Research Article

Development and Evaluation of Internal and External Predictability of Metoclopramide Hydrochloride Modified Release Formulations: An Establishment of Level A *In vitro* and *In vivo* Correlation

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ABSTRACT

The objective of this study was to develop an *in vitro–in vivo* correlation (IVIVC) model for hydrophilic matrix sustained-release (SR) Metoclopramide formulations. The *in vitro* release characteristics of the drug were determined using USP apparatus II at 50 rpm, pH 6.8. *In vivo* plasma concentrations and pharmacokinetic parameters in healthy human subjects were obtained after administering oral dose, developed SR formulations and marketed immediate-release (IR) products. The similarity factor *f*2 was used to compare the dissolution data. The IVIVC model was developed using pooled fraction dissolved and fraction absorbed of developed SR formulations i.e. fast, medium and slow release and marketed immediate-release (IR) products. An *in vitro-in vivo* correlation (IVIVC) was established for sustained release tablet by deconvolution using data from an immediate-release treatment as the characteristic response. To assess the correlation between *in vitro* dissolution uniqueness and *in vivo* absorption performance of Metoclopramide sustained release (SR) and immediate release (IR) tablet in human subjects. The established IVIVC was evaluated internally by predicting data used to develop and externally by predicting data not originally included in developing the IVIVC model. The observed low prediction errors for Cmax and AUC demonstrated that the Metoclopramide IVIVC model was valid.

Keywords: Metoclopramide Hydrochloride, Modified Release, Level A Correlation, Internal And External Prediction Errors.

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INTRODUCTION

In vitro in vivo correlation is a predictive mathematical model describing the relationship between the *in vitro* property of dosage form and *in vivo* response. *In vitro* property is a rate of drug release and *in vivo* response is the amount of drug absorbed. *In vitro in vivo* correlation plays a vital role in the early stage of product development, substitute for *in vivo* bioavailability and to support biowaivers, to facilitate scale-up and post approval changes, and to set significant dissolution specifications. *In vitro in vivo* correlation is established to facilitate dissolution test to be used as surrogacy for bio study and it benefit the pharmaceutical manufacturer in terms of time and cost on bioequivalence study.[7-10]

In vitro in vivo correlation models will be useful for optimizing the SR (sustained release) dosage forms, otherwise predict *in vivo* performance of the SR dosage forms based on *in vitro* dissolution data. The FDA guidance has identified three *In vitro in vivo* correlation models: namely, level A, B, and C models.[6] Numerous investigations have been undertaken to develop *In vitro in vivo* correlation models, a level A correlation utilize the complete time course of *in vitro* dissolution and *in vivo* input and recognized model of choice for achieve biowaivers or

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setting of dissolution specifications.[7-16] However, level B and C models may be used in the initial stages of formulation development to inspect level A *In vitro in vivo* correlation models shall be used.[6]

The present work aim was to develop and establish the internal and external predictability of level A *In vitro in vivo* correlation models for the three Metoclopramide sustained release and one immediate release formulation were evaluated. The rationale behind the study was develop Metoclopramide *In vitro in vivo* correlation models, so that it can serve as a surrogate for *in vivo* bioavailability and to support biowaivers and which in turn reduce the cost of the optimization process.

MATERIALS AND METHODS

Chemicals and Reagents

Metoclopramide Hydrochloride & Cisapride were supplied by Adcock Ingram Healthcare Pvt. Ltd. (Bangalore, India). The HPMCK100M supplied by Colorcon Asia Pvt Ltd. (Goa, India). Avicel, Magnesium stearate and talc were procured from local supplier.

Formulation of Tablets

Sustained release formulations, each tablet containing 10 mg of Metoclopramide hydrochloride, prepared by weighing the amount of active ingredients, polymers (HPMC), avicel magnesium stearate and talc in different ratio and blended to get homogeneous mixture. Tablets were prepared by direct compression on a single punch machine (10 mm bi-flat round shaped punches). The Metoclopramide hydrochloride formulations were prepared at three different release rates i.e. fast, medium and slow sustained release tablets.[1-3]

In vitro release studies

The dissolution performance of Metoclopramide hydrochloride determined by using a USP dissolution apparatus 2 (paddle method). The release studies were performed in pH 1.2, 4.5, 5.5, 6.8, 7.4 (900 ml) at 37.0 ± 0.5 °C and a rotation speed of paddle was 50 & 75 rpm. Samples (5 ml) were collected at 0.0, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 12.0, 18.0 and 24.0 hours. and replaced with fresh medium at various interval times. The amount of released Metoclopramide hydrochloride was analyzed by using UV-Visible spectrophotometric at a wavelength of 309 nm.

Clinical pharmacokinetic study

This was an open-label, single dose, four-treatment crossover study by using six healthy volunteers under fasting condition. Subjects were given informed consent prior to participation and study was approved by the ethics committee. Subjects randomly allocated treatment as per the randomization schedule, such a way all subjects would receive four formulations upon completion of the study. Blood samples were collected at predetermined time 0.0, 0.33, 0.67, 1.0, 1.33, 1.67, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0, 12.0, 16.0, 18.0 and 24.00 hours post-dose. Washout period of at least 07 days between dosage.[4] Plasma samples were stored in an upright position below -20°C till completion of analysis. Plasma samples were estimated for Metoclopramide by using a validated LCMS/MS with liquid liquid extraction method. Chromatographic separation achieved on a Eclipse XDB C18 (100 mm x 4.6 mm, 3.5 µm) column using a mobile phase consisting of methanol and ammonium acetate buffer 5 mM (50:50 v/v). The RT of Metoclopramide hydrochloride and internal standard was 1.1 and 2.1 minutes. The method was validated over a concentration range of 0.532 ng/mL to 201.005 ng/mL for Metoclopramide. The lower limit of quantization (LLLQ) was established at 0.532 ng/mL for Metoclopramide. Plasma sample analysis were performed by using a validated high performance liquid chromatography mass spectrometric method.[5]

Dissolution data analysis

The *in vitro* drug release was calculated by using similarity factor (*f*2) and dissolution profile of all formulations were determined by scheming the cumulative percent of Metoclopramide hydrochloride versus time. The in-vitro drug release profile of the three SR formulations were compared using the similarity factor (*f*2) as mentioned in the following equation:

$$f2 = 50 \log \left[\begin{matrix} n \\ 1 + 1/n \sum_{t=1} (Rt - T_t)^2 \end{matrix} \right]^{-0.5} \times 100 \quad \text{(Equation 1)}$$

where R_t and T_t are the percent dissolved at each time point for the reference and test product respectively.

The f_2 equation is a logarithmic transformation of the sum of squares of the difference between test and reference formulation. The f_2 average difference between 0 and 100. If the value of f_2 is less than 50 dissolution profiles wee considered dissimilar. If f_2 value 50 suggests that the reference and test product release curves differ by at least 10% and f_2 values greater than 50 (between 50 and 100) make sure similarity or uniformity between two dissolution profiles. The percent drug dissolved versus time calculated by using weibull method.

Clinical pharmacokinetic data analysis

The plasma concentration-time data were estimated by using Phoenix 6.4.0 version software. Maximum plasma

concentration observed for the subject was Cmax and time to take place was Tmax. Area under the plasma concentration-time profile estimated by the trapezoidal method. The rate constant (K) describing the terminal slope of mean plasma concentration- time profile was determined by linear least squares fitting of the natural logarithm (Ln) transformed terminal plasma concentrations vs. time to the equation for a straight line. The elimination rate constant was estimated by linear regression of linear portion of logarithm of the concentrations versus time. The percent of drug absorbed versus time estimated by using deconvolution technique.

In vitro In vivo Correlation

Level A correlation was developed as mentioned in the FDA and USP guidelines by using the data obtained in the *in vivo* study. The percent of drug dissolved was estimated by plotting time points against the drug release profile and the fraction of drug absorbed was derived by using Wagner–Nelson method. Linear regression analysis was used to inspect the relationship between percent of drug dissolved and percent of drug absorbed. The correlation developed by using *in vitro* and *in vivo* data.

IVIVC Model validation

The objective is to validate the prediction of *in vivo* performance from *in vitro* data. The validation explain how well the developed IVIVC model predicts the *in vivo* data when *in vitro* data applied. The percentage prediction error of Cmax and AUC estimated by using equation mentioned below:

$$\%\ PE_{Cmax} = \frac{observed - predicted}{observed} \times 100$$

Internal validation includes the cross validation of one formulation with other three formulation used for IVIVC model development. Internal validation is optional for IVIVC analysis and provides base for the suitability of the model. and external validation is based on how good IVIVC predicts the additional set of data. As described on FDA guidance IVIVC model is valid when the internal predictability (absolute PE) of Cmax and AUC for each formulation should not exceed 15% and with the average should not exceed 10% (percentage), and for external validation the prediction errors of Cmax and AUC should not exceed 10% and values between 10% to 20% and more than20% is not acceptable.

RESULTS

In vitro studies

The *in vitro* drug release studies were conducted at different pH conditions (namely pH 1.2, 4.5, 5.5, 6.8, 7.4) to check the release and to select the optimum pH condition for the drug. The percentage fraction dissolved for Metoclopramide hydrochloride of marketed immediate release, fast, medium and slow sustained release tablets and percentage drug release at different time points are presented in Table 1 & Figure 1.

DISCUSSION

In vitro and *in vivo* studies were performed for the developed sustained release formulation i.e. fast, medium and slow sustained release tablets. Dissolution studies were performed at pH 1.2 for 50 rpm, the release of the drug was unfinished and showed maximum up to 31.98%, pH 4.5 at 50 rpm, the drug release was slow over the last time point and maximum release up to 85.64% pH 5.5 at 50 rpm drug release was homogeneous and observed 94.50-99.23% over 24 hours, pH 6.8 and pH 7.4 at 50 rpm, drug released up to 91.08-95.21% and 89.57-90.47%. To check rpm effect dissolution tests performed at pH 1.2 , pH 4.5, pH 5.5, pH 6.8 and pH 7.4 at 75 rpm not much variations observed. Therefore for pH 5.5 at 50 rpm formulation optimized as slow, medium and fast release. The *in vitro* release characteristics Metoclopramide hydrochloride Sustained Release formulations were determined and the percentage drug release at various time intervals. The optimized formulation at pH 5.5 were reformulated i.e. Metoclopramide hydrochloride SR Tablets (Slow, Medium & Fast) and dissolution test performed for test and reference formulation at pH 6.8 as per the USP method. The percentage fraction dissolved for Metoclopramide hydrochloride are in the rank order of marketed immediate release, fast, medium and slow sustained release tablets and percentage drug release at different time points are presented in Table 1 & Figure 1. The f2 values are calculated for fast versus slow f2 value is 47.12 at the borderline, fast versus medium and medium versus slow formulation observed higher than 50 confirms and for formulation that these dissolution mediums are indistinguishable and ensures sameness or equivalence between the two dissolution profiles.

The mean Metoclopramide pharmacokinetic parameters AUClast and AUCINF_obs were reasonably higher for marketed release tablet compare to the test formulation. Followed by lower elimination rate and higher half life for fast and medium release observed compare to slow release and marketed tablet. The mean pharmacokinetic results are presented in Figure 2 and Table 2.

A Level A correlation was developed by building IVIVC plot using percent dissolved versus percent absorbed for all three formulations. The slope of the best-fit line was

observed for percentage of drug dissolved and the percentage of drug absorbed. The correlation coefficient (r^2) for was 0.9025, 0.8905 and 0.8685 are presented in Figure 3, 4 and 5 respectively. A good linear regression relationship was observed.

IVIVC Validation

The percentage prediction errors for C_{max} and AUC were calculated by using marketed Metoclopramide hydrochloride immediate release tablets as a target formulation. The C_{max} prediction errors for the fast, medium and slow

Table 1: Percentage Cumulative Release of Metoclopramide Hydrochloride SR Tablets (E2 Slow, E4 Medium & E5 Fast)

Time	Slow (E2)		Medium (E4)		Fast (E5)	
	% Release	% Cumulative Release	% Release	% Cumulative Release	% Release	% Cumulative Release
0.0	0.00	0.0	0.00	0.00	0.00	0.00
0.50	12.60	10.70	15.40	13.00	19.20	11.90
1.00	17.70	17.77	20.50	20.59	24.20	24.31
1.50	26.10	26.20	31.70	31.81	34.30	34.43
2.00	32.90	33.05	38.50	38.68	42.60	42.79
2.50	39.60	39.78	49.70	49.91	53.50	53.74
3.00	48.00	48.22	57.40	57.68	61.00	61.30
4.00	53.90	54.17	63.40	63.72	67.70	68.04
6.00	58.10	58.40	69.30	69.65	76.10	76.48
8.00	66.50	66.82	76.20	76.59	79.40	79.82
10.00	73.30	73.67	80.50	80.92	85.20	85.64
12.00	78.30	78.71	86.50	86.95	89.40	89.87
18.00	85.10	85.54	92.50	92.98	95.30	95.80
24.00	91.80	92.27	95.00	95.51	98.60	99.13

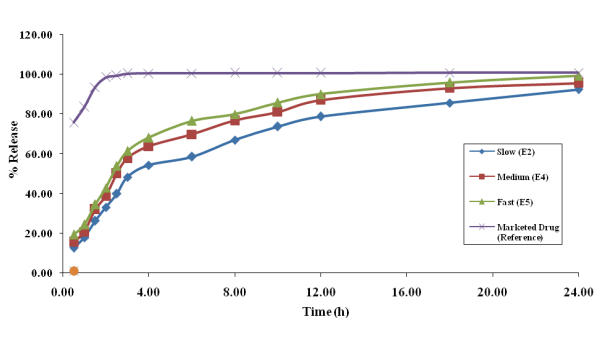

Figure 1: Percentage Cumulative Release of Metoclopramide Hydrochloride SR Tablets (Slow, Medium, Fast & Marketed Drug)

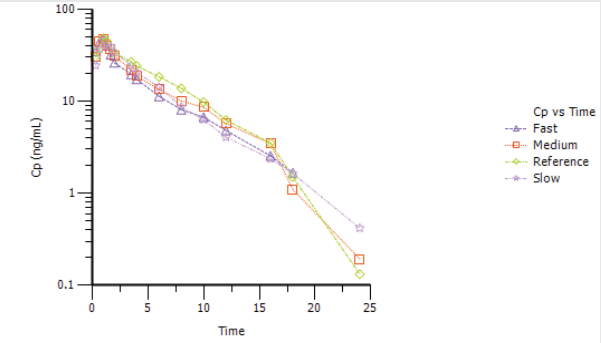

Figure 2: Mean plasma concentrations versus time of Metoclopramide Hydrochloride for Marketed Immediate Release and Sustained Release Tablet (Fast, Medium & Slow)

Table 2: Mean Pharmacokinetic Parameter of Metoclopramide Hydrochloride for Marketed Immediate Release and Sustained Release Tablet (Fast, Medium & Slow)

Formulation	Kel (1/hr)	Half life (hr)	Tmax (hr)	Cmax (ug/mL)	AUClast (hr*ug/mL)	AUCINF_obs (hr*ug/mL)
Immediate Release Tablet	0.195	3.587	1.11	49.06	267.393	273.694
Fast Sustained Release Tablet	0.186	3.749	0.89	48.433	205.467	214.506
Medium Sustained Release Tablet	0.199	3.729	0.89	48.004	230.418	238.671
Slow Sustained Release Tablet	0.221	3.564	1.00	48.366	222.996	230.834

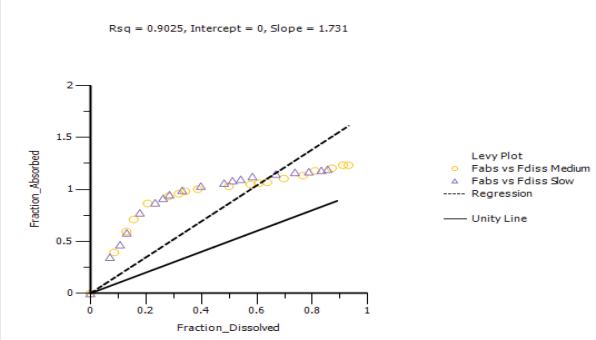

Figure 3: IVIVC Model Linear Regression Percentage Dissolved and Percentage Absorbed for Metoclopramide Hydrochloride and Slow SR Tablet

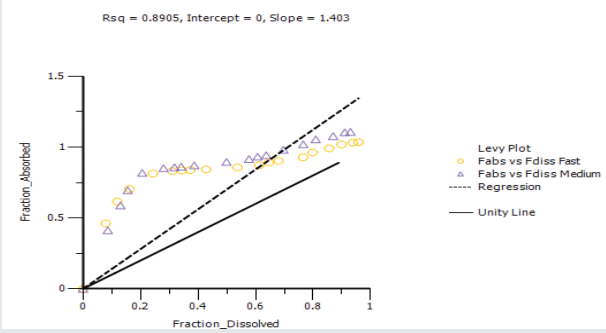

Figure 4: IVIVC Model Linear Regression Percentage Dissolved and Percentage Absorbed for Metoclopramide Hydrochloride Fast and Medium SR Tablet

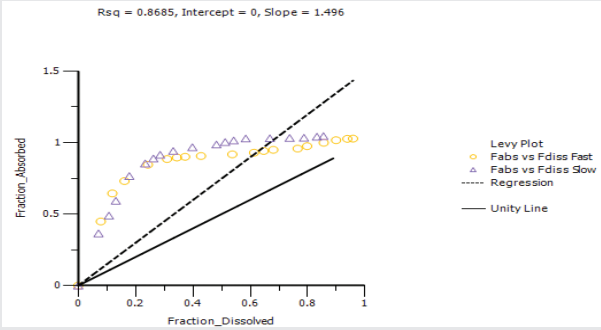

Figure 5: IVIVC Model Linear Regression Percentage Dissolved and Percentage Absorbed for Metoclopramide Hydrochloride Fast and Slow SR Tablet

formulations were found to be -12.356%, -16.049% and -24.612% respectively and AUC values were -22.484%, 7.461% and 7.349% for respectively refer Table 3.

The C_{max} prediction errors prediction errors for Metoclopramide hydrochloride SR tablets i.e. fast, medium, slow and marketed immediate release formulations were estimated and results are observed -56.178, -58.401, -62.407 and 3.349, respectively and for AUC values are -38.758, -40.158, -41.836 and -27.405 respectively. The

Table 3: Prediction errors (%) associated with Cmax and AUC for Metoclopramide Hydrochloride Fast, Medium and Slow SR Tablets

Formulation	Parameter	%PE
Fast Internal	AUClast	-22.484
Fast Internal	Cmax	-12.356
Medium Internal	AUClast	7.461
Medium Internal	Cmax	-16.049
Slow Internal	AUClast	7.349
Slow Internal	Cmax	-24.612

study was conducted to examine, Level A IVIVC relating the complete time-course of plasma concentrations was developed and validated internally and externally for Metoclopramide hydrochloride formulation i.e. fast, medium and slow SR tablets and marketed immediate release tablet. The validation of internal and external predictability was successfully accomplished. The prediction error for C_{max} and AUC was within the specified limit as per the FDA guidance hence, the IVIVC is well thought-out as validated both in terms of internal and external validation. Thus, this IVIVC model may be used during process change, site change, to reduce the number of human studies during the formulation development and serve as a surrogate for *in vivo* bioavailability, to support biowaivers, to support dissolution methods and specification settings. It can also assist in quality control for during the scale-up and post-approval changes (SUPAC).

CONCLUSION

The developed *in vitro* methods can act as a surrogate for *in vivo* bioavailability study and support biowaivers, assist in quality control during scale-up and post-approval changes. It may be useful in predicting the variation in site change, process changes and to predict the absorption performance of Metoclopramide Hydrochloride products with different release rates.

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CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ABBREVIATION USED

nm: Nanometer, Liquid chromatography with tandem mass spectrometry (LC-MS-MS); **°C:** Degree Celsius; **ml:** Milliliter; **ng/mL:** Nanogram/milliliter; **HPMC:** Hydroxypropyl methylcellulose; **mm:** Millimeter; **USP:** United State of Pharmacopeia: **FDA:** Food and Drug Administration; **Cmax:** Maximum plasma concentration; **Tmax:** Time of the maximum plasma concentration; **AUClast:** Area under the plasma concentration-time curve from time zero to the time of the last quantifiable concentration; **AUC INF_obs:** Area under the plasma concentration-time curve from time zero extrapolated to the infinite time; **Kel:** Elimination rate constant; **%:** Percentage.

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SUMMARY

- Level A *in vitro* and *in vivo* correlation was developed by comparing percent dissolved versus the percent absorbed of Metoclopramide Hydrochloride fast, medium and slow sustained release and marketed immediate release tablets.
- *In vitro* and *in vivo* correlation can be used in the development of new drug to decrease the number of human studies during the formulation development and optimization.

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