D, and E (Table VI). These formulations were significantly different in their release mechanisms. In the case of Test product B, the drug contained a combination of both the immediate release and extended release pellets within the tablet. This combination creates an *in vivo* profile that is significantly different than the extended release formulation and immediate release formulations alone. The model seemed to have over-predicted the initial drug concentration resulting from the immediate release pellets and did not fully account for the contribution of the extended release pellets (13). This indicates that even though the model can predict slow and fast release profiles of the extended release tablets, the combination of both pellets within one tablet renders it unpredictable.

Test B, D, and E products have significantly different release mechanisms than the reference product, and serve to challenge the limits of generalizing IVIVC to multiple products. Unlike the Test B tablets, the Test D tablets are comprised of only one type of extended-release drug beads. However, the process utilized to create the Test D drug product was significantly different from the rest of the

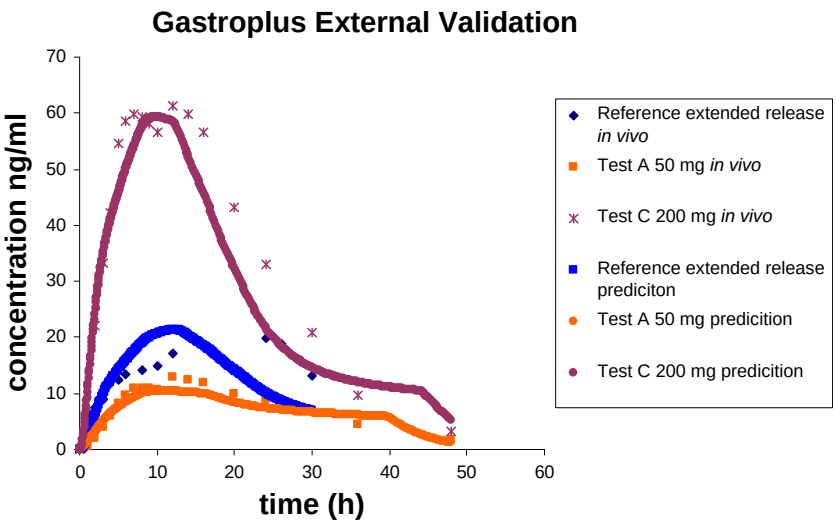

Fig. 11 Prediction of reference (100 mg) up to 30 h, Test A 50 mg up to 48 h and Test C 200 mg up to 48 h (all slow extended release) using Gastroplus.

formulations. While the reference and other products used only one extended-release coating on the API bead, Test D used four iterative coats on the pellets (14,15). The resulting profile from the multiple coatings accounted for a different release profile which resulted in reduced predictability of the model (16). Having multiple coatings creates larger pellets, which have a smaller surface area to volume ratio than smaller pellets for the same mass. This smaller surface area ratio would lead to a slower rate of diffusion of drug product into the blood stream. The Test E tablets also contain only one type of extended release pellet; however they are manufactured in yet another manner. Unlike the reference, the Test E pellets are manufactured by initially coating the API onto a drug layering substrate, sugar cubes, to create an initial drug pellet. These pellets are coated once again with an API and then given an extended release covering (17). This process of secondary drug layering and coating is markedly different than process utilized by the reference and Test products A and C (18,19). This may explain the difficulty in accurately predicting the *in vivo* profile of Test E.

A linear regression analysis of the predicted FRA *vs.* observed FRA (Wagner-Nelson) was performed for all the formulations including Test products D and E. The resulting correlation coefficient indicated a strong correlation (Table IV). This leads to the question as to why the convolution would be so poor when the predicted FRA appears to correlate well. The linear regression of FRA observed *vs.* FRA predicted in fact doesn't indicate the similarity of the curves in terms of an f_2 test or percent difference. Rather it indicates the similarity in the shape of the curves. This is a small but a significant difference (19). The *in vivo* profiles can have different C_{max} and AUC values but as long as the overall shape is similar they will have a linear regression of FRA observed *vs.* FRA predicted with a high R^2 value.

The results obtained by using the conventional model were compared with the results generated by utilizing GatstroPlus™ simulation software. The results from the two models compared favorably. The model from GatstroPlus™ exhibited greater prediction accuracy. Another advantage of the GatstroPlus™ was its ability to predict the *in vivo* drug concentration throughout the entire *in vivo* time course profile. This is possible because the GatstroPlus™ uses an ACAT model (6,10,13).

CONCLUSION

The data presented in this manuscript indicate that an IVIVC model created for one product may be able to predict *in vivo* profiles of formulations with a similar release mechanism. These preliminary findings suggest that substantially different release mechanisms are beyond the scope of a single IVIVC. More investigations are underway to further build on this premise. Different deconvolution techniques, simulation methods and *in vitro* methods will be utilized in order to better understand and improve the predictability of the IVIVC models. Furthermore, exploration of additional BCS Class 1, and possibly Class 2, drugs are logical avenues of additional consideration.

ACKNOWLEDGMENTS AND DISCLOSURES

The findings and conclusions in this presentation have not been formally disseminated by the Food and Drug Administration and should not be construed to represent any Agency determination or policy.

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