Application of in vitro – in vivo Correlation as a Predictive Tool for Bioequivalence of Generic Paracetamol Immediate Release Oral Tablets

R. ATEBE[1]*, L. J. TIROP[2], S. M. MARU[2] AND K.A.M. KURIA

[1]*Regal Pharmaceuticals Ltd, P.O. Box 44421-00100 Nairobi GPO*
[2]*Department of Pharmaceutics and Pharmacy Practice; University of Nairobi, P. O. Box 19676 – 00202, Nairobi.*

An *in vitro – in vivo* correlation (IVIVC) model can predict the bioavailability of some drug substances, and mitigate against the high cost of bioequivalence studies, long generic product development lead times, and the exposure of human subjects to drug substances they do not need. In this study a total of three batches of generic paracetamol immediate release oral tablets and one batch of the comparator product, were subjected to dissolution testing to generate a dissolution profile from which the blood drug concentration – time profile and specifically the bioavailability parameters AUC and C_{max} were computed using an IVIVC tool. Statistical analysis demonstrated sameness between the generic product and the reference product. The IVIVC method can thus be a surrogate for *in vivo* human studies, providing a scientific justification for biowaiver for generic products of candidate drug substances.

Key words: paracetamol, *in-vitro in-vivo* correlation, generic, bioequivalence, dissolution profile

INTRODUCTION

The bio-pharmaceutical properties of a drug substance as elucidated in the WHO Biopharmaceutics Classification System (BCS) and the drug product characteristics determine whether an *in vivo* or *in vitro* method should be used to demonstrate interchangeability of a generic drug product with the innovator product [1].

Both BCS Class I (high aqueous solubility and high permeability) and to a lesser extent Class III (high aqueous solubility and low permeability) APIs, where sufficient information concerning the API is available to complete an accurate risk-based assessment for the use of this approach, are considered to be eligible for biowaiver. Products containing BCS class IV (low aqueous solubility and low permeability) APIs are excluded from the BCS-based biowaiver procedure. In the EU and countries using the WHO criteria, products containing Class III APIs are only eligible for biowaiver if they are very rapidly dissolving. Class II APIs (low aqueous solubility and high permeability) are only eligible for the biowaiver procedure in countries using the WHO criteria and even then only in the case of weak acids that are highly soluble at pH 6.8. A drug with a narrow therapeutic range cannot be considered for a biowaiver due to safety considerations [2].

The *in vitro – in vivo* co-relationship (IVIVC) is "a predictive mathematical model describing the relationship between an in vitro property of a dosage form and a relevant in vivo response such as plasma drug concentration or the amount of drug absorbed". There is no causality relationship [3]. Three levels of the IVIVC relationship, A, B and C are reported [4]. Level A correlation is "generally linear representing a point-by-point relationship between *in vitro* dissolution and the *in vivo* input rate (such as *in vivo* dissolution of the drug from the dosage form)'. This level predicts "the entire *in vivo* time course from the *in vitro* data" and is also recommended by the US FDA, the EMEA and Canada's Therapeutic Products Directorate as being the most useful for medicines evaluation and registration; discouraging the use of human and animal studies for evaluation of pharmaceutical products [5, 6].

*Author to whom correspondence may be addressed. Email: rogers.atebe@gmail.com

Paracetamol, the model drug selected for this study, has been used extensively for its analgesic and antipyretic activity for over fifty years. The paracetamol biowaiver monograph though available in some regulatory jurisdictions, has not been adopted locally. Studies have reported that paracetamol is a BCS Class III drug that possesses borderline BCS Class I properties. Kalantzi, *et al*, in a 2006 review of the biowaiver monograph for paracetamol immediate release oral tablets, recommended that the biowaiver could be accepted if the test product contains the same excipients *in their usual amounts and is rapidly dissolving*, and also *fulfills the criterion of similarity of dissolution profiles to the reference product* [7]. The main objective of this study was to determine whether there was any difference between the pharmacokinetic parameters obtained from dissolution data using the IVIVC predictive model for generic paracetamol tablets and those obtained for a registered reference product under identical test conditions.

METHODS AND MATERIALS

Product selection

The selected generic product, manufactured at a licensed site within the East African Community (EAC), was formulated using optimal quantities of prequalified excipients and manufactured via a validated procedure [8].The reference product possessed marketing authorizations in the EAC.

Equipment, reagents and dissolution method

The dissolution test was carried out as per the procedure specified in the BP monograph for the *Dissolution Test Tablets and Capsules (Dissolution Test for Solid Dosage Forms)*, which is *PhEur method 2.9.3*. The test was conducted using the *Electrolab TDT-06P* tablet dissolution tester manufactured by *Electrolab* of *401, Tripati Industrial Estate, I. B. Patel Road, Bombay India*, that complied with USP, IP and BP specifications. Dissolution apparatus type 2 (paddle type) was employed at a speed of 50 rpm.

The media pH specifications prescribed for dissolution testing are pH 1.2 simulating fasted state gastric content, pH 4.5 for fed state and pH 6.8 simulating the pH of the small intestine. Phosphate buffer 900mL of pH 6.8 was selected for the present study. The pH of the entire gastrointestinal tract is lower than the model drug's pKa of 9.5, ranging from 1 in the stomach to nearly 8 in the distal end of the small intestinal. When given orally, paracetamol, which is a weak organic acid, is mostly un-ionized in the stomach, favoring diffusion through the gastric mucosa. However, most drug absorption occurs in the small intestine because the surface area is larger (over 94% of gastrointestinal surface area), and membranes are more permeable than those in the stomach [9].

Potassium Phosphate monobasic reagent used was supplied by *Finar Limited, 184-186 ChacharwadiVasna, Ahmedabad 382110, Gujarat, India*. Sodium Hydroxide Pellets used was sourced from *Central Drug House (P) Ltd, 7/28 Vardaan House, Daryaganj, New Delhi – 11002, India.* The drug absorbance was determined at a wavelength of 257nm using *Shimatzu UV1700* spectrophotometer supplied by *Shimatzu Corporation, 3 Kanda-Nishikicho 1-Chome, Chiyoda-ku, Tokyo 101-8448, Japan.*

Methods of comparing dissolution profile data

The dissolution profiles of twelve tablets per batch of either the test product or the reference product were obtained under identical testing conditions and compared. ANOVA univariate and multivariate analysis were used to quantify differences in dissolution percentages at each sampling time point. The model independent fit factors as described by Moore and Flanner (1996); the difference factor f_1 and the similarity factor f_2 were calculated.

Prediction of pharmacokinetic parameters

The IVIVC tool used was a spreadsheet software (*Microsoft Excel*, by *Microsoft Corporation*) in

which the computation formulae are embedded [10]. In this model, *in-vitro* data were converted into a mathematical equation expressing a linear relationship represented by the general formula shown in Equation 1:

$$Y = mX + C \qquad \text{Equation 1}$$

Where Y is the *in vivo* absorbed drug, X the *in vitro* drug dissolved, m is the slope of the relationship, and C is the Y-intercept. For a linear relationship, as is the case in immediate release formulations, m=1 and C=0. For modified release formulations, the IVIVC model will require time-scaling and time-shifting parameters added.

Further, Equation 2 below was used to compute the percentage of paracetamol dissolved:

The dissolution profile was then converted into discrete dosage segments and the amount of drug in the blood calculated (Equation 3).

The amount of drug in the blood was computed using the bioavailability factor (F) for the drug (75.5%) using Equation 4:

$$\%\ Paracetamol\ dissolved = \frac{SmpAbs}{StdAbs} \times WtStd \times \frac{9}{5} \times \frac{Av.Wt}{Wt\ of\ tab} \times \frac{Potency}{100}$$

Equation 2

$$Amt\ of\ drug\ released = \%\ Drug\ released \times \frac{Product\ strength}{100}$$

Equation 3

$$Amt\ of\ drug\ in\ the\ blood = Amt\ of\ drug\ released \times Bioavailability\ factor\ (F)$$

Equation 4

The blood drug concentration was then calculated using the Equation 5:

$$C_t = \frac{Amt\ of\ drug\ in\ blood\ x\ F}{V_d\ x\ Body\ weight}$$

Equation 5

where C_t is the drug concentration in blood, V_d the apparent volume of distribution for the drug, and F is the bioavailability factor of the drug in blood.

From the above calculations, the drug elimination constant, k_{el}, was obtained from the drug elimination half-life using their reciprocal relationship.

The blood drug concentration versus time profile was obtained readily from the dissolution curve using widely reported pharmacokinetic parameters (k_{el}, V_d and F) to predict corresponding blood drug levels and compute the bioavailability parameters C_{max} and AUC. Paracetamol pharmacokinetic parameters reported separately by Gilman *et al* [11], namely a k_{el} of 0.2235h^{-1}, an F value of 75.5% at normal doses and V_d value of 1.025L/Kg were used in the present study. A body weight of 70Kg for a "physiological man" was used [11].

Statistical analysis

Both the *in vitro* dissolution data and the predicted bioavailability parameters were subjected to statistical analysis, using open source *IBM STATISTICS (SPSS) Version 21* statistical analysis software, to compare the test product with the reference product at within 95% confidence interval at a significance level, *p*-value, of 0.05.

RESULTS AND DISCUSSION

Dissolution profiles

The dissolution profiles for batches A1, A2 and A3 for the generic Paracetamol formulations and the dissolution performance of the reference product are presented in Table 1.

Pharmacokinetic profile predicted using IVIVC

The amount of drug dissolved and the predicted drug absorption, in mg, was calculated for each of the batches of the test product and the reference product, at the end of each sampling time, i.e. 5min, 15min, 25min, 35min and 45min (Table 2).

The predicted drug absorption was calculated taking into account the percentage dissolution, product strength, and the absolute bioavailability (F of 75.5%). Table 3 presents the predicted pharmacokinetic parameters computed using drug parameters from literature [11]; k_{el} of $0.2235h^{-1}$ and V_d of 1.025L/Kg.

Figures 1 and 2 present the average blood drug concentration versus time profiles for the three test product batches, and that of the reference product batch respectively.

Table 1: Dissolution profile data (% API released) for generic and reference product [Standard deviation]

	Mean dissolution % (Standard deviation)					
	0	**5min**	**15min**	**25min**	**35min**	**45min**
Generic, Batch A1	0	70.39 (6.79)	82.50 (4.49)	88.80 (3.72)	92.90 (3.01)	96.59 (1.95)
Generic, Batch A2	0	71.29 (8.09)	84.32 (7.33)	89.30 (2.85)	92.67 (2.11)	96.30 (2.87)
Generic, Batch A3	0	64.71 (9.53)	82.42 (0.95)	91.06 (3.21)	94.76 (1.89)	96.29 (1.80)
Generic, Mean	0	68.80 (8.50)	83.08 (4.93)	89.72 (3.33)	93.44 (2.51)	96.39 (2.20)
Reference Batch	0	71.45 (3.54)	83.45 (2.91)	90.05 (2.99)	93.98 (2.08)	97.51 (1.56)

Table 2: Amount of drug dissolved and predicted drug absorption calculated at the end of each sampling time interval

Time (h)	% Drug dissolved (Cumulative)	% Drug dissolved (within sampling interval)	Amount of drug dissolved (mg) (within sampling interval) Tablet Strength (500 mg)	Predicted drug absorption (mg) corrected for bioavailability (F)
Batch A1				
0	0.0	0.00	0.00	0.000
0.08	70.4	70.39	351.95	265.719
0.25	82.5	12.11	60.57	45.731
0.42	88.8	6.29	31.46	23.751
0.58	92.9	4.11	20.53	15.500
0.75	96.6	3.69	18.45	13.933
Batch A2				
0	0.0	0.00	0.00	0.000
0.08	71.3	71.29	356.46	269.126
0.25	84.3	13.03	65.15	49.188
0.42	89.3	4.97	24.87	18.774
0.58	92.7	3.37	16.86	12.731
0.75	96.3	3.63	18.15	13.706
Batch A3				
0	0.0	0.00	0.00	0.000
0.08	64.7	64.71	323.53	244.268
0.25	82.4	17.72	88.58	66.874
0.42	91.1	8.63	43.17	32.591
0.58	94.8	3.71	18.53	13.990
0.75	96.3	1.53	7.63	5.757
Reference batch				
0	0.0	0.00	0.00	0.000
0.08	71.5	71.45	357.27	269.739
0.25	83.5	12.00	60.00	45.300
0.42	90.0	6.59	32.95	24.880
0.58	94.0	3.94	19.68	14.861
0.75	97.5	3.53	17.63	13.307

Table 3: Predicted pharmacokinetic profiles of batches

Batch A1									
Dissolution sampling time (h)	0	0.08	0.25	0.42	0.58	0.75			
Amt. (mg) equivalent	0.00	265.72	45.73	23.75	15.50	13.93			
Time after absorption (h)	**Blood Amt after Absorption, mg**						**Cumulative blood amt after absorption (mg)**	**Conc. (ng/ml)**	**AUC (ng.h/ml)**
0	0.00						0.00	0.00	
0.08	0.00						0.00	0.00	0.00
0.25	0.00	265.72					265.72	3703.40	308.62
0.42	0.00	246.64					246.64	3437.53	595.08
0.58	0.00	237.62	45.73				283.36	3949.20	615.56
0.75	0.00	228.94	40.90				269.83	3760.71	642.49
								3949.20	**2161.75**
Batch A2									
Dissolution sampling time (h)	0	0.08	0.25	0.42	0.58	0.75			
Amt. (mg) equivalent	0.00	269.13	49.19	18.77	12.73	13.71			
Time after absorption (h)	**Blood Amt after Absorption, mg**						**Cumulative blood amt after absorption (mg)**	**Conc. (ng/ml)**	**AUC (ng.h/ml)**
0	0.00						0	0.00	
0.08	0.00						0.00	0.00	0.00
0.25	0.00	269.13					269.13	3750.89	312.57
0.42	0.00	249.80					249.80	3481.60	602.71
0.58	0.00	240.67	49.19				289.86	4039.85	626.79
0.75	0.00	231.87	43.99				275.86	3844.71	657.05
								4039.85	**2199.11**
Batch A3									
Dissolution sampling time (h)	0	0.08	0.25	0.42	0.58	0.75			

Amt. (mg) equivalent	0.00	244.27	66.87	32.59	13.99	5.76			
Time after absorption (h)	**Blood Amt after Absorption, mg**						**Cumulative blood amt after absorption (mg)**	**Conc. (ng/ml)**	**AUC (ng.h/ml)**
0	0.00						0	0.00	
0.08	0.00						0.00	0.00	0.00
0.25	0.00	244.27					244.27	3404.43	283.70
0.42	0.00	226.73					226.73	3160.01	547.04
0.58	0.00	218.44	66.87				285.31	3976.51	594.71
0.75	0.00	210.45	59.80				270.26	3766.65	645.26
								3976.51	**2070.71**
Reference Batch									
Dissolution sampling time (h)	0	0.08	0.25	0.42	0.58	0.75			
Amt. (mg) equivalent	0.00	269.74	45.30	24.88	14.86	13.31			
Time after absorption (h)	**Blood Amt after Absorption, mg**						**Cumulative blood amt after absorption (mg)**	**Conc. (ng/ml)**	**AUC (ng.h/ml)**
0	0.00						0.00	0.00	
0.08	0.00						0.00	0.00	0.00
0.25	0.00	269.74					269.74	3759.44	313.29
0.42	0.00	250.37					250.37	3489.54	604.08
0.58	0.00	241.22	45.30				286.52	3993.30	623.57
0.75	0.00	232.40	40.51				272.91	3803.62	649.74
								3993.30	**2190.68**

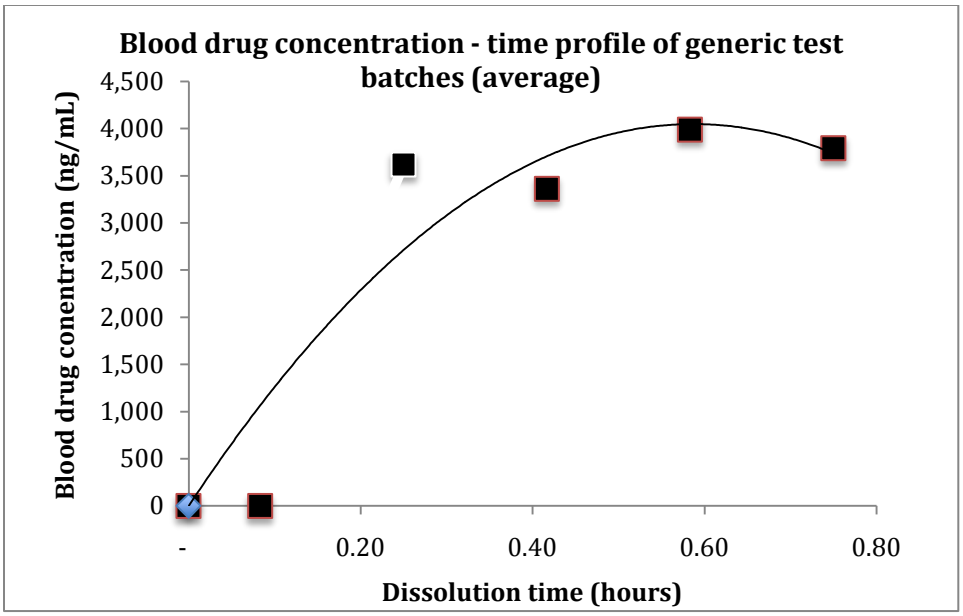

Figure 1: Predicted blood drug concentration-time profile for three generic batches (Average)

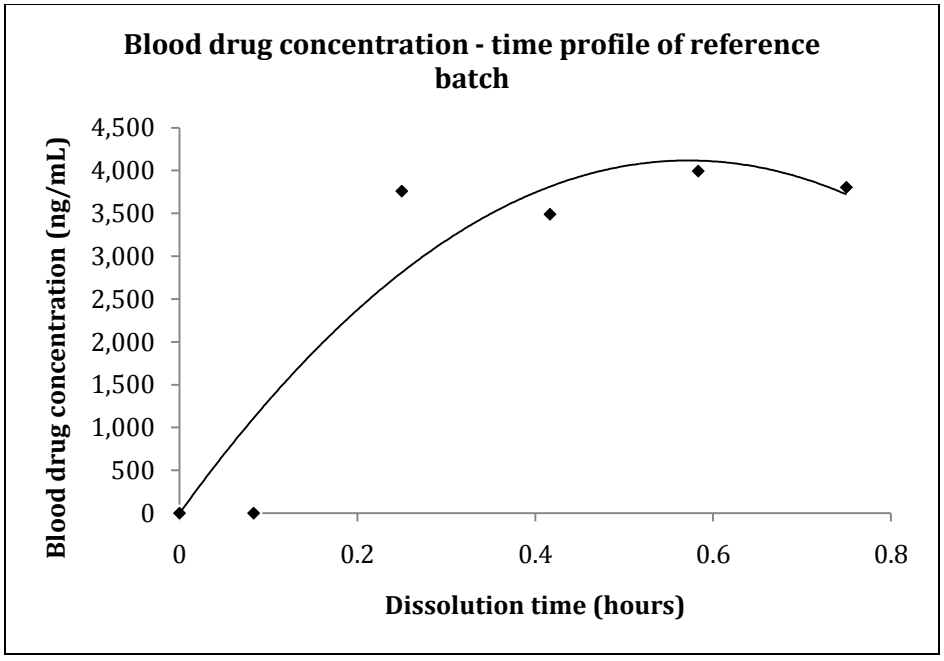

Figure 2: Predicted blood drug concentration-time profile for reference product

Statistical analysis

Intra-batch variability

Analysis of intra-batch variability was done for each of the three batches of the test product; and the intra-batch variability outcome per batch was compared among the three batches. The obtained *p*-value of 0.102 suggests that there is no difference in the dissolution values among the tablets within the same batch, across all batches.

Inter-batch variability

The statistical difference in dissolution between reference and the test product batches at the different time points gave *p*-values of ≥ 0.05 at each time point for each of the three experimental batches, indicating that there was no difference in dissolution time profile between the experimental batches and the reference product.

Comparison of dissolution profiles between test product and reference product

From the p-values obtained across the dissolution sampling time points for the test product and the reference product ($p \geq 0.05$ in all cases), it can be concluded that there was no statistical difference between the results obtained for the test and generic products.

Analysis of variance

ANOVA was performed on the data obtained from dissolution tests on the reference and the experimental products and the statistics obtained a *p*-value of 0.528 suggesting that the reference and experimental products have comparable dissolution values across the five sampling time periods.

Calculation of comparative dissolution profile fit factors (f_1 and f_2)

Following the model independent method by Moore and Flanner [12] using fit factors; the difference factor f_1 and the similarity factor f_2 were calculated as per the US FDA and the European Medicines Agency guidelines. For curves to be considered similar, f_1 values should be close to 0 (0 to 15), and f_2 values should be greater than 50 (50 to 100).

The calculated f_1 and f_2 values (Table 4) for the three experimental batches fell within the aforementioned acceptance ranges, indicating that the three batches of test product had similar dissolution-time behavior to that of the reference product under identical test conditions.

Table 4: Fit factors for the dissolution profiles

	Batch A1	Batch A2	Batch A3
f_1 value	0.94	1.04	0.83
f_2 value	65.01	66.12	68.33

The predicted C_{max} and AUC values were compared by ANOVA to determine variability between the batches of the experimental product and the reference product. C_{max} values gave a *p*-value (two tailed) of 0.95 while the AUC values gave a *p*-value (two-tailed) of 0.99. ANOVA suggests that the reference and experimental products have comparable C_{max} and AUC values predicted using the IVIVC tool.

CONCLUSION

The predictive value of the IVIVC tool has been demonstrated for generic paracetamol tablets, when compared to a reference product. The p-values obtained from the dissolution data depict that there is no statistical difference between the dissolution behavior of the test product and that of the reference product. There was also no difference between the test product and reference product with respect to the pharmacokinetic parameters predicted using an IVIVC tool. Furthermore, paracetamol has a wide therapeutic window and the public health consequences for any comparative dissolution differences are not serious. Thus refraining from *in vivo* bioequivalence studies can be scientifically justified. IVIVC provides both ethical and economic benefits to the pharmaceutical product development process. It also makes the exposure of human subjects to drug substances they do not need unnecessary. It

is *a simple and practical approach* for demonstrating drug bioavailability [9]. IVIVC is recommended for routine use in the evaluation and registration of applicable medicines.

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