Once-a-Day Extended-Release Dosage Form of Divalproex Sodium III: Development and Validation of a Level A *In Vitro–In Vivo* Correlation (IVIVC)

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ABSTRACT: Defining a quantitative and reliable relationship between *in vitro* drug release and *in vivo* absorption is highly desired for rational development, optimization, and evaluation of controlled-release dosage forms and manufacturing process. During the development of a once-daily extended-release (ER) tablet of divalproex sodium, a predictive *in vitro* drug release method was designed and statistically evaluated using three formulations with varying release rates. In order to establish an internally and externally validated Level A IVIVC, a total of five different ER formulations of divalproex sodium were used to evaluate a linear IVIVC model based on the *in vitro* test method. For internal validation, a single-dose four-way crossover study ($N = 16$) was performed using fast-, medium-, and slow-releasing ER formulations and a 12-h IV infusion of valproic acid as reference. To validate the IVIVC externally, a second three-way crossover study ($N = 36$) was performed using slightly-fast-, medium-, and slightly-slow-releasing ER formulations. The *in vivo* absorption–time profile was inferred by deconvolution of the observed plasma concentration–time profiles against the unit disposition function (UDF). A linear IVIVC model was established in which the *in vivo* absorption was expressed as a function of *in vitro* drug release. Plasma profiles of ER formulations were estimated via convolution of *in vitro* release profiles with the UDF. Successful internal and external validations of the model were demonstrated by individual and average absolute percent prediction errors of $\leq 9\%$ for both C_{max} and AUC_{∞}. In conclusion, a Level A IVIVC describing the entire time-course of plasma concentrations was developed and validated, both internally and externally, for ER formulations of divalproex sodium.
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Keywords: controlled-release; dissolution; matrix formulation; *in vitro/in vivo* correlations (IVIVC); pharmacokinetics; modified-release

INTRODUCTION

The challenges and values of developing *in vitro/in vivo* correlations (IVIVC) have undergone extensive debates and discussions since the 1980s.[1–6] With controlled- or extended-release (ER) products, patients are often exposed to specific plasma levels over an extended period of time (e.g. up to 24 h); biorelevant *in vitro* methods are desired to assure the consistent *in vivo* performance. Over the past decade, there has been increased confidence and success in using *in vitro* dissolution to evaluate and predict *in vivo* performance of modified release drug product based on IVIVC, particularly since the publication of a guidance on IVIVC by the FDA in 1997.[7] The guidance provides a comprehensive perspective and framework on development, validations and applications of IVIVC.

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Establishing IVIVC is highly dependent on the ability of *in vitro* tests to correlate with oral absorption and quantitatively predict *in vivo* performance. The state-of-the-art is such that there is no universal *in vitro* model that can mimic highly complex gastrointestinal environment and predict *in vivo* performance. Thus, IVIVC development is often carried out on a case-by-case basis.[8] Once properly established and validated, IVIVC can be utilized to guide formulation and process development in the early stages of product development, to facilitate scale-up and post-approval changes, to set meaningful dissolution specifications, and to use dissolution as a surrogate for bioequivalency studies.[7]

The commercially available ER tablet of divalproex sodium (Depakote ER®, Abbott Laboratories, IL, USA) was developed using controlled-release matrix technology. It provides nearly 24 h of apparent zero-order *in vivo* absorption of divalproex sodium that allows once daily administration.[9,10] In our previous work on the ER matrix system of divalproex sodium, a predictive *in vitro* method was designed and evaluated using a linear model that accounts for inter and intra-subject variability in the *in vivo* absorption.[11] The differences in the release rate were correlated quantitatively with the *in vivo* differences in performance of three different formulations. In order to establish an internally and externally validated Level A IVIVC in the present study, five different divalproex-ER formulations were designed by varying the percent of the release-controlling polymer, hydroxy propyl methyl cellulose (HPMC), for obtaining faster and slower release rates. Two *in vivo* studies were performed to investigate these ER formulations in healthy volunteers. The first study was used to develop and internally validate the IVIVC model. The second study was carried out to externally validate the IVIVC model.

METHODS

Materials and Equipment

The following materials and equipment were used in the study: Divalproex sodium (Pharmaceutical Products Division, Abbott Laboratories, North Chicago, IL), Methocel K15MP CR (Dow Chemical Co., Midland, MI), Depacon® (Abbott Laboratories), Collette high shear mixers (model Gral-75, Gral-600), Stokes tablet machine (model B-2), Fette tablet machine (model 2090), Vanderkamp dissolution tester (model 600) and Abbott TDx® Analyzer.

Test Formulations

Three ER hydrophilic matrix tablets of divalproex sodium, designated as medium-, fast- and slow-releasing tablets (Table 1), used in this study have been discussed previously.[9,11] Two additional formulations, that were slightly-slow- and slightly-fast-releasing relative to the medium-releasing tablets, were designed for external validation of the IVIVC. Tablets were prepared using the same methods previously described at 15 kg scale.[9] Two separate lots of medium-releasing tablets were manufactured at 120 kg

Table 1. ER Formulations of Divalproex Sodium Used in the Development and Validations of IVIVC

	Formulations				
	Fast[a]	Slightly Fast[b]	Medium[a,b,c]	Slightly Slow[b]	Slow[a]
Tablet strengths (mg)	500	500	500	500	500
Biostudy	Study 1	Study 2	Study 1 Study 2	Study 2	Study 1
Bioequivalency	no	yes	Reference	yes	yes
Ingredients					
Divalproex sodium	53.8%	53.8%	53.8%	53.8%	53.8%
Methocel K15M	20%	27%	30%	33%	40%
Fillers/lubricant	26.2%	19.2%	16.2%	13.2%	8.0%
Tablet weight	1.00 g	1.00 g	1.00 g	1.00 g	1.02 g

[a]Internal validation (study 1); formulations F, B, and G from Ref. 9 were designated as fast-, medium-, and slow-releasing tablets, respectively.
[b]External validation (study 2).
[c]Two registration lots.

granulation scale and used in two separate validation studies.

In Vitro Drug Release

In vitro release rates from the divalproex-ER formulations were determined using a predictive test method.[11] The test uses USP Apparatus II operating at 100 rpm in 500 mL of 0.1N HCl for 45 min followed by 900 mL of 0.05 M phosphate buffer containing 75 mM SLS, pH 5.5, $37 \pm 0.5^{\circ}C$ for up to 24 h. Samples were taken at predetermined time intervals, filtered through a 35 μm polyethylene filter, and assayed for valproate by TDx® fluorescence polarization immunoassays after dilution with dissolution medium.[9]

In Vivo Studies

Internal Validation (Study 1)

To develop and internally validate the IVIVC model, a single-dose four-way crossover study was performed in 16 healthy volunteers (10 males and 6 females) using fast-, medium-, and slow-releasing ER formulations and a 12-h infusion of intravenous (IV) valproic acid (VPA) (Depacon®) as reference. Subjects were randomly assigned in equal numbers to four sequences of formulations such that they would receive all four formulations upon completion of the study. Doses in the consecutive periods were separated by one week. The four treatments were 500 mg doses of: (1) fast-releasing ER; (2) medium-releasing ER; (3) slow-releasing ER, and (4) 12-h IV-VPA infusion.

External Validation (Study 2)

To validate the IVIVC externally, a second single-dose three-way crossover study was performed in 36 healthy volunteers (30 males and 6 females) using slightly-fast-, medium-, and slightly-slow-releasing ER formulations. Subjects were randomly assigned in equal numbers to three sequences of formulations such that they would receive all three formulations upon completion of the study. Doses in the consecutive periods were separated by one week. The three treatments were 500 mg doses of: (1) slightly-fast-releasing ER, (2) medium-releasing ER, and (3) slightly-slow-releasing ER.

For both studies, blood samples (5-mL) were collected into heparinized collection tubes prior to dosing (0-h) and at 1, 2, 3, 4, 5, 6, 7.5, 9, 10.5, 12, 15, 18, 24, 36, 48, and 72 h after dosing in each period. Plasma samples were assayed for valproate content using a validated gas chromatography with flame ionization detection method.[12] The lower limit of quantitation was 0.5 mg/L.

Development and Validations of IVIVC Model

Deconvolution

The percent absorbed versus time profiles obtained for the fast-, medium-, and slow-release ER formulations were calculated from the plasma concentration–time data by deconvolution using the Wagner–Nelson method (Eq. 1).

$$\%\ In\ Vivo = \text{Percent absorbed} = \frac{(C_t/\mathrm{k}) + \mathrm{AUC}_t}{\mathrm{AUC}_\infty} \quad (1)$$

The terminal elimination rate constant (k) obtained after IV–VPA administration was used for the calculation of the percent absorbed. AUC_t and AUC_∞ represent the area under the curve from time zero to time "t" and infinity, respectively.

IVIVC Model

To establish the *in vivo–in vitro* correlation, the percent absorbed (*% in vivo*) for the ith measurement of the jth subject was modeled as a function of the mean percent dissolved (*% in vitro*) using the following linear model.

$$\%\ In\ Vivo_{(i,j)} = \alpha_{(j)} + \beta * \%\ In\ Vitro_{(i,j)} + \varepsilon_{(i,j)} \quad (2)$$

The analysis was carried out using the following assumptions and definitions:

(1) α and β are the intercept and slope, respectively, of the correlation line.
(2) Only *in vivo* data obtained at times corresponding to those for which there were *in vitro* measurements were used.
(3) The intercept was assumed to vary among subjects. This variability was modeled using an additive error model with a normal probability distribution. Initially, the slope was likewise assumed to vary among subjects, but the estimate of the variance of the slope from the fitting of the data was essentially zero. Therefore, the slope was assumed to be the same for all subjects.
(4) Intrasubject variability, represented by ε, was assumed to have a normal probability distribution with mean 0 and variance σ^2.

(5) All observations from a subject were assumed to be independent of observations from other subjects. All intrasubject errors were assumed to be mutually independent and also independent of the random component of the intercept.

A full model with separate intercepts and slopes for each formulation, as well as a reduced model with the same intercept and slope for all three formulations, were fitted to the data using the NONMEM software.[13] Under the null hypothesis that the *in vivo–in vitro* relationship is the same for all three formulations, the difference in the minimum value of the objective function (MVOF) of the full and reduced models have an approximate chi-square distribution with four degrees of freedom. A reduction of more than 9.5 in the MVOF when the additional four parameters are included in the model is considered to be statistically significant at the 0.05 level.

Convolution

To generate the predicted VPA concentration–time profiles, *% in vivo* versus time profile was calculated from the *in vitro* data based on IVIVC model and then convolved with the Unit Disposition Function (UDF). The following closed form equation (Eq. 3) was used to perform the convolution using Microsoft® Excel 2000.

$$Cp(t) = \sum_{i=1}^{n} \sum_{j=1}^{m} \left[\frac{R_j C_i}{\lambda_i} \left\{ e^{-\lambda_i (t-\tau_{j2})^*} - e^{-\lambda_i (t-\tau_{j1})^*} \right\} \right] \quad (3)$$

where C_p (t) is the plasma concentration at time "t" for a subject who has "i" number of exponentials for the UDF ($i = 1$ in this case) and;

$$R_j = \text{jth input rate}$$
$$\tau_{j1} = \text{Start time of the jth input rate}$$
$$\tau_{j2} = \text{Stop time of the jth input rate}$$
$$C_i = \text{IV intercept for the ith exponential}$$
$$\lambda_i = \text{rate constant for the ith exponential}$$
$$(t-\tau)^* = t - \tau \quad \text{for } t > \tau$$
$$= 0 \quad \text{for } t \le \tau$$

Internal Validation of IVIVC Model (Study 1)

Using the IVIVC equation (Eq. 2) and the *% in vitro* values (Figure 1), *% in vivo* values were calculated for fast-, medium-, and slow-releasing ER formulations. Subsequently, the piece-wise continuous zero-order absorption rate was determined from the slope of the IVIVC model predicted *% in vivo* versus time profile. A slope was calculated for each pair of consecutive (on time scale) *% in vivo* values; time values served as the start and stop times for the piece-wise constant zero-order absorption rate.

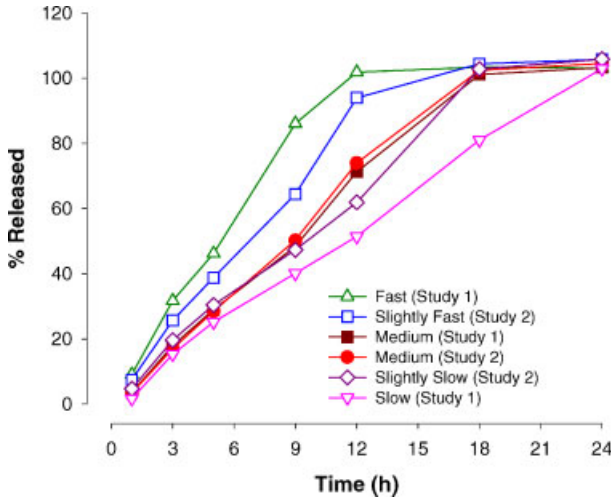

Figure 1. Mean *in vitro* release of five ER formulations of divalproex sodium used in development and validations of IVIVC.

The UDF parameters (IV intercept and first-order elimination rate constant for an unit dose) were estimated by fitting a one-compartment model to the IV-VPA plasma concentration–time data using WinNonlin (Pharsight Corporation, Mountain View, CA). The IV intercept, C_1, for the UDF was calculated as the reciprocal of the volume of distribution for a one-compartment model. The IVIVC model predicted *% in vivo* vs. time profile was convolved with the UDF to generate the predicted plasma VPA concentration–time profile.

External Validation of IVIVC Model (Study 2)

Using the IVIVC equation (Eq. 2) and the *% in vitro* values (Figure 1), *% in vivo* values were calculated for slightly-fast-, medium-, and slightly-slow-releasing ER formulations. Subsequently, the piece-wise continuous zero-order absorption rate was determined from the slope of the *% in vivo* vs. time profile as described for the internal validation. Convolution of IVIVC model predicted *% in vivo* vs. time profile with the UDF was used to generate the predicted VPA concentration–time profiles as described for the internal validation.

Table 2. Mean (SD) VPA Pharmacokinetic Parameters for Internal and External Validation Studies

Formulations	C_{max} (mg/L)	T_{max} (h)	AUC_{∞} (mg · h/L)	λ_z (1/h)	CL/F (L/h)	V/F (L)	$t_{1/2}$ (h)	Abs F
Study 1: internal validation study								
Fast	39.3 (8.8)	9 (3)	1148 (311)	0.046 (0.009)	0.47 (0.14)	10.1 (1.6)	15.7 (3.4)	1.01 (0.10)
Medium	29.7 (7.9)	16 (5)	1056 (347)	0.047 (0.009)	0.56 (0.33)	11.9 (6.2)	15.5 (3.1)	0.92 (0.19)
Slow	31.1 (9.4)	21 (6)	1040 (367)	0.047 (0.009)	0.56 (0.29)	11.9 (5.5)	15.2 (3.1)	0.90 (0.18)
IV-VPA	41.8 (8.5)	13 (1)	1141 (308)	0.046 (0.008)	0.47 (0.13)	10.2 (1.8)	15.7 (3.2)	—
Study 2: external validation study								
Slightly-fast	31.0 (6.9)	14 (5)	935 (293)	0.050 (0.009)	0.60 (0.28)	11.8 (3.9)	14.4 (3.6)	—
Medium	33.1 (7.1)	15 (4)	942 (319)	0.049 (0.010)	0.59 (0.22)	11.7 (2.5)	14.6 (3.8)	—
Slightly-slow	31.2 (10.3)	16 (6)	930 (338)	0.050 (0.010)	0.63 (0.33)	12.3 (4.6)	14.1 (3.6)	—

C_{max}, maximum observed plasma VPA concentration; T_{max}, time to C_{max}; AUC_{∞}, area under the curve from time zero to infinity; λ_Z, terminal elimination rate constant; CL/F, apparent oral clearance; V/F, apparent volume of distribution; $t_{½}$, terminal elimination half-life; and Abs F, absolute bioavailability.

IVIVC Model Evaluations

For both internal and external validations, the absolute % prediction error, was calculated as:

$$\%\ PE_{abs} = \frac{|\text{Predicted} - \text{Observed}|}{\text{Observed}} \times 100 \qquad (4)$$

The C_{max} and AUC_{∞} values predicted by the convolution of the IVIVC model predicted % *in vivo* (Eq. 2) with the UDF was compared with the observed values using % PE_{abs}.

RESULTS

The results of the *in vitro* release from the five ER formulations are shown in Figure 1.

For the internal validation study (study 1), all 16 enrolled subjects (10 males, 6 females) completed the study. The mean ± SD (range) age, weight, and height were 33.9 ± 11.3 (18–50) years, 73.0 ± 9.3 (54.9–88.9) kg, and 173.4 ± 9.9 (160.0–190.5) cm, respectively. The mean (SD) VPA pharmacokinetic parameters are presented in Table 2 and the plasma VPA concentration–time profiles are shown in Figure 2.

For the external validation study (study 2), 36 healthy subjects (30 males, 6 females) were enrolled into the study, and 34 subjects (29 males, 5 females) completed the study. Two subjects missed one or more periods during the study. The mean ± SD (range) age, weight, and height of the 34 subjects completing the study were 32 ± 10 (19–51) years, 75.5 ± 8.1 (57.6–88.0) kg, and 176.7 ± 8.0 (160–190.5) cm, respectively. The mean (SD) VPA pharmacokinetic parameters are presented in Table 2 and the plasma VPA concentration–time profiles are shown in Figure 2.

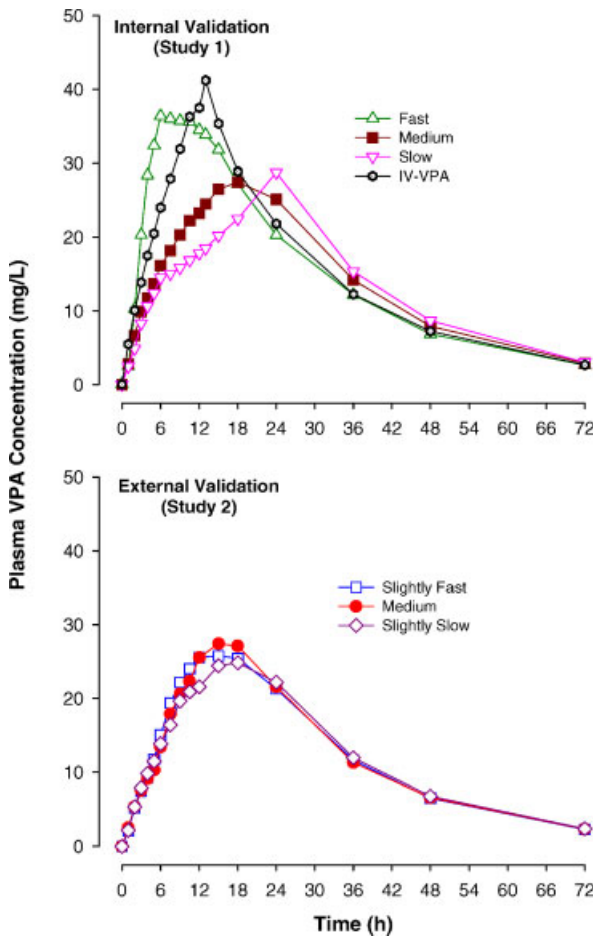

Figure 2. Mean plasma VPA concentration–time profiles of five ER formulations of divalproex sodium tested in internal and external validation studies.

Table 3. Estimates of Intercepts and Slopes for Reduced and Full Regression Models

	Estimate of Central Value	SE	MVOF
Reduced model			
Slope	0.900	0.020	1849.9
Intercept	8.73	2.46	
Full model			
Slope/intercept—fast	0.908/10.8	0.048/3.09	
Slope/intercept—medium	0.875/8.61	0.022/3.09	
Slope/intercept—slow	0.912/7.74	0.029/3.22	1844.9

SE, standard error; MVOF, minimum value of NONMEM objective function.

The estimates of intercepts and slopes for each formulation of the full model, as well as the reduced model with the same intercept and slope for all three ER formulations tested in Study 1 are provided in Table 3. Based on the difference in MVOF between the full and reduced model (5 units), it is concluded that the three regression lines are not statistically significantly ($p = 0.284$) different at the significance level of 0.05, and that a single regression line (Figure 3 and Table 3) adequately fits the combined data from the three ER tablet formulations.

The VPA concentration–time profiles predicted by the IVIVC model for both the internal and external validation studies are shown in Figure 4 and the $\%PE_{abs}$ values are provided in Table 4.

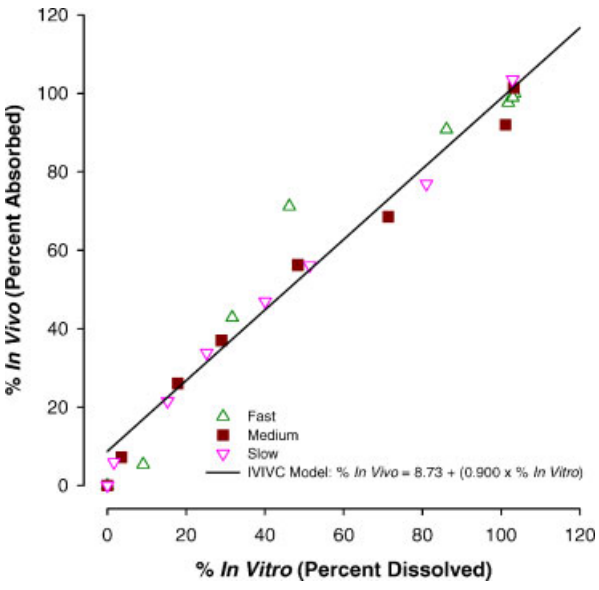

Figure 3. IVIVC model for ER formulations of divalproex sodium.

DISCUSSION

The analysis of the percent absorbed data of the individual subjects by NONMEM with a mixed effects model supports the conclusion that the relationship between *in vivo* percent absorbed and *in vitro* percent dissolved is the same for all three ER formulations (same regression line for all three) in Study 1. For the average subject, the relationship was estimated as:

$$\text{Percent absorbed } (\%\ in\ vivo) = 8.73 + 0.900 \times \text{Percent dissolved } (\%\ in\ vitro) \quad (5)$$

Complete *in vivo* release of the drug occurred within approximately 12, 18, and 24 h for the fast-, medium-, and slow-releasing ER formulations used for IVIVC model development and internal validation (Study 1), respectively. The absolute bioavailability of the fast-, medium-, and slow-releasing ER formulations was about 90% or higher with average T_{max} values of 9, 16, and 21 h (Table 2), respectively. The VPA concentration–time profiles predicted by the IVIVC model adequately described the observed VPA concentration–time course as shown in Figure 4 and quantitated using $\%\ PE_{abs}$ (Table 4). The IVIVC model attains a Level A correlation based on the internal validation since the average $\%\ PE_{abs}$ is less than 10% with no formulation having a $\%\ PE_{abs}$ greater than 9% for either C_{max} or AUC_{∞}.[7]

The slightly-fast-, medium-, and slightly-slow-releasing ER formulations used for the external validation (Study 2) had average T_{max} values of 14, 15, and 16 h (Table 2), respectively. The VPA concentration–time profiles predicted by the IVIVC model adequately described the observed VPA concentration–time course as shown in Figure 4 and quantitated using $\%\ PE_{abs}$ (Table 4). The IVIVC model attains a Level A correlation

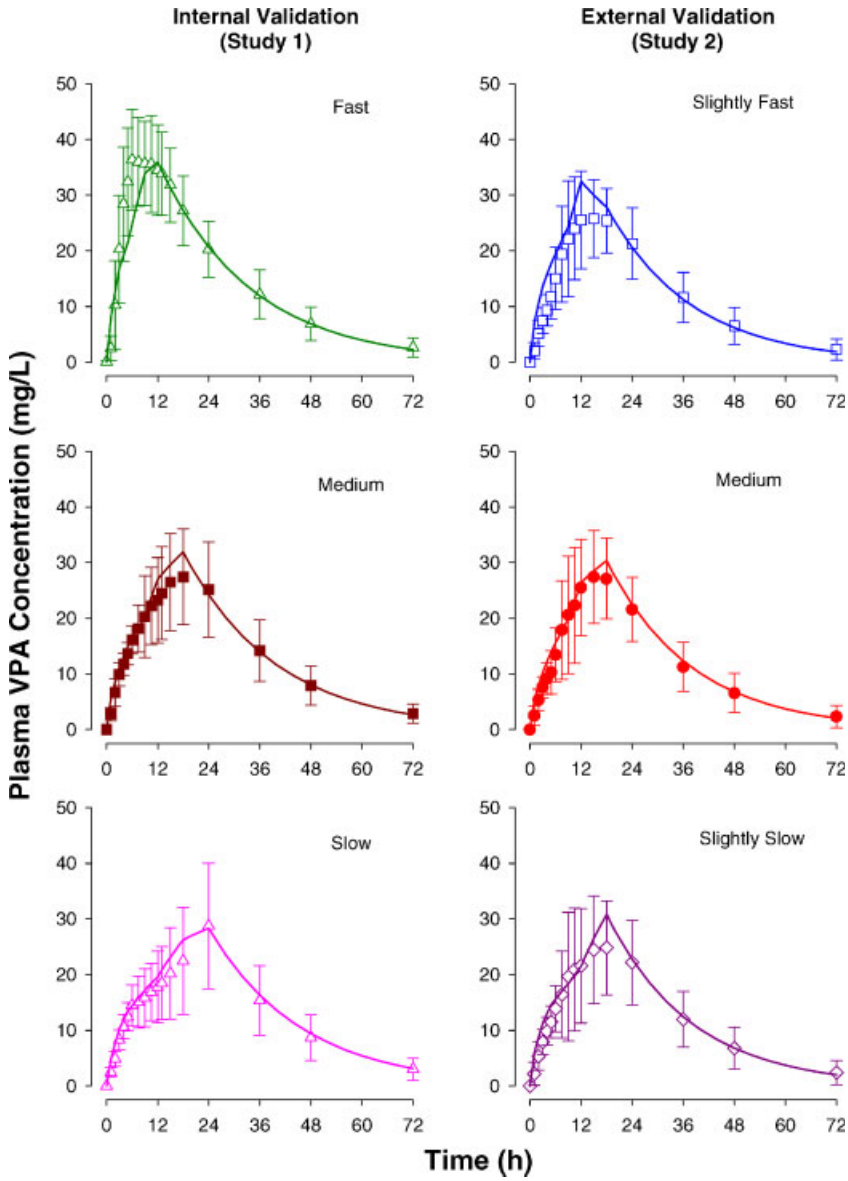

Figure 4. Observed versus predicted plasma VPA concentration–time profiles of ER formulations of divalproex sodium based on the IVIVC model. Symbols represent mean, and error bars represent standard deviation of observed plasma VPA concentrations. The continuous lines represent the IVIVC model predicted plasma VPA concentration–time profiles.

Table 4. IVIVC Model Predictions

	Parameters of IVIVC Predicted Curves		Mean Observed Parameters		% PE_{abs}	
Formulations	C_{max}	AUC_{∞}	C_{max}	AUC_{∞}	C_{max}	AUC_{∞}
Study 1: internal validation study						
Fast	35.95	1066	39.30	1148	9	7
Medium	31.91	1060	29.74	1056	7	0
Slow	28.31	1077	31.13	1040	9	4
Mean % PE_{abs}					8	4
Study 2: external validation study						
Slightly-fast	32.47	962	30.98	935	5	3
Medium	30.32	960	33.08	942	8	2
Slightly-slow	30.79	943	31.15	930	1	1
Mean % PE_{abs}					5	2

based on the external validation since the average % PE_{abs} is less than 10% with no formulation having a % PE_{abs} greater than 8% for either C_{max} or AUC_{∞}.[7]

In conclusion, a Level A IVIVC describing the entire time-course of plasma concentrations was developed and validated, both internally and externally, for ER formulations of divalproex sodium. This IVIVC has been applied to setting bio-relevant dissolution specifications, guiding new product development, supporting SUPAC change, waiving bioequivalence study and, more importantly, ensuring commercial product quality over the years.

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