APPLICATION OF IN VITRO – IN VIVO CORRELATION (IVIVC) AS A PREDICTIVE TOOL FOR BIOEQUIVALENCE STUDIES FOR GENERIC PARACETAMOL IMMEDIATE RELEASE TABLETS

A dissertation submitted in partial fulfillment of the requirements for the award of the degree of Master of Pharmacy in Industrial Pharmacy of the University of Nairobi

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DECLARATION

This dissertation is my original work and has not been presented elsewhere for award of any degree.

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Supervisors' approval

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DEDICATION

To my mother Esther Mbera and my father Jackson Atebe who raised me up with affirmation on the principles of integrity and doing my honest part in life.

To my dear wife Grace Nyansiaboka and our children Brian Mogoi, Sharon Osebe, Craig Ong'ang'a and Charles Onsinyo, who have always urged me to carry on to completion.

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Except the Lord build the house, they labor in vain that build it: except the Lord keep the city, the watchman wakes but in vain [Psalm 127:1].

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I treasure the unwavering support I received from my dear wife and our beloved children who urged me on throughout the project and the program.

Above all, I give all honor and glory to the Almighty God through whose providence this project was completed.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
API	Active Pharmaceutical Ingredient
Av	Average
Av.Wt	Average weight
BCS	Biopharmaceutics Classification System
BE	Bioequivalence
BP	British Pharmacopoeia
C_t	Drug concentration at time t
DDSolver	A freely available add-in program for modeling and comparison of drug dissolution profiles
EAC	East African Community
EAC-MRH	East African Community Medicines Regulatory Harmonization
EMEA	European Medicines Agency
EU	European Community
F	Bioavailability factor
FaSSIF	Fasted state simulated intestinal fluid
FeSSIF	Fed state simulated intestinal fluid
FBE	Fluidized Bed Equipment
FDA	Food and Drugs Authority
GMP	Good Manufacturing Practices
HBSS	Hank's balanced salt solution

IBM	International Business Machines Corporation
IR	Immediate release
IMI	The [European Union] Innovative Medicines Initiative (IMI)
IVIVC	In vitro – in vivo correlation
MA	Marketing authorization
Max	Maximum
MER	Medicines Evaluation and Registration
Min	Minimum
MR	Modified release
NCE	New Chemical Entity
NDA	National Drug Authority
NLT	Not less than
NMRA	National Medicines Regulatory Authority
NMT	Not more than
OrBiTo	Oral Biopharmaceutical Tools
PE	Prediction error
PhEur	European Pharmacopoeia
PK	Pharmacokinetic
RLD	Reference Listed Drug
RPM	Revolutions per minute
SmpAbs	Absorbance of the sample preparation
SPSS	An IBM software package for statistical analysis
SR	Sustained release
StdAbs	Absorbance of the standard preparation
TDR	(United Nations Development Program /World Bank/WHO Special Program for Research and Training in Tropical Diseases).

UNDP	United Nations Development Program
USP	United States Pharmacopoeia
UV	Ultraviolet
V_d	Apparent volume of distribution
VS	Volumetric solution
WHO	World Health Organization

DEFINITION OF TERMS

Bioavailability

The rate and extent to which the active moiety is absorbed from a pharmaceutical dosage form and becomes available at the site(s) of action or in the general circulation.

Bioequivalence

Pharmaceutical products are bioequivalent if they are pharmaceutically equivalent or pharmaceutical alternatives, and they display comparable bioavailability, when studied under similar experimental conditions. Bioequivalence is considered proven, in case the bioavailability parameters, in terms of peak (Cmax and Tmax) and total exposure (area under the curve (AUC) after administration of the same molar dose under the same conditions are similar to such a degree that the effects of the studied products can be expected to be essentially the same.

Biopharmaceutics Classification System (BCS)

The BCS is a scientific framework for classifying active pharmaceutical ingredients based upon their aqueous solubility and intestinal permeability. When combined with the dissolution of the pharmaceutical product, the BCS takes into account three major factors that govern the rate and extent of drug absorption (exposure) from immediate-release oral solid dosage forms: dissolution, solubility, and intestinal permeability.

Biowaiver

The term bio waiver is applied to a regulatory drug approval process when the efficacy and safety part of the dossier is approved based on evidence of equivalence other than through in vivo equivalence testing. A biowaiver can be applied only for products which meet requirements on pharmaceutical similarity, as well as similarity in comparative dissolution tests.

Convolution (with regards to IVIVC model)

Convolution is the process of obtaining blood drug concentration – time profile from dissolution results. It employs dissolution data to predict the blood drug amounts by the use of the intrinsic pharmacokinetic parameters of the drug.

Deconvolution (with regard to IVIVC model)

Deconvolution is the process of using drug concentration – time profile to derive a dissolution profile. It is most suited to establish dissolution test specifications (method, apparatus)

Generic product

A generic product is a medicinal product which has the same qualitative and quantitative composition in active substances and the same pharmaceutical form as the reference medicinal product, and whose bioequivalence with the reference medicinal product has been demonstrated by appropriate bioavailability studies.

Highly soluble drug substance

A drug substance is considered highly soluble when the highest dose strength is soluble in not more than 250 mL water over a pH range of 1 to 7.5 at 37^{o}C.

High permeability drugs

A drug substance is considered highly permeable when the extent of absorption in humans is determined to be at least 85% of an administered dose, based on mass-balance or in comparison to an intravenous reference dose.

Pharmaceutical equivalence

Products are pharmaceutical equivalents if they contain the same molar amount of the same active pharmaceutical ingredient (s) in the same dosage form, if they meet comparable standards, and if they are intended to be administered by the same route.

Rapidly dissolving drug

A drug product is considered to be RAPIDLY DISSOLVING when > 85% of the labeled amount of drug substance dissolves within 30 minutes using USP apparatus I or II in a volume of < 900 mL buffer solutions.

Reference listed drug (RLD)

Reference listed drug is an approved drug product to which new generic versions are compared to show bioequivalence. A drug company seeking approval to market a generic equivalent must refer to the Reference Listed Drug in its Abbreviated New Drug Application. By designating a single reference-listed drug as the standard to which all generic versions must be shown to be bioequivalent, FDA hopes to avoid possible significant variations among generic drugs and their brand-name counterpart.

Therapeutic equivalence

Pharmaceutical products are considered to be therapeutically equivalent if they are pharmaceutically equivalent or pharmaceutical alternatives and after administration in the same molar dose, their effects, with respect to both efficacy and safety, are essentially the same.

LIST OF PHARMACOKINETIC PARAMETERS

$AUC_{(o \rightarrow t)}$ The area under the plasma concentration versus time curve, from time zero to the last measurable concentration, as calculated by the linear trapezoidal method.

$AUC_{0 \rightarrow \infty}$ The area under the plasma concentration versus time curve, from time zero to infinity. $AUC_{0 \rightarrow t}$ plus the ratio of the last measurable plasma concentration to the elimination rate constant.

C_{max} Maximum measured plasma concentration over the time span specified.

T_{max} Time of the maximum measured plasma concentration. If the maximum value occurs at more than 1 time point, T_{max} is defined as the first time point with this value.

K_{el} Apparent first-order terminal elimination rate constant calculated from a semi-log plot of the plasma concentration versus time curve.

$T_{½}$: The apparent first-order terminal elimination half-life was calculated as $\frac{0.693}{k_{el}}$.

ABSTRACT

Medicines regulatory authorities among developing countries, particularly in Africa, are increasingly demanding that data on bioequivalence studies should be included when applying for marketing authorization for some generic products. Generic products for some of the drug substances for which BE data are demanded include common substances which have been in use for decades with good safety and efficacy profile. In addition, biowaiver monographs are available for some of the listed generic formulations, including Paracetamol immediate release tablets. Studies have been carried out to demonstrate that a simple mathematical model, the in vitro – in vivo correlation (IVIVC), can be used to predict bioavailability profile of a drug substance from in vitro dissolution data. The IVIVC tool has not been put into widespread use in some parts of the world especially the poorer countries where the greatest benefits would result, avoiding incurring the high cost of BE studies and reducing generic product development lead time. This study demonstrated how dissolution data are used to predict drug bioavailability by employing an IVIVC tool. Generic Paracetamol immediate release tablets were compared to a registered reference formulation using an IVIVC tool as a surrogate human bioequivalence studies. Three batches of a generic product, and one batch comparator product, were subjected to dissolution testing to generate a dissolution profile. The dissolution profile data were subjected to computation using an IVIVC tool to predict the blood drug concentration time profile, and specifically compute the AUC and C_{max} values. The AUC and C_{max} values obtained for the generic product and those obtained for the comparator product were subjected to statistical analysis to evaluate sameness. On this basis, the usefulness in the application of an IVIVC in generic product development was demonstrated with a possibility of wider application of this model by the drug regulatory authorities and marketing authorization (MA) applicants as a justification for biowaiver for generic formulations of candidate drug substances.

1.0 INTRODUCTION

Medicines regulatory authorities are increasingly requiring that applicants for marketing authorization of generic products must demonstrate pharmaceutical and therapeutic equivalence of their formulations to the corresponding reference innovator products. Four ways are available for demonstrating interchangeability of a generic drug product with the innovator product, and also interchangeability with comparable generic products that are already legitimately on the market. These include the conduct of clinical trials, conduct of comparative pharmacodynamics studies, the conduct of in vivo bioequivalence studies, and also the conduct of comparative dissolution kinetics. The choice of the method to use depends on the bio-pharmaceutical properties of the drug substance and the drug product characteristics (WHO Expert Committee on Pharmaceutical Preparations, 2015). Some classes of generic medicines qualify for waiver of BE requirements (biowaiver).

The target region of interest for this project is specifically the East African Community where the medicines evaluation and registration guidelines are due for harmonization under the East African Community Medicines Regulatory Harmonization (EAC-MRH) project (EAC, 2013). Uganda's national medicines regulatory agency, the National Drug Authority, specified in September 2015 that *exemption of BE studies must be justified even where an oral solid dosage form is a tablet or capsule containing an high solubility API with high permeability, and where the pharmaceutical product has a high dissolution rate* (NDA, 2015).

As late as 2015, regulators in Africa have demanded the conduct of BE studies for commonly used generic products including Paracetamol tablets before registration of the product. Generic Paracetamol tablets have been in use for a long time with no significant safety and efficacy concerns.

Generally, the generic drug products are developed with the pharmacokinetic profile in mind. Developers of generic products use the same excipients and as much as possible similar levels. They set the specifications of their products, including dissolution test specifications, to be as those of the innovator or reference product. They also attempt to use similar manufacturing processes to the RLD. It would therefore be broadly expected that results obtained from in vitro test results, including those for dissolution, conducted under the same conditions as those conducted on the innovator products, could give an assessment of sameness to innovator product (World Health Organization, 2006).

The dissolution test values obtained have a predictive value for the pharmacokinetic (PK) profile of the drug substance. The absorption of a drug and its availability in the blood following oral administration of solid dosage form is important for drug action, and depends on how the drug product releases the active substance, its solubility and its ability to cross the biological membranes into the blood circulation. The aqueous solubility and the membrane permeability of the active pharmaceutical ingredient (API) and its pharmacokinetics depend on its physicochemical properties (EMEA, 2007). The API properties form the basis for the Biopharmaceutics Classification System (BCS) which groups the APIs into broad categories: BCS Class 1 which have high solubility and high permeability, BCS Class II comprising low solubility high permeability drugs, BCS Class III to which belong the high solubility low permeability APIs, and BCS Class IV that has the low solubility and low permeability substances (World Health Organization, 2006). The BCS classes are summarized in Table 1.

Table 1: Biopharmaceutics Classification System

Class	Aqueous solubility	Membrane permeability
I	High	High
II	Low	High
III	High	Low
IV	Low	Low

In the development of anew drug products of new chemical entities (NCE) clinical trials are conducted to demonstrate how safe and efficacious the drug is in the body. The human study test results together with the BCS concept are employed as an aid in setting the dissolution test specifications. The pH solubility profile and pKa of the drug substance are also considered in setting the dissolution characteristics (FDA, 2000).

Medicines regulators in the US and the European Union have discouraged the use of human and animal studies for evaluation of pharmaceutical products where in vitro methods are available to simulate bioequivalence studies (Suankara, 2008; Ostrowski & Baczek, 2010).

2.0 LITERATURE REVIEW

2.1 Quality of generic products

The quality of generic products in Kenya and other parts of the world has been of great concern to the consumers and also to the regulators of medicines.

A study conducted in Kenya between 1983 and 1986, and later in 2007 reported that 46% of generic products in the market did not meet the required quality standards (Kibwage et al., 1992; Chepkwony et al., 2007). A similar study conducted later between 2001 and 2005 reported a lower figure of about 6.1% (Thoithi et al., 2008). In Nigeria, a study of Sulfadoxine-Pyrimethamine antimalarials in the market reported that 41.7% did not meet the required quality standards (Ochekpe et al., 2012).

Various laboratory tests are available for checking the quality of pharmaceutical products depending on their dose forms and the route of administration. Routine quality testing of oral immediate release tablets includes tests for identification, uniformity of weight, disintegration time, dissolution testing and assay (Kibwage et al., 1992).

These tests are not sufficient to demonstrate pharmaceutical equivalence of generic products versus the innovator products on which in vivo bioequivalence studies have been conducted. Comparative dissolution profiles between generics and innovator products and generic versus generics are usually considered useful in demonstrating pharmaceutical equivalence (Manani, 2014).

2.2 In vitro dissolution profile

Dissolution profiles conducted between the generic formulation or lower strengths of a dosage form and the innovator or reference listed drug can be used to waive BE requirements. In principle, the highly soluble drug substances with documented favorable absorption characteristics are eligible for waiver of human BE studies (FDA, 2000).

BCS Class I and to a lesser extent Class III APIs, subject to sufficent risk-based assessment may be considered for biowaiver. Excluded from consideration are BCS class IV APIs. (World Health Organization, 2006).

Despite the stringent requirements for generic medicines to reach the threshold for biowaiver; the high cost of in vivo human bioequivalence studies demand that a cost-beneficial analysis be carried out to avoid the relegation of beneficial generic products to the orphan drug list and infliction of public health concern over the lack of manufacturer interest in drug products which are non-viable commercially due to high development costs.

The dissolution test determines consistent drug release of the product over the entire shelf life, and it can also be used to demonstrate that minor changes in formulation or manufacturing process do not adversely affect the drug release characteristics. It can also be employed to predict the drug bioavalability profile, particularly where solubility is the limiting step. The predictive value of in vitro dissolution in determining in vivo drug concentration-time profiles is also based on the design of the test method itself. Attempts have been made at simulating the complex in vivo physiological system such as the adding appropriate amounts of enzymes and setting the pH level, the disposal of digestive products (sink

conditions), physiological mixing of chime, transit time and peristaltic motion (Lue et al., 2008).

Kostewicz, and others, have provided the background of the various techniques for simulation of gut conditions. They have indicated that the need for better understanding of drug formulation requires development of tools that evaluate the formulation in a bio-relevant and mechanistic manner. This requires the use of complex intraluminal processes (such as solubilization, supersaturation and precipitation) calling for "*development and optimizing innovative, predictive Oral Biopharmaceutical Tools as the main target of the OrBiTo project within the [European Union] Innovative Medicines Initiative (IMI) framework*" (Kostewicz et al., 2014). For research and new drug development purposes, it is important to use bio-relevant media to characterize new drug candidates and screen new formulations. Some of these media that reflect actual physiological conditions such as the fasted state simulated intestinal fluid (FaSSIF), Hank's balanced salt solution (HBSS) and fed state simulated intestinal fluid (FeSSIF).

Simpler dissolution media area needed for routine laboratory use for dissolution testing. A lower degree of simulation of physiological systems has given simpler standardized dissolution test media, and test apparatus operation. These are presented in compendia such as the USP and BP. The in vitro dissolution method used for batch testing for different products of the same API should remain the same (Cardot et al., 2007). Where the same standard method is used then the in vitro dissolution curve will depend upon the release characteristics of the formulation of the drug product and its manufacturing process.

Comparative dissolution profiling of generic formulations against innovator products has been identified as an indicator of pharmaceutical equivalence; and it

in turn reduces the cost of pharmaceutical care through interchangeability with innovator products (Anand et al., 2001; FDA, 1997; Shah, 2001). Comparative dissolution can also be used to determine pharmaceutical equivalence between generic products.

The dissolution test and dissolution profile of a drug product and its absorption are assumed to have a linear relationship (Qureshi, 2010b). The dissolution time profile is a function of the formulation of the solid dose form while pharmacokinetic-time profile depends on the physico-chemical properties of the API. In turn dissolution profile will affect the drug concentration in the blood over time. Conducting dissolution tests makes it possible to draw some inferences with respect to how the drug will behave in vivo. A dissolution test result that does not predict the drug blood concentration – time profile is, indeed, incomplete.

The value of dissolution testing extends beyond formulation development for generic pharmaceuticals and is beneficial for establishing the how changes in formulation and manufacturing process may affect the drug blood concentration (World Health Organization, 2006).

Computational tools have been developed for comparison of the therapeutic usefulness between generic formulations and the innovator products. The *DDSolver* has been cited as being particularly useful as a one stop model that provides effective comparative dissolution statistics including the fit factors (f_1 and f_2), univariate ANOVA among others. Zhang et al., in their research into the DDSolver model concluded that the computer program was capable of computing statistics arising from in vitro comparative dissolution profile data between two formulations (Zhang et al., 2010).

Another useful tool is the IVIVC employed to predict drug concentrations from dissolution test results (Qureshi, 2010b).

Whereas the two tools are of value in evaluation of in vitro dissolution data, DDSolver focuses on comparing the dissolution profiles of two formulations. The IVIVC model is used for its predictive value to estimate the blood drug concentration.

Application of IVIVC tool to a generic formulation was the subject of the present study.

2.3 In vitro – in vivo correlation

Continuous drug release, absorption and disposition has an impact on drug plasma levels (Dressman & Lennernäs, 2000). The mathematical model that links the in vitro dissolution data to the in vivo drug dissolution of the drug prior to its absorption is termed in vitro – in vivo co-relationship (IVIVC). Modi et al., have reported that there is *good fit between predicted and the actual observed levels* (Modi et al., 2000). IVIVC has been employed in product development and for justifying biowaiver applications. The greatest impact of IVIVC has been demonstrated in justifying biowaiver applications in medicine evaluation and registration (Suankra et al., 2008).

IVIVC is employed to give a high degree of assurance on product quality and also make the need to undertake human BE studies less necessary (Qureshi, 2010b). In this way authentic predictions of drug PK characteristics can be made using a predictive model that employs in vitro dissolution data. The latter provide both ethical and economic benefits to pharmaceutical product development. The IVIVC modeling is simpler than in vivo BE study and reduces product development costs and lead times. It also makes the exposure of human subjects to drug substances they do not need unnecessary.

However, an IVIVC may only be expected to be useful among low solubility APIs such as BCS Class II drugs. IVIVC may not be satisfactorily demonstrable in BCS Class III drugs since the rate-limiting step to bioavailability is permeability and intestinal residency time is a key factor affecting the absorption (FDA, 1997). However, biowaiver monographs have been developed for some generic product formulations, including Acetaminophen, which is BCS Class III drug bordering on BCS Class I (Kalantzi et al., 2006).

The value of dissolution profile data lies in their predictive nature when fed into an appropriate tool designed for that purpose. This technique has been referred to as the convolution method, and it is applicable in predicting the blood drug concentrations for sustained release (IR), and immediate release (SR) solid dosage forms.

The FDA has defined IVIVC as "*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"* (FDA, 1997). There's no causality relationship (Cardot et al., 2007). There are three levels in the IVIVC relationship. The first is level A correlation which represents a direct relationship between the in vitro data and the in vivo characteristic for each sampling time point leading to a mathematical relationship for each of the corresponding points. This level will predict. The second, Level B *employs statistical moment analysis that compares in vitro dissolution with in vivo dissolution.* The third is Level C *relates only to an individual time point.* The fourth, Multiple Level C seeks to establish a relationship between PK parameters and several points of the in vitro dissolution profile.

FDA recommends the use of Level A as it is perceived give the most comprehensive information. A Level A IVIVC makes it possible for the in vitro dissolution data obtained to be used on its own in predicting in vivo pharmacokinetic behavior (FDA, 2000). Level A is also the most useful for medicines evaluation and registration (MER). He other levels of IVIVC, namely A, B and C, can be used for developing product formulation, excipient selection, process optimization, and establishing product specifications and quality control standard test procedures. A multiple Level C IVIVC gives a map of several sampling time points of the dissolution profile and one or several of the PK parameters.

IVIVC has been employed for its predictive capacity for IR or modified release (MR) oral solid dosage forms. The complexity in the product design for polymer-based delivery systems and other long term delivery systems limit its general application and calling for sophisticated modeling techniques.

2.4 IVIVC development

Development of a mathematical model that predicts in vivo drug pharmacokinetic behavior from in vitro dissolution profile involves collection of in vivo absorption data after administering a number of formulation strengths of IR and extended release drug product orally and subjecting the same to residual regression analysis. The model is then subjected to both internal and external validation (*see section 2.4.2)*, followed by further confirmation using computer software such as the *GastroPlus* used by Mirza and others (Mirza et al., 2012).

The development of such predictive model, IVIVC, has two main components: IVIVC model development and IVIVC model validation. Development of an accurate IVIVC depends on the formulation matrix, the combination of excipients used, the manufacturing process, the physicochemical properties of the active pharmaceutical ingredient and the dissolution test system employed. A well-developed IVIVC makes it possible for it to be employed as a surrogate for in vivo bioequivalence testing (Suankra Gangadhar, 2008). Kovacevic and others have summarized IVIVC development graphically (Kovaĉević et al., 2009) as shown in Figure 1.

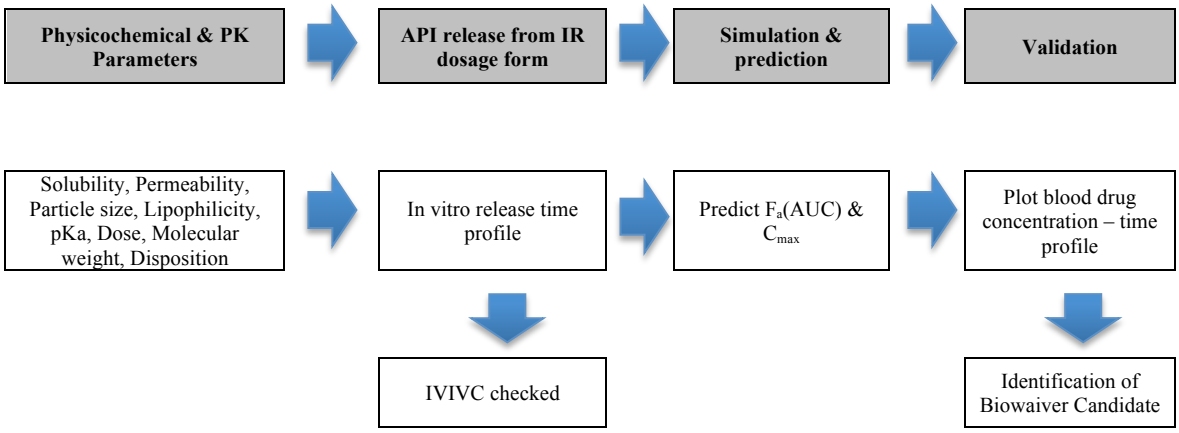

Figure 1: IVIVC development process

2.4.1 IVIVC model development

IVIVC model development has two approaches. The first approach is a two-stage de-convolution method that uses the Wagner-Nelson or the Loo-Riegelman methods to estimate in-vivo absorption profile from concentration-time data as the first step. The second stage is the establishment of the relationship between the estimated in-vivo absorption profile with the in vitro dissolution. Another approach is a one stage convolution-based and includes the computation of in vivo dissolution while concurrently modeling the in vitro-in vivo data. This approach requires intravenous

administration of the drug, or its administration as an oral solution or as immediate release bolus dose (Suankra. Gangadhar, 2008).

The data are then converted into a mathematical equation expressing a linear relationship represented by the general formula:

$$Y = mX + C$$

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 addition of parameters that will scale and shift time commensurately.

2.4.2 IVIVC model validation

In order for the IVIVC to predict the in vivo pharmacokinetic profile, it must be subjected to a validation process. Internal validation involves the use of the same data that was used to build the model to predict the in vivo PK parameters C_{max} and AUC. The values are then compared to the actual values. The model is validated if the mean absolute % prediction error (%PE) for the model is not more than 10% and the %PE for the single formulation is NMT 15%. For non-narrow therapeutic index drugs, an internal validation is considered satisfactory (FDA, 1997). However, external validation is mandatory for drugs with a narrow therapeutic index. In such cases, the acceptance criteria for %PE for C_{max} and AUC are as for internal validation. If the %PE falls between 10% and 20%, then additional data are required for further study in order for the external validation to be conclusive (Sunkara, 2008).

Kostewicz and others (Kostewicz et al., 2014) have reviewed the background on establishment of IVIVC predictive model and the working of the model.

It is important to design an effective IVIVC model as it could provide an alternative to bioequivalence studies to save both time and cost.

Demonstration of bioavailability similar to the reference listed drug (RLD) is necessary following formulation stability problems, manufacturing technology changes, change of excipients or any other change that may affect product quality.

2.5 Product selection

2.5.1 Pain management

Pain is an unpleasant sensation that signals actual or potential tissue damage. It is both subjective and complex. It can be acute or chronic. Pain is also categorized as "nociceptive," "neuropathic," and "psychogenic" pain. It is useful in diagnosing a disease condition or other medical problem that needs to be corrected. Without pain, one might hurt oneself unknowingly or suffer a medical condition that needs treatment, without knowing it. The pain can be resolved once the underlying problem is resolved. It is sometimes necessary to relieve the pain. Pain can be treated using different methods, such as use of pain relievers (analgesics), acupuncture and sometimes surgery.

2.5.2 Paracetamol in pain management

Paracetamol is also referred to as acetaminophen and bears the chemical name $N-(4-hydroxyphenyl)acetamide$. It is a white odorless crystalline powder; with large monoclinic prisms crystallizing from water.

It has the molecular formula $C_8H_9NO_2$, and the structure given below:

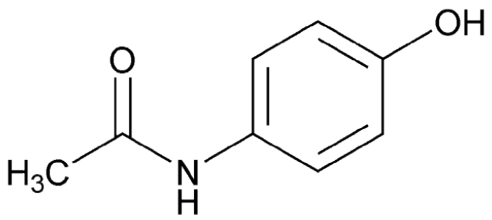

Figure 2: Chemical Structure of Paracetamol

Paracetamol is widely used for its analgesic and antipyretic activity. It has weak anti-inflammatory activity. It is recommended to be taken at a dose of 100-500mg, and is usually taken by adults as a single dose of 1g every eight hours. It is safe when given within the recommended dose, but serious toxicity and fatality may result from acute overdose. Severe hepatocellular necrosis may result from 10-15g of Paracetamol with 20-25g giving fatal results (Jiang et al., 2013).

2.5.2.1 Physicochemical properties of Paracetamol

Polymorphism

Paracetamol is known to exist in three crystalline forms. The monoclinic (Form I) that is stable with a melting range of 167–169 °C is commercially used; the orthorhombic Form II, that is metastable has a melting point of 156 °C; the third polymorphic form, Form III is unstable and poorly characterized (Bashpa et al., 2014).

Stability

The dry, pure Paracetamol is stable at 45°C. Degradation increases if the drug substance is contaminated with paraaminophenol. Moisture causes Paracetamol to undergo hydrolysis to paraaminophenol, which causes degradation and discoloration. Paracetamol is slightly light sensitive in solution; acids or bases catalyze its degradation.

Solubility

Solubility of Paracetamol in water has been reported at 14,000mg/L at 25^{o}C and 23.7mg/mL at 37^{o}C. The partition coefficient of 0.2 is reported depicting absorption by passive diffusion. Paracetamol has a pKa of 9.5 at 25^{o}C (Kalantzi et al., 2006).

2.5.2.2 Pharmacokinetic properties of Paracetamol

The pharmacokinetic parameters for Paracetamol have been reported widely from several sources (Kovaĉević et al., 2009). Following oral administration, Paracetamol has been found to have an absolute bioavailability of between 62% and 89% (average of 75.5%). Absolute bioavailability for Paracetamol does not vary at doses of between 5mg/kg and 20mg/kg body weight. Food increases the T_{max} and reduces the C_{max}, but not AUC. The T_{max} varies from 0.17 hours to 1.2 hours post-dosing. Protein binding occurs between 20 to 25%. It has an apparent volume of distribution of between 0.69 and 1.36L/kg (average of 1.025L/kg) and an elimination rate constant of 0.2235h^{-1}. About 85-90% undergoes glucoronization and sulfation to inactive metabolites; 5% is found unchanged in urine. It has a clearance of 11.8 to 22.3L/h. Typically, its half-life is 1.9h to 4.3h (Gilman et al., 2013).

2.5.2.3 In vitro in vivo correlation of Paracetamol

There have been previous studies on the bioavailability of orally administered Paracetamol tablets. Kalantzi reported correlation between in vitro dissolution with in

vivo dissolution in the canine stomach (Kalantzi et al., 2005). Others have reported this correlation as well. Consistent results of this in vitro in vivo correlation for Paracetamol tablets were demonstrated in humans (Kalantzi et al., 2006)

2.5.2.4 BCS Classification of Paracetamol

According to the WHO BCS criteria, Paracetamol falls within Class III. Lindenberg and others in their review (Lindenberg et al., 2004) and Kalantzi state that it possesses borderline BCS Class I properties (Kalantzi et al., 2006). This had been reported earlier (Dressman & Lennernäs, 2000). Kalantzi, *et al.,* 2006, in their Paracetamol biowaiver monograph review recommended that the biowaiver could be accepted upon fulfillment of some conditions: the product under consideration should contain the same excipients that are contained in the formulation of the comparator product. This is important for manufacturers of generic products of innovator products, in an effort to demonstrate sameness. The comparator product used should hold a marketing authorization from a stringent NMRA such as Germany, Finland, Greece and the Netherlands (Kalantzi et al., 2006). The generic product, which should be rapidly dissolving, should have a dissolution profile that comparable to the reference product.

2.6 Justification

Much time and resources are spent by generic manufacturers in Africa on fulfilling the demands of NMRAs and regional MER guidelines by conducting BE studies; and sometimes giving up on generic drug development and registration effort due to costly BE study requirements. There exist alternative in vitro methods of demonstrating in vivo drug release and bioavailability (WHO Expert Committee on Pharmaceutical Preparations, 2015).

Qureshi, (2010b) has summarized the role of IVIVC in "determining drug concentrations in blood from dissolution testing", from the perspective of one of the

stringent drug regulatory authorities, *Therapeutics Products Directorate, Health Canada* and termed it as *a simple and practical approach* for demonstrating drug bioavailability. Kalantzi et al., 2006, have reviewed the Paracetamol biowaiver monograph that is already available and conclude: "… *in view of its therapeutic use, its wide therapeutic index and its uncomplicated pharmacokinetic properties, in vitro dissolution data collected according to the relevant "Guidances" can be safely used for declaring bioequivalence (BE) of two acetaminophen formulations. Therefore, accepting a biowaiver for immediate release (IR) acetaminophen solid oral drug products is considered scientifically justified, if the test product contains only those excipients reported in this paper in their usual amounts and the test product is rapidly dissolving, as well as the test product fulfills the criterion of similarity of dissolution profiles to the reference product"* (Kalantzi et al., 2006).

An NMRA has requested for BE study on Paracetamol IR tablets. Paracetamol has been in use for over 50 years thus giving a wide experience on its therapeutic application and therefore becomes a test case in applicability of IVIVC computational predictive tool for bioavailability. Its biowaiver monograph is also available and has been reviewed (Kalantzi et al., 2006).

The application of the IVIVC tool as an alternative to expensive BE studies, as done by stringent regulatory authorities, in the developed world would reduce the drug development hurdles of cost and lead time in the poorer countries of the world currently facing convoluted regulatory demands for generic product registration (Ostrowski & Baczek, 2010).

The present study will serve as a baseline survey on comparative dissolution profiling of a generic product and how it predicts bioavailability of the drug substance following oral administration.

The results obtained from this study will serve to inform regulatory authorities on the bioavailability of oral generic formulations as a basis for medicines evaluation and

registration. The study can also used as a starting point for future studies on in vitro methods of determining bioavailability of qualifying generic formulations.

2.7 Objectives

2.7.1 General objective

The main objective of the present study was to demonstrate that there is no difference between the pharmacokinetic parameters obtained from dissolution data using the IVIVC predictive model for generic Paracetamol tablets and the pharmacokinetic profile obtained in a similar manner for a registered reference product. The primary end-point is to obtain the time-dependent concentration of Paracetamol in blood/plasma using in vitro dissolution data.

2.7.2 Specific objectives

- To obtain dissolution data for the generic product batches and the reference product batch;
- To determine intra-batch and inter-batch variability from the dissolution behavior;
- To compare the dissolution behavior of the generic formulation with the dissolution behavior of the registered reference product.
- To determine the Cmax, and AUC of Paracetamol using a computational method (IVIVC);
- To determine the nature of relationship between results obtained using computational methods for generic product and those obtained using a registered reference product; and

- To propose the use of IVIVC in medicines evaluation and registration for qualifying generic products within the East African Community for which IVIVC tool is applicable.

2.8 Limitations

This project does not intend to redesign an IVIVC tool, but rather to use an already developed tool (Qureshi, 2010a) that employs in vitro dissolution data for computation of in vivo drug blood concentration profile.

2.9 The Null Hypothesis

There is no difference between the pharmacokinetic parameters predicted using the in vitro – in vivo correlation (IVIVC) tool of Paracetamol 500 mg immediate release tablets (as computed for a generic product) and the pharmacokinetic parameters predicted using a registered reference product).

The working title for the project was: *Application of in vitro – in vivo correlation (IVIVC) as a predictive tool for bioequivalence studies for generic Paracetamol immediate release tablets*

3.0 MATERIALS AND EXPERIMENTAL METHODS

3.1 Product selection

Some basic requirements for a manufacturer's product to be included in the study were that the manufacturer possessed a valid pharmaceutical manufacturing license issued by the competent regulatory authority, had a GMP certificate, provided its name and physical address and evidence that the identified product was manufactured at their licensed manufacturing site. In addition, the manufacturer also provided evidence that all its suppliers of the ingredients used in manufacturing the product had been prequalified and appeared on the approved vendor list. The product manufacturing process had been qualified as evidenced by a process validation report. It was also confirmed that a change control procedure was in place ascertaining that no changes could be made on validated processes and the approved suppliers without appropriate authorization. For the product, the specification was provided, as were also specifications for the API and excipients. The specific inclusion and exclusion criteria that were used are summarized below.

3.1.1 Product inclusion criteria

In selecting the suitable product for this study, it is essential that it was a generic product manufactured at a licensed site within the East African Community (EAC). The product was also confirmed as having been evaluated and registered by a competent National Medicines Regulatory Authority (NMRA) of a partner state within the EAC. The API of the product also belonged to BCS Class II or Class III and fell within the jurisdiction of an EAC member state NMRA or EAC-MRH requirements. The API and the excipients used in the manufacture were also subject to a documented and authorized change control procedure for the material specifications and the suppliers. The method of

manufacture/process was held the same for all the batches of the generic product used in the study as evidenced by the process flow chart. It was also confirmed that the product manufacturer held a current cGMP compliance certificate and a valid manufacturing license.

3.1.2 Product exclusion criteria

Drug products containing APIs that fell into certain criteria are excluded from the study. BCS class IV drug substances were excluded since BE was mandatory for this class. Antiretroviral drugs and products used in the treatment of malaria and those for tuberculosis were also excluded since the WHO prequalification requirements demand human bioavailability study (BE). Drug products containing active pharmaceutical ingredients with narrow therapeutic index were excluded. Products that are intended for absorption in the oral cavity were excluded. Similarly, products with documented evidence for bioavailability or efficacy problems based on post-marketing pharmacovigilance reports were not to be included. Also excluded were those products on which there was scientific evidence that the API undergoes polymorphic change or where process excipients affect bioavailability. Products that did not have a biowaiver monograph in any known jurisdiction were not considered for the study.

3.2 Drug product

3.2.1 Paracetamol immediate release (IR) tablet formulation

The drug product was selected using the laid out inclusion criteria set out earlier. The formulation was the same for all the batches used in the study, and the supplier of each excipient was also the same. The selected product had the ingredients identified and used in the amounts recommended from experience and well documented (Rowe, et al., 2009). Further, the same ingredients used in the selected formulation were contained in Paracetamol IR oral drug formulations registered in some member sates of the European Union (Kalantzi et al., 2006). Biowaiver monographs that existed for acetaminophen were reviewed by Kalantzi and others and a list of approved ingredients for formulation of Paracetamol IR tablets made (Kalantzi et al., 2006). The generic product used for the study had only the ingredients appearing on the approved list, and its formulation is shown in Table 2.

Table 2: List of ingredients used in generic Paracetamol immediate release tablets

Ingredient	Specification	Amount per tablet, mg	Purpose of ingredient
Paracetamol	BP	500.00	Active pharmaceutical ingredient
Starch	BP	92.50	Binder, diluent, disintegrant
Gelatin	BP	7.50	Binder
Potassium Sorbate	BP	2.20	Antimicrobial preservative
Magnesium stearate	BP	5.00	Lubricant

3.2.2 Paracetamol immediate release tablet manufacturing process

The manufacturing process for the product selected was held the same for all the batches used in the study. Wet granulation using standard equipment followed the flow chart as illustrated in Figure 3.

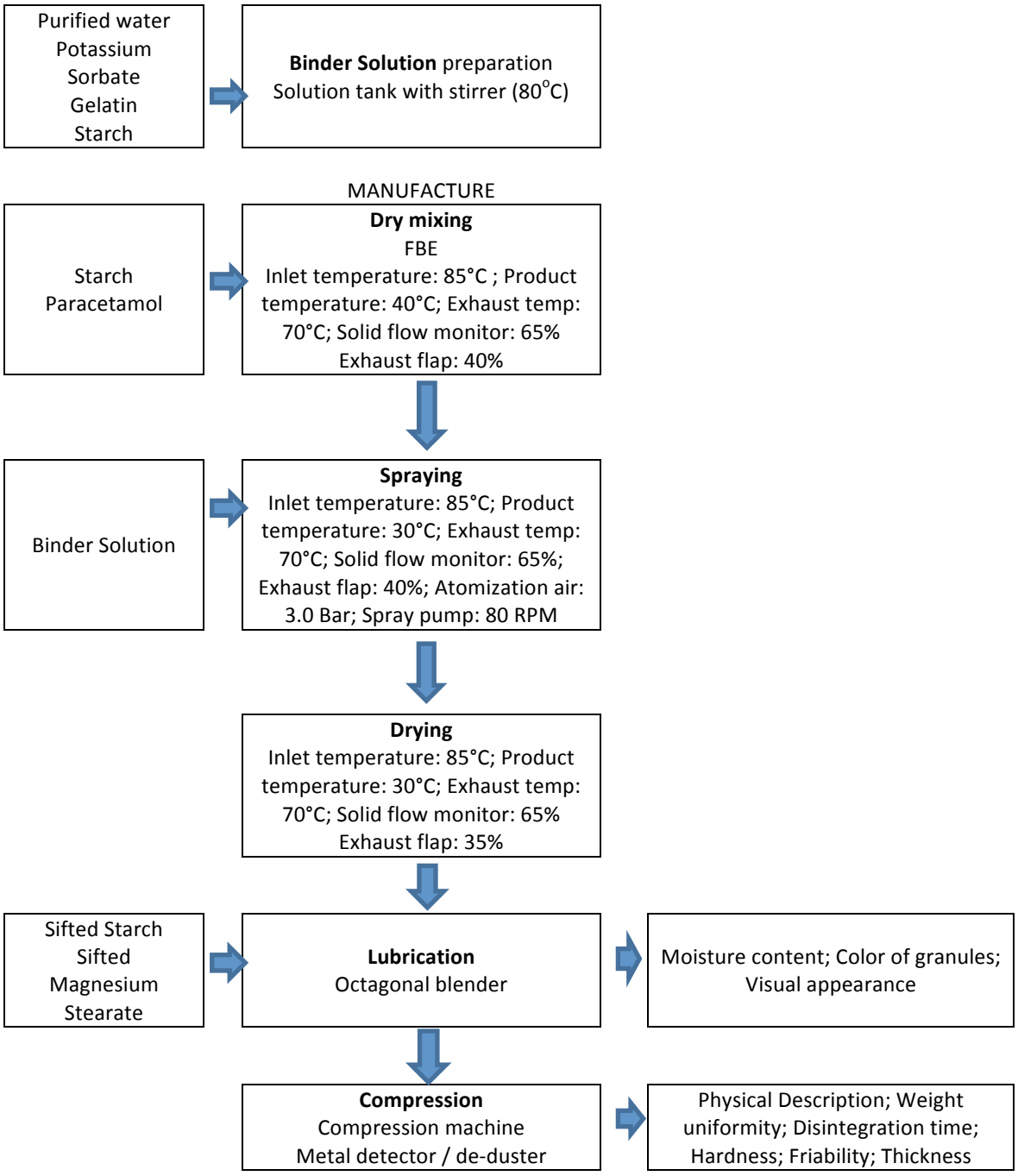

Figure 3: Flow chart for manufacturing process for Paracetamol tablets

3.3 Dissolution method

The dissolution method used in the study was as per the British Pharmacopoeia. The dissolution test was conducted using the *Electrolab* tablet dissolution tester model No. *TDT-06P* manufactured by *Electrolab* of 401, Tripati Industrial Estate, I. B. Patel Road, Bombay India, that complied with USP, IP and BP specifications. The media employed was 900mL at pH 6.8 representing the intestinal content.

The phosphate buffer pH 6.8 dissolution medium was prepared following the BP method. For each amount of 200mL phosphate buffer solution, 50mL of 0.2M *potassium dihydrogen orthophosphate* was mixed with 23.65mL 0.2M *sodium hydroxide VS* and diluting to 200mL with distilled water. The pH was confirmed at 20^{o}C. Potassium Phosphate monobasic reagent used to prepare the 0.2M *potassium dihydrogen orthophosphate* was supplied by Finar Limted of 184-186 Chacharwadi Vasna, Ahmedabad 382110, Gujarat, India. The Soium Hydroxide Pellets used to prepare 0.2M *sodium hydroxide VS* and 0.1M *sodium hydroxide* was sourced from Central Drug House (P) Ltd, 7/28 Vardaan House, Daryaganj, New Delhi – 11002, India.

Apparatus type 2 (paddle type) was employed at a speed of 50 rpm. The test was conducted as per the procedure specified in BP monograph for the "*Dissolution Test Tablets and Capsules (Dissolution Test for Solid Dosage Forms)",* which is *Ph Eur method 2.9.3.*

The sample solution and standard solution were prepared as prescribed in the BP dissolution method using 0.1M *sodium hydroxide*. The absorbance at a wavelength of 257nm was determined using *Shimatzu UV1700* spectrophotometer.

The dissolution tests were carried out following the same method, and same aqueous medium of pH 6.8 at a temperature of 37^{o}C with low stirring to simulate intestinal physiological kinetics. Paracetamol is a weak organic acid, that exists un-ionized and ionized forms in an aqueous environment. It is the un-ionized form, being lipophilic, that

passively diffuses across the cell membrane while the ionized form is hydrophilic. The pH of the entire gastrointestinal tract is lower than the 9.5 ranging from 1 in the stomach to nearly 8 in the distal end of the small intestinal. When given orally paracetamol is mostly unionized. Most of it is absorbed in the small intestine because the surface area is larger (which accounts for over 94% of gastrointestinal surface area). 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 intestinal pH. In view of the foregoing, the phosphate buffer of pH 6.8 was selected for the present study. (Helander & Fandriks, 2014; Le, 2016).

3.3.1 Dissolution data

One batch of the comparator product was used. A total of three batches of the test product were used for the dissolution profile. Each of the batches was made using the same ingredients (each ingredient of which was obtained from the same supplier and manufacturer). The process employed in manufacturing was the same validated one held constant for all the batches that were used; using the same equipment and the same process parameters. The same analyst was also retained throughout the study and carried out all the dissolution tests. The percentage dissolution was calculated as per the formula given below:

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

Equation 1: Calculation of % Paracetamol dissolved

Twelve tablets were used from each batch to carry out the dissolution profile tests as per the sampling specified times in minutes: 5, 15, 25, 35 and 45 as shown in Table 3.

Table 3: In vitro % drug release per tablet

pH		6.8				
Time, minutes		5	15	25	35	45
Comparator	1					
	2					
	3					
	4					
	5					
	6					
	7					
	8					
	9					
	10					
	11					
	12					
	Av					
Test Product Batch 1	1					
	2					
	3					
	4					
	5					
	6					
	7					
	8					
	9					
	10					
	11					
	12					
	Av					

pH		6.8				
Time, minutes		5	15	25	35	45
Test Product Batch 2	1					
	2					
	3					
	4					
	5					
	6					
	7					
	8					
	9					
	10					
	11					
	12					
	Av					
Test Product Batch 3	1					
	2					
	3					
	4					
	5					
	6					
	7					
	8					
	9					
	10					
	11					
	12					
	Av					

3.4 Methods of comparing dissolution profile data

Three methods are available for dissolution profile comparison of products of the same API, when obtained under similar testing conditions. The first is the ANOVA using univariate and multivariate to quantify differences in dissolution percentages at each sampling time point. The model dependent methods include the cubic root law (Hixson and Crowell) mathematical model, the Weibull distribution model and the logistics (Rowlings) model for sigmoidal dissolution curves (Yuksel et al., 2000).

The model independent method by Moore and Flanner (Moore & Flanner, 1996) uses the difference factor, f_1 and the similarity factor, f_2 (Ma, Lin, & Liu, 1999).

The difference factor is the percentage difference between the two curves, at each time point; the mean dissolution profile curve of the experimental product on the one hand, and the dissolution profile curve of the reference product on the other. This is a measure of relative error between the two curves.

The f_1 values were worked out as shown below (Equation 2).

$$f_1 = \{(\sum_{t=1}^{n} |R_t - T_t|] / \sum_{t=1}^{n} R_t]\} \times 100$$

Equation 2: Calculation of difference factor f_1

where *n* is the number of time points, R_t is the dissolution value of the reference batch at time t, and T is the dissolution value of the test batch at time t. The acceptance range for the difference factor f_1 as prescribed by the US FDA is between 0% to 50% (Yuksel et al., 2000).

The similarity factor (f_2) is a logarithmic reciprocal square root transformation of the sum of squared errors, and is a measurement of the similarity in the percentage (%) dissolution between the two curves. An f_2 value between 50 to 100 is an indication that the two curves are similar.

The f_2 values were worked out using the formula indicated in Equation 3.

$$f_2 = 50 \log\{[1 + (1/n) \{(\sum_{t=1}^{n} (R_t - T_t)^2]^{-0.5} \times 100\}$$

Equation 3: Calculation of f_2 value

The acceptance criteria for the f_1 and f_2 values are $0 < f_1 < 15$ and $50 < f_2 < 100$, respectively (FDA, 1997). Values obtained between the two products product therefore infer that the two curves are equivalent or similar.

Noting that both products (test and reference) contained similar excipients in the usual amounts; using these two values, and noting that the dissolution tests were carried out under the same test conditions, at the same sample time points, we can make a declaration of similarity if the values lie within the limits prescribed by the US FDA and by the EMEA.

The US FDA and the European Medicines Agency EMEA have adopted the f_2 value, with acceptance limits at between 50% and 100% (EMEA, 2010; FDA, 1997). The f_2 factor was adopted for dissolution profile comparison since it has been found to be "more sensitive for dissolution profile than the f_1 factor" (Yuksel et al., 2000).

3.5 IVIVC data analysis

The IVIVC tool uses spreadsheet software in which the computation formulae are embedded. In this model the drug concentration time profile is obtained readily from the dissolution curve. Human study of the test product is not required, as only the dissolution profile and some PK parameters (k_{el}, V_d, F and body weight) are needed for the prediction of drug blood levels.

The tool also contains embedded logarithmic formulae for working out the C_{max} values, and employs the trapezoidal rule to compute the AUC.

The tool used to predict the in vivo pharmacokinetic profile has been elucidated and is summarized. The dissolution profile is converted into discrete dosage segments and the amount of drug in the blood is calculated.

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

Equation 4: Calculation of Amount of Drug Released

The amount of drug in the blood is computed using the bioavailability factor (F) for the drug, which is 75.5% using the simple formula:

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

The blood drug concentration is then calculated using the equation:

$$Ct\ = \frac{Amt\ of\ drug\ in\ blood\ x\ F}{Vd\ x\ Body\ weight}$$

Equation 5: Calculation of Blood Drug Concentration

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, can be obtained from the drug elimination half-life using their reciprocal relationship, $k = \frac{0.693}{t_{1/2}}$.

The pharmacokinetic parameters required are the C_{max} and AUC, which are referred to as the bioavailability parameters. It is then possible to compare the calculated (predicted) C_{max} and AUC to the numbers of these parameters obtained and reported from in vivo human studies. A similarity denotes IVIVC validity. With a valid finding then in vitro dissolution profile may be used to compute in vivo drug amounts, the C_{max} and the AUC, which enable conclusions on bioavailability and bioequivalence to be drawn.

The IVIVC model application is summarized in the flow chart in Figure 4 (Qureshi, 2010b).

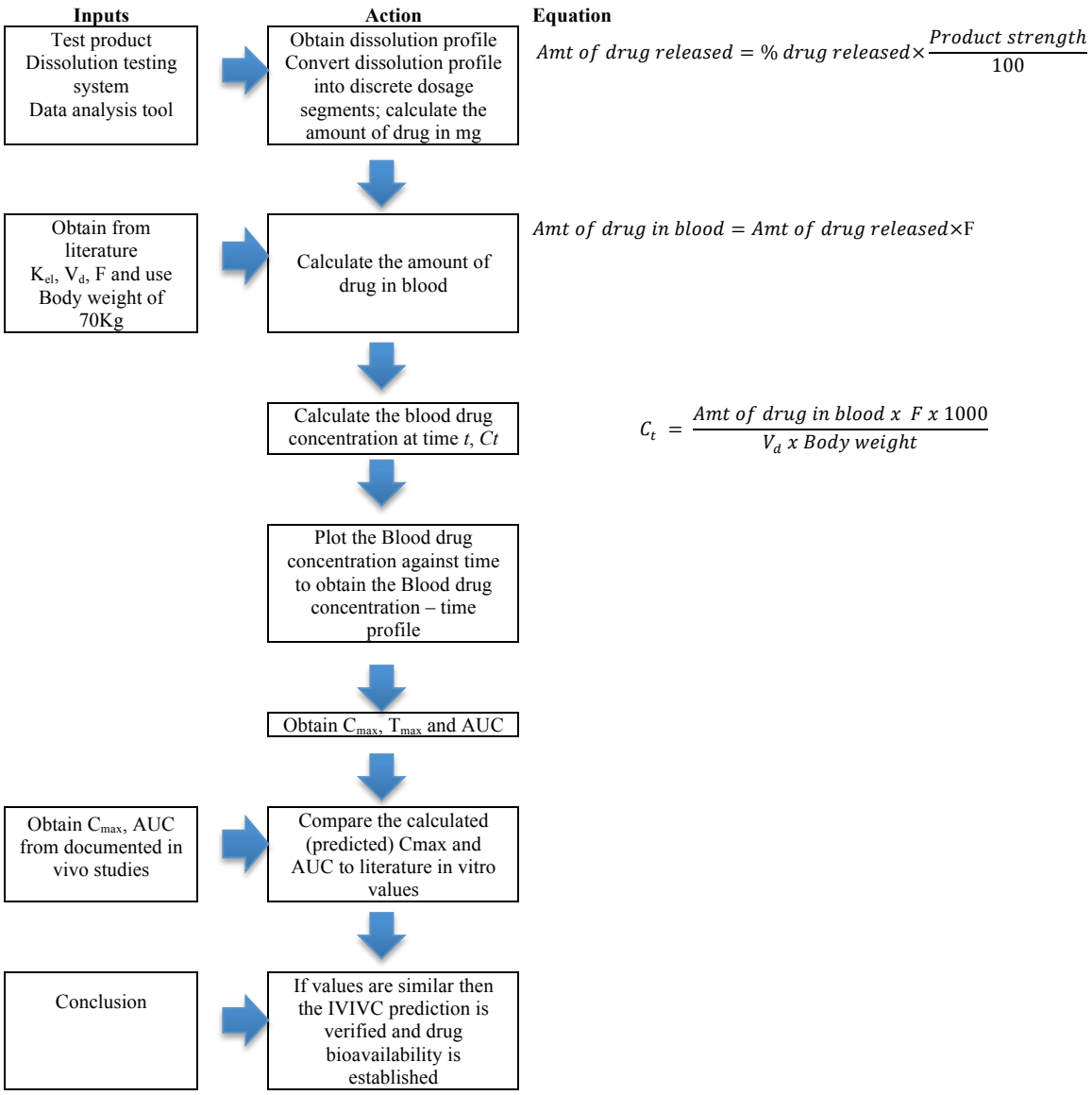

Figure 4: Flow Chart of the IVIVC Model Computation Process

3.6 Documentation, archiving and confidentiality of data

The test product identity was encoded and was retained confidentially by the principal investigators.

3.7 Statistical analysis

Both the in vitro dissolution data and the predicted pharmacokinetic parameters were subjected to statistical analysis using IBM STATISTICS (SPSS) VERSION 21 statistical analysis software.

3.8 Ethical considerations

3.8.1 Basic principles

It was established that the manufacturer of the product selected for this study met the regulatory requirements of manufacture of pharmaceutical products, possessing a valid manufacturing license and GMP certificate issued in accordance with applicable national legislation (Probitts & Wiley, 2000). In addition, the relevant requirements of Good Laboratory Practice were met (UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR), n.d.). Applicable aspects of Good Clinical Practice (WHO, 2002) were also complied with.

3.8.2 Institutional Review Board

This study was based on a computational method and did not directly use human subjects. No application was made to the institutional review board for approval.

4.0 RESULTS

4.1 Dissolution profiles

The dissolution data obtained by conducting dissolution tests at different time points for the three batches of the generic Paracetamol formulation were presented in tabular form and subjected to statistical analysis to determine the intra-batch variation and also inter-batch variation within the same product formulation.

4.1.1 Dissolution data

The performance of the individual tablets in the three batches A1, A2 and A3 are summarized in Table 4, Table 5 and Table 6.

Table 4: Dissolution profile (%API released) of individual tablets of generic product Batch A1

Tablet	5min	15min	25min	35min	45min
1	65.24	79.91	82.37	88.41	95.06
2	70.71	79.42	91.47	92.23	94.61
3	71.99	80.43	90.64	95.14	96.81
4	66.33	84.28	88.73	91.96	94.08
5	69.36	84.10	92.69	97.72	99.77
6	65.16	79.55	89.05	91.89	95.13
7	70.94	77.86	84.64	90.60	97.79
8	69.77	80.64	85.33	88.48	97.48
9	63.38	80.79	84.97	94.46	98.79
10	73.13	80.51	89.76	92.61	94.76
11	68.94	92.10	94.68	97.48	99.04
12	89.72	90.45	91.21	93.83	95.78
Min	**63.38**	**77.86**	**82.37**	**88.41**	**94.08**
Max	**89.72**	**92.10**	**94.68**	**97.72**	**99.77**
Av	**70.39**	**82.50**	**88.80**	**92.90**	**96.59**
SD	**6.79**	**4.49**	**3.72**	**3.01**	**1.95**

Table 5: Dissolution profile (% API released) of individual tablets of generic product Batch A2

Tablet	5min	15min	25min	35min	45min
1	70.93	91.34	87.96	90.93	96.78
2	75.47	93.39	90.12	93.26	95.12
3	79.78	91.69	87.56	90.39	92.34
4	78.02	95.61	88.42	92.63	99.08
5	69.78	88.48	90.08	94.58	99.95
6	75.64	75.89	88.20	94.05	97.56
7	71.12	78.46	81.83	89.87	95.48
8	62.02	75.72	91.55	93.98	97.97
9	85.59	83.64	90.23	89.97	94.73
10	66.04	78.03	91.86	96.74	98.94
11	63.24	80.46	92.04	91.97	90.25
12	57.87	79.15	91.69	93.64	97.38
Min	**57.87**	**75.72**	**81.83**	**89.87**	**90.25**
Max	**85.59**	**95.61**	**92.04**	**96.74**	**99.95**
Av	**71.29**	**84.32**	**89.3**	**92.67**	**96.3**
SD	**8.09**	**7.33**	**2.85**	**2.11**	**2.87**

Table 6: Dissolution profile (% API released) of individual tablets of generic product Batch A3

Tablet	5min	15min	25min	35min	45min
1	57.88	81.58	84.25	94.05	95.74
2	72.43	82	95.71	96.24	98.53
3	75.65	82.05	92.59	94.58	97.71
4	67.23	83.74	94.14	95.7	97.82
5	40.51	83.22	90.42	98.16	99.76
6	59.43	84.48	89.74	97.82	94.74
7	58.5	81.96	90.94	93.15	94.88
8	70.25	82.33	87.67	94.5	96.64
9	68.74	82.17	91.19	92.21	95.86
10	65.39	81.23	88.72	94.03	94.64
11	68.49	81.74	94.34	94.07	95.12
12	71.98	82.56	92.95	92.62	93.99
Min	**40.51**	**81.23**	**84.25**	**92.21**	**93.99**
Max	**75.65**	**84.48**	**95.71**	**98.16**	**99.76**
Av	**64.71**	**82.42**	**91.06**	**94.76**	**96.29**
SD	**9.53**	**0.95**	**3.21**	**1.89**	**1.80**

The dissolution performance at each sampling time point for the individual tablets of the reference Paracetamol formulation is presented in Table 7.

Table 7: Dissolution profile (% API released) of individual tablets of the reference formulation

Tablet	5min	15min	25min	35min	45min
1	68.97	83.14	88.46	92.78	97.51
2	73.13	87.62	92.01	96.4	99.52
3	69.4	82.64	90.14	93.45	97.38
4	68.31	86.11	92.81	94.66	98.06
5	73.52	86.24	93.86	96.25	100.93
6	64.78	79.02	87.25	94.36	98.09
7	74.01	79.2	82.56	88.76	95.26
8	75.96	82.69	91.16	95.11	97.08
9	74.3	84.09	89.08	93.67	96.45
10	68.01	79.8	91.32	95.37	97.47
11	71.24	85.64	90.35	92.19	96.68
12	75.82	85.26	91.54	94.78	95.65
Min	**64.78**	**79.02**	**82.56**	**88.76**	**95.26**
Max	**75.96**	**87.62**	**93.86**	**96.4**	**100.93**
Av	**71.45**	**83.45**	**90.05**	**93.98**	**97.51**
SD	**3.54**	**2.91**	**2.99**	**2.08**	**1.56**

The average dissolution performance for batches A1, A2 and A3 for the generic Paracetamol formulations; and the dissolution performance of the reference product are presented in Table 8.

Table 8: 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	0	71.45 [3.54]	83.45 [2.91]	90.05 [2.99]	93.98 [2.08]	97.51 [1.56]

The dissolution values at the sampling time points, for Batch A1 of the generic product are shown in Figure 5.

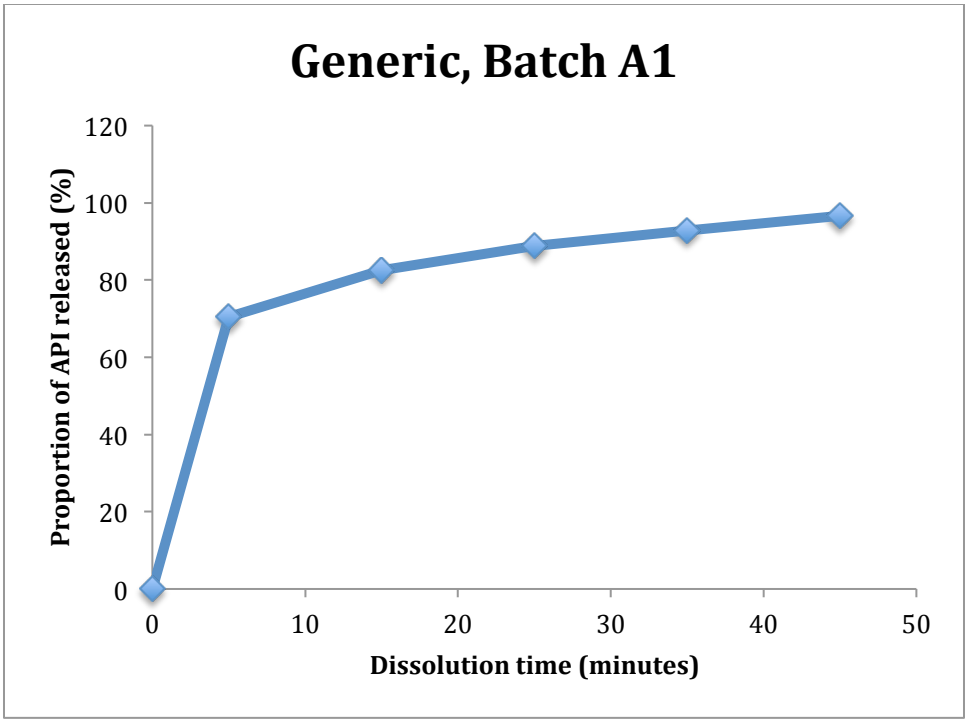

Figure 5: Dissolution values for Batch A1 at the sampling time points

4.1.2 Statistical analysis of the dissolution data

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 results obtained are presented in Table 9 below.

Table 9: Intra-batch variability statistics for all the three batches

	tab1	tab2	tab3	tab4	tab5	tab6	tab7	tab8	tab9	tab10	tab11	tab12	p-value
Mean	84.7	88.5	87.6	87.9	87.5	85.1	83.9	85.8	86.7	85.9	87	87.9	0.102
Std. Deviation	2.6	1.9	1	2.3	3.4	0.9	0.4	2	1.9	0.8	2.8	3.4	

From the p-value calculated there is no difference in the dissolution values among the tablets within the same batch, across all batches.

Inter-batch variability

The distribution of dissolution values at different time points for each batch is given in Table 10.

Table 10: Distribution of dissolution values at different time points for each batch

Time	Mean [Standard Deviation]			
	Reference	Batch 1	Batch 2	Batch 3
5 minutes	71.5 [3.5]	70.4 [6.8]	71.3 [8.1]	64.7 [9.5]
15 minutes	83.5 [2.9]	82.5 [4.5]	84.3 [7.3]	82.4 [0.9]
25 minutes	90 [3]	88.8 [3.7]	89.3 [2.9]	91.1 [3.2]
35 minutes	94 [2.1]	92.9 [3]	92.7 [2.1]	94.8 [1.9]
45 minutes	97.5 [1.6]	96.6 [2]	96.3 [2.9]	96.3 [1.8]

From the table above the variation among specific tablets within the same batch was minimal as demonstrated by the SD values.

Statistical difference in dissolution between reference and the batches at different time points has been shown in Table 11.

Table 11: Statistical difference in dissolution between reference and the generic batches at different time points.

Batch	Mean dissolution in (g/mL) at specific time points (minutes)				
	5	15	25	35	45
Reference	**71.5**	**83.5**	**90**	**94**	**97.5**
Batch 1	70.4	82.5	88.8	92.9	96.6
p-value	*0.679*	*0.59*	*0.35*	*0.252*	*0.26*
Batch 2	71.3	84.3	89.3	92.7	96.3
p-value	*0.95*	*0.652*	*0.56*	*0.172*	*0.15*
Batch 3	64.7	82.4	91.1	94.8	96.3
p-value	*0.049*	*0.557*	*0.386*	*0.377*	*0.147*

The p-values are 0.05 and above at each time point for each of the 3 experimental batches.

Comparison of dissolution profiles between test product and reference product

Table 12 shows the statistical difference in dissolution between reference and the generic batches at different time points.

Table 12: Statistical difference in dissolution between reference and the generic batches (average) at different time points.

Batch	Mean dissolution in (g/mL) at specific time points (minutes)				
	5	15	25	35	45
Reference	**71.5**	**83.5**	**90**	**94**	**97.5**
Experimental	68.8	83.1	89.7	93.4	96.4
p-value	*0.296*	*0.802*	*0.757*	*0.498*	*0.115*

From the p-values obtained across the dissolution sampling time points for the test product and the reference product, it can be concluded that there was no statistical difference between the data obtained from the dissolution – time profile tests conducted in similar conditions on the experimental product and on the reference product.

Analysis of variance

ANOVA was performed on the data obtained from dissolution tests on the reference and the experimental products and the statistics obtained are presented in Table 13.

Table 13: Analysis of Variance between the experimental product and reference product

	N	Mean	Std. Deviation	95% Confidence Interval for Mean		P value
				Lower Bound	Upper Bound	
Reference	60	87.288	9.6295	84.801	89.776	0.528
Experimental	180	86.286	10.9561	84.674	87.897	

ANOVA suggests that the reference and experimental products have comparable dissolution values across the 5 time periods.

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

The model independent method by Moore and Flanner 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 (EMEA) guidelines. For curves to be considered similar, f_1 values should be close to 0 (0 to 15), and f_2 values should be close to 100 (50 to 100).

Calculation of the difference factor, f_1

The calculation formula for f_1 is as shown below

$$f_1 = \{(\sum_{t=1}^{n} |R_t - T_t|] / \sum_{t=1}^{n} R_t]\} \times 100$$

Where *n* is the number of time points, R_t is the dissolution value of the reference batch at time t, and T_t is the dissolution value of the test batch at time t.

Calculation of f_1 for Batch A1

$\sum R_{45} = 1170.08$

$\sum T_{45} = 1159.10$

$|R_t - T_t| = (1170.08 - 1159.10) = 10.98$

$$= \frac{10.98}{1170.08} \times 100$$

$$= \underline{\mathbf{0.94}}$$

The f_1 factor was computed for dissolution profile comparison between the experimental product and reference product. Each batch was considered separately, and the f_1 values obtained are summarized and presented in Table 14.

Table 14: Difference factor f_1 for each experimental batch

	Batch A1	**Batch A2**	**Batch A3**
f_1 value	0.94	1.04	0.83

Calculation of the similarity factor f_2

The f_2 factor was computed for dissolution profile comparison between the experimental product and reference product.

$$f_2 = 50 \log\{[1 + (1/n)\{(\sum_{t=1}^{n}(R_t - T_t)^2]^{-0.5} \times 100\}$$

Calculation of f_2 for Batch A1

$$f_2 = 50 \log\left[\frac{1}{\sqrt{[1+(1/n)(\sum R_t - T_t)^2]}} \times 100\right]$$

$$f_2 = 50 \log\left[\frac{1}{\sqrt{[1+(^1/_5)(10.98)^2]}} \times 100\right] = 50 \log(^1/_{5.01} \times 100)$$

$$= 50 \log(19.96)$$

$$= 50 \times 1.3002$$

$$= \underline{\mathbf{65.01}}$$

The USFDA and EMEA have adopted the f_2 value, with acceptance limits at between 50% and 100% (EMEA 2010, FDA 1997). The f_2 factor was adopted for dissolution profile comparison, as per USFDA and EMEA guidelines.

Each batch was considered separately, and the f_2 values obtained are summarized and presented in Table 15.

Table 15: Similarity factor f_2 for each experimental batch

	Batch A1	Batch A2	Batch A3
f_2 value	65.01	66.12	68.33

The f_2 values obtained for each experimental batch was within the tolerance limits prescribed by the US FDA and EMEA, indicating that the dissolution profiles obtained for the experimental batches were similar to the dissolution profile obtained from the reference product in each case.

4.2 Prediction of bioavailability using in vitro dissolution profile data

The in vitro dissolution profile data for each batch were subjected to a two-step process. First the data were converted into discrete dosage segments between the dissolution sampling time points. The amount of Paracetamol in blood was then computed from the in vitro data using the Excel spreadsheet formatted for that purpose.

Paracetamol is eliminated following first order kinetics. To cater for the amount eliminated after absorption following each sampling point, further computation was done using the first order elimination formula, Equation 6.

$$C_t = C_0 e^{-0.2235t}$$

Equation 6: Calculation of remaining blood concentration of Paracetamol at time t

Following first order kinetics for Paracetamol, the amount in blood, the absolute bioavailability factor (F), volume of distribution (V_d), and the body weight of the 'physiological man' (70kg) the blood drug concentration after each sampling time point was calculated. A conversion factor of 1,000 was used in Equation 7, in order to report the blood drug concentration in ng per mL instead of μ/mL.

$$C_t = \frac{Amt\ of\ drug\ in\ blood\ x\ F\ x\ 1000}{V_d\ x\ Body\ weight}$$

Equation 7: Calculating blood concentration in ng/mL

Batch A1

The amount of drug in mg was calculated at the end of each sampling time as represented in Table 16

Table 16: Amount of drug calculated at the end of each sampling time interval for Batch A1

Time (h)	% Released (Cumulative)	% Released (within sampling interval)	Amt. (mg) released (within sampling interval) Tablet Strength (500 mg)	Amt. (mg) corrected for bioavailability (F)
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

The amount in blood was calculated from the percentage of the product strength dissolved, and also corrected using the absolute bioavailability, F, obtained from literature as 75.5% (Gilman et al., 2013). The other parameters for Paracetamol that were employed in the computation include k_e=0.2235 and V_d=1.025. The data was presented in Table 17

Table 17: Predicted pharmacokinetic profile for Generic 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**						**Total Blood Amt. after Absorption**	**Conc. (ng/mL) at Times**	**AUC**
0	0.00						0	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**

From Table 17, the C_{max} is 3949.20ng/mL and the AUC at 45 minutes is 2161.75 ng.h/mL.

The blood drug concentrations – time profile is predicted as shown in Figure 6.

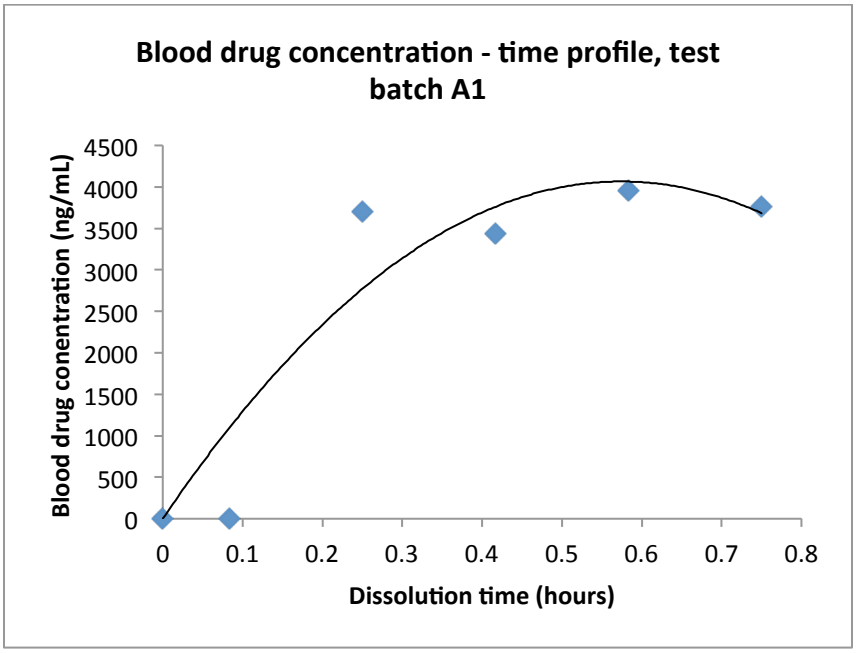

Figure 6: Predicted concentration-time profile for Generic Batch A1

The dissolution profile data for Batch A2, Batch A3 and the Reference batch were each in turn subjected to similar statistical treatment and the resultant tables are represented in the relevant tables and figures in the respective sections. The dissolution data average for the three generic batches was subjected to the same treatment and the PK parameters predicted.

Batch A2

Table 18: Amount of drug calculated at the end of each sampling time interval for Batch A2

Time (h)	% Released (Cumulative)	% Released (within sampling interval)	Amt. (mg) released (within sampling interval) Tablet Strength (500 mg)	Amt. (mg) corrected for bioavailability (F)
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

Predicted PK for Batch A2

Table 19: Predicted pharmacokinetic profile for Generic 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**						**Total Blood Amt. after Absorption**	**Conc. (ng/mL) at Times**	**AUC**
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**

From Table 19, the Cmax is **4039.85**ng/mL and the AUC at 45 minutes is **2199.11** ng.h/mL.

The blood drug concentrations – time profile is predicted as follows:

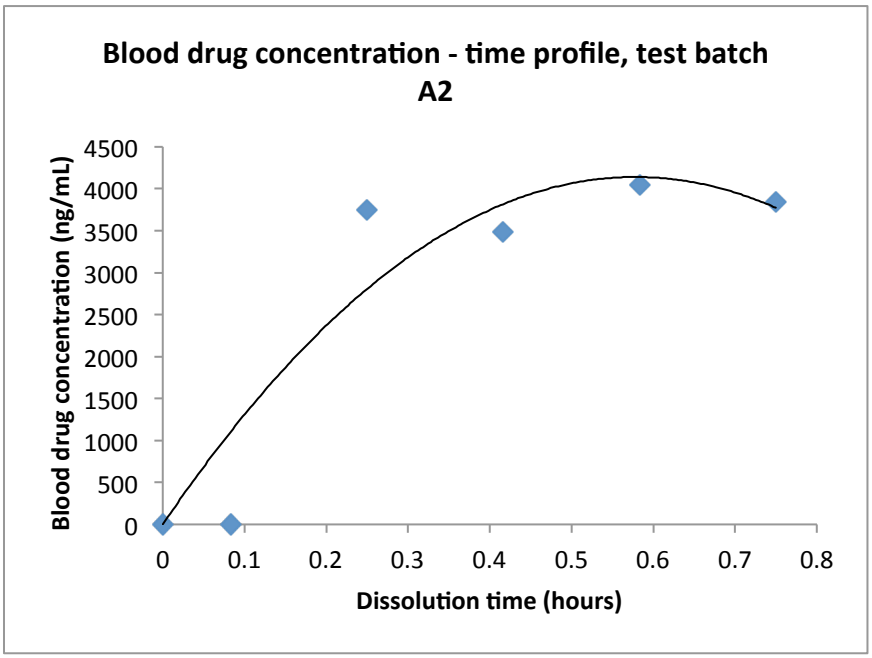

Figure 7: Predicted concentration-time profile for Generic Batch A2

Batch A3

Table 20: Amount of drug calculated at the end of each sampling time interval for Batch A3

Time (h)	% Released (Cumulative)	% Released (within sampling interval)	Amt. (mg) released (within sampling interval) Tablet Strength (500 mg)	Amt. (mg) corrected for bioavailability (F)
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

Predicted PK for Batch A3

Table 21: Predicted pharmacokinetic profile for Generic product 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**						**Total Blood Amt. after Absorption**	**Conc. (ng/mL) at Times**	**AUC**
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**

From Table 21, the C_{max} is **3976.51**ng/mL and the AUC at 45 minutes is **2070.71** ng.h/mL. The blood drug concentrations – time profile is predicted as follows:

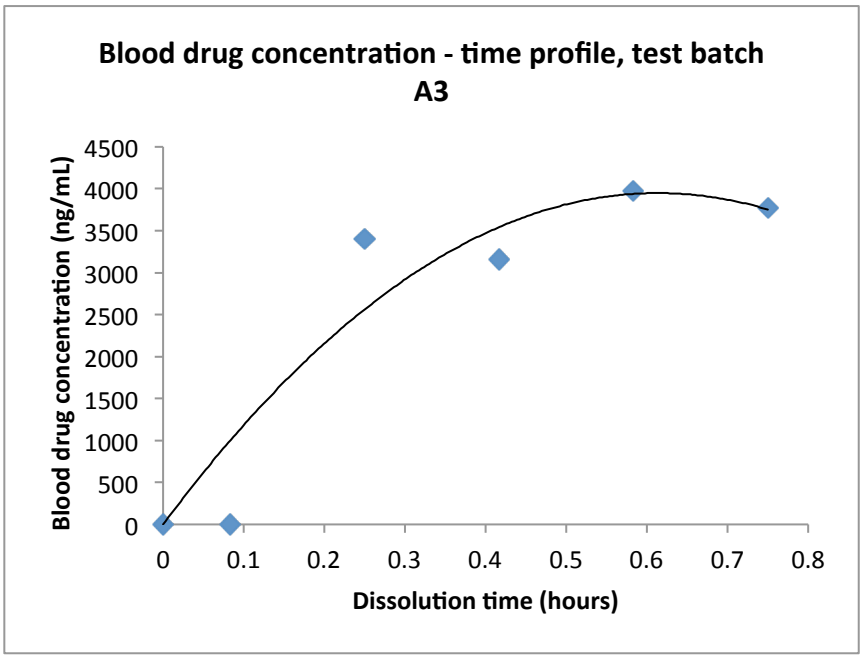

Figure 8: Predicted concentration-time profile for Generic Batch A3

Three batches PK (A1, A2 and A3) average

Table 22: Amount of drug calculated at the end of each sampling time interval for average of the three batches of generic product

Time (h)	% Released (Cumulative)	% Released (within sampling interval)	Amt. (mg) released (within sampling interval) Tablet Strength (500 mg)	Amt. (mg) corrected for bioavailability (F)
0	0.0	0.00	0.00	0.000
0.08	68.8	68.80	343.98	259.704
0.25	83.1	14.29	71.43	53.931
0.42	89.7	6.63	33.16	25.039
0.58	93.4	3.73	18.64	14.073
0.75	96.4	2.95	14.74	11.132

Table 23: Amount of Paracetamol in blood computed from in vitro data average (Generic product)

Dissolution Sampling Time (h)	0	0.08	0.25	0.42	0.58	0.75			
Amt. (mg) equivalent	0.00	259.70	53.93	25.04	14.07	11.13			
Time after absorption (h)	**Blood Amt after Absorption**						**Total Blood Amt. after Absorption**	**Conc. (ng/mL) at Times**	**AUC**
0	0.00						0	0.00	
0.08	0.00						0.00	0.00	0.00
0.25	0.00	259.70					259.70	3619.57	301.63
0.42	0.00	241.06					241.06	3359.71	581.61
0.58	0.00	232.25	53.93				286.18	3988.52	612.35
0.75	0.00	223.75	48.23				271.98	3790.69	648.27
								3988.52	**2143.86**

From Table 23, the C_{max} is 3988.52ng/mL and the AUC at 45 minutes is 2143.86 ng.h/mL.

The above-calculated PK parameters are represented in Figure 9.

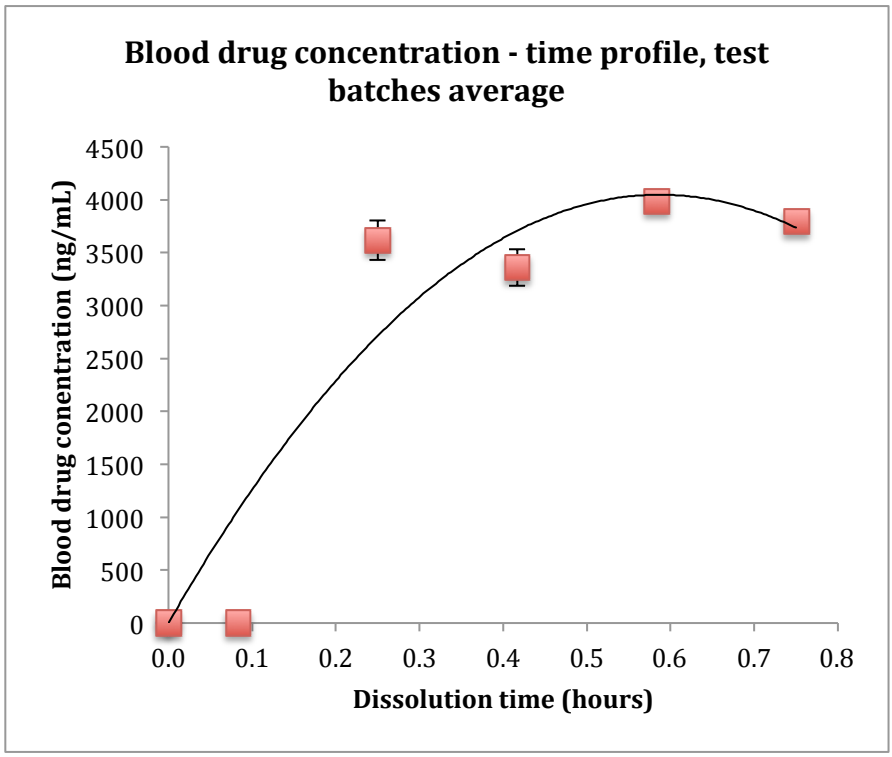

Figure 9: Predicted concentration-time profile for three Generic Batches (averaged)

Computed PK values for reference product

The dissolution profile data for the reference product was also subjected to similar treatment and results obtained are presented in Table 24.

Table 24: Amount of drug calculated at the end of each sampling time interval for the Reference product

Time (h)	% Released (Cumulative)	% Released (within sampling interval)	Amt. (mg) released (within sampling interval) Tablet Strength (500 mg)	Amt. (mg) corrected for bioavailability (F)
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

The corresponding computations and representation of various parameters and values for the reference product are shown in Table 25.

Table 25: Amount of Paracetamol in blood computed from in vitro data for the Reference product

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**						**Total Blood Amt. after Absorption**	**Conc. (ng/mL) at Times**	**AUC**
0	0.00						0	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**

From Table 25, the C_{max} is 3993.3ng/mL and the AUC at 45 minutes is 2190.68 ng.h/mL. The blood drug concentrations – time profile is predicted as follows:

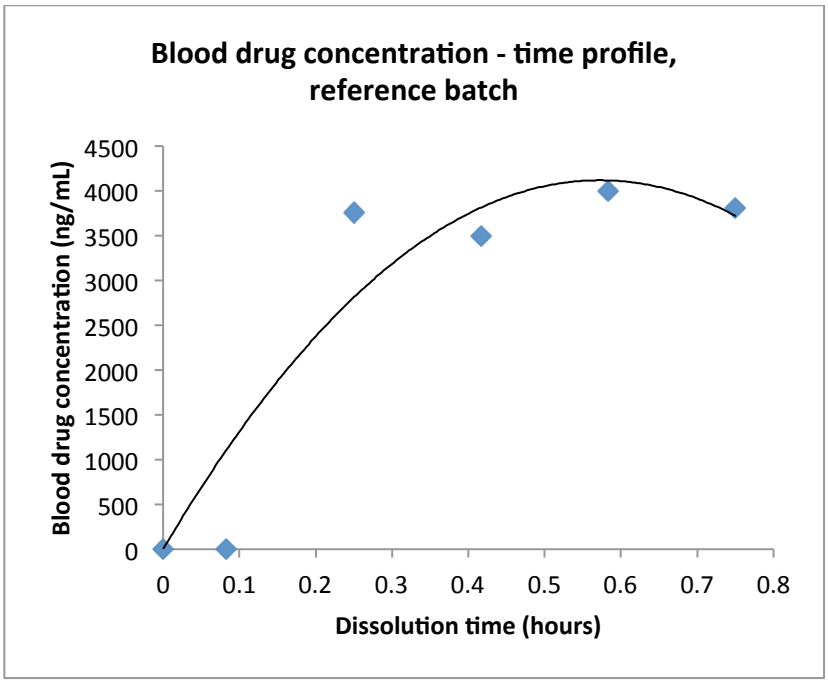

Figure 10: Predicted concentration-time profile for Reference Product

4.2.1 C_{max} and AUC

In vitro conditions being dissimilar to in vivo conditions, measures that are more standard than those obtained so far are desired. These standard measures are the C_{max} and the AUC. These are normalized parameters derived from drug concentration time profiles. An evaluation of these two bioavailability parameters determines bioequivalence between two formulations. For the two formulations under study, these factors were computed and tabulated.

C_{max}

The comparative C_{max} values are presented in the Table 26 and Figure 11.

Table 26: IVIVC-predicted C_{max} values for generic and Reference Product

	Generic Batch A1	Generic Batch A2	Generic Batch A3	Generic batches Av	Reference Product
Cmax (ng/mL)	3,949.20	4,039.85	3,976.51	3,988.52	3,993.30

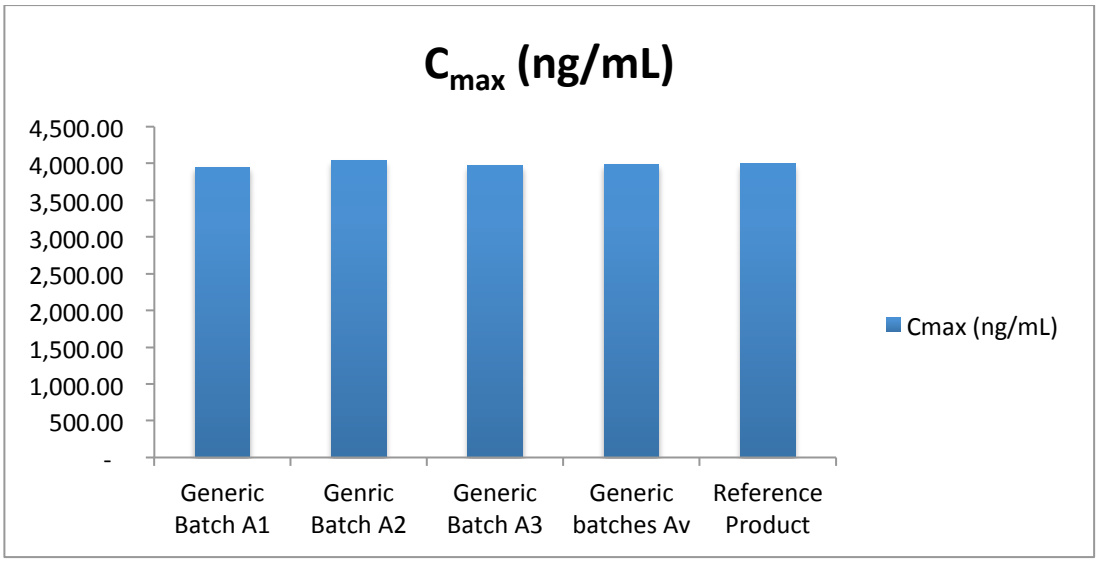

Figure 11: Comparative C_{max} between generic batches and the Reference product.

The Cmax values were treated to ANOVA to determine variability between the batches of the experimental product and the reference product.

Table 27: Analysis of Variance (ANOVA) of C_{max} values

ANOVA Table

Source of variation	Sum of squares	d.f	Mean square	*F* statistic	p-value[1]
Between Groups	21423.8	3	7141.25	0.110195	0.952893
Within Groups	1036890	16	64805.5		
Total	1058310	19			

[1] p-value (two-tailed)

ANOVA suggests that the reference and experimental products have comparable Cmax values.

AUC

The AUC values obtained from each of the batches of the generic product are summarized in Table 28.

Table 28: IVIVC-predicted Cmax values for generic and Reference Product

	Generic Batch A1	Generic Batch A2	Generic Batch A3	Generic batches Av	Reference Product
AUC (ng.h/mL)	2,161.75	2,199.11	2,070.71	2,143.86	2,190.68

The AUC values are compared and presented in Figure 12.

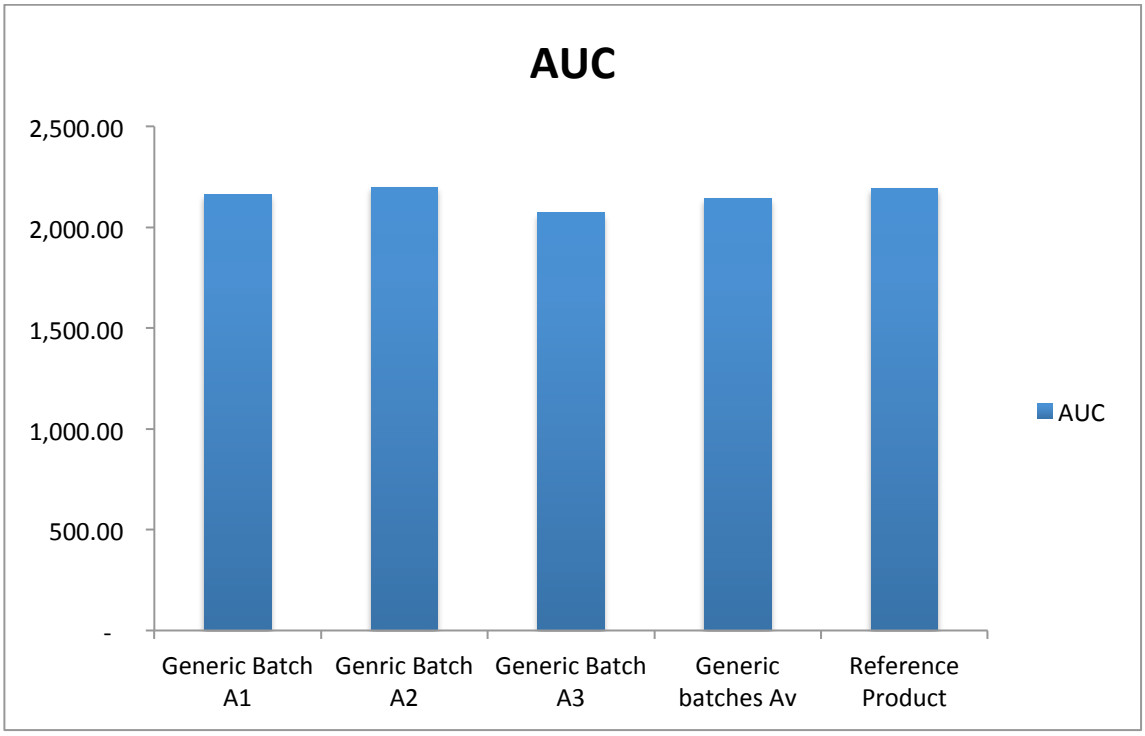

Figure 12: AUC of the Generic batches, their average, and the Reference Product

The AUC computed for the experimental batches and the reference product were subjected to ANOVA. The results obtained are summarized in Table 29:

Table 29: Analysis of Variance (ANOVA) of AUC values

Analysis of Variance (ANOVA) of AUC values
ANOVA Table

Source of variation	Sum of squares	d.f	Mean square	*F* statistics	p-value[1]
Between Groups	51263.8	3	17087.9	0.0487994	0.985202
Within Groups	5602660	16	350167		
Total	5653930	19			

[1] p-value (two-tailed)

ANOVA suggests that the reference and experimental products have comparable AUC values.

5.0 DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

Single point dissolution tests have been used in SUPAC for validating changes such as batch size, change of manufacturing site, formulation changes, and equipment and process changes. Dissolution profile comparisons under identical conditions are undertaken when major changes have taken place, in which case a demonstration of overall profile similarity and at every dissolution sample time point are essential to demonstrate comparability to the reference product or reference batch or formulation.

The *p*-values calculated for each sampling time point were generally $p \geq 0.05$, indicating that there was no difference in dissolution time profile between the experimental batches and the reference product. Further, the calculated similarity factor f_2 values for the three experimental batches fell within the acceptance range as per US FDA and EMEA. These two analytical approaches indicate that the three batches of experimental product that were subjected to the dissolution time profile test using similar test conditions as those used to test the reference product, were similar in dissolution-time behavior under the dissolution test conditions.

5.2 Conclusions

From the results obtained, it may be concluded that the C_{max} and the AUC values obtained from IVIVC for both the generic Paracetamol product indicate similarity to those obtained from the reference product that has been evaluated and already registered by the national drug regulatory authority.

Further, the therapeutic indications of Paracetamol are not considered critical (Kalantzi, 2006). There is also a wide difference between the therapeutic dose and the toxic dose,

indicating that Paracetamol does not have a narrow therapeutic window. On this basis and on the basis of the similarity established between the test product and the reference product, and also that the public health consequences for any comparative dissolution differences are not serious (Kalantzi, 2006), refraining from in vivo BE studies can be scientifically justified.

The in vitro dissolution profile test may be used to predict the drug concentration-time profiles on the basis of C_{max} and AUC. The IVIVC tool is much simpler that BE studies. The tool can therefore be used to obtain the blood drug concentration-time profile for Paracetamol when comparative dissolution time profile test results are compared to those of an already evaluated and registered reference product.

The benefits of the IVIVC tool are summarized: IVIVC is an appropriate tool for simulating BE studies. It saves the high cost of BE studies and reduces product development lead-time particularly for qualifying generic products. In this way, the IVIVC lowers the cost of generic drugs, and can therefore be recommended for use in MER. It is also clear that IVIVC eliminates the exposure of human subjects to drug substances that are not required thereby addressing the ethical question.

5.3 Recommendations

The predictive value of the IVIVC tool has been demonstrated for generic Paracetamol tablets, when compared to a reference product. In vitro dissolution profile data have been used in this particular case and a comparison to the PK parameters obtained from data generated from a reference product tested under similar conditions.

Employment of IVIVC will help reduce product development costs and lead times, and also medicines evaluation lead times. It will also provide a basis for biowaiver for applicable generics.

IVIVC should be employed for routine use in medicines evaluation and registration.

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7.0 APPENDICES

7.1 Timelines

The timeframes that were followed in the study project are given in Figure 13.

Month	**February 2016**				**March 2016**					**April 2016**				**May 2016**				
Week in 2016	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
First Monday	1	8	15	22	28	7	14	21	28	4	11	18	25	2	9	16	23	30
Concept development																		
Supervisor Report																		
Literature search																		
Proposal writing																		
Data Collection																		

Month	**June 2016**				**July 2016**					**August 2016**				**September 2016**				**Oct**
Week in 2016	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	39	39	40
First Monday	5	12	19	26	3	10	17	24	31	7	14	21	28	4	11	18	25	2
Supervisor Report																		
Data Computation																		
Draft dissertation writing																		
Presentation of dissertation																		
Final dissertation preparation																		
Dissertation defense																		

Figure 13: Project Timelines