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Carbamazepine Level-A *In Vivo–In Vitro* Correlation (IVIVC): A Scaled Convolution Based Predictive Approach

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ABSTRACT: A method is presented for prediction of the systemic drug concentration profile from *in vitro* release/dissolution data for a drug formulation. The method is demonstrated using four different tablet formulations containing 200 mg carbamazepine (CZM), each administered in a four way cross-over manner to 20 human subjects, with 15 blood samples drawn to determine the resulting concentration profile. Amount versus time dissolution data were obtained by a 75 rpm paddle method for each formulation. Differentiation, with respect to time, of a monotonic quadratic spline fitted to the dissolution data provided the dissolution rate curve. The dissolution curve was through time and magnitude scaling mapped into a drug concentration curve via a convolution by a single exponential, and the estimated unit impulse response function. The method was tested by cross-validation, where the *in vivo* concentration profiles for each formulation were predicted based on correlation parameters determined from *in vivo–in vitro* data from the remaining three formulations. The mean prediction error (MPE), defined as the mean value of 100% x(observed − predicted)/observed was calculated for all 240 cross-validation predictions. The mean values of MPE were in the range of 10–36% (average 22%) with standard deviations (S.D.s) in the range of 9–33% (average 13%), indicating a good prediction performance of the proposed *in vivo–in vitro* correlation (IVIVC) method. Copyright © 2000 John Wiley & Sons, Ltd.

Key words: carbamazepine; convolution; cross-validation estimation; dissolution rate; *in vivo–in vitro* correlation

Introduction

One of the challenges of biopharmaceutics research is correlating *in vitro* drug release information of various drug formulations to the *in vivo* drug profiles. Limited research has been performed in establishing a predictive *in vivo–in vitro* correlation (IVIVC) [1,2]. Descriptive analysis has been used most frequently to compare *in vitro* and *in vivo* results. With better understanding of the effect of the slow release of a drug on the ultimate therapeutic requirements, focus has been directed on drug formulation development. Thus, the necessity for a tool to reliably correlate *in vitro* and *in vivo* drug release data has increased many-fold. Such a tool would shorten the drug development period and economize the resources.

A novel IVIVC analysis methodology is presented, aimed at establishing predictive, level-A type correlation, enabling drug level profiles to be predicted from *in vitro* dissolution data. The use of a general representation for the *in vivo* and the *in vitro* drug transport functions facilitates the applicability of IVIVC analysis. Modern system analysis principles [3,4], such as convolution and deconvolution, are employed in the analysis to make it as general and comprehensive as possible.

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Theory

The IVIVC analysis method in this work makes use of the following *in vitro* to *in vivo* prediction model:

$$c(t) = s \cdot v \cdot u \cdot s_t \cdot r(s_t \cdot u \cdot t) * f \cdot e^{-ft} * \mathrm{UIR}(t) \qquad (1)$$

where: $c(t) = in\ vivo$ drug concentration at time t, $s =$ scale parameter, $v =$ amount unit conversion factor, $u =$ time unit conversion factor, $s_t =$ time scale parameter, $r(t) = in\ vitro$ release rate at time t, $* =$ convolution operation, $f =$ convolution mapping parameter, and $\mathrm{UIR}(t) =$ unit impulse response function at time t.

The conversion factors (v, u) in the equation are included to allow the *in vivo* and *in vitro* data to use different measurement units. For example, the *in vitro* release is frequently measured using minutes, while *in vivo* drug level data typically use hours as time units. The time scale parameter, s_t, basically provides time-directed stretching, or contraction of the *in vitro* release rate function $r(t)$ to match the *in vivo* release rate. The magnitude scaling via the scale parameter, s, enables adaptation to differences in dose amounts, or in the extent of *in vivo* release, content uniformity or availability. The convolution operation with the exponential function provides basic shape changes in the mapping of the *in vitro* drug release function. Finally, the convolution with the unit impulse response function (UIR) provides the additional mapping step to produce the drug level profile, $c(t)$.

If the UIR function is defined from an oral solution, then the total expression preceding UIR(t) in the above IVIVC model is simply the rate of gastro-intestinal release. If the UIR function is defined from a direct, known vascular input (e.g. iv bolus or infusion), then the preceding expression is the rate of drug input (absorption) into the general systemic circulation.

If data from a known reference input (solution or iv administration) are not available, then a normalized drug input is evaluated, based on drug disposition information extracted from terminal drug level data. For drugs with a linear disposition the drug level, $c(t)$, and the rate of systemic drug input, $f(t)$, are linked together through a convolution with the unit impulse response, UIR(t):

$$c(t) = f(t) * \mathrm{UIR}(t) \equiv \int_0^t f(t-u)\,\mathrm{UIR}(u)\,\mathrm{d}u$$

The UIR is considered to be well described by a sum of exponentials:

$$\mathrm{UIR}(t) = \sum A_j\, e^{-\alpha_j t}$$

If the input process is negligible in the terminal phase ($t > T$), then the terminal drug level is given by the expression:

$$c(t) = \sum A'_j e^{-\alpha_j (t-T)} \qquad (t > T)$$

where

$$A'_j = \int_0^T e^{\alpha_j t} f(t)\,\mathrm{d}t$$

Comparison of the UIR and the terminal 'post-absorption' phase reveals a great degree of similarity between the two. In particular, the exponential time coefficients (α) of the UIR are preserved in the terminal phase.

The UIR analysis consists of two parts. Part one extracts preliminary information about the UIR from the terminal portion of the drug level data. Part two determines the drug release/delivery from the whole dataset, by making use of the UIR information obtained in part one as an initial start in an iterative refinement procedure. The method extracts the disposition information (α) from the terminal drug level data by fitting a hybrid spline to the terminal data points. This is done in a two-step process.

In step one, the terminal drug level data used for extracting the UIR information are determined. This is done by scanning the data backwards to identify the peak level data point that precedes the terminal data. The terminal data used in the initial analysis then consist of the peak data point, together with its immediately preceding point, and the data following the peak point. In step two, the terminal data are fitted by a hybrid least squares spline, $s(t)$. The spline consists of a quadratic polynomial, $q(t)$, joined together smoothly with a polyexponential expression at the knot time t_k, i.e.:

$$s(t) = q(t) = k_0 + k_1(t - t_k) + k_2(t - t_k)^2 \qquad (t < t_k)$$

$$s(t) = \sum A_j e^{-\alpha_j (t - t_k)} \qquad (t \geq t_k)$$

The transition between the spline segments is subject to two smoothness constraints; namely, function value and derivative (slope) which result in the following equality constraints:

$$k_0 = \sum A_j$$

$$k_1 = -\sum \alpha_j A_j$$

The spline function, $s(t)$, defined above, is fitted by least squares to the terminal data. The object of the fitting process is to estimate the 'post-absorption' phase, to estimate the disposition parameters of the exponential UIR. This is done iteratively by adjusting the parameters of the quadratic segment, in addition to varying the position (t_k) where the segments are joined together.

The fitting of the hybrid spline to the terminal data also serves the purpose of estimating the terminal inflection point, t_{flx}, of the drug level curve, which is automatically determined according to the following definition:

$$s''(t_{\text{flx}}) = 0$$

The t_{flx} parameter is used initially to determine approximately the 'start' of the disposition phase ('post-absorption phase'). Simulation studies, using dimensionless analysis principles, have shown that, in many cases, it is often a good approximation to consider the disposition phase region to be $t > 1.5\ t_{\text{flx}}$. The exact determination of the disposition phase region is not critical for the analysis, nor is the determination of the α parameters. The terminal analysis serves mainly to provide an initial estimate for the α disposition parameters of the UIR. In fact, the final estimates determined in part two of the analysis may yield α values significantly different from the ones determined in the preliminary terminal phase analysis. This difference is commonly caused by the 'spillover' of remaining drug input into the terminal phase, which is not detrimental to the analysis.

The *in vivo–in vitro* mapping function (Equation (1)) is, subsequently, fitted by leased squares to the *in vivo* drug level data. This is done by iteratively adjusting the scaling parameters s and s_t, and the convolution mapping parameter f, while the *in vitro* release function, $r(t)$, previously determined, is kept constant.

Methods

The analysis is implemented in a Windows-based computer program PC_IVIVC, which, based on user supplied *in vitro* and *in vivo* data, automatically performs the procedures described in the 'Theory' section. The analysis is illustrated using four different tablet formulations (A, B, C and D) of Carbamazepine (CZM), of 200 mg each, when no reference administration (e.g. iv or oral solution) was available.

In vitro data and conversion to in vitro rate function

Four different formulations of CZM (200 mg each) were subjected to dissolution testing using 900 mL of 1% sodium lauryl sulphate in water, at 75 rpm paddle speed. The *in vitro* release data were, as typically seen, in the form of amount versus time data. The data were converted into a dissolution rate function by a non-parametric, two-step process. The first step checks for inconsistent data points (i.e. non-monotonic data points) that, if identified, are corrected by an Iman–Konover monotonic regression procedure [5]. The monotonic data are then fitted by an interpolating, shape preserving (here monotonic) quadratic spline. The quadratic spline, when differentiated, provides the required rate function $r(t)$ that is used in the correlation function (Equation (1)) in a time-scaled form.

In vivo data

The *in vivo* behaviour of the four formulations of CZM was studied in 20 healthy volunteers, using a four way cross-over design. Blood samples were collected at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 25, 49, 97, 121, 145 and 169 h.

The correlation parameters, which include f, s_t and s, are estimated using the *in vitro* and *in vivo* data from a particular tablet formulation. Subsequently, the *in vivo* drug level profiles resulting from the remaining three formulations were predicted using the previously estimated correlation parameters. This cross-validation prediction is repeated for all the four formulations, and among the 20 subjects. The goodness of fit of each cross-validation predicted curve was determined by the mean prediction error (MPE) defined as:

$$\mathrm{MPE} = \frac{\sum^{n} \frac{|\mathrm{Observed} - \mathrm{Predicted}|}{\mathrm{Observed}}}{n} \cdot 100$$

In this equation, 'Observed' is an observed CZM concentration value, 'Predicted' is the cross-validation predicted value (curve value), and n is the number of observed/predicted concentration values in the subject receiving the particular formulations. For example, three MPE values can be calculated for each of the three predictions in Figures 3–6. Accordingly, with 20 subjects in the study, there are $3 \times 20 = 60$ MPE values for each formulation, i.e. with four formulations, the proposed method is tested using 240 MPE evaluations.

Results

Figure 1 presents the *in vitro* amount dissolved, and rate of dissolution versus time data, in a typical subject for formulation D. Figure 2 shows the corresponding plot of the fitted *in vivo–in vitro* relationship (Equation (1)), determined for the same formulation.

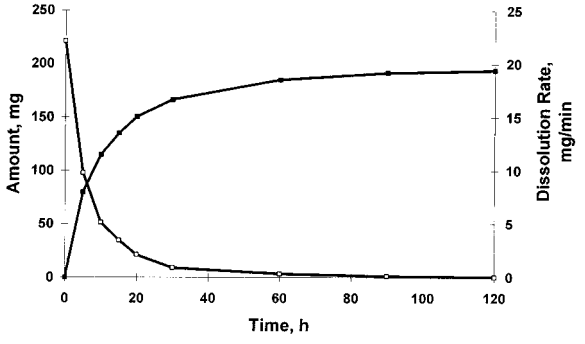

Figure 1. *In vitro* amount dissolved, rate of dissolution and time data in a typical subject for formulation D

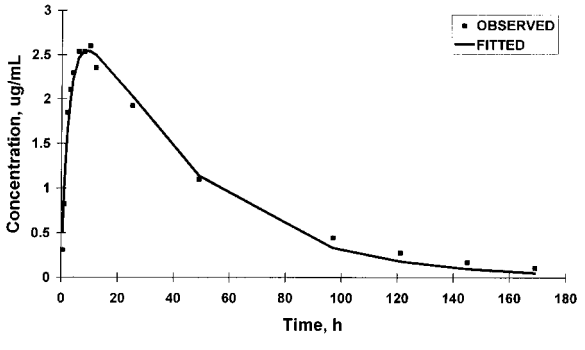

Figure 2. Plot of the fitted *in vivo–in vitro* model (Equation (1)) determined for formulation D

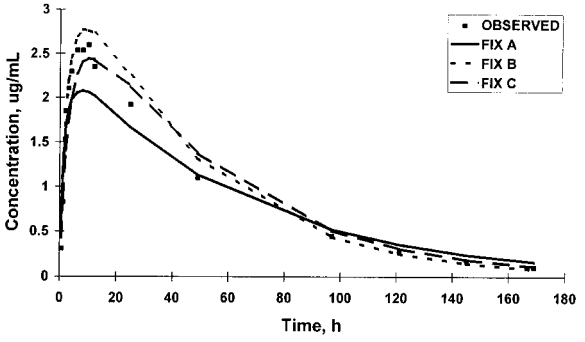

Figure 3. The *in vivo* predictions of formulation D, when the mapping parameters were estimated from the formulations A, B and C in a typical subject

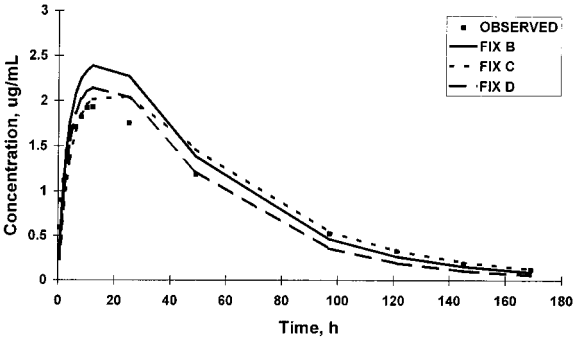

Figure 4. The *in vivo* predictions of formulation A, when the mapping parameters were estimated from the formulations B, C and D in a typical subject

The *in vivo–in vitro* mapping parameters, determined for each formulation, were used to predict the CZM concentrations for the remaining formulations. Accordingly, Figure 3 shows *in vivo* predictions of formulation D, when the *in vivo–in vitro* mapping parameters were estimated from formulations A, B and C in a typical subject.

Similar analysis is shown in Figures 4–6. Thus, Figure 4 shows the formulation A predictions determined from formulations B, C and D. Similarly, Figure 5 shows CZM predictions for formulation B, using three sets of mapping parameters that were determined by fitting Equation (1) to CZM *in vivo* data from formulations A, C and D in a typical subject. In Figure 6,

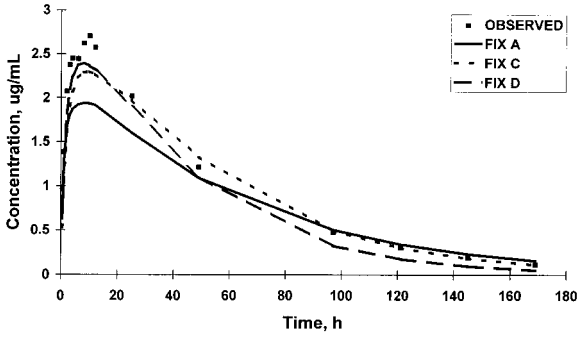

Figure 5. The *in vivo* predictions of formulation B, when the mapping parameters were estimated from the formulations A, C and D in a typical subject

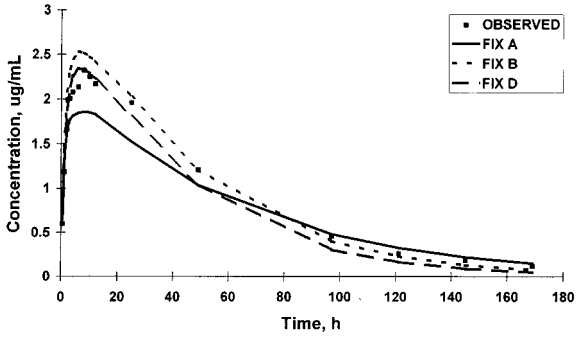

Figure 6. The *in vivo* predictions of formulation C, when the mapping parameters were estimated from the formulations A, B and D in a typical subject

the drug concentration profile for formulation C is predicted in a similar fashion, based on the mapping parameters separately determined from the *in vivo–in vitro* data for formulations A, B and D, using Equation (1). Note that the curves in Figures 3–6 are not fitted curves, but represent cross-validation predictions for a typical subject selected from the 20 subjects, who all received the four formulations in a standard four way cross-over design. Table 1 gives MPE ± standard deviation (S.D.) values of the predictions.

Table 1. Mean MPE (±S.D.) for the different tablet formulations over 20 subjects[a]

	A	B	C	D
	MPE (%)	MPE (%)	MPE (%)	MPE (%)
A	17 ± 10	26 ± 14	22 ± 15	25 ± 12
B	31 ± 18	14 ± 9	17 ± 12	24 ± 13
C	36 ± 33	22 ± 15	10 ± 5	23 ± 9
D	29 ± 16	20 ± 12	18 ± 8	12 ± 5

[a] Letters A, B, C and D in the column 1 indicate the four tablet formulations used to estimate the *in vivo–in vitro* mapping parameters. For example, the cell column 3, row 3 (26 ± 14) corresponds to MPE in predicting *in vivo* profile for formulation B, with mapping parameters estimated from formulation A.

Discussion

The proposed IVIVC analysis method is based on a model (Equation (1)) that assumes that the rate of *in vitro* drug release differs from *in vivo* drug release in two ways. First, the rate of release is operating on different time scales, mainly as a result of different agitation conditions. Variations of such factors as degree of sink condition and pH may also contribute to this difference. Second, the food composition and the consistency of the gastro-intestinal content represent a different vehicle for the drug transport. As stochastic principles are involved in the mass transfer operation in both the *in vitro* and the *in vivo* systems, it is justified to propose that the effect of this difference in vehicle composition may be accounted for by a convolution operation. Furthermore, a 'magnitude' scale factor (s) is included to add additional flexibility for considering different *in vivo* and *in vitro* doses, formulation content variability, and different availability. Also, the rate of release in the gastro-intestinal tract will not be equal to the absorption rate. In the simplest way, this difference between *in vivo* release rate and absorption rate may be considered by a simple single exponential convolution ($\exp(-ft)$ function in Equation (1)) to correct for this difference. All these considerations are incorporated in the *in vivo–in vitro* prediction model Equation (1).

This procedure permits the investigation, whether the cross-validation predictions are consistent with the actual drug level data for the other tablet formulations. As evident from Figures 3–6 and Table 1, the predictions from the analysis are in good agreement with the actual observed concentration data. The mean values of MPE (Table 1) were in the range of 10–36% (average 22%), with S.D.s in the range of 9–33%

(average 13%), indicating a good prediction performance of the proposed *in vivo–in vitro* correlation method.

The *in vivo* performance may be measured in terms of *in vivo* drug release and *in vivo* drug delivery, or may, more ultimately, be defined in terms of the resulting *in vivo* drug level profile, as done here, which is the highest and most meaningful form of correlation. The analysis is primarily aimed at a functional (level-A) type correlation that provides a mapping from *in vitro* release/dissolution measurements to the *in vivo* drug level profile. Typically, the analysis is done to predict what effect formulation changes may have on the *in vivo* performance. Once a viable level-A correlation is established, such predictions may be made simply from *in vitro* release/dissolution measurements. Accordingly, the analysis may be used for quality assurance purposes, or may be used to develop drug formulations with more optimal *in vivo* performance. The methodology promises to reduce the need for costly and time-consuming *in vivo* testing, and provides a less empirical and more rational, *in vivo*-directed drug formulation development.

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