An-Najah National University

Faculty of Graduate Studies

Evaluation of Food Effect on the Absorption of Clarithromycin using Physiological Modeling

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Dedication

All praise to Allah, today I fold the days tiredness and the errand summing up between the cover of this humble work…

To the utmost knowledge lighthouse, to our greatest and most honored **prophet Mohammad**- may peace from Allah be upon him

To the spring that never stops giving, to whom she weaves my happiness, with strings from her merciful heart… To my beloved **mother**

To whom he strives to bless comfort and welfare, never stints what he owns to push me in the success way… To my beloved **father**

To whose loves flows in my veins, and my heart always remembers them…

to my dearest **brothers** and beloved **sisters**

To all who gave me help and support throughout my life

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Finally, my great thanks and supplication to my mother,

father, sisters and brothers

And I can't forget my beloved friends, their encouragement was my motivation.

Rand Jayyousi

الإقرار

أنا الموقع أدناه موقع الرسالة التي تحمل العنوان:

Evaluation of Food Effect on the Absorption of Clarithromycin using Physiological Modeling

أقر بأن ما اشتملت عليه الرسالة هو نتاج جهدي الخاص، باستثناء ما تمت الإشارة إليه حيثما ورد، وأن هذه الرسالة ككل، أو أي جزء منها لم يقدم من قبل لنيل أي درجة أو لقب علمي أو بحثي لدى أي مؤسسة تعليمية أو بحثية أخرى.

Declaration

The work provide in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

Student's name: **اسم الطالب:**

Signature: **التوقيع:**

Date: **التاريخ:**

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List of Abbreviations

ACAT	Advanced Compartmental Absorption and Transit model
API	Active pharmaceutical ingredient
AUC	Area under plasma drug concentration-time curve
BA	Bioavailability
BCS	Biopharmaceutical classification system
BE	Bioequivalence
C_{max}	Maximum plasma drug concentration
CLM	Clarithromycin
Conc.	Concentration
ER	Extended release
FaSSGF	Fasted State simulated Gastric Fluid
FaSSIF	Fast state simulated intestinal fluid
FDA	Food and drug administration
FeSSIF	Fed state simulated intestinal fluid
GI	Gastrointestinal
GIT	Gastrointestinal tract
H	Hour
HCl	Hydrochloric acid
HPLC	High performance liquid chromatography
IR	Immediate release
IVIVC	In vitro in vivo correlation
L	Liter
µl	Microliter
MDT	Mean dissolution time
Mg	Milligram
mg/ml	Milligram per milliliter
Min	Minute
mM	Millimolality
mPa.s	Millipascal second
MRT	Mean residence time
NaCl	Sodium chloride
$NaH_2PO_4*H_2O$	Sodium dihydrogen phosphate monohydrate
NaOH	Sodium hydroxide
PE	Prediction error
P_{eff}	Effective permeability
PK	Pharmacokinetic
PTFE	Polytetrafluoro ethylene
Rpm	Round per minute

SD	Standard deviation
SGF	Simulated gastric fluid
SI	Small intestine
SIF	Simulated intestinal fluid
SUPAC	Scale up and post approval changes
susp	Suspension
Tab	Tablet
$T_{1/2}$	Half-life
T_{max}	Time to reach maximum plasma concentration
USP	United State Pharmacopeia

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Evaluation of Food Effect on the Absorption of Clarithromycin using Physiological Modeling

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Abstract

Background: Food may affect the oral absorption of drugs by inducing physiological changes in the gastrointestinal physiology, such as: gastrointestinal pH, gastric residence time, bile salt secretion and drug metabolism.

Purpose: The aim of the present study was to investigate the influence of food on the oral absorption of Clarithromycin by evaluating the effect of media parameters such as; pH, bile secretions and food composition, on the release of the drug from immediate release tablet, using *in vitro* and *in silico* assessments

Method: The solubility, disintegration and dissolution profiles of Clarithromycin 500 mg immediate release tablets in compendial media with/without the addition of homogenized FDA meal as well as in biorelevant simulated intestinal media mimicking fasting and fed conditions were determined. These *in vitro* data were input to GastroPlus™ to make computational simulation in order to anticipate the effect of food on Clarithromycin absorption profiles under fasted and fed states. *In vivo* plasma concentration curves were used for compartmental modeling of pharmacokinetic data. Level A *in vitro – in vivo* linear correlations were established using mechanistic absorption modeling based deconvolution

approach. Gastroplus™ was used for developing a physiological absorption model for Clarithromycin, which is capable of predicting the *in vivo* performance

Results: Media pH has a profound effect on drug solubility, tablet disintegration and drug release. Clarithromycin has lower solubility in the biologically biorelevant media compared to other media, due to complex formation with bile salts. Clarithromycin tablets exhibited prolonged disintegration times and reduced dissolution rates in the presence of the standard FDA meal. The simulation model predicted no significant food effect on the oral bioavailability of Clarithromycin. The developed IVIVC model considered SIF, acetate, and FaSSIF buffer media to be the most relevant from the physiological standpoint.

Conclusion: the intake of standard FDA meal with Clarithromycin may have no significant effect on oral bioavailability of Clarithromycin from immediate release tablets. This may suggest that the dissolution conditions recommended by ICH are sufficient to demonstrate interchangeability between generic and brand Clarithromycin, a class II drug, especially during the developmental phase of the generic product.

Chapter One

1. Introduction

Oral administration is the most convenient route of drug delivery, because of its safety, effectiveness and feasibility. Immediate release tablet is the most popular dosage forms given by this route. For these formulations, tablet disintegration and drug dissolution are essential steps in the absorption process.

Drug release from the solid dosage form into the GI (Gastrointestinal) fluids is the first step in the absorption process. After being dissolved, the active pharmaceutical ingredient (API) will be absorbed from the GI into the systemic circulation before reaching the target site.

In this thesis, the effect of food on CLM bioavailability (BA) is investigated. The study is prepared to evaluate the effect of media parameters such as; pH, bile secretions and food composition, on CLM release from immediate release tablet.

In the introduction, a description of food effect on the gastrointestinal tract (GIT) physiology is presented, with a special concern of the mechanisms underlying positive food effect. Furthermore, *in silico* and *in vitro* modeling for predicting the *in vivo* performance of the drug are discussed.

1.1 Food effect

Food may affect the rate and extent of drug BA by inducing physiological changes in GIT such as: lumen pH, gastric emptying time, bile salts

secretions and drug metabolism [1]. Food effect may have clinical significance in term of drugs' safety and/or efficacy [2]. Food may have positive or negative or no effect on drug absorption [3].

Positive food effect

A drug exhibits positive food effect if its extent of absorption -area under plasma drug concentration-time curve (AUC) - is enhanced by 25% or more when administered with food. Biopharmaceutical classification system (BCS) class II, for which drug dissolution is the rate limiting step, are likely to have positive food effect. About 71% of BCS II drugs have shown to exhibit positive food effect while 29% have no effect [4]. Several mechanisms have been identified for the observed positive effect on BCS Class II drugs, which include the enhanced solubility of these agents by high fat meal and micelles formation.

Negative food effect

A drug is described of having negative food effect if the rate and extent of its absorption were reduced by 20% -or more upon its administration with food. This can be attributed to delayed tablet disintegration and drug dissolution, prolonged drug transit time and enhanced metabolism of the drugs [5].

The positive food effect and the mechanisms behind it depend on:

1.1.1 Gastrointestinal pH

The pH along the human GIT varies greatly. In fact, in the upper part, acidic conditions dominate, whereas; in the small intestine, the conditions are more alkaline. The luminal pH is largely affected by the fed state. In fact, food intake would induce changes in the luminal pH by inducing acidic secretion in the stomach as well as the pancreatic bicarbonate secretion in the small intestine. The gastric pH was shown to be elevated to pH close to 6.7 after meal ingestion [6], due to the foods' buffering capacity of the gastric secretions [2], while the duodenal pH was shown to decrease to reach a pH value of 5.4 [6].

Table 1 shows the pH of the different regions of the GIT in the fast vs. fed state. Such variability in the GI pH may have impact on the rate of drug dissolution and absorption. This is of especial importance for both acidic and basic drugs having pH dependent solubility. Weak basic drugs readily dissolve in the acidic media, whereas weak acidic drugs dissolve more in the alkaline environment of the small intestine.

Table 1: The pH of the different parts of the GI in the fasted and fed states

GI part	pH in the fast state	pH in the fed state
Stomach	1.5-2	4.3-5.4 [6]
Duodenum	6.5	4.5-5.5 [7]
Jejunum	6-7	5.2-6 [7]
Ileum	6.5-8	6.8-8 [8]

1.1.2 Gastric residence time

Food intake may delay the gastric emptying rate, the degree of which depends on the composition and the volume of the ingested meal. Fiber rich food was shown to retard the gastric emptying and prolong the residence time to a great extent [9,10]. Solid components of food have larger residence time than liquids. Whenever meal size become greater the residence time increased [11]. Prolonged residence time may enhance the dissolution of low soluble drug.

1.1.3 Drug metabolism

Certain types of food may influence drug metabolism due to its effect on the metabolic enzymes, especially Cytochrome P450 enzyme, which is highly expressed in the intestine and has a broad substrate specificity [12]. Broiled food or cruciferous vegetables can induce the metabolism of some drugs, where grapefruit juice may interfere with the metabolizing enzymes, this increase the bioavailability of this enzyme substrate [13].

1.1.4 Bile salts secretion

Bile salts, which are excreted from the gall bladder, are important in the digestion and absorption of nutrients [14]. The concentration of bile salt in fast state is in the range of 0.6–17 mM [15]. Food intake stimulates the secretion of bile salts, and increases its concentration to become in the range of 1.6–40 mM [16, 17].

Bile salts may affect drug absorption by different mechanisms; bile may enhance the solubility of poorly soluble drugs and enhance the absorption through micelles formation, on the other hand, bile salts may form complex with some drugs, such as atenolol reducing its BA [18].

In the fasting state, bile salts play a role in wetting more than the solubilization effect, while in higher concentration like in fed state the solubilization effect was predominant [19].

1.1.5 Food composition

Food composition is one of the important parameters controlling food-drug interactions. The intake of fatty meal was shown to improve the solubility and the bioavailability of lipophilic drugs by simulating bile salts secretions. For instance: The bioavailability of griseofulvin was enhanced by intake of a fat rich meal, however, no effect was observed when protein or carbohydrate rich diets were ingested [20]. Another study on the effect of food on ketoconazole dissolution has shown that protein components of food may have positive food effect on BCS class II drug, due to dissolution enhancement. The dissolution rate of ketoconazole in medium containing whole fat milk was better than that in semi-skimmed milk, and skimmed milk [21].Another mechanism that was recently estimated by Radwan et al, consists of the effect of viscous food such as Jute [28].

1.2 Models for predicting Food Effect

Several Approaches have been used for prediction of food effect on drug absorption. These include:

- Biopharmaceutical Classification Systems (BCS) approach, which predicts food effects based on the physicochemical characteristics of the drug such as solubility and drug permeability.
- *In vitro* dissolution testing: Physiologically based media approach
- *In silico* method using GastroPlus™ software

1.2.1 Biopharmaceutical Classification Systems (BCS)

Biopharmaceutical classification system (BCS), was firstly introduced by Gordon Amidon in 1996. It is a scientific framework that classifies the drugs according to their solubility and permeability into 4 classes (Figure 1):

- BCS Class I: High solubility-high permeability drugs,
- BCS Class II: Low solubility-high permeability drugs,
- BCS Class III: High solubility-low permeability drugs, and
- BCS Class IV: Low solubility-low permeability drugs.

The BCS is based on recognizing that drug dissolution and GI permeability as the basic parameters controlling drug absorption. It classifies the drugs in a way to ease correlating *in vitro* drug dissolution and *in vivo* bioavailability.

BCS system helps in prediction food effect on drug [22]. Accordingly, BCS Class I drugs are predicted to have no food effect, since gastric emptying is the rate limiting step for their absorption. While, BCS Class II

are likely to have positive food effect, since the dissolution is considered as the rate limiting step for their absorption. On the other hand, BCS Class III drugs would be expected to exhibit negative food effect, especially that drug permeation is the rate-controlling step. For BCS Class IV drugs, food effect is unpredictable [4].

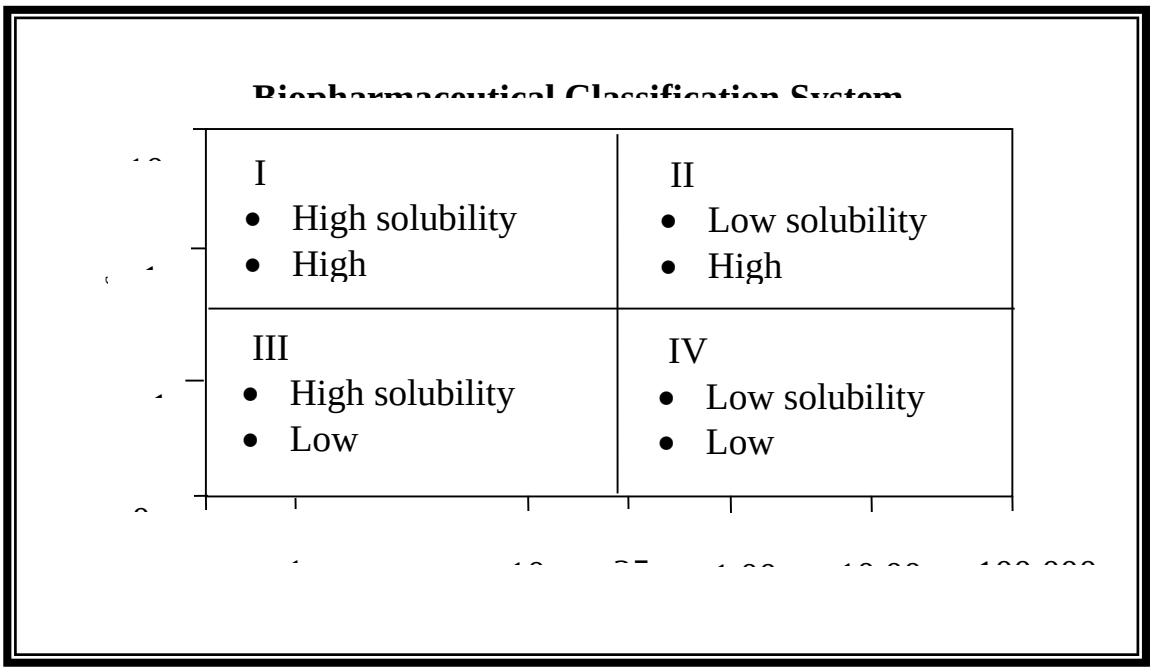

Fig 1: Biopharmaceutical Classification System

1.2.2 Dissolution test

Dissolution testing is an important tool, which is useful in many pharmaceutical fields such as: drug development, for quality control and, determination of bioequivalence. It is important in the regulation of post-approval changes [23]. In addition, it can be used to compare the release of same active ingredient for different formulations [24].

In vitro dissolution profiles give information about the rate of drug release in the dissolution medium, and it is a vital tool in predicting the *in vivo*

performance of drugs. Since *In vivo* BA and bio equivalence (BE) studies are expensive and invasive, dissolution could be used as a surrogate for bioequivalence (BE) studies in certain cases, especially for class I drugs.

1.2.2.1 Dissolution media

The available compendial media are not representative of the *in vivo* environment of the GI physiology. These include three different pH media that cover the entire pH of the GIT 1.2, 4.5 and 6.8. Various physiologically biorelevant media were developed to simulate the gastric and intestinal fluids under fed conditions. These systems were reported to be superior in predicting *in vivo* performance especially for low soluble drugs [25].

They were suggested as an alternative to the homogenized standard meal. These models consider factors such as: media pH, osmolality, surface tension, bile salts, lipolytic enzymes and phospholipids contents. However, the effect of standard food composition on drug release was not well defined. An overview of the composition of the compendial and biorelevant media and their applications follows:

1.2.2.2 Compendial Dissolution Media

- *Simulated Gastric Fluid* (SGF): is a medium that simulates the pH of gastric environment in the fast state. It consists of hydrochloric acid and sodium chloride, pepsin and water, the pH of this media is 1.2 [25].

- *Simulated Intestinal Fluid (SIF):* is a medium simulates the small intestinal (SI) conditions in the fast state. The pH of this medium is 6.8 [25].

1.2.2.3 Biorelevant Dissolution Media

- *Fasted State simulated Gastric Fluid (FaSSGF):*

This medium was developed to simulate the gastric conditions under fasted state. It contains pepsin, lecithin, and bile salt in the physiological concentration [25].

- *Fasted State simulated Intestinal Fluid (FaSSIF):*

This medium was proposed to mimic the fasting conditions in the proximal small intestine. FaSSIF consists of phosphate buffer with a pH of 6.5, it contains bile salts and phospholipids (lecithin) in a ratio of 4:1. Sodium taurocholate was selected to represent the bile salts in concentration about 3–5 mM. These compounds enhance the solubility of lipophilic drugs more than the observed value in simple aqueous solutions.

The buffer capacity in fed state or in SIF is much higher than the fast state. The volume of dissolution test is ≤500 ml, because the maximum total volume of proximal SI is 300–500 ml according to pharmacokinetic (PK) studies of drug absorption in the fasted state [25].

- *Fed State Small Intestinal Fluid (FeSSIF)*

This system was designed to mimic the conditions in the SI after meal ingestion. The pH of FeSSIF is 5 since the pH of chyme is less than that in the fast state, while the osmolality, buffer capacity and bile concentration

increased sharply, taurocholate and lecithin are present in FeSSIF in higher concentration compared to FaSSIF. The volume of the dissolution media is up to 1 L; because the food induce the secretions [25].

Table 2: Sample Composition for Biorelevant Dissolution Media

FaSSGF	**Sodium taurocholate**	**Lecithin**	**Pepsin**	**NaCl**	**HCl conc.**	**Deionized water**	**pH**	**Osmolality (mOsmol/kg)**	**Buffer capacity (mEq/pH/L)**	**Surface tension (mN/m)**
	80 µM	20 µM	0.1 mg/ml	34.2 mM	*qs ad* pH 1.6	*ad* 1 L	1.6	120.7 ± 2.5	_____	42.6
FaSSIF	**Sodium taurocholate**	**Lecithin**	**NaH2PO4**	**NaCl**	**NaOH**	**Deionized water**	**pH**	**Osmolality (mOsmol/kg)**	**Buffer capacity (mEq/pH/L)**	**Surface tension (mN/m)**
	3 mM	0.75 mM	3.438 g	6.186 g	*qs ad* pH 6.5	*qs ad* 1 L	6.5	~270	~12	54
FeSSIF	**Sodium taurocholate**	**Lecithin**	**CH3COOH**	**NaCl**	**NaOH pellets**	**Deionized water**	**pH**	**Osmolality (mOsmol/kg)**	**Buffer capacity (mEq/pH/L)**	**Surface tension (mN/m)**
	15 mM	3.75 mM	8.65 g	11.874 g	4.04 g	*qs ad* 1 L	5.0	~670	~72	48

These media neglected food composition and its effect on drug absorption, so *in vivo* fed BA or BE studies recommended to be conducted using meal that provide the greatest effect on GI physiology. A high- fat (approximately 50 percent of total caloric content of the meal) and high-calorie (approximately 800 to 1000 calories) meal is recommended as a test meal for fed BE studies. An example test meal would be two eggs fried in butter, two slices of toast with butter, four ounces of hash brown potatoes and eight ounces of whole milk [26].

Simulation of the *in vivo* conditions is important for the prediction of drug absorption. Food effect can be predicted by using *in vitro* dissolution in a medium simulates the luminal composition following food intake. Accordingly, these tools could be of great importance in the IVIVC studies.

1.2.3 *In* silico method

Simulation technology has proven its usefulness in many fields of pharmaceutical sciences during the process of developing formulation. Simulation technology was effective in predicting food effect on the GI absorption of many poorly soluble drug [27,28]. Gastroplus™ software is an example, which has applications based on BCS theory and the advanced compartmental transit and absorption model. Gastroplus™ can aid in the development of new formulations and the selection of biorelevant dissolution conditions with IVIVC, as well as in the assessment of waiving BE studies and the determination of IVIVC for class II and III drugs, reducing both research time and the cost of developing new generic

medicines [29]. *In silico* method, was used to predict the *in vivo* behavior of many drugs and establish IVIVC for them, such as; etoricoxib, and montelukast (Singulair®) [30].

In silico modeling can be coupled with *in vitro* dissolution test for better prediction of the *in vivo* performance under fed conditions. In fact, Food effect on the absorption of low soluble celecoxib was better predicted by coupling the *in silico* simulation with biorelevant dissolution testing compared to compendial media [27]. Another study on the *in vivo* delivery of nifedipine previous reports suggested the potential of coupling *in silico* method with *in vitro* dissolution data for identification of the *in vivo* drug product behavior under fed condition [31].

1.3 *In Vitro In Vivo* Correlation (IVIVC)

IVIVC is defined by (FDA) as ‘a predictive mathematical model describing the relationship between an *in vitro* property of a dosage form and an *in vivo* response’.

Generally, *in vitro* dissolution test can be used as the *in vitro* property, whereas, the *in vivo* plasma drug concentration or amount of drug absorbed as the *in vivo* response [32]. There are four levels of IVIVC:

-Level A correlation: is considered as the most useful and informative type. It provides a point-to-point relationship linking the *in vitro* dissolution and the *in vivo* absorption rate of a drug from the dosage form. This level can be used as a surrogate for *in vivo* performance of a drug product [33].

- Level B correlation: This level of correlation involves a relationship between *in vitro* mean dissolution time (MDT) with either the mean *in vivo* residence time (MRT) or mean *in vivo* dissolution time. It is considered the least useful among the different types for regulatory purposes since it is not a point-to-point correlation [33].
- Level C Correlation: This type represents a single-point relationship between a dissolution parameter (e.g., $t_{1/2}$) and a pharmacokinetic parameter (e.g., AUC, T_{max}, C_{max}). A Level C correlation does not reflect the complete shape of the plasma concentration time curve [33].
- Multiple Level C correlation: A multiple level C correlation compares one or more pharmacokinetic parameters of interest (e.g., C_{max}, AUC) to the amount of drug dissolved [33].

Many researchers aim to develop IVIVC for drugs in order to predict the *in vivo* performance according to *in vitro* dissolution test [32]. Developing such an IVIVC would reduce the number of BE studies required during the drug approval process [34,35,36], which is time consuming and very costly.

1.4 Clarithromycin

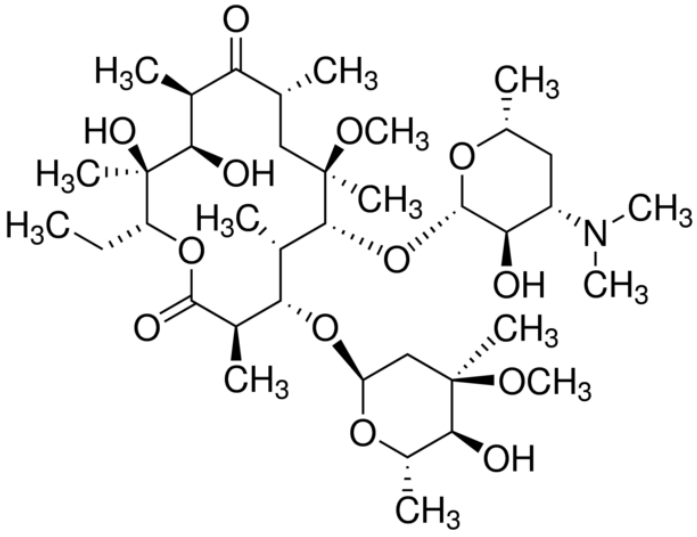

Figure 2: CLM chemical structure

CLM is a broad-spectrum antibiotic belongs to macrolide group [37]. It is used in treatment of lower respiratory tract infection, sinusitis, pharyngitis, and skin infections [38].

CLM is rapidly absorbed, it take 2 h after oral administration to reach the peak concentration. Its protein binding about 72%, but with increasing the drug conc. the binding decreases. CLM conc. in the lung are higher than in the serum by 5 times [39].

CLM oral BA is 52 to 55%, and it has an active metabolite, which is 14-hydroxy due to first-pass metabolism. CLM is eliminated by nonlinear PKs, such that with increasing the dose, the total clearance decreases, while the elimination half-life and AUC increases. The volume of distribution ranges from 191-306 L. The drug is excreted out the body in faeces (40%) , and (53%) in the urine, Therefore, there is no need for dose adjustment in elderly or hepatic disease patients, however, it is necessary in renal failure patients due to the increased level of the parent drug and its active metabolite. CLM

is excreted in nursing mothers milk, who treated with the drug [40]. The $t_{1/2}$ of CLM is from 3.3-4.9 h [41].

CLM metabolism is affected by drugs inducing CYP450 enzyme, therefore, it has several drug-drug interaction, since it increases the plasma conc. of these drugs due to its effect on their metabolic enzymes: with theophylline, cyclosporin, tacrolimus, carbamazepine, digoxin and rifabutin [40].

CLM classified as BCS class II due to its very low solubility and good permeability [42]. This drug has low bioavailability and its absorption is dissolution rate limited [37]. It is a weak basic drug with a pka value of approximately 9 [43], with pH-dependent solubility; highly soluble at low pH and low soluble at high pH. The variability in the GI pH may have significant effect on this drug solubility. Food was shown to have positive effect on CLM absorption [44].

CLM antibiotic presents in Palestinian market in different trade names and pharmaceutical dosage forms like: Klaricare 500 tab, Klaricare XL 500 tab, Klaricare 125 mg susp, Klaricare 250 mg susp, Klacid 500 tab, Klacid XL 500 tab, Klacid 125 mg susp, Klarimax 500 tab, Klarimax 125 mg susp, Klarimax 250 mg susp, Laricid 500 tab. We used Klaricare 500 tab as a model drug in our experiments.

1.4.1 Clarithromycin stability and solubility

The stability of CLM was studied in aqueous solution and in human gastric juice, CLM was stable at pH range 5-8 in aqueous solution study, but it rapidly degrades at gastric pH (1-2). At pH below 5 the degradation rate

increased. About 90% of the drug lost from aqueous sample at pH 1.2, while 41.1% was lost at pH 2.0. The kinetic of degradation of CLM was pseudo-first order [45]. The $T_{1/2}$ of CLM degradation at pH 1.39 was 17 min, and 10 min at pH 1.2. CLM was stable at pH 3.0 [46].

The solubility of CLM was shown to be pH-dependent, i.e.: it decreases with increasing pH and kept constant at pH 9.The solubility of CLM in water is affected by temperature increasing, it decreased with increasing temperature [46]. CLM was freely soluble in chloroform and sparingly soluble in methanol [47].

1.4.2 Food effect on Clarithromycin

Literature data on food effect on the oral BA for CLM is contradictory. A preclinical study in dogs demonstrated a pronounced decrease in C_{max} and AUC values in fed state compared to the fasted state. *In vivo* human study reported an increase in the bioavailability of CLM by 25% upon co-administration of CLM immediate-release tablets with food. However, this increase was considered to be non-clinically significant effect. They concluded that immediate-release CLM could be taken with or without food [44].

Positive food effect on CLM extended release (ER) has been demonstrated in several studies. In Guay et al study, the oral bioavailability (AUC) of an extended release tablet containing 500 mg of CLM administered in the fasted state was found to be 30% lower than in the fed conditions [48]. Similarly, Sanjay J. Gurule reported significant positive-food effect on the rate and extent of absorption of CLM 500 mg extended

release tablet when comparison is made between fasting and different types diets (high-fat and low-fat vegetarian and non-vegetarian) [49]. Moreover, a bioequivalence study on CLM extended 500 mg tablet under fasting and fatty meal conditions in 38 Jordanian volunteers has shown an increase in both C_{max} and AUC for the test and reference formulations in fed condition compared to the fasting conditions [50].

1.5 Aims of the study

The general aims of this work were to get an insight on the effect of food on the oral absorption of CLM from immediate release formulations as well as to study the influence of media related parameters on CLM release such as: food composition, bile salts concentration and media pH.

The specific goals were to:

1. Characterize the effect of food composition on the release process by conducting *in vitro* dissolution study in media containing standard meal.
2. Check the feasibility of biorelevant media in predicting food effect by comparing drug release profiles in these systems with that in compendial buffers with/without homogenized fatty meal.
3. Develop a physiological absorption model for CLM capable of predicting the *in vivo* performance of the drug using computational technology.
4. In an attempt to develop a correlation between the *in vitro* data and *in vivo* profiles.

5. Select the most suitable dissolution test that could be used as surrogate for *in vivo* bioavailability testing.

Chapter Two

2. Methodology

2.1 Materials, Equipment and tools.

Hydrochloric acid (Israel), glacial acetic acid (Frutarom), monobasic potassium phosphate (Israel), sodium acetate (Israel), phosphoric acid (Frutarom), methanol HPLC-grade (LAB-SCAN, Ireland) , sodium hydroxide (Israel), sodium hydroxide (pellets) (Israel), sodium chloride (Israel), sodium phosphate monobasic monohydrate (Sigma-Aldrich), Acetonitrile HPLC-grade (Sigma-Aldrich) and Biorelevant media powder (Biorelevant Company, UK) were used during the study.

Klaricare® 500 mg immediate release tablets were purchased from local Pharmacies. CLM API was obtained as a gift sample from Pharmacare Company, Palestine.

- Equipment and tools:

The equipment that were used during this work include: Centrifuge (Hettich zentrifugen universal 320), thermo shaker (BOECO TS-100), pH meter-JENWAY 3510, dissolution tester BTC-9100 (Hsiang taimachinery industry co, ltd), tablet disintegration tester (µP, 1901), rotational rheometer (Brookfield DV1 viscometer), GastroPlus™ software (version 9.0, Simulations Plus Inc., Lancaster, CA, USA).

2.2 Media composition and preparation.

Different types of media with various pH were used to simulate the fasted state:

- Simulated Gastric Fluid (SGF) without pepsin, which has pH=1.2, was prepared by diluting 9.7 ml of 32% HCl in one Liter distilled water.
- Acetate buffer (pH=4.5) was prepared by mixing 2.99 g of sodium acetate, and 1.66 g of glacial acetic acid, then distilled water added up to 1 L, the pH adjusted to 4.5. [64]
- Simulated Intestinal Fluid (SIF), which has a pH =6.8, was prepared by dissolving 40.83 g monobasic potassium phosphate and 2.4 g sodium hydroxide in 1000 ml distilled water. [64]
- FDA standard breakfast was prepared to investigate the effect of food composition on the *in vitro* release of CLM. FDA standard meal was selected as a test meal since it is the recommended food to be administered to the healthy volunteers in Fed bioequivalence and Fed bioavailability studies. FDA food were prepared by mixing: two strips of hot dogs (44g), two eggs fried in a butter, two slices of toast with butter, eight ounces of whole milk (226.8 g), four ounces of hash brown potato (113.39 g), and 240 ml of tap water in a mixer until being homogenized [26]. Then, 100 ml of this homogenized meal were mixed thoroughly with 800 ml of the different buffers (SGF, acetate buffer, SIF) using a mixer.
- FaSSIF (pH=6.5), was prepared in two steps. In the first step, Phosphate buffer was prepared by dissolving 3.95 g of $NaH_2PO_4*H_2O$

(monohydrate), 0.42 g sodium hydroxide (pellets) and 6.19 g of sodium chloride in 0.9 L of purified water. The pH was adjusted to 6.5 with either 1 N HCl or 1N NaOH. Purified water was added to make the volume up to one Liter. The second step entailed dissolving 2.24 g of biorelevant powder in 500 ml of the buffer, and stirring until the powder has dissolved and the volume was made up to one Liter. FaSSIF was left to stand for 2 h before being used. [65]

- FeSSIF media (pH=5), was prepared using acetate buffer, in which 8.65 g of glacial acetic acid, 4.04 g of sodium hydroxide (pellets), and 11.87 g of sodium chloride were dissolved in 0.9 L of purified water. The pH was adjusted with 1N HCl/1N NaOH to the desired value (pH=5). Purified water was added to make the volume up to one L. 11.2 g of the biorelevant powder was then added to 500 ml of the acetate buffer, and stirred until all the powder has dissolved, then the volume was made up to one Liter with purified water. [65]

2.3 pH measurement

All measurements of pH values of different media were determined using pH meter- JENWAY 3510. The pH meter was calibrated between pH (4) and pH (7).

2.4 Solubility study

Flask shake method was used to determine the equilibrium solubility of CLM in the different media. CLM powder was added in excess amount to 10 ml of the investigated media in test tubes, which were kept in a shaker (memmert, Germany) at 37 ºC for 48 h. Thereafter, the samples were centrifuged, filtered by using 0.45 µm PA membrane filter, and analyzed by HPLC. Samples containing homogenized FDA meal were treated by the same method used in drug release study.

2.5 Rheological measurement

The rheological behavior of the diluted FDA meal was characterized using a rotational rheometer (Brookfield DV1 viscometer) spindle 7, within the shear rate range (0-100 rpm). The measurements were made in triplicates at 37°C.

2.6 Disintegration test

Tablet disintegration rates in the different media were determined using tablet disintegration tester (µP, 1901) at 37°C. All the tests were carried out according to the USP, in 800 ml of the various media, using six tablets, one per vessel, for each test. Individual disintegration times were measured and means ±SDs were reported.

2.7 Drug release study

CLM release from the IR tablets was determined using apparatus type II (BTC – 9100, Hsiang taimachinery industry co, ltd) with a paddle rotating at a speed of 50 rpm. The tests were conducted in 500 ml dissolution media at 37ºC. Five ml samples were withdrawn at predetermined time intervals: 0, 5, 10, 15, 30, 45, 60, 90 and 120 minutes, filtered using a 0.45-µm PTFE syringe filter and assayed for CLM concentrations using HPLC. Samples containing FDA meal were treated before further analysis in order to precipitate protein. The samples were centrifuged at 4000 rpm for 5 min, then 100 µl of acetonitrile was added to precipitate protein after which, centrifuged again at 15000 rpm for 10 minutes. The supernatant layer was taken for analysis, and subsequently analyzed using HPLC.

Klaricare product which has a batch number of 117K15 and expired in Nov 2018 was used in this study.

2.7.1 The HPLC analysis

The HPLC analysis of CLM in the various samples was performed using a Waters HPLC system, equipped with Waters 1525 binary pump, and a Waters 2998 photodiode array Detector.

The chromatographic separation of CLM was achieved using stainless steel column (25 cm x 4.6 mm) packed with base-deactivated octadecylsilyl silica gel chromatography (5µm). The mobile phase was prepared by mixing methanol and 0.067M monobasic potassium phosphate (650:350), the pH was adjusted to 4 by phosphoric acid. The mobile phase was filtered by using

0.45 μm microporous PTFE filter and was degassed by sonication for 5 minutes prior to use. A wavelength of 210 nm was used. The flow rate was set at 1.1 ml/minute and the injection volume was 20 μl. The standard solution was prepared by dissolving 150 mg accurately weighed of CLM in 50 ml acetonitrile, and then diluted with water up to 100 mL [64].

The retention time for CLM was about 8 minutes. The concentrations of CLM in the unknown samples were determined by comparing the peak areas to the response of the standard curve.

2.7.2 Statistical analysis

Drug release data in the different media were assessed using similarity and difference factors (f_2 and f_1) respectively as reported in equations 1 and 2. f_2 factor measures the closeness between two profiles while f_1 measures how much different difference the two profiles:

$$f_2 = 50 \cdot \log\left\{\left[1 + \frac{1}{n}\sum_{t=1}^{n}(R_t - T_t)^2\right]^{-0.5} \times 100\right\} \quad \ldots\ldots\ldots (1)$$

$$f_1 = \left\{\left[\sum_{t=1}^{n}|R_t - T_t|\right] \Big/ \left[\sum_{t=1}^{n} R_t\right]\right\} \times 100 \quad \ldots\ldots\ldots\ldots (2)$$

Where R_t and T_t are the percentages of drug dissolved at each time point for the reference (fast state) and test products (fed state), respectively. An f_1 value greater than 15 indicates non-similarity, and an f_2 value greater than 50 indicates significant similarity between the two analyzed products

2.8 Simulation using Gastroplus™

GastroPlus™ software (version 9.0, Simulations Plus Inc., Lancaster, CA, USA) was used for building an absorption model for CLM under fasted and fed states. This software is based on the Advanced Compartmental Absorption and Transit (ACAT) model and is comprised of three modules: compound, physiology, and pharmacokinetics. For the compound and pharmacokinetics modules, the input parameters were either taken from the literature or determined experimentally such as solubility and dissolution results. The *in vivo* concentration-time profile under fast state of the product (Klaricare®) was obtained from Pharmacare Ltd. Due to the lack in BE data at fed state for Klaricare, the *in vivo* data in fed state was taken from Chu et al article [44]. The summary of the input parameters used in the simulation is given in Table 3. In the physiology module, the Human Physiology Fasted mode was selected for predicting the absorption profile under fasted state, while the Human Physiology Fed mode was used when conducting the simulations upon the concomitant intake of FDA meal. All the physiological parameters were fixed at default values in both states. The simulations were conducted using the Johnson model as a dissolution model and the "IR tablet mode" to refers for immediate (IR)-release tablets. The predictability of the simulation was measured by the percent prediction error (% PE) between the predicted and *in vivo* observed data, which can be calculated using the following equation:

$$\%PE = \frac{PK_{predicted} - PK_{\text{observed}}}{PK_{\text{observed}}} \times 100\%$$

Table 3: Input Data for Simulation

Parameters	**Value**
Molecular weight (g/mol)	747.95 [51]
Log P	1.7 at pH 7.4[51]
pKa	9 [52]
Dose (mg)	500
Formulation	Tablet
Dose volume	250 (a)
Solubility	At pH 4.5= 1.04 mg/ml (b) pH 6.8= 0.8 mg/ml (b)
Precipitation time (s)	900 (a)
Particle density (g/mL)	1.2 (a)
Physiology	Fasted
P_{eff} (cm/s*10^4)	2.91 (a)
Body weight (kg)	70
Clearance (L/h)	25 [53]
Volume of distribution (L/Kg)	1.8 [45]
$t_{1/2}$ (h)	3.49(c)
Bioavailability (F)	55% [41]
C_{max} (µg/ml)	3.12 (d)
T_{max} (h)	2.85 (d)
AUC (ng-h/ml)	26020 (d)

(a): Gastroplus standard

(b): Solubility test data

(c): Estimated by Gastroplus software

(d): Pharmacare *in vivo* data

2.8.1 In Vitro–In Vivo Correlation

Level A IVIVC was developed using the IVIVC Plus Module™ integrated within the GastroPlus™ Software. The *in vivo* fraction of CLM absorbed was estimated using the mechanistic absorption deconvolution approach. The fraction of drug absorbed at specific time points were plotted against the percentage of drug dissolved *in vitro* at the same time points. Regression analysis was used to evaluate the obtained correlations.

Chapter Three

3. Results

3.1. Solubility study

The results of the solubility determinations of CLM in the different media are presented in Table 4. The solubility of CLM, which is a weak base (pka=9), was shown to be pH dependent; i.e.: An increase in the pH, lead to a decrease in the solubility of the drug. Food has an apparent effect on CLM solubility. The solubility of CLM in the presence of food was greater when comparing results in buffer media alone. The high fat content in the FDA meal would play a crucial role in enhancing the solubility of lipophilic drugs, such as BCS class II drug. Interestingly, the solubility of CLM in FeSSIF was lower than that in FaSSIF, despite the higher bile salts content in this medium, which would be expected to aid solubility of this lipophilic drug.

Table 4: the solubility of CLM in fast and fed media

Media	pH	S1	S2	S3	Average Solubility mg/ml	SD
SGF	1.2	*	*	*	*	*
SGF with FDA meal	1.4	3.1	1.1	1.2	1.8	0.95
Acetate Buffer	4.5	1.3	0.8	0.9	1.04	0.22
Acetate Buffer with FDA meal	4.6	1.4	1.4	1.5	1.5	0.05
SIF	6.8	0.9	0.9	0.6	0.8	0.15
SIF with FDA meal	6.7	4.2	5.5	3.7	4.5	0.77
FaSSIF	6.5	1.3	1.5	1.4	1.43	0.09
FeSSIF	5	0.98	1.3	0.95	1.07	0.16
* The solubility of the drug was not be determined at pH 1.2 due to its decomposition at that pH						

3.2. Rheological measurement

The viscosity of the buffer media (SGF, acetate buffer and SIF) was shown to follow Newtonian behavior (0.8mPa.s). The rheogram of the diluted FDA meal (in acetate buffer and SIF) at the different shear rates is shown in (Figure 3 and table 5). The rheological behavior of this food is pseudo-plastic, i.e, the viscosity decreases with an increase in the shear rate. At low shear rate (0.3 rpm), the viscosity was 13330 mPa.s as the shear rate, increased

Table 5: average viscosity for FDA food containing media at different shear rate

shear rate (RPM)	Acetate buffer with FDA food	SIF medium with FDA food
	Average viscosity (cP)	Average viscosity (cP)
0	0	0
0.3	13330	13330
0.5	8000	800
0.6	6667	6667
1	4000	4000
1.5	2667	2667
2	2000	2000
2.5	1600	1600
3	1333	1330
4	1000	1000
5	800	800
6	666.7	666.7
10	400	400
12	333.3	333.3
20	200	200
30	133.3	133.3
50	80	80
60	66.67	66.67
100	40	40

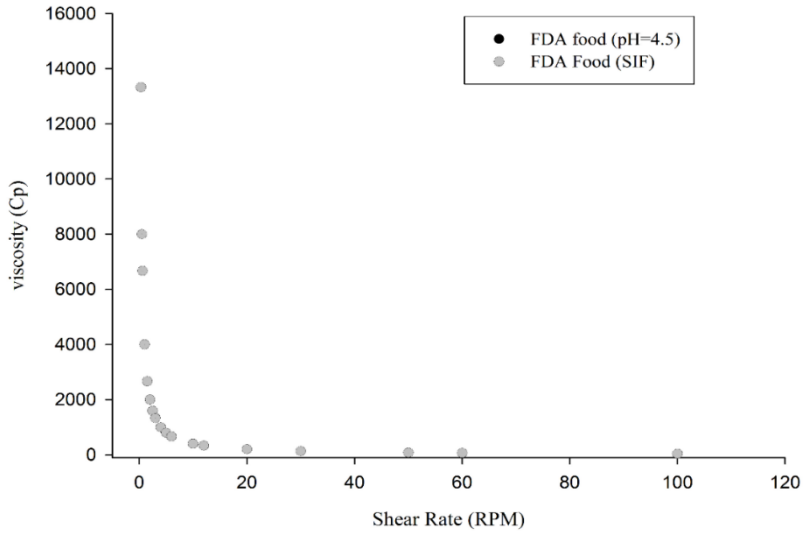

Fig 3: The viscosity of FDA food containing media

the viscosity decreased to 40 mPa. Elevated viscosity within the GI tract would be expected to reduce drug release and delay tablet disintegration. The rheogram for FDA meal in both SIF and acetate buffer were superimposed. However, the viscosity of homogenized FDA meal (pH=1.2) was lower than that at pH= 4.5 and 6.8 at the various shear rates. This viscosity–pH dependent behavior is in line with the previous reports [55, 28] who demonstrated reduced viscosity of polymeric solutions and soups at low pH [55].

3.3. Disintegration study

The average disintegration times of the CLM tablets in the different media are shown in (Figure 4 and table 6). Media pH showed significant effect on tablet disintegration, were P_{value} = 0.0022, 0.43 and 0.0002 for SGF, acetate buffer and SIF respectively. In acidic media, CLM tablet exhibited longer disintegration times compared to more alkaline media. The mean

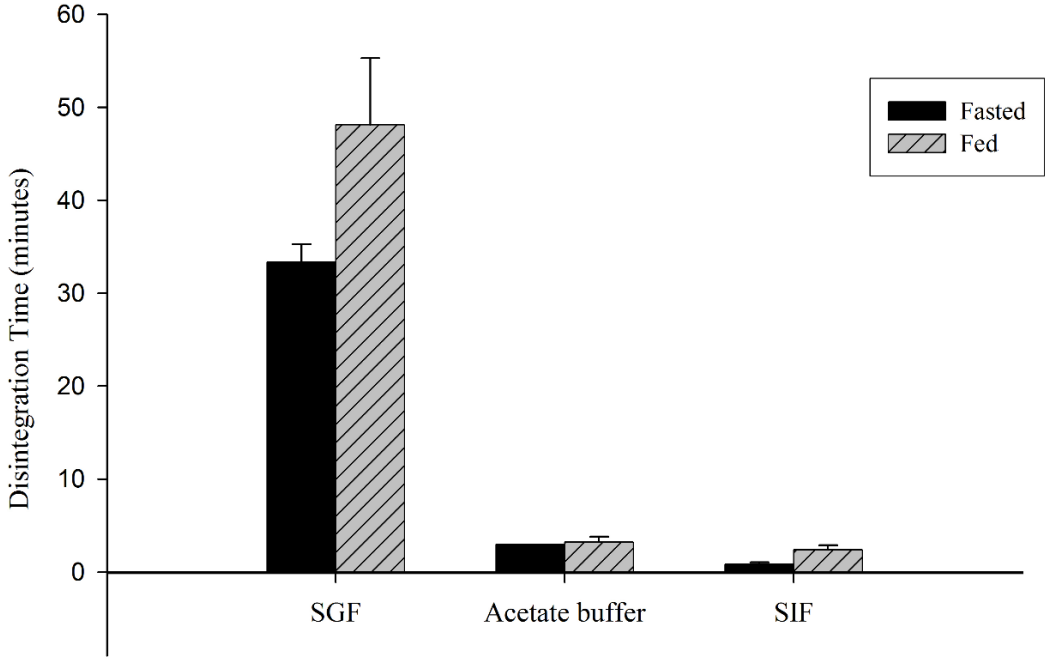

Fig 4: CLM tablet disintegration in the various media

disintegration times were increased from an average value of 0.8 and 3 min in SIF and Acetate buffers to 33.4 min in SGF media. These findings are in agreement with a previous report, which reported a delay in the *in vitro* disintegration of CLM tablet in media of acidic pH [56,57]. This delay in tablet disintegration in acidic media was attributed to gel formation on the tablet surface. Inclusion of FDA meal in the different buffers has delayed

Table 6: average disintegration time for different media

	HCl buffer	HCl buffer with food	acetate buffer	acetate buffer with food	phosphate buffer	phosphate buffer with food
Av. Time (min)	33.38	48.12	3	3.22	0.806	2.4
SD	1.89	7.17	0	0.59	0.24	0.49

tablet disintegration. Prolonged disintegration times in media containing FDA food reflect the high viscosity of the media.

3.4. Dru g release study

3.4.1. The effect of media pH on the release of Clarithromycin

The *in vitro* release of CLM from immediate release tablet was investigated in media with different pH (1.2, 4.5 and 6.8). Media pH has a profound effect on the drug release profiles (Figure 5 and table 7). In SGF (pH=1.2), the total amount of CLM released was less than 20% within 120 minutes. However, the dissolution rate of the drug was higher than 88% in SIF (pH =6.8) and almost complete in the acetate buffer (pH=4.5).

Table 7 : The average percentage release for CLM in different media

% Released in different media at different time						
Media	**GIF**	**SD**	**Acetate buffer**	**SD**	**SIF**	**SD**
5 min	12.55	**5.64**	55.2	**11,0**	37.34	**6.095**
15 min	10.69	**3.55**	76.88	**8,4**	72.85	**2.61**
30 min	11.72	**3.97**	75.8	**3,2**	85.53	**8.68**
45 min	11.86	**3.07**	87.2	**13,3**	88.32	**1.87**
60 min	13.82	**2.72**	88.5	**5,3**	88.83	**3.44**
90 min	13.38	**1.54**	93.2	**0,9**	88	**0.83**
120 min	44.18	**1.53**	94.3	**4,8**	88.93	**8.41**

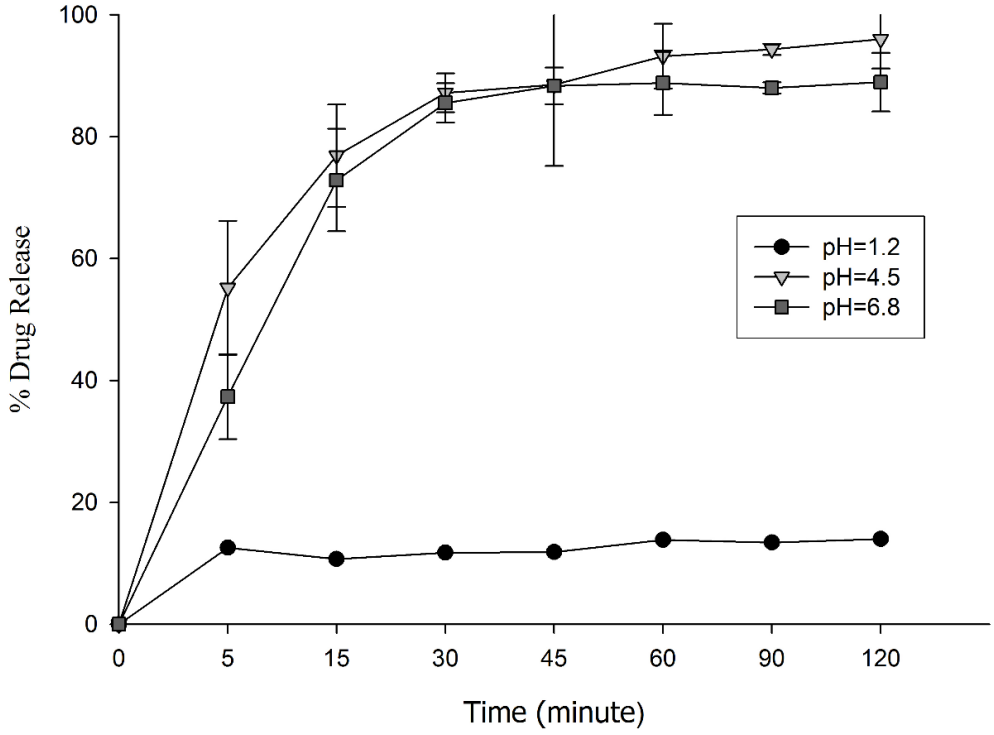

Fig 5: The effect of media pH on the release of CLM

3.4.2.The effect of food on the release of CLM

FDA food presence in the media was shown to have a marked effect on CLM release. A great difference was obvious between the *in vitro* drug release profiles under fed conditions at the various pH. In SGF at fed state, the percentage of CLM released (after addition of FDA meal to SGF) was higher compared to that in the fasted state, because of the slower degradation rate. However, at higher pH (pH=4.5 and 6.8), the release of the drug in the presence of FDA meal was negatively affected as can be shown in Figures (6,7 and 8) . The dissolved amount of CLM in the dissolution medium with added FDA diet did not reach 60% or 74% of the total tablet content of the

drug at (pH=4.5 and 6.8) respectively. This delay in dissolution rates reflects the slower disintegration. Results of similarity were lower than 50 at the various dissolution conditions

Table 8: The average percentage release for CLM in different fed state media

% Released in fed state media at different time						
Media	**GIF +food**	**SD**	**Acetate buffer +food**	**SD**	**SIF +food**	**SD**
5 min	14,5	**17**	19,5	**10,3**	38.9	**31.17**
15 min	17,7	**10**	59,92	**1,1**	60.2	**14.63**
30 min	18,7	**9**	60,48	**0,3**	67	**3.38**
45 min	19,5	**7**	60,8	**0,46**	68.2	**2.097**
60 min	21,06	**7**	60,97	**0,95**	68.8	**7.33**
90 min	22,36	**5**	62,87	**0,39**	69.2	**1.35**
120 min	22,8	**3**	62,09	**0,33**	69.5	**1.24**

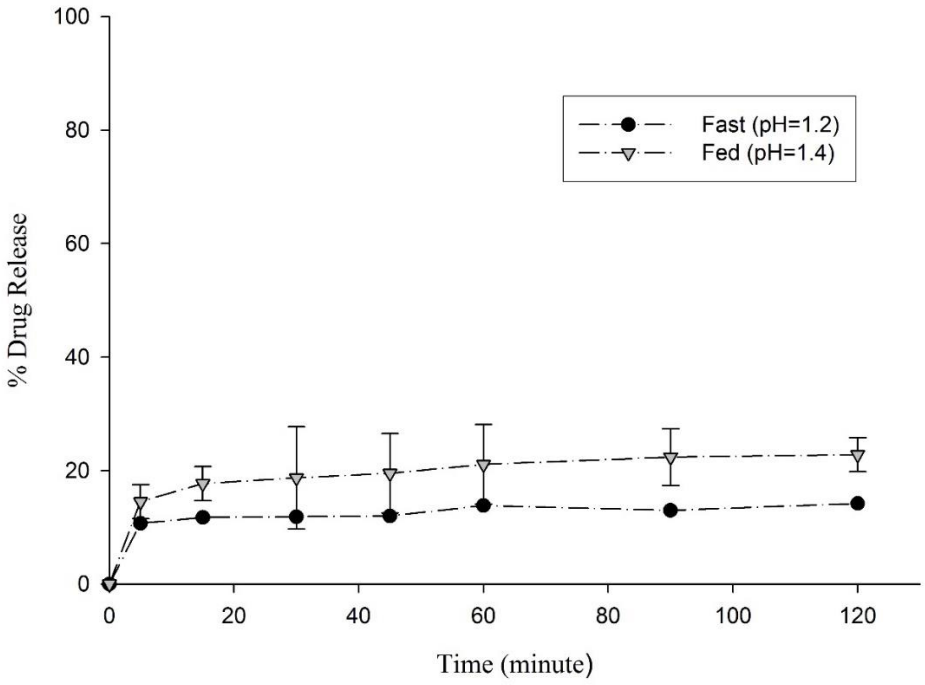

Fig 6: The effect of food on CLM release in SGF medi**a**

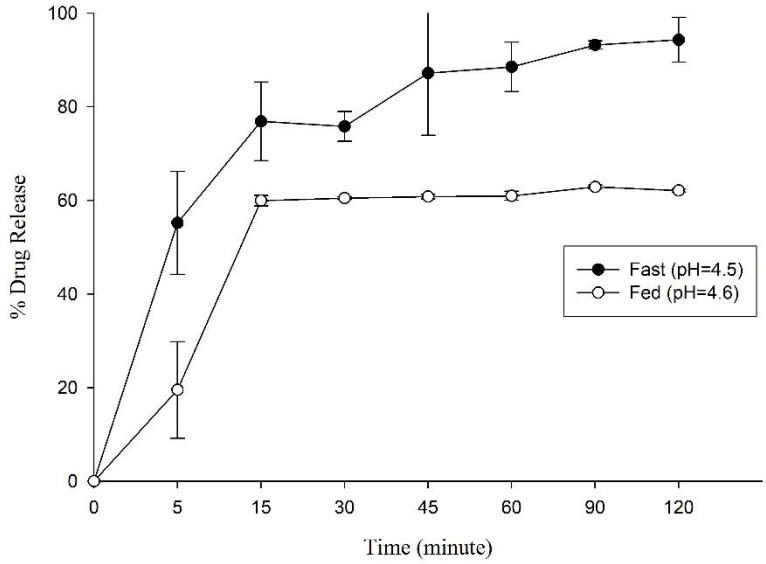

Fig 7: The effect of food on CLM release in acetate buffer media

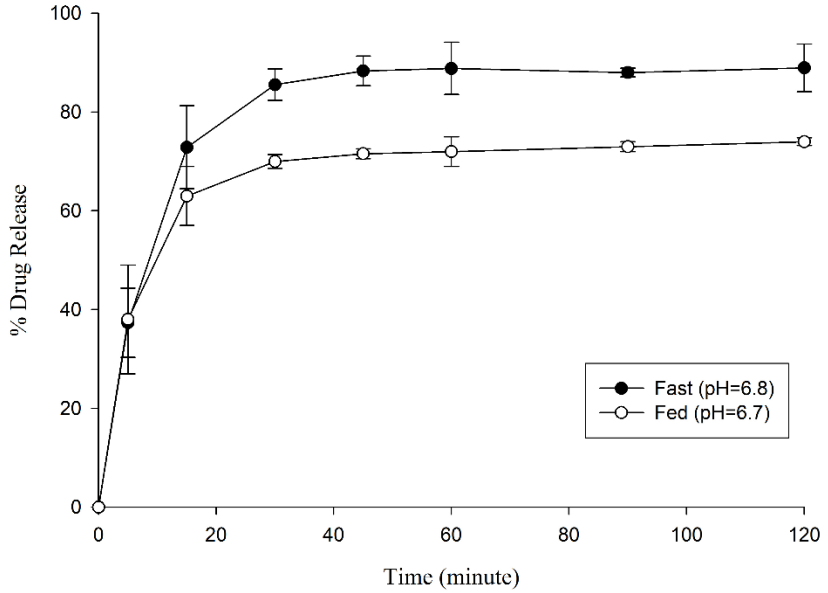

Fig 8: The effect of food on CLM release in SIF media

CLM release from immediate release tablet was determined in biorelevant media and compared to that in compendial buffers. The percentages of drug released in FaSSIF and FeSSIF were lower than that in blank buffers. In

FaSSIF (pH= 6.5), 60% of the drug was dissolved within 120 minutes compared to 88% in SIF (pH=6.8). Obviously, there is a great difference between the release profiles of the drug in FaSSIF and SIF, despite the small change in the pH between FaSSIF and SIF (difference in pH=0.3). In FeSSIF (pH=5), the dissolution rate was very poor, i.e., only 10 % of the drug dissolved within 120 minutes however, the drug release was almost complete in acetate buffer (pH=4.5). (Figure 9 and table 9)

Table 9: The average percentage release for CLM in FaSSIF and FeSSIF media

% Released in FaSSIF and FeSSIF media at different time				
Media	**FaSSIF**	**SD**	**FeSSIF**	**SD**
5 min	21,8	4,4	2,06	1,4
15 min	33,9	6,8	3,26	1,5
30 min	41,1	8,2	3,8	1,7
45 min	42,1	8,4	3,83	2,8
60 min	54.0	10,8	5,06	3,6
90 min	54,3	10,9	9,67	4,3
120 min	59,9	12,0	10	3

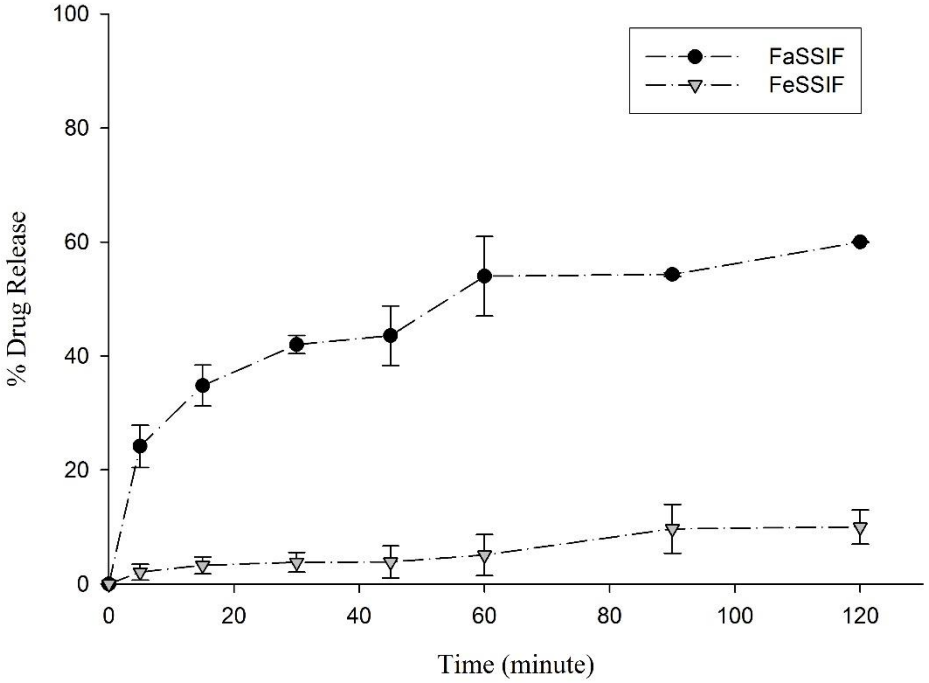

Fig 9: The percentage of drug released in FaSSIF and FeSSIF media

f_1 and f_2 value were calculated for each medium, were the fast state in each buffer was the reference and the fed state was the test medium, the results are shown in table 10

	SIF	**Acetate buffer**	**SGF**	**FaSSIF**
***f*$_1$ value**	160.28	198.35	52.37	763.83
***f*$_2$ value**	14.40	15.93	45.22	21.52

3.5. Gastrointestinal simulation

3.5.1. Drug absorption simulation

The results of the *in silico* simulation for CLM using GastroPlus™ are shown in Figures (10 and 11). The simulated plasma concentration time profile was in good agreement with the *in vivo* absorption curve following the intake of 500 mg oral dose CLM immediate release tablet under fasting conditions. The simulated pharmacokinetic parameters and those observed *in vivo* are presented in Table 11. The percent prediction errors obtained were less than 10% for all pharmacokinetic parameters, indicating good predictability. The simulated absorption profile predicted a prolonged T_{max} in the fed state compared to that observed *in vivo* under fasted state. This can be attributed to the delay in tablet disintegration. However, no significant changes in the AUC (0-infinity) values were associated with CLM administration with FDA meal.

Table 11: *In silico* predicted and *in vivo* observed Pharmacokinetics parameter

Study	C_{max} (µg/ml)			AUC $_{0\text{-}t}$(µg h/ml)		
	Observed	**Simulated**	**%PE**	**Observed**	**Simulated**	**%PE**
Fasted	2.48	2.5576	3.1	18.366	19.922	8.5
Fed	2.5	2.35	6.4	15.67	16.65	6.2

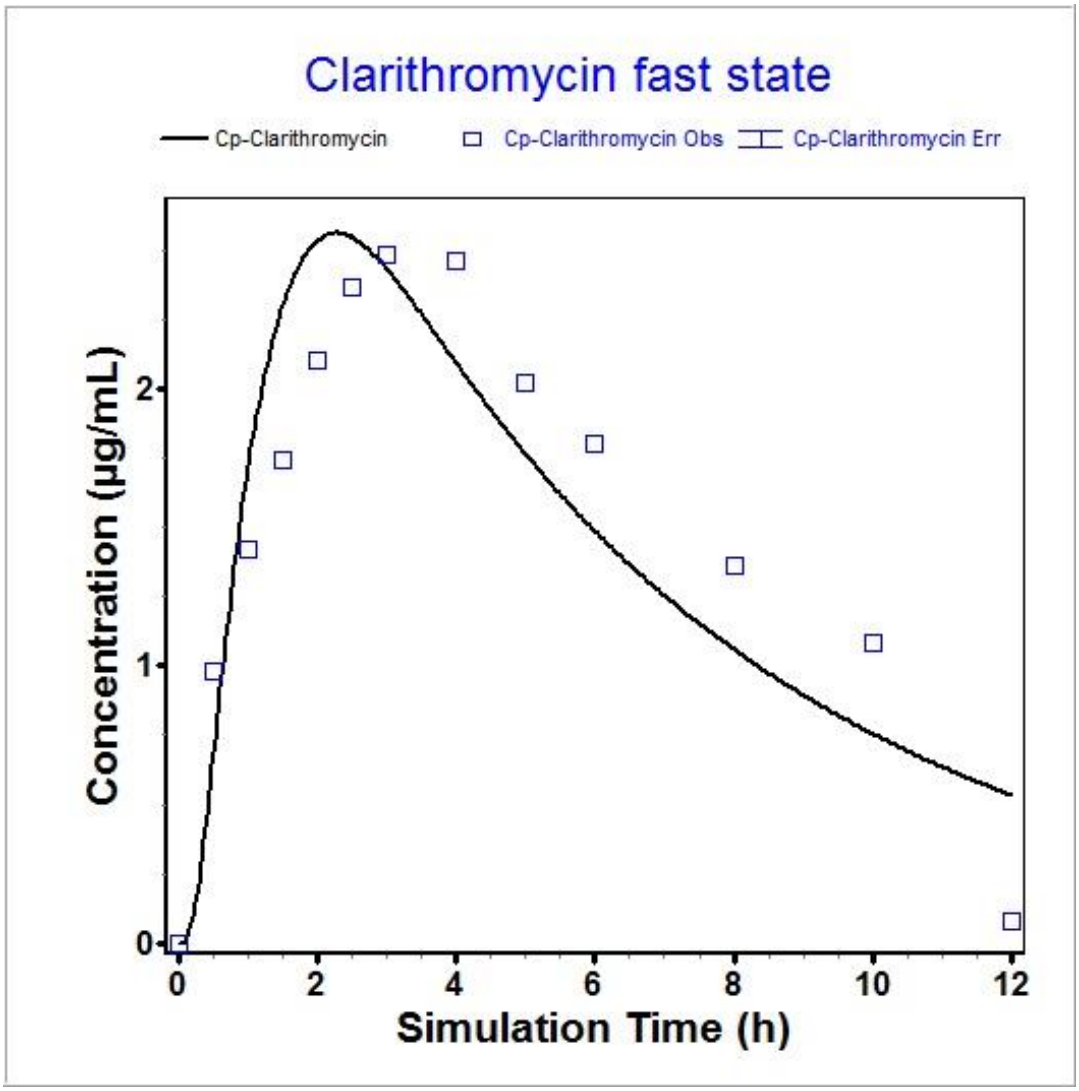

Fig 10: CLM simulation in fast state

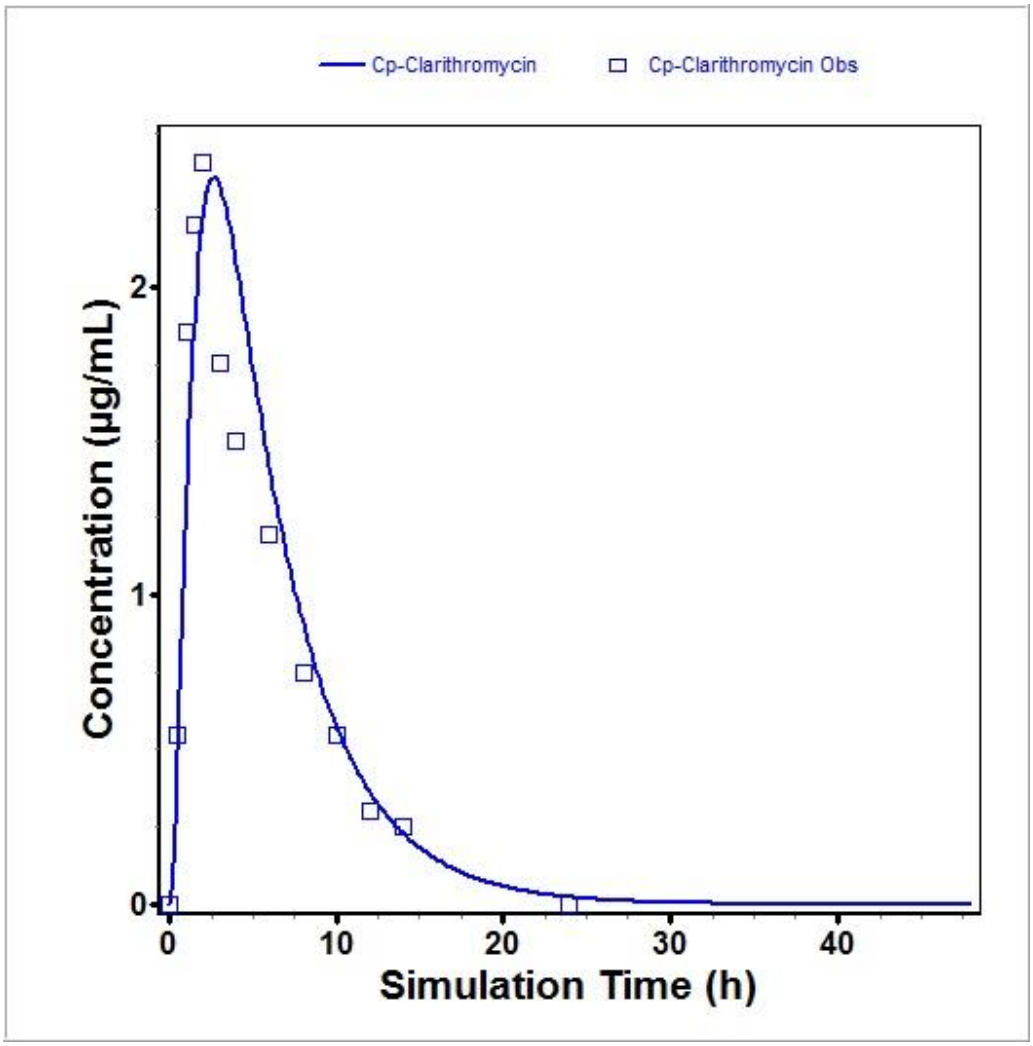

Fig 11: CLM simulation in fed state

The regional absorption distribution simulated by GastroPlus™ suggested that the majority of CLM was absorbed in the Jejunum 55%, while 23% of the dose was absorbed in duodenum, and 13% absorbed in ileum and regions (Fig12).

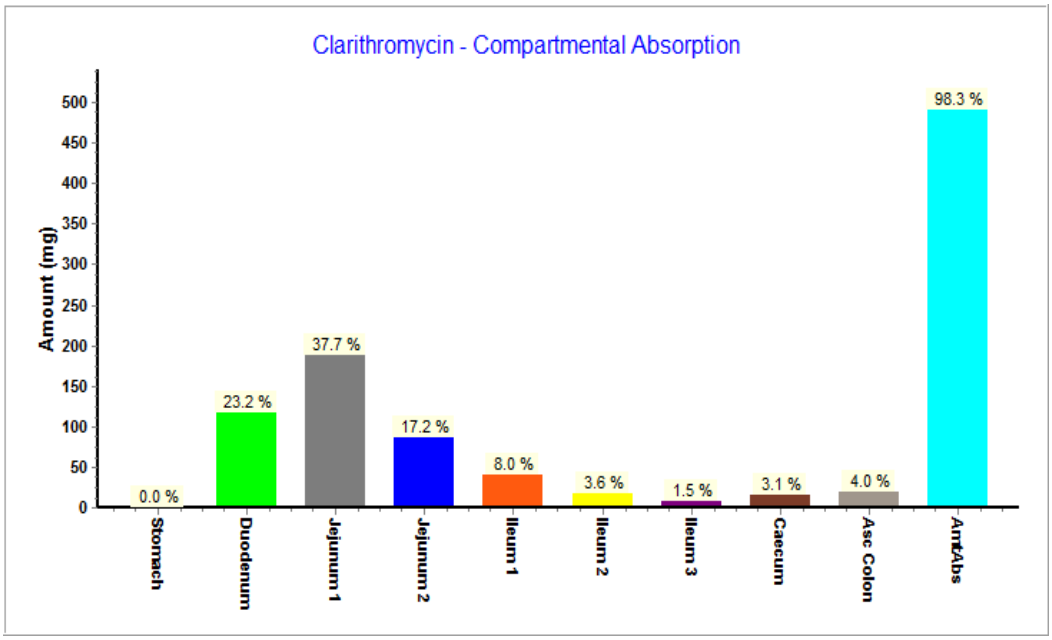

Figure 12: The regional absorption distribution of CLM

3.6. IVIVC

GastroPlus™ was used to establish an *in vitro–in vivo* correlation using mechanistic absorption model based on deconvolution approach to correlate the *in vitro* dissolution data and the corresponding *in vivo* plasma concentration. Figure (14) shows that *in vivo* correlation was successfully established in SIF acetate and FaSSIF media, since the value of correlation coefficient (r^2) was close to 1 Table 12.The correlation value (r^2) showed that using the HCl medium resulted in a low correlation coefficient (r^2=0.3). whereas, the *in vitro* dissolution data were not correlated with *in vivo* dissolution values in HCl media. The mechanistic absorption model was the most suitable model for use in the simulation of the convolution. which indicated that it was the preferable method for establishing *in vitro–in vivo* correlation for the CLM tablet.

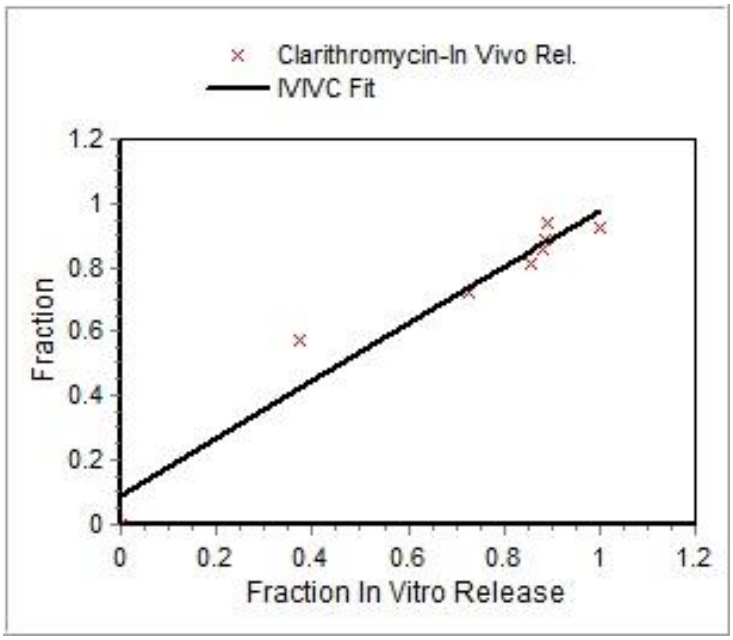

Fig 13: IVIVC plot for CLM dissolution in acetate buffer

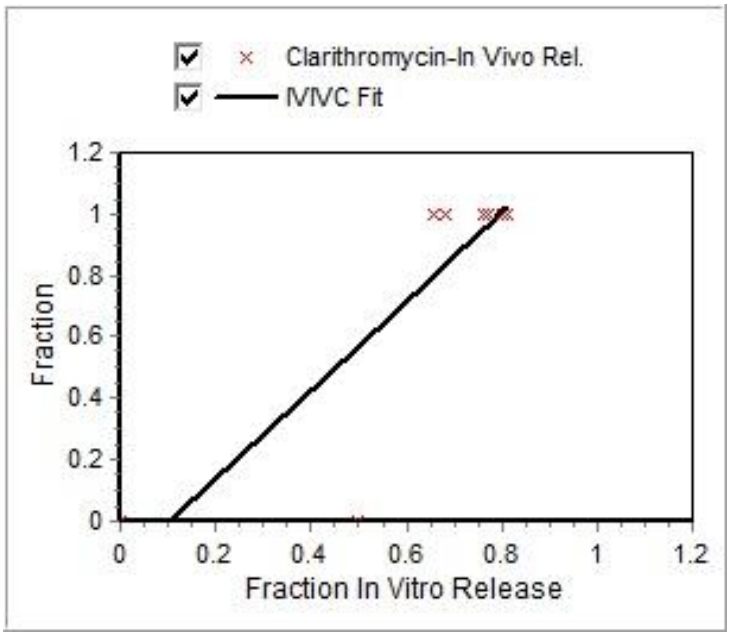

Fig 14:IVIVC plot for CLM dissolution in SIF

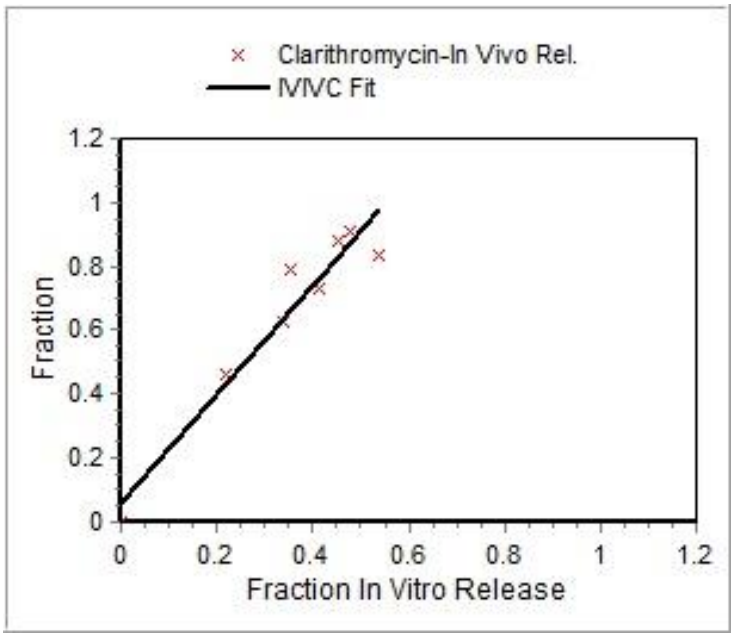

Fig 15:IVIVC plot for CLM dissolution in FaSSIF

Table 12.: Statistical Parameters of the Obtained IVIVC

Media	**Slope**	**r^2 Value**
SGF	1.451	0.346
Acetate buffer	1.445	0.721
SIF	0.889	0.942
FaSSIF	1.694	0.927
FeSSIF	0.026	0.093

Chapter Four

4. Discussion

In the present work, the effect of food on the release of CLM antibiotic from immediate release tablet has been investigated. FDA standard breakfast was used as a model meal, especially that it is the recommended food in the fed bioequivalence and bioavailability study. *In silico* modeling was employed to build `model describing the *in vivo* performance of CLM under fasted and fed conditions

Media pH had a pronounced effect on CLM release from the formulations. The acidic environment dominating in the stomach reduced the released fraction of the drug available for absorption. The cumulative percentage of drug released at pH=1.2 was not more than 20% within 120 minutes. This can be explained by the decomposition of CLM under acidic pH conditions. These findings are in agreement with Fujiki et al 2011 , who investigated the effect of the media acidity on the stability and release of CLM from immediate release tablets. Fujiki reported rapid decomposition and slow dissolution of CLM with no more than 20% of the API was dissolved at pH 1.2 [56], Similarly, Manani et al, 2014 have reported a degradation of 64% of CLM active ingredient within 1 h at pH=1.2 [58]. Delayed tablet disintegration under acidic condition can be another explanation which can be attributed to the gelling tendency of tablet ingredient at pH 1.2 results in increasing the viscosity around drug particles and hence retarding the dissolution [56].

Therefore, the intake of the CLM under fasted state could reduce the fraction available for absorption and hence, drug bioavailability. The decrease in the dissolution rate of this drug at the low pH confirms the potential of the gastric acidity on reducing the bioavailability of CLM. At the luminal pH, CLM is presumably stable and did not undergo degradation (pH more than 3). The cumulative amount of drug released at pH =4.5 and pH=6.8 was more than 94% and 88%, respectively.

FDA food has a significant effect on the dissolution and disintegration rates of CLM tablet. In media simulating the fed gastric conditions, the dissolution rate of the CLM was enhanced. In fact, FDA meal has a neutralization capacity for the acidic secretions of the stomach. A previous study reported a rapid rise in the gastric pH from an initial value of 1.2 under fasted state to about 3-7 following food intake [6]. Postprandial elevation in gastric pH could reduce CLM degradation in the stomach and enhance its dissolution rate. Based on this, CLM bioavailability is expected to increase when dosed in the fed state. On the other hand, at the luminal pH (pH= 4.5, 6.8), the homogenized FDA meal caused a marked reduction in drug dissolution and prolonged tablet disintegration rates compared to that in the fasted states. These observations can be explained by the high viscosity of the medium contains FDA meal. These results are in line with previous reports, which ascribed the reduced disintegration and dissolution rates in various types of beverages, soups and FDA food to the elevated viscosity, slow water uptake rates into tablets and film precipitation on tablets [28,59,60,61,62].

Biorelevant systems contain bile salts in concentration within the physiological range. Bile salts are responsible for solubilization of lipid soluble drugs and their transport. In contrast to expectation, CLM release in the presence of bile salts was much lower than that in compendial media. This can be attributed to the complexation interaction between macrolide antibiotic and bile salts. CLM was reported to strongly bind to bile acids cholate and deoxycholate micelles, which form a complex of low solubility and diffusivity but faster uptake through the body [63]. This complex formation between the drug with bile decrease the release of the drug, but bile re-uptake increase the CLM particle that inter the blood. Dissolution study in biorelevant media does not reflect the *in vitro* dissolution in media containing FDA meal or simple buffers. This makes the feasibility of using these models on predicting food effect questionable.

GI simulation was used to build an absorption model for predicting the *in vivo* performance of CLM from immediate release tablet under fasted and fed states. The simulated concentration- time profile under fasted and fed conditions matched well with the *in vivo* observed data. The calculated percent prediction error values for the *in silico* data indicates good predictability. These findings are in agreement with a previous clinical data showing that the concomitant intake of FDA food with CLM caused a delay in the onset of action with no significant effect on the extent of absorption [44].

A level A Linear correlation was established between the actual *in vivo* data and the *in vitro* dissolution data in the acetate and SIF media. The

developed IVIVC model considered SIF, acetate, and FaSSIF buffer media to be the most relevant from the physiological standpoint.

Conclusion

Food effects on the oral absorption of CLM from an IR tablet, is most likely to be as a result from a complex combination of many factors, which include: media pH, bile salts, viscosity, and food composition. Food may retard the *in vitro* disintegration and dissolution rates of the drug product, but increase the drug uptake through bile. The intake of standard FDA meal may have no significant effect on oral BA of CLM IR tablet. The effect of food on CLM BA was possible to predict using *in silico* and *in vitro* methods without the need for conducting fed BE studies, which may increase the cost and time required for development of a new generic CLM solid products. In fact, FDA is attempting to revise the guidelines that are currently in use to reduce the obstacles and to encourage the development of new generic products. For this purpose, FDA implemented a new policy to accelerate the review of generic drug applications where competition is limited.

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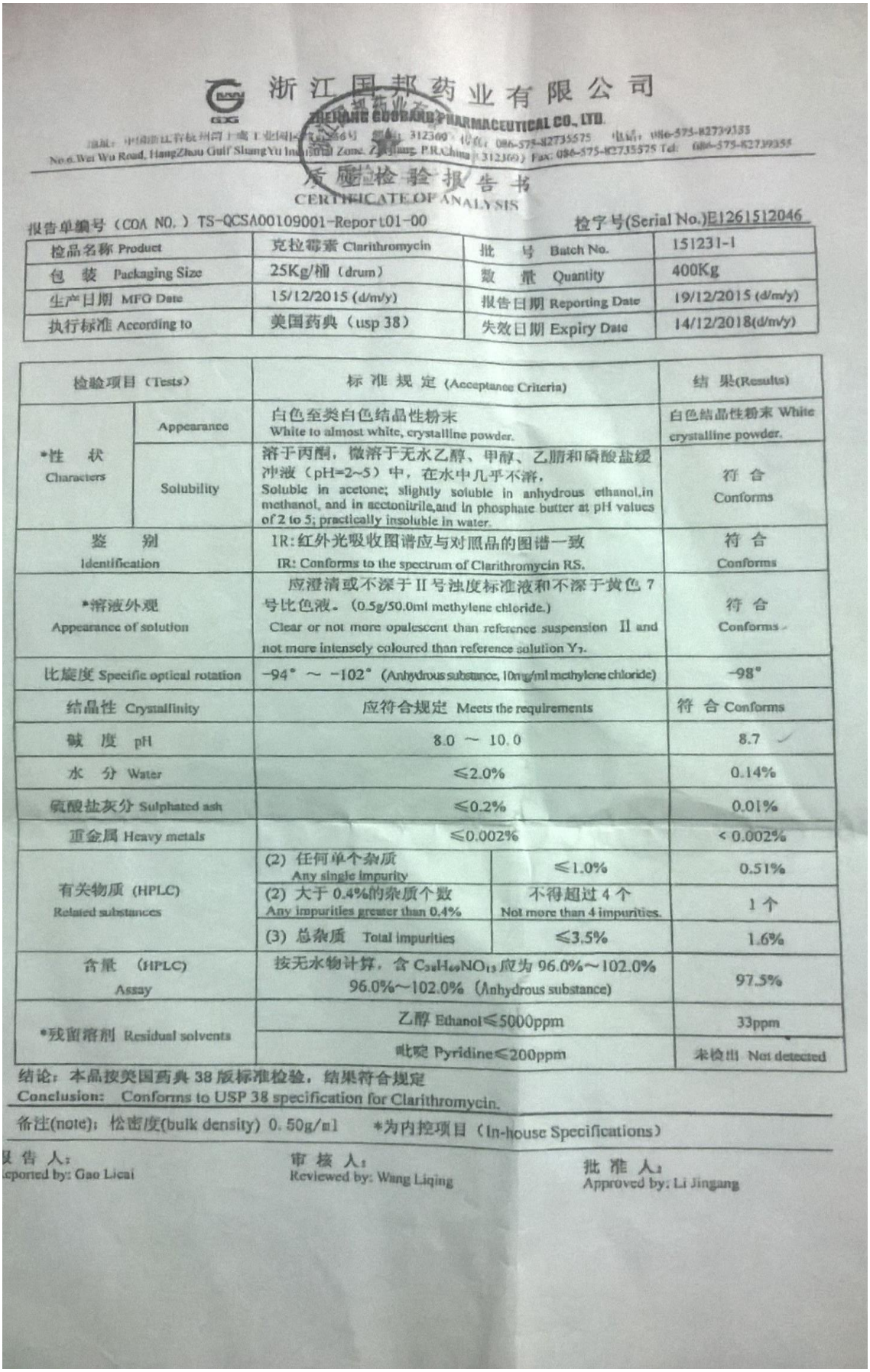
Appendix



浙江国邦药业有限公司

ZHEJIANG GUOBANG PHARMACEUTICAL CO., LTD.

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质量检验报告书

CERTIFICATE OF ANALYSIS

报告单编号（COA NO.）TS-QCSA00109001-Report01-00　　检字号(Serial No.)E1261512046

检品名称 Product	克拉霉素 Clarithromycin	批　号 Batch No.	151231-1
包　装 Packaging Size	25Kg/桶（drum）	数　量 Quantity	400Kg
生产日期 MFG Date	15/12/2015 (d/m/y)	报告日期 Reporting Date	19/12/2015 (d/m/y)
执行标准 According to	美国药典（usp 38）	失效日期 Expiry Date	14/12/2018(d/m/y)

检验项目（Tests）		标准规定 (Acceptance Criteria)		结　果(Results)
*性　状 Characters	Appearance	白色至类白色结晶性粉末 White to almost white, crystalline powder.		白色结晶性粉末 White crystalline powder.
	Solubility	溶于丙酮，微溶于无水乙醇、甲醇、乙腈和磷酸盐缓冲液（pH=2~5）中，在水中几乎不溶。 Soluble in acetone; slightly soluble in anhydrous ethanol,in methanol, and in acetonitrile,and in phosphate butter at pH values of 2 to 5; practically insoluble in water.		符　合 Conforms
鉴　别 Identification		IR:红外光吸收图谱应与对照品的图谱一致 IR: Conforms to the spectrum of Clarithromycin RS.		符　合 Conforms
*溶液外观 Appearance of solution		应澄清或不深于II号浊度标准液和不深于黄色7号比色液。（0.5g/50.0ml methylene chloride.) Clear or not more opalescent than reference suspension II and not more intensely coloured than reference solution Y_7.		符　合 Conforms
比旋度 Specific optical rotation		-94° ~ -102° (Anhydrous substance, 10mg/ml methylene chloride)		-98°
结晶性 Crystallinity		应符合规定 Meets the requirements		符　合 Conforms
碱　度 pH		8.0 ~ 10.0		8.7
水　分 Water		≤2.0%		0.14%
硫酸盐灰分 Sulphated ash		≤0.2%		0.01%
重金属 Heavy metals		≤0.002%		< 0.002%
有关物质 (HPLC) Related substances		(2) 任何单个杂质 Any single impurity	≤1.0%	0.51%
		(2) 大于0.4%的杂质个数 Any impurities greater than 0.4%	不得超过4个 Not more than 4 impurities.	1个
		(3) 总杂质 Total impurities	≤3.5%	1.6%
含量 (HPLC) Assay		按无水物计算，含 $C_{38}H_{69}NO_{13}$ 应为 96.0%~102.0% 96.0%~102.0% (Anhydrous substance)		97.5%
*残留溶剂 Residual solvents		乙醇 Ethanol≤5000ppm		33ppm
		吡啶 Pyridine≤200ppm		未检出 Not detected

结论：本品按美国药典38版标准检验，结果符合规定
Conclusion: Conforms to USP 38 specification for Clarithromycin.

备注(note)：松密度(bulk density) 0.50g/ml　*为内控项目（In-house Specifications）

报告人：
Reported by: Gao Licai

审核人：
Reviewed by: Wang Liqing

批准人：
Approved by: Li Jingang

Certificate of Analysis

Document Name: *FaSSIF/FeSSIF/FaSSGF CoA* *Document Version:* *1.6*

Document Reference: *CoA 01-1608-02 NP (01)* *Replaced* *CoA 01-1608-01 NP (01)*

Product : FaSSIF/FeSSIF/FaSSGF (FFF)

Batch number : 01-1608-02 NP (01)

Packaging : HDPE bottle

Long-term storage : 2 – 8 °C, protect from light and humidity

Expiry date : 28 April 2018 for unopened bottle

Test	Test Method	Target Specification	Result
Appearance	Visual inspection	White to slightly yellow powder	Complies
Sodium taurocholate content (based on dry weight)	^{1}H-NMR	73.2± 5 (% w/w)	70.4 %
Lecithin content (based on dry weight)	^{31}P-NMR	26.8± 3 (% w/w)	26.8%
Water content	Karl Fisher	≤ 3.0 %	0.22 %
FaSSIF particle size	Z_{av} PCS	≤ 70.0 nm	≤ 70.0 nm
FeSSIF particle size	Z_{av} PCS	≤ 10.0 nm	< 7 nm

Result : All results are within the target specifications

Date of Release : 01 October 2016

Responsible Person : Daryl Leigh — Quality Control — Signature — 01 October 2016 — Date

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كلية الدراسات العليا

جامعة النجاح الوطنية

تقييم تأثير الغذاء على امتصاص الكلاريثرومايسين باستخدام النمذجة الفسيولوجية

إعداد

رند خليل عبداللطيف جيوسي

إشراف

د.أسماء رضوان

قدمت هذه الأطروحة استكمالاً لمتطلبات درجة الماجستير في العلوم الصيدلانية بكلية الدراسات العليا في جامعة النجاح الوطنية في نابلس، فلسطين.

2017

تقييم تأثير الغذاء على امتصاص الكلاريثرومايسين باستخدام النمذجة الفسيولوجية

إعداد
رند خليل عبد اللطيف جيوسي
إشراف
د. أسماء رضوان

الملخص

قد يؤثر الغذاء في امتصاص الدواء عن طريق تحفيز تغييرات فسيولوجية في الجهاز الهضمي، كتغيير درجة الحموضة، ومدة بقاء الدواء في المعدة، وإفراز العصارة الصفراء ، والعمليات الأيضية للدواء.

الهدف: الهدف من هذه الدراسة هو التحقق من تأثير الغذاء في امتصاص دواء الكلاريثرومايسين، عن طريق تقييم تأثير بعض العوامل مثل: درجة الحموضة، وإفراز العصارة الصفراء، ومكوّنات الطعام في إفراز المادة الفعالة من قرص الدواء.

الطريقة: فحص الذائبية، والتحطيم، والانحلال للكلاريثرومايسين 500 ملغم في الأوساط التقليدية؛ بإضافة الFDA food، أو عدمه، وفي ال Biorelevant media لمحاكاة حالتيّ الإفطار والصيام في الجسم. فهذه البيانات التي تم جمعها من فحوصٍ خارج الجسم استُخدمت كمدخلات لبرنامج Gastroplus™؛ لعمل نمذجة فسيولوجية تهدف إلى توقع تأثير الغذاء في امتصاص الكلاريثرومايسين في حالتيّ الإفطار والصيام. استُخدم منحنى تركيز الدواء في البلازما لعمل نموذج بناءً على بيانات الحركة الدوائية، وتم إنشاء المستوى أ في العلاقة الخطية مابين in vitro-in vivo، باستخدام نموذج mechanistic absorption معتمداً على أسلوب ال deconvolution. وقد تم استخدام برنامج ال Gastroplus™ ، لتطوير نموذج امتصاص فسيولوجي للكلاريثرومايسن، وهو بدوره قادر على التنبؤ بسيْر الدواء داخل الجسم.

النتائج: الكلاريثرومايسين له ذائبيه قليله في ال Biorelevant media مقارنة مع غيرها من الأوساط؛ وذلك بسبب تكوين مركب مع ال العصارة الصفراء. أظهرت التجارب أن الكلاريثرومايسين يستغرق فترة طويلة في التحطم، وبطء في الانحلال عند وجودFDA food. وكما بيّن النموذج الفسيولوجي عدم وجود تأثير ذي أهمية للغذاء على التوافر البيولوجي للكلاريثرومايسين.

خاتمة: تناول ال FDA food مع الكلاريثرومايسين قد لايكون له تأثير على التوافر البيولوجي لأقراص الكلاريثرومايسن.