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DEVELOPMENT OF *IN VITRO-IN VIVO* CORRELATION FOR ENCAPSULATED METOPROLOL TARTRATE

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Abstract: This study was aimed to develop level A, B and C *in vitro-in vivo* correlation (IVIVC) for encapsulated metoprolol tartrate (T1, T2 and T3 having metoprolol tartrate/polymer ratio of 1 : 1, 1 : 1.5 and 1 : 2, w/w). The *in vitro* data were correlated with *in vivo* data. For level A IVIVC, drug absorption data were calculated using Wagner-Nelson method. In addition, convolution approach was used to approximate plasma drug levels from *in vitro* dissolution data. The coefficient of determination (R^2) for level A IVIVC was 0.720, 0.905, 0.928 and 0.878 for Mepressor®, T1, T2 and T3 formulations, respectively, with acceptable percent error (< 15%). The value of R^2 for level B and C IVIVC was 0.231 and 0.714, respectively. It is also concluded that level A IVIVC is a proficient mathematical model for biowaiver studies involving study parameters as those implemented for T1S (T1 formulation tested for dissolution in the presence of sodium lauryl sulfate) revealing that IVIVC level A is dosage form specific, rather than to be drug specific.

Key words: metoprolol tartrate; Eudragit® FS; convolution; IVIVC

Based on FDA guidelines, level A *in vitro–in vivo* correlation (IVIVC) is expected for modified release formulations of BCS class I drugs (like metoprolol tartrate), where dissolution is the rate limiting step. Level A IVIVC is the highest correlation for the submission of New Drug Application (NDA) and Abbreviated New Drug Application (ANDA) (1). Level B and C IVIVC is also of remarkable importance in biowaiver studies. An increasing trend of IVIVC development has been observed in recent research. The main advantage of IVIVC is the prediction capability of *in vivo* performance of an alternative formulation of predefined nature from specific dissolution characteristics and IVIVC function (2). According to Food and Drug Administration (FDA) guidelines for the establishment of IVIVC, three formulations of the subject drug with different release rates are required followed by the internal or external validation of IVIVC (3). Previously, many studies have been conducted for the formulation of metoprolol tartrate sustained release solid oral dosage form (1-4). However, present polymer for enteric delivery of drug is not available in literature except our work (5-7).

This article is a part of our study that was designed to develop metoprolol tartrate-Eudragit® FS modified release pH-dependent formulations i.e., tabletted microparticles using various concentrations of polymer. Then, the *in vitro* and *in vivo* evaluation of the prepared and reference formulations (Mepressor® 200 mg, Novartis Pharmaceuticals, Karachi, Pakistan) was conducted followed by the development of IVIVC.

MATERIALS AND METHODS

Materials

Metoprolol tartrate (Novartis Pharmaceuticals, Karachi, Pakistan), Eudragit® FS (Rohm Pharma, Germany) as well as analytical grade liquid paraffin, methanol, and petroleum ether (Merck, Germany) were employed in this study. Mepressor® 200 mg SR tablets (Batch No. 457X, Novartis Pharma, Pakistan) was used as reference formulation.

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Encapsulated metoprolol tartrate

Encapsulated metoprolol tartrate (T1, T2 and T3 having metoprolol tartrate/polymer ratio of 1 : 1, 1 : 1.5 and 1 : 2, w/w) was synthesized by solvent evaporation method and then was compressed into adequately hard tablets so that each tablet contained 200 mg of metoprolol (5).

For drug-polymer compatibility analysis, Fourier transform infra-red spectroscopy, x-ray diffractometry and differential scanning calorimetry of un-compressed T2 formulation was conducted. Compatibility analysis showed that metoprolol tartrate maintained its chemical integrity in the form of encapsulated metoprolol tartrate (5). Other compendial tests like weight variation, tablet hardness, friability, disintegration and dissolution for the reference and test formulations were also carried out, which was in accordance with the compendial criteria (5). The dissolution test was performed by sequential pH change technique and high-performance liquid chromatography was employed for the analysis of metoprolol samples (5, 6). Drug release kinetics was calculated using various kinetic models like zero-order, first-order, Higuchi and Korsmeyer-Peppas models. The *in vivo* study as well as the calculation of pharmacokinetic parameters has been narrated previously (7). Zero order kinetic model best explained the *in vitro* dissolution data of developed formulations; zero order model illustrates the concentration independent release of drug (7).

Computation of absorption data and IVIVC development

In order to establish a level A IVIVC, *in vivo* absorption (%) data were calculated using Wagner-Nelson equation. Firstly, the area under the plasma concentration-time curve from zero to time "t" ($AUC_{0\text{-}t}$) was evaluated from plasma drug concentration (C_t) data using the trapezoidal rule, and then the area under the plasma concentration-time curve from zero to time infinity ($AUC_{0\text{-}\infty}$) was calculated by adding $AUC_{0\text{-}t}$ to the last log-linear concentration divided by the terminal disposition rate constant. Secondly, elimination rate constant (K_e) was multiplied with $AUC_{0\text{-}t}$ (resulting in $K_e \times AUC_{0\text{-}t}$) as well as with $AUC_{0\text{-}\infty}$ (resulting in $K_e \times AUC_{0\text{-}\infty}$) and then the product of $K_e \times AUC_{0\text{-}t}$ was added to the respective C_t at each time point [resulting in $C_t + (K_e \times AUC_{0\text{-}t})$]. Finally, each $C_t + (K_e \times AUC_{0\text{-}t})$ was divided by the product of $K_e \times AUC_{0\text{-}\infty} \times 100$ to calculate the percentage of drug absorbed (F) at each time point using following equation 1 (8):

$$F = \{[C_t + (K_e \times AUC_{0\text{-}t})] / [K_e \times AUC_{0\text{-}\infty}]\} \times 100 \quad \text{(Eq. 1)}$$

Level A IVIVC was developed by drawing a plot between the percentage drug absorbed (along x-axis) of a formulation and its percentage drug dissolved (along y-axis) followed by the regression analysis of each curve to evaluate the strength of correlation determining whether the curve is linear or non-linear. The closer the value of determination coefficient to 1, the stronger is the correlation and linear is the curve. Level B IVIVC is developed by plotting the values of MDT (along x-axis) against MRT (along y-axis) of a formulation followed by the regression analysis of the curve. Level C IVIVC is a single point correlation which is developed by plotting $t_{50\%}$ (along x-axis) and pharmacokinetic parameter like AUC (along y-axis) followed by the regression analysis of the curve (8).

Convolution of *in vitro* data to approximate plasma drug levels

Convolution of *in vitro* dissolution data was done to get the c(t) (predicted plasma drug concentration) from the dissolution data utilizing (unit impulse response which is found from the intravenous bolus dose data or standard oral solution data) and (drug input rate *in vitro* from oral solid dosage form) as follows:

$$C(t) = \int_0^t C_\delta(t-u)X'_{vitro}(u)du \quad \text{(Eq. 2)}$$

The function *"u"* indicates the variable of integration.

To predict plasma drug concentration from the *in vitro* dissolution profiles, the distinct drug concentrations, obtained from the percentage *in vitro* dissolution data during each sampling interval, were converted into the bioavailable drug concentrations utilizing the published bioavailability data of the drug. Then, the calculation of reducing levels of plasma drug concentrations during each interval utilizing the published elimination data of drug was carried out. All the determined drug concentrations for each time point were added, and finally the predicted plasma drug level at each time point was determined using the published values of volume of distribution of drug as well as the adult body weight (70 kg in average) (6).

Predictability of IVIVC

Predictability of IVIVC was determined using the following formula (4):

$$\text{Prediction error } (\%)_{C_{max}} = \frac{C_{maxObserved} - C_{maxPredicted}}{C_{maxObserved}} \quad \text{(Eq. 3)}$$

Statistical analysis

The experimentally obtained results were stated as the average ± standard deviation (SD). The sta-

Table 1. *In vitro in vivo* correlation data

Level of *in vitro in vivo* correlation	Factor	Result
IVIVC level A	R^2 for Mepressor®	0.720
	R^2 for T1	0.928
	R^2 for T2	0.905
	R^2 for T3	0.878
	R^2 for T1S	0.973
	Prediction error (%)	14
IVIVC level B	R^2	0.231
IVIVC level C	R^2	0.714

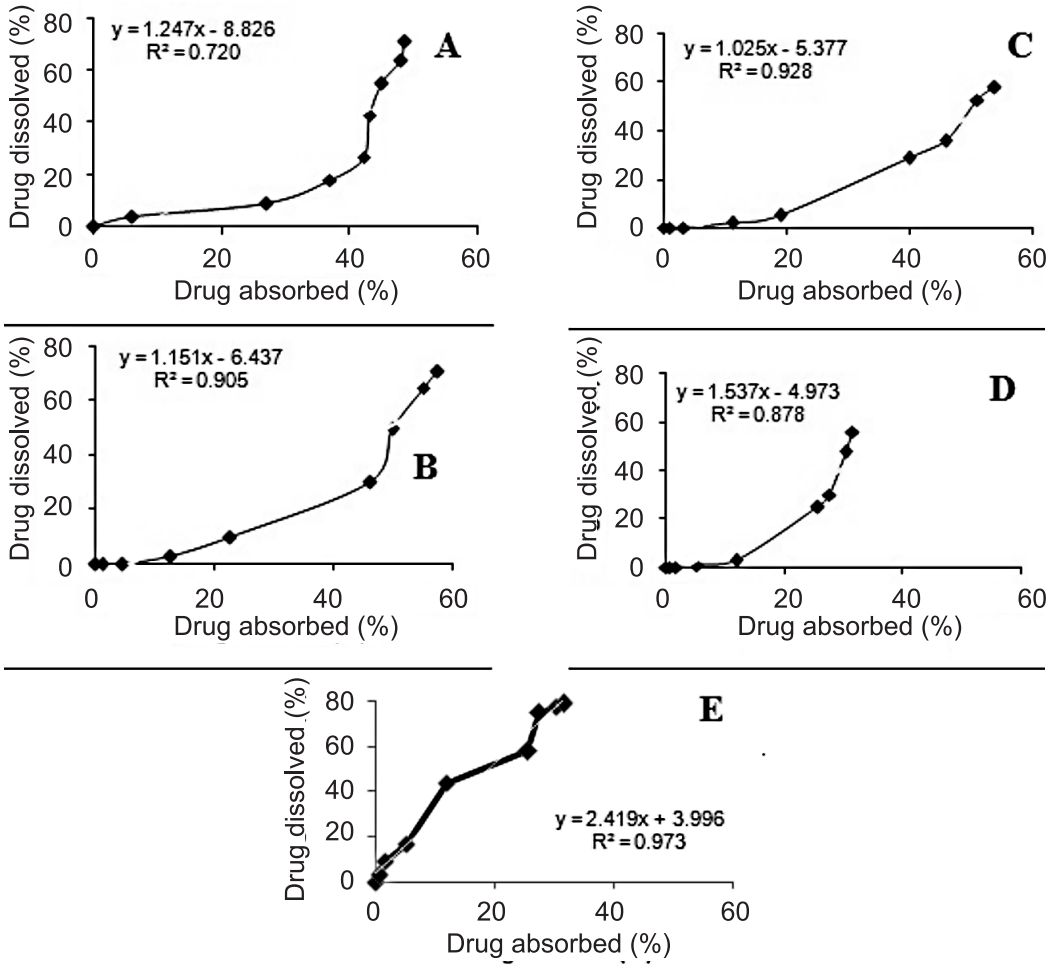

Figure 1. Level A IVIVC for formulations Mepressor® (**A**), T1 (**B**), T2 (**C**), T3 (**D**) and T1S (**E**)

tistical analysis was conducted by one way analysis of variance using software, SPSS version 13.0 (IBM, USA). The level of significance was set at 0.05.

RESULTS AND DISCUSSION

In this article, the evaluation and application methods of IVIVC in setting *in vitro* release specifi-

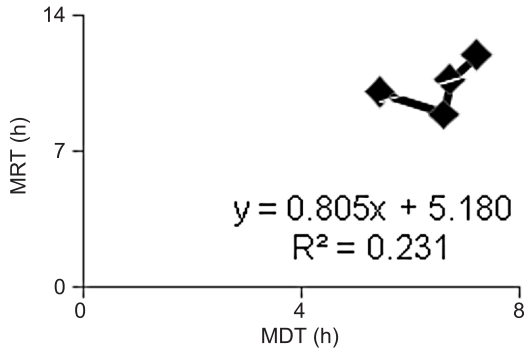

Figure 2. Level B IVIVC

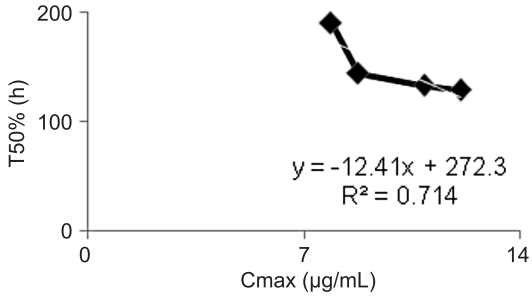

Figure 3. Level C IVIVC

cation for a product are given. For biowaiver studies, comparative dissolution analysis is employed in addition to routine quality control tests, and subsequently the obtained dissolution data are evaluated. Biowaiver study is usually carried out for formulations with different strengths (thus different release rates) (4). To approximate *in vivo* activity of a formulation, the application of dissolution testing as a quality control tool has considerably increased after developing IVIVC. There are many applications of IVIVC such as the selection of the biorelevant *in vitro* dissolution medium in bioequivalence studies, use of validated IVIVC for providing details for a biowaiver in scale-up or post approval changes. Indeed, a biowaiver is only granted if the prediction of *in vivo* performance of the formulation with the modified *in vitro* release rate remains bioequivalent with that of the originally tested formulation (4).

Figure 1 and Table 1 exhibits an effort at a level A IVIVC for encapsulated metoprolol tartrate formulation. The IVIVC is considered as the most valuable tool for the approximation of *in vivo* activity from dissolution profiles. It is also remarkable to identify the subsistence of a superb relationship between drug absorbed (%) *in vivo* and drug dissolved (%) *in vitro* in this article, which also involves the prediction of plasma drug concentration and absorption kinetics from dissolution data as well as the drug release kinetics. This feature is evidently expressed in Figure 1 reflecting the release behaviors for *in vitro* as well as the relevant *in vivo* absorption processes. It is apparent from the data that the developed formulations have revealed insensitivity to the hydrodynamic conditions, which is a climatic feature of gastrointestinal tract; this characteristic helps in the prediction of *in vivo* plasma drug levels. The IVIVC for T2 revealed a good correlation coefficient ($R^2 = 0.928$) followed by the T1 ($R^2 = 0.905$) and T3 ($R^2 = 0.878$) (Table 1). It clearly indicates the enteric nature of formulated products which is further confirmed from a weaker IVIVC ($R^2 = 0.720$) for Mepressor® (Figure 1) as it a non-enteric formulation performing unlikely in sequential pH change dissolution test. In addition, the value of $R^2 = 0.973$ is significantly ($p < 0.05$) higher for T1S which involved the dissolution in the presence of 0.1% sodium dodecyl sulfate. This use of surfactant enhanced the rate of dissolution which resulted in the close resemblance of dissolution conditions to that of normal physiology. In addition, percentage prediction error was found to be 14% exhibiting convolution technique as a proficient procedure for predicting plasma drug levels.

There was a very week correlation coefficient ($R^2 = 0.231$) for level B IVIVC, while 0.714 was the correlation coefficient in case of level C IVIVC (Figure 2 and 3).

In short, Wagner-Nelson equation for the development of IVIVC was revealed using a targetted release formulation as a model system. The technique possesses the benefit of tolerating the data characteristically obtainable from a formulation development program to be used for establishing IVIVC.

CONCLUSION

This study corroborates that there is an excellent *in vitro-in vivo* correlation for metoprolol tartrate formulations encapsulated into Eudragit® FS, principally for T1S. It is also concluded that level A IVIVC is a proficient mathematical model for biowaiver studies involving study parameters as those implemented for T1S revealing that IVIVC level A is dosage form specific, rather than to be drug specific.

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