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Biowaiver monograph for ascorbic acid immediate release solid oral dosage forms

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Dedication

To my parents

To my precious husband

To my adorable daughters

To my lovely sons

To my wonderful sisters

To my friends

Without whom none of my success would be possible

Acknowledgement

Greeting goes to my supervisors Dr. Ramzi Shawahna and Dr. Amjad Hussien for their sincere encouragement, helpful, and close supervision which has been invaluable for me. Thanks to my family with all my love, especially my mother, father, husband and my sons and daughters who stood with me throughout my study and provided me with psychological support and encouragement.

الاقرار

أنا الموقعة أدناه,مقدمة الرسالة التي تحمل عنوان:

Biowaiver monograph for immediate release solid oral dosage forms containing ascorbic acid

أقر بأن ما أشتملت عليه هذه الرسالة هو نتاج جهدي الخاص,بأستثناء ما تمت الأشارة اليه حيث ورد,وأن هذه الرسالة كاملة,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 provided in this thesis, unless otherwise referenced, is the researchers own work and has not been submitted elsewhere for any other degree or qualification.

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Abstract

Background: Demonstrating similarity in terms of safety and efficacy between innovator pharmaceutical products and their generic versions is a critical step in granting marketing authorizations (MAs) for generics. Similarity often proved by conducting in vivo bioequivalence (BE) studies in healthy volunteers. Health regulatory bodies issue MAs for generic versions after furnishing a proof of similarity with their innovator counterparts. BE studies are expensive, time consuming and risky studies since they are conducted in healthy volunteers. Today, the biopharmaceutical classification system (BCS) introduced by Amidon and adapted by various regulatory authorities and organizations like the US Food and Drug Administration (FDA), European Medicines Agency (EMA) and the World Health Organization (WHO) has significantly changed the processes of drug development and approval. Regulatory bodies now allow waiver of in vivo BE studies using surrogate in vitro dissolution testing for immediate release (IR) solid oral dosage forms containing high solubility and high permeability (BCS class I) drugs.

Objectives: The objectives of this thesis were to systematically evaluate the possibility of granting biowaiver for immediate release (IR) formulations containing ascorbic acid as an active pharmaceutical ingredient (API). The release characteristics of two formulations containing ascorbic acid were also assessed.

Methods: Solubility studies were conducted to determine the aqueous solubility of ascorbic acid at different pH points in the range of 1-7.5 at 37 ºC and to assign a correct solubility class for ascorbic acid. According to the BCS, and the FDA guidelines, a drug substance is considered highly soluble when the highest dosestrength is soluble in 250 ml or less of aqueous media over the pH range of 1–7.5. A standard shake-flask method was applied using three different aqueous media with pH values of 1.0 (maleate buffer), 4.5 (acetate buffer), and 7.5 (phosphate buffer) at 37 °C. The establishment of equilibrium was confirmed by comparing the solubility at 24 h and 48h.Drug levels in the samples were analyzed using UV spectrophotometric assay of ascorbic acid at 260nm. Dissolution experiments on two IR tablets containing ascorbic acid 500 mg were carried out. Dissolution profiles of ascorbic acid 500 mg tablets were generated in 900 ml of deionized water at pH points of 1.2, 4.5 and 6.8 adjusted using 0.1 N HCl or NaOH solutions. A paddle type dissolution apparatus was used and dissolution was tested in according to the USP type-II method. Paddles rotated at 75 rpm and the temperature of the dissolution media was maintained at 37 ± 0.5 ºC. Aliquots of 5 mL were withdrawn at predetermined time intervals of 5, 10, 15, 20, 25, 30, 45

and60 min.Samples were suitably diluted and analyzed at260 nm using UV spectrophotometer. Molecular descriptors like polar surface area (PSA), *n*-octanol/water partition coefficient (log *P*), distribution-coefficient at pH 7.4 (log $D_{7.4}$), number of hydrogen bond acceptors, number of hydrogen bond donors and p*K*a were calculated using software packages. Literature databases were searched for solubility, permeability and dissolution related parameters.

Results: The solubility measurements show that the maximum dose listed on the WHO's EML list was soluble in less than 250 mL of water over the pH range specified by the regulatory agencies at 37 °C. The calculated dose number was in the range 0.00011 of 0.00029 in the pH range of 1.2-7.5. These results suggest that ascorbic acid should unequivocally be assigned a "high solubility" BCS class. Based on the predicted physicochemical properties and observed in vivo behavior, ascorbic acid behave like high permeability BCS class drugs. Therefore, we suggest that ascorbic acid should be assigned to BCS class I drugs.

Visual as well as similarity (f_2) and difference (f_1) factors comparisons between the two IR oral formulations containing ascorbic acid(C-Tamin tablets, and Vitamin C tablets) as a single API showed the ascorbic acid was released differently in the tested dissolution media.

Conclusions: Ascorbic acid is a high solubility and high permeability drug, and therefore is classified as a BCS class 1 compound. The risk of bio*in*equivalence is manageable as long as the use of ascorbic acid is safe. For these reasons, we consider ascorbic acid to be a good candidate for

waiver of *in vivo* BE studies. Conducting in vitro dissolution could reveal the quality of IR oral formulations.

Key words:absorption; bioavailability; bioequivalence; biopharmaceutical classification system (BCS); biowaiver; ascorbic acid; pharmacokinetics; permeability; solubility

1 CHAPTER ONE: INTRODUCTION

1.1 Introduction

Demonstrating similarity in terms of safety and efficacy between innovator pharmaceutical products and their generic versions is a critical step in granting marketing authorizations (MAs) for generics [1]. Similarity often proved by conducting in vivo bioequivalence (BE) studies in healthy volunteers. BE is defined as the absence of a significant difference in the rate and extent to which the active ingredient in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study [2]. Health regulatory bodies issue MAs for generic versions after furnishing a proof of similarity with their innovator counterparts. BE studies are expensive, time consuming and risky studies since they are conducted in healthy volunteers. Today, the biopharmaceutical classification system (BCS) introduced by Amidon and adapted by various regulatory authorities and organizations like the US Food and Drug Administration (FDA), European Medicines Agency (EMA) and the World Health Organization (WHO) has significantly changed the processes of drug development and approval [3]. Regulatory bodies now allow waiver of in vivo BE studies using surrogate in vitro dissolution testing for immediate release (IR) oral solid dosage forms containing high solubility high permeability (BCS class I) drugs [4]. Guidelines issued by some agencies are continually revised and now accept to evaluate dossiers containing BCS class III drugs. Dissolution studies are cheaper and less time consuming than BE studies and moreover, conducted

in vitro, therefore, can be used as affordable surrogates for the in vivo BE studies [2].

1.2 Background

The present monograph addresses ascorbic acid as an active pharmaceutical ingredient (API). This monograph is one of a series of biowaiver monographs by the International Pharmaceutical Federation (FIP) that has published over 40 monographs; these monographs can be found at (http://www.fip.org/bcs). These monographs aim to assess whether in vivo pharmacokinetic BE studies can be safely waived in favor of in vitro dissolution studies for the approval of new or reformulated IR solid oral dosage forms of a given API.

This monograph evaluates data related to the application of the biowaiver principles for the approval of IR solid oral dosage forms containing ascorbic acid. Clinical and biopharmaceutical data of ascorbic acid will be assessed in order to take a decision to allow waiver of in vivo BE testing for the approval of IR solid oral dosage forms containing ascorbic acid. Pharmacokinetic properties, therapeutic index, therapeutic use, solubility and permeability, and any possibility of excipient interactions are taken into consideration. The purpose, aim, and scope of this series of monographs have been previously discussed [5]. Briefly, to evaluate the risks associated with a biowaiver decision after evaluation of all pertinent data available from literature sources for a given API. Risk is defined as the probability of an incorrect biowaiver decision as well as the consequences of the decision in terms of public health and individual patient risks. On the

basis of these considerations, a recommendation can be made as to whether a biowaiver approval is advisable or not.

This systematic approach to recommend or advice against a biowaiver decision is referred to in the recently published WHO guideline [6]. These monographs do not intend to simply apply the WHO, FDA, and/or EMA Guidance, but aim also as a critical evaluation of these and other countries' regulatory documents [6-8].

1.3 General characteristics

1.3.1 Scope

This monograph refers solely to ascorbic acid. The analysis is pertinent primarily for IR solid oral formulations in which ascorbic acid is the only API. It may also be applied for combination products containing ascorbic acid. However, for combination products, the other APIs and their possibility of interaction with ascorbic acid should be analyzed as well. Modified/extended release formulations are out of the scope of this analysis.

1.3.2 Name

The IUPAC name of ascorbic acid is {(5R)-5-[(1S)-1,2-dihydroxyethyl]-3,4-dihydroxy-2,5-dihydrofuran-2-one}[9].

Ascorbic acid has the chemical formula of $C_6H_8O_6$[9]. The CAS number for ascorbic acid is 50-81-7. Ascorbic acid has an average molecular weight of 176.12 with a melting point of 190-192 °C.The structure of ascorbic acid is shown in Figure 1.

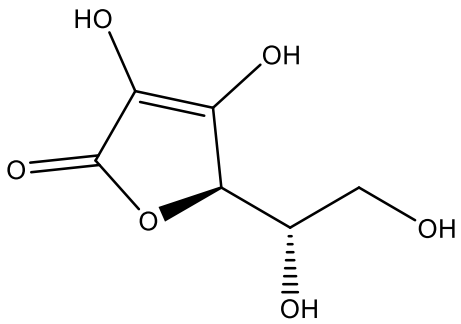

Figure 1: The structural Formula of ascorbic acid. The chemical structure was drawn using ChemBioDraw Ultra (Cambridge Soft Corporation, USA)

1.3.3 Importance, therapeutic indication and dose

Ascorbic acid, is an agent that is essential for proper health, and important component of human diet; therefore, it must be supplied in the diet, in proper amounts for the prevention of scurvy and to limit the risk of developing chronic diseases such as heart disease and cancer [10]. Daily consumption of fresh fruit and vegetables is recommended for adequate intake of ascorbic acid.Dietary ascorbic acid can come from foods, plant or animal in origin and intake depends on the type of food eaten.

Ascorbic acid is essential for both connective tissue formation and bone formation. Its role as an antioxidant is invaluable [11], because it helps to protect the cells from oxidative damage resulting from free-ion radicals [12]. It also protects low-density lipoprotein from oxidation, which reduces the risk of heart disease [13]. Individuals with atherosclerosis may benefit from ascorbic acid intake (1-3 g/day), because it may lower their risk of heart attack [14]. Also, individuals who consume higher amounts of ascorbic acid in their diet may have a lower risk of developing cancer [15]. The effect of ascorbic acid on cancer is more preventive than therapeutic. It

also aids the absorption of iron from the gastrointestinal tract, and as a result, it is important for individuals with iron deficiency to have ascorbic acid in their diet [16].

Humans cannot synthesize ascorbic acid; due to mutation in the gene for L-gulonolactone oxidase, the enzyme that catalyzes the terminal steps in the biosynthetic pathway [17]. Most animals make ascorbate in the liver and from there it is transported around the body via the circulation and taken up into other tissues [18, 19].

Ascorbic acid is an antioxidant which acts as a free radical scavenger[20]. It is used in the prophylaxis and treatment of scurvy [21].Ascorbic acid is used to prevent and to treat scurvy [22]. Scurvy may be treated with dietary ascorbic acid; however, administration of therapeutic doses of ascorbic acid probably results in more prompt saturation of tissue stores. The recommended dietary allowance for ascorbic acid is 60 mg/day for the prevention of scurvy[23].

The highest single dose recommended for ascorbic acid in IR solid oral dosage forms is 500mg. Extended-release dosage forms are available in 1000 mg [24]. Ascorbic acid is also indicated in a dose of 200 mg along with desferrioxamine in the treatment of thalassemia to enhance its chelating effects and improve excretion of iron [25]. In iron deficiency, ascorbic acid may be given in oral doses as a supplement[26]. Eye drops containing potassium ascorbate (ascorbic acid 10%) have been used for the treatment of chemical eye burns [27]. Ascorbic acid may also be used as antioxidants in pharmaceutical manufacturing of foods and drug [28].

A beneficial effect of ascorbic acid therapy has been claimed for an extraordinary number of conditions, including age-related macular degeneration[29], Alzheimer's disease [30], atherosclerosis [31], cancer [32], the common cold, and idiopathic thrombocytopenic purpura [33]. Other conditions claimed to benefit from ascorbic acid administration include asthma, wound healing, psychiatric disorders [34], infections due to abnormal leukocyte function, infertility, pain in Paget's disease[35], and tetanus[36]. Generally there are few properly controlled studies to substantiate these claims.

Antioxidant effects have been demonstrated as increased resistance of red blood cells to free radical attack in elderly persons and reduced activated oxygen species in patients receiving chemotherapy and radiation [21]. Antioxidant mechanisms have been shown in the reduction of low density lipoprotein (LDL) oxidation as well, though studies on the prevention of heart disease and stroke are conflicting [38].

Ascorbic acid facilitates absorption of iron by keeping iron in reduced form. A few microcytic anemia’s respond to ascorbic acid treatment, which may be due to improved absorption of iron [39].

1.3.4 Therapeutic index and toxicity

Ascorbic acid is of both natural and synthetic origin, natural origin of ascorbic acid is found in fresh fruit and vegetables[40]. Citrus fruits are a particularly good source of ascorbic acid and also hip berries, acerola and fresh tea leaves.

The main target organs for toxicity are found in the gastrointestinal, renal and hematological systems [41]. In individuals with glucose-6-phosphate dehydrogenase (G-6-PD) deficiency, hemolytic anemia may develop after administration of ascorbic acid [42].

In individuals predisposed to renal stones, chronic administration of high doses may lead to renal calculi formation. In some cases, acute renal failure may be observed under both conditions [43]. So Ascorbic acid is contraindicated in patients with hyperoxaluria and G-6-PD deficiency.

When the body is saturated with ascorbic acid and blood concentrations exceed the threshold, unchanged ascorbic acid is excreted in the urine [44]. Inactive metabolites of ascorbic acid such as ascorbic acid-2-sulfate and oxalic acid are excreted in the urine.

Over doses may acidify the urine, cause nausea, diarrhea, interfere with the healthy antioxidant-prooxidant balance in the body, and, in patients with thalassemia or hemochromatosis, promote iron overload[45]. Intake below the upper limit does not have toxic effects in healthy adults.

The recommended daily intake by the US Food and Nutrition Board of the Institute of Medicine for men more than 18 years old is 90 milligrams of ascorbic acid daily[46]; for women more than 18 years old, it is 75 milligrams daily; for pregnant women more than 18 years old, it is 85 milligrams daily; and for breastfeeding women more than 18 years old, it is 120 milligrams daily. The upper limit of intake should not exceed 2,000 milligrams daily[47].

1.4 Physicochemical properties

1.4.1 Salt, Esters, Polymorphs

Ascorbic acid is available as an acid and in magnesium phosphate (magnesium ascorbyl phosphate) and sodium (sodium ascorbate) salts. The acid and sodium salts are often given orally while the magnesium phosphate salt is given topically. Dose calculation is often made using the free acid form. For example, 1mg of free acid is equivalent to 1.1248 mg of the sodium salt [48] and1mg of the sodium salt is equivalent to 0.8890 mg of free acid. There was no evidence of polymorphism of ascorbic acid [49].

1.4.2 Dosage form strengths and dose

Ascorbic acid is listed on the WHO's Model List of Essential Medicines (EML) [50]. MAs were issued for ascorbic acid IR tablet, capsules, powder, powder for solution, powder for suspension, liquid filled, chewable tablet, granule, syrup, liquid, solution, extended release tablet, lozenge/troche, and extended release capsule [9]. The maximum dose strength listed on the WHO's essential medicines list is 50 mg. Dosage strengths are available in the range of 125 to 1000 mg [51, 52]. The dose and duration of time used for treatment by ascorbic acid differ according to the therapeutic goal. The usual dose begins from 125 mg to 1000 mg once daily for adults.

2 CHAPTER TWO: LITERATURE REVIEW

1.1 Literature review

Several studies have evaluated the possibility of granting a biowaiver decision for medications, here are examples of these studies:

1. Kortejarvi et al evaluated the possibility of granting a biowaiver decision for ranitidine hydrochloride [53],according to Literature and experimental data relevant to the decision, to allow a waiver of *in vivo*BE testing for the approval of immediate release (IR) solid oral dosage forms containing ranitidine hydrochloride are reviewed. According to the current Biopharmaceutics Classification System (BCS), ranitidine hydrochloride should be assigned to Class III. However, based on its therapeutic and therapeutic index, pharmacokinetic properties and data related to the possibility of excipient interactions, a biowaiver can be recommended for IR solid oral dosage forms that are rapidly dissolving and contain only those excipients as reported in this study.
2. Potthast et al evaluated the possibility of granting a biowaiver decision for Ibuprofen [54], according to literature data are reviewed on the properties of ibuprofen related to theBCS. Ibuprofen was assessed to be a BCS class II drug. Differences in composition and/or manufacturing procedures were reported to have an effect on the rate, but not the extent of absorption; such differences are likely to be detectable by comparative *in vitro* dissolution tests. Also in view of its therapeutic use, its wide therapeutic index and uncomplicated pharmacokinetic properties, a biowaiver for IR ibuprofen solid oral

drug products is scientifically justified, provided that the test product contains only those excipients reported in this paper in their usual amounts, the dosage form is rapidly dissolving (85% in 30 min or less) in buffer pH 6.8 and the test product also exhibits similar dissolution profiles to the reference product in buffer pH 1.2, 4.5, and 6.8.

3. Kalantzi et al evaluated the possibility of granting a biowaiver decision for acetaminophen (paracetamol) [55]according to the Literature data,they reviewed on the properties of acetaminophen (paracetamol) related to the biopharmaceutics classification system (BCS). According to the current BCS criteria, acetaminophen is BCS Class III compound. Differences in composition seldom, if ever, have an effect on the extent of absorption. However, some studies show differences in rate of absorption between brands and formulations. In particular, sodium bicarbonate, present in some drug products, was reported to give an increase in the rate of absorption, probably caused by an effect on gastric emptying. In view of Marketing Authorizations (MAs) given in a number of countries to acetaminophen drug products with rapid onset of action, it is concluded that differences in rate of absorption were considered therapeutically not relevant by the Health Authorities. Moreover, in view of its therapeutic use, its wide therapeutic index and its uncomplicated pharmacokinetic properties, *in vitro* dissolution data collected according to the relevant Guidancescan be safely used for declaring 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 fulfils the criterion of similarity of dissolution profiles to the reference product.

4. Jantratid et al evaluated the possibility of granting a biowaiver decision for cimetidine [56],according to literature data relevant to the decision to allow a waiver of *in vivo* BE testing for the approval of immediate release (IR) solid oral dosage forms containing cimetidine are reviewed. According to the current Biopharmaceutics Classification System (BCS), cimetidine would be assigned to Class III. Cimetidine's therapeutic use and therapeutic index, its pharmacokinetic properties, data related to the possibility of excipient interactions, and reported BE/bioavailability (BA) problems were also taken into consideration. On the basis of the overall evidence, a biowaiver can be recommended for cimetidine IR products, provided that the test product contains only those excipients reported in this paper in their usual amounts, and that the test and the comparator drug products both are "rapidly dissolving" as per BCS.
5. Granero et al evaluated the possibility of granting a biowaiver decision for acetazolamide [57]. Literature data relevant to the decision to allow a waiver of *in vivo*BE testing for the approval of immediate release (IR) solid oral dosage forms containing acetazolamide werereviewed. Acetazolamide's solubility

and permeability characteristics according to the Biopharmaceutics Classification System (BCS), as well as its therapeutic use and therapeutic index, its pharmacokinetic properties, data related to the possibility of excipient interactions and reported BE/bioavailability (BA) problems were taken into consideration. The available data on solubility, on oral absorption and permeability were not sufficiently conclusive to classify acetazolamide with certainty. Taking a conservative approach, no biowaiver was considered justified for the registration of new multisource drug products. However, SUPAC level 1 and level 2 post-approval changes and most EU Type I variations can be approved waiving *in vivo* BE studies.

6. Dressman et al evaluated the possibility of granting a biowaiver decision for acetylsalicylic acid [58].A biowaiver monograph for acetylsalicylic acid (ASA) was presented. Literature and experimental data indicated that ASA is a highly soluble and highly permeable drug, leading to assignment of this active pharmaceutical ingredient (API) to Class I of the Biopharmaceutics Classification System (BCS). Limited bioequivalence (BE) studies reported in the literature indicated that tested products were bioequivalent. Most of the excipients used in products with a marketing authorization in Europe were not considered to have an impact on gastrointestinal motility or permeability. Furthermore, ASA has a wide therapeutic index. Thus, the risks to the patient that might occur if a non-bioequivalent product were to be incorrectly deemed bioequivalent according to the

biowaiver procedure appear to be minimal. As a result, they concluded that the BCS-based biowaiver procedure can be recommended for approval of new formulations of solid oral dosage forms containing ASA as the only API, including both multisource and reformulated products, under the following conditions: (1) excipients are chosen from those used in ASA products already registered in International Conference on Harmonization and associated countries and (2) the dissolution profiles of the test and the comparator products comply with the BE guidance.

7. Manzo et al evaluated the possibility of granting a biowaiver decision for amitriptyline hydrochloride [59]. Literature data relevant to the decision to allow a waiver of *in vivo* bioequivalence (BE) testing for the approval of immediate release (IR) solid oral dosage forms containing amitriptyline hydrochloride were reviewed. Its therapeutic uses, its pharmacokinetic properties, the possibility of excipient interactions and reported BE/bioavailability (BA) problems were also taken into consideration. Literature data indicated that amitriptyline hydrochloride is a highly permeable active pharmaceutical ingredient (API). Data on the solubility according to the current Biopharmaceutics Classification System (BCS) were not fully available and consequently amitriptyline hydrochloride could not be definitively assigned to either BCS Class I or BCS Class II. But all evidence taken together, a biowaiver can currently be recommended provided that IR tablets are formulated with excipients used in existing approved

products and that the dissolution meets the criteria defined in the guidances.

8. Arnalet al evaluated the possibility of granting a biowaiver decision for acyclovir [60].Literature data relevant to the decision to allow a waiver of *in vivo* bioequivalence (BE) testing (biowaiver) for the approval of immediate release (IR) solid oral dosage forms containing acyclovir were reviewed. Acyclovir therapeutic use and therapeutic index, pharmacokinetic properties, data related to the possibility of excipient interactions and reported BE/bioavailability (BA) studies were also taken into consideration in order to ascertain whether abiowaiver can be recommended. According to the Biopharmaceutics Classification System (BCS) and considering tablet strengths up to 400 mg,acyclovir would be BCS Class III. However, in some countries also 800 mg tablets are available which fall just within BCS Class IV. Acyclovir seems not to be critical with respect to a risk for bioinequivalence, as no examples of bioinequivalence have been identified. It has a wide therapeutic index and is not used for critical indications. Hence, if: (a) the test product contains only excipients present in acyclovir solid oral IR drug products approved in ICH or associated countries, for instance as presented in this article; and (b) the comparator and the test product both are very rapidly dissolving, a biowaiver for IR acyclovir solid oral drug products is considered justified for all tablet strengths.

9. Nair et al evaluated the possibility of granting a biowaiver decision for amodiaquine hydrochloride [61]. The present monograph reviewed data relevant to applying the biowaiver procedure for the approval of immediate release (IR) multisource solid dosage forms containing amodiaquine hydrochloride (ADQ) as the single active pharmaceutical ingredient (API). Both biopharmaceutical and clinical data of ADQ were assessed. Solubility studies revealed that ADQ meets the "highly soluble" criteria according to WHO and EMA but fails to comply with the FDA specifications. Although metabolism hints at high permeability, available permeability data were too scanty to classify ADQ inequivocably as a Class I drug substance. According to WHO and EMA guidances, ADQ would be conservatively categorized as a Class III drug, whereas according to the US FDA specifications, it would fall into Class IV. ADQ has a wide therapeutic index. Furthermore, no cases of bioinequivalent products have been reported in the open literature. As risks associated with biowaiving appear minimal and requirements for "highly soluble" API are met in the WHO and EMA jurisdictions, the biowaiver procedure can be recommended for bioequivalence (BE) testing of multisource IR products containing ADQ as the only API, provided the test product contains excipients used in ADQ products approved in International Conference of Harmonisation and associated countries, and in similar amounts. Furthermore, both comparator and test should conform to "very rapidly dissolving" product criteria (≥85% dissolution of the

API in 15 min at pH 1.2, 4.5, and 6.8) and the labeling should specify that the product not be co administered with high-fat meals. If the comparator and/or test product fails to meet these criteria, BE needs to be established by pharmacokinetic studies in humans.

10. Vogelpoel et al evaluated the possibility of granting a biowaiver decision for verapamil hydrochloride, propranolol hydrochloride, and atenolol [5]. Literature data related to the Biopharmaceutics Classification System (BCS) were presented on verapamil hydrochloride, propranolol hydrochloride, and atenolol in the form of BCS-monographs. Data on the qualitative composition of immediate release (IR) tablets containing these active substances with a Marketing Authorization (MA) in the Netherlands (NL) were also provided; in view of these MA's the assumption was made that these tablets were bioequivalent to the innovator product. The development of a database with BCS-related data was announced by the International Pharmaceutical Federation (FIP).
11. Charooet al evaluated the possibility of granting a biowaiver decision for bisoprolol [62] .Literature data relevant to the decision to allow a waiver of *in vivo* bioequivalence (BE) testing for the approval of immediate-release (IR) solid oral dosage forms containing bisoprolol as the sole active pharmaceutical ingredient (API) were reviewed. Bisoprolol was classified as a Class I API according to the current Biopharmaceutics Classification System (BCS). In addition to the BCS class, its therapeutic index,

pharmacokinetic properties, data related to the possibility of excipient interactions, and reported BE/bioavailability problems were taken into consideration. Qualitative compositions of IR tablet dosage forms of bisoprolol with a marketing authorization (MA) in ICH (International Conference on Harmonisation) countries were tabulated. It was inferred that these tablets had been demonstrated to be bioequivalent to the innovator product. No reports of failure to meet BE standards have been made in the open literature. On the basis of all these pieces of evidence, a biowaiver can currently be recommended for bisoprolol fumarate IR dosage forms if (1) the test product contains only excipients that are well known, and used in normal amounts, for example, those tabulated for products with MA in ICH countries and (2) both the test and comparator dosage form are very rapidly dissolving, or, rapidly dissolving with similarity of the dissolution profiles demonstrated at pH 1.2, 4.5, and 6.8.

12. Verbeeck et al evaluated the possibility of granting a biowaiver decision for chloroquine sulfate [63]. Literature data on the properties of chloroquine phosphate, chloroquine sulfate, and chloroquine hydrochloride related to the Biopharmaceutics Classification System (BCS) were reviewed. The available information indicated that these chloroquine salts can be classified as highly soluble and highly permeable, i.e., BCS class I. The qualitative composition of immediate release (IR) tablets containing these Active Pharmaceutical Ingredients (APIs) with a Marketing Authorization

(MA) in Belgium (BE), Germany (DE), Finland (FI), and The Netherlands (NL) were provided. In view of these MA's and the critical therapeutic indication of chloroquine, it is assumed that the registration authorities had evidence that these formulations are bioequivalent to the innovator. It was concluded that IR tablets formulated with these excipients are candidates for a biowaiver.

13. Oliveraet al evaluated the possibility of granting a biowaiver decision for Ciprofloxacin Hydrochloride [64]. Literature data relevant to the decision to allow a waiver of *in vivo* bioequivalence (BE) testing for the approval of new multisource and reformulated immediate release (IR) solid oral dosage forms containing ciprofloxacin hydrochloride as the only active pharmaceutical ingredient (API) were reviewed. Ciprofloxacin hydrochloride's solubility and permeability, its therapeutic use and index, pharmacokinetics, excipient interactions and reported BE/bioavailability (BA) problems were taken into consideration. Solubility and BA data indicated that ciprofloxacin hydrochloride is a BCS Class IV drug. Therefore, a biowaiver based approval of ciprofloxacin hydrochloride containing IR solid oral dosage forms cannot be recommended for either new multisource drug products or for major scale-up and postapproval changes (variations) to existing drug products.

14. Dahanet al evaluated the possibility of granting a biowaiver decision for codeine phosphate [65].The monograph reviewed data relevant to applying the biowaiver procedure for the approval of immediate-

release multisource solid dosage forms containing codeine phosphate. Both biopharmaceutical and clinical data of codeine were assessed. Solubility studies revealed that codeine meets the "highly soluble" criteria according to WHO, EMA, and the FDA. Codeine's fraction of dose absorbed in humans was reported to be high (>90%) based on cumulative urinary excretion of drug and drug-related material following oral administration. The permeability of codeine was also assessed to be high in both Caco-2 monolayers and rat intestinal perfusion studies. The main risks associated with codeine, that is, toxicity (attributed to CYP2D6 polymorphism) and its abuse potential, are present irrespective of the dosage form, and do not need to be taken into account for bioequivalence (BE) considerations. Taken together, codeine is a class 1 drug with manageable risk and is a good candidate for waiver of *in vivo* BE studies.

15. Chuasuwan et al evaluated the possibility of granting a biowaiver decision for diclofenac potassium and diclofenac sodium [66]. Within the biopharmaceutics classification system (BCS), diclofenac potassium and diclofenac sodium are each BCS class II active pharmaceutical ingredients (APIs). Due to their therapeutic use, therapeutic index, pharmacokinetic properties, potential for excipient interactions, a biowaiver can be recommended for IR drug products of each salt form, and performance in reported BE/bioavailability (BA) studies, provided that test and comparator contain the same diclofenac salt; the dosage form of the test and comparator is identical; the test

product contains only excipients present in diclofenac drug products approved in ICH or associated countries in the same dosage form, test drug product and comparator dissolve 85% in 30 min orless in 900 mL buffer pH 6.8, using the paddle apparatus at75 rpm or the basket apparatus at 100 rpm; and test product and comparator show dissolution profile similarity in pH 1.2, 4.5, and 6.8.

16. Jantratid et al evaluated the possibility of granting a biowaiver decision for Doxycycline Hyclate[67]. Literature data relevant to the decision to allow a waiver of *in vivo* bioequivalence (BE) testing for the approval of immediate release (IR) solid oral dosage forms containing doxycycline hyclate were reviewed. According to the Biopharmaceutics classification System (BCS), doxycycline hyclatecan be assigned to BCS Class I. No problems with BE of IR doxycycline formulations containing different excipients and produced by different manufacturing methods have been reported and hence the risk of bioinequivalence caused by these factors appears to be low. Doxycycline has a wide therapeutic index. Further, BCS-based dissolution methods have been shown to be capable of identifying formulations which may dissolve too slowly to generate therapeutic levels. It was concluded that a biowaiver is appropriate for IR solid oral dosage forms containing doxycycline hyclate as the single Active Pharmaceutical Ingredient (API) provided that (a) the test product contains only excipients present in doxycycline hyclate IR solid oral drug products approved in the International Conference on

Harmonization (ICH) or associated countries; and (b) the comparator and the test products comply with the BCS criteria for ‘‘very rapidly dissolving’’ or, alternatively, when similarity of the dissolution profiles can be demonstrated and the two products are ‘‘rapidly dissolving.’’

17. Cristofoletti et al evaluated the possibility of granting a biowaiver decision for Efavirenz[68]. Literature data pertaining to the decision to allow a waiver of *in vivo* bioequivalence testing for the approval of immediate-release (IR) solid oral dosage forms containing favirenz as the only active pharmaceutical ingredient (API) were reviewed. Because of lack of conclusive data about efavirenz’s permeability and its failure to comply with the “high solubility”criteria according to the Biopharmaceutics Classification System (BCS), the API can be classified as BCS Class II/IV. In line with the solubility characteristics, the innovator product does not meet the dissolution criteria for a “rapidly dissolving product.” Furthermore, product variations containing commonly used excipients or in the manufacturing process have been reported to impact the rate and extent of efavirenz absorption. Despite its wide therapeutic index, sub therapeutic levels of efavirenz can lead to treatment failure and also facilitate the emergence of efavirenz-resistant mutants. For all these reasons, a biowaiver for IR solid oraldosage forms containing efavirenz as the sole API is not scientifically justified for reformulated or multisource drug products.

18. Becker et al evaluated the possibility of granting a biowaiver decision for EthambutolDihydrochloride[69]. Literature data relevant to the decision to allow a waiver of *in vivo* bioequivalence (BE) testing for the approval of immediate release (IR) solid oral dosage forms containing ethambutoldihydrochloride as the only active pharmaceutical ingredient (API) were reviewed. Ethambutoldihydrochloride is a Biopharmaceutics Classification System (BCS) Class III drug with permeability properties approaching the border between BCS Class I and III. BE problems of ethambutol formulations containing different excipients and different dosages forms have not been reported and hence the risk of bioinequivalence caused by excipients is low. Ethambutol has a narrow therapeutic index related to ocular toxicity. However, as long as the prescribers' information of the test product stipulates the need for regular monitoring of ocular toxicity, the additional patient risk is deemed acceptable. It was concluded that a biowaiver can be recommended for IR solid oral dosage forms provided that the test product (a) contains only excipients present in ethambutol IR solid oral drug products approved in ICH or associated countries, for instance as presented in this paper, (b) complies with the criteria for ''very rapidly dissolving'' and (c) has aprescribers' information indicating the need for testing the patient's vision prior to initiating ethambutol therapy and regularly during therapy.

19. Charoo et al evaluated the possibility of granting a biowaiver decision for Fluconazole [70]. Literature data pertaining to the decision to allow a waiver of *in vivo* bioequivalence (BE) testing requirements for the approval of immediate release (IR) solid oral dosage forms containing fluconazole as the only active pharmaceutical ingredient (API) were reviewed. The decision was based on solubility, dissolution, permeability, therapeutic index, pharmacokinetic parameters, pharmacodynamic properties, and other relevant data. BE/bioavailability (BA) problems and drug-excipients interaction data were also reviewed and taken into consideration. According to the biopharmaceutics classification system (BCS), fluconazole in polymorphic forms II and III is a BCS class I drug and has a wide therapeutic index. BE of test formulations from many different manufacturers containing different excipients confirmed that the risk of bioinequivalence because of formulation and manufacturing factors is low. It was inferred that risk can be further reduced if *in vitro* studies are performed according to biowaiver guidelines. Thus, it was concluded that a biowaiver can be recommended for fluconazole IR dosage forms if (a) fluconazole is present as polymorphic form II or III or any other form/mixture showing high solubility, (b) the selection of excipients be limited to those found in IR drug products approved in International Conference on Harmonisation (ICH) countries for the same dosage form and used in their usual amounts, and (c) both the test and comparator dosage form are very rapidly dissolving, or,

rapidly dissolving throughout the shelf life with similar dissolution profiles at pH 1.2, 4.5, and 6.8.

20. Granero et al evaluated the possibility of granting a biowaiver decision for Furosemide [71].Literature and new experimental data relevant to the decision to allow a waiver of *in vivo* bioequivalence (BE) testing for the approval of immediate release (IR) solid oral dosage forms containing furosemide were reviewed. The available data on solubility, oral absorption, and permeability were sufficiently conclusive to classify furosemide into Class IV of the Biopharmaceutics Classification System (BCS). Furosemide's therapeutic use and therapeutic index, its pharmacokinetic properties, data related to the possibility of excipient interactions and reported BE/bioavailability (BA) problems were also taken into consideration. In view of the data available,itwas concluded that the biowaiver procedure cannot be justified for either the registration of new multisource drug products or major postapproval changes (variations) to existing drug products.

21. Beckeret al evaluated the possibility of granting a biowaiver decision for Isoniazid [72].Literature data relevant to the decision to allow a waiver of *in vivo* bioequivalence (BE) testing for the approval of immediate release (IR) solid oral dosage forms containing isoniazid as the only active pharmaceutical ingredient (API) were reviewed. Isoniazid's solubility and permeability characteristics according to the Biopharmaceutics Classification System (BCS), as well as its therapeutic use and therapeutic index, its pharmacokinetic properties,

data related to the possibility of excipient interactions and reported BE/bioavailability (BA) problems were taken into consideration. Isoniazid is ‘‘highly soluble’’ but data on its oral absorption and permeability were inconclusive, suggesting this API to be on the borderline of BCS ClassI and III. For a number of excipients, an interaction with the permeability is extreme unlikely, but lactose and other deoxidizing saccharides can form condensation products with isoniazid, which may be less permeable than the free API. A biowaiverwas recommended for IR solid oral drug products containing isoniazid as the sole API,provided that the test product meets the WHO requirements for ‘‘very rapidly dissolving’’and contains only the excipients commonly used in isoniazid products, as listed in this article. Lactose and/or other deoxidizing saccharides containing formulations should be subjected to an *in vivo*BE study.

22. Shohinet al evaluated the possibility of granting a biowaiver decision for Ketoprofen[73].Literature and experimental data relevant to the decision to allow a waiver of *in vivo* bioequivalence (BE) testing for the approval of immediate-release (IR) solid oral dosage forms containing ketoprofenwere reviewed. Ketoprofen’s solubility and permeability, its therapeutic use and therapeutic index, pharmacokinetic properties, data related to the possibility of excipient interactions, and reported BE/bioavailability (BA)/dissolution data were taken into consideration. The available data suggested that according to the current Biopharmaceutics Classification System (BCS) and all

current guidances, ketoprofen is a weak acid that would be assigned to BCS Class II. The extent of ketoprofen absorption seems not to depend on formulation or excipients, so the risk of bioinequivalence in terms of area under the curve is very low, but the rate of absorption (i.e., BE in terms of peak plasma concentration, *C*max) can be altered by formulation. Current *in vitro* dissolution methods may not always reflect differences in terms of *C*max for BCS Class II weak acids; however, such differences in absorption rate are acceptable for ketoprofen with respect to patient risks. As ketoprofen products may be taken before or after meals, the rate of absorption cannot be considered crucial to drug action. Therefore, a biowaiver for IR ketoprofen solid oral dosage form was considered feasible, provided that (a) the test product contains only excipients present also in IR solid oral drug products containing ketoprofen, which are approved in International Conference on Harmonisation or associated countries, for instance, as presented in this paper; (b) both the test drug product and the comparator dissolve85% in 30 min or less in pH 6.8 buffer; and (c) test product and comparator show dissolution profile similarity in pH 1.2, 4.5, and 6.8. When one or more of these conditions are not fulfilled, BE should be established *in vivo*.

23. Strauch et al evaluated the possibility of granting a biowaiver decision for Lamivudine [74].Literature data relevant to the decision to allow a waiver of *in vivo* bioequivalence(BE) testing for the approval of immediate release (IR) solid oral dosage forms containing lamivudine

as the only active pharmaceutical ingredient were reviewed. The solubility and permeability data of lamivudine as well as its therapeutic index, its pharmacokinetic properties, data indicating excipient interactions, and reported BE/bioavailability (BA) studies were taken into consideration. Lamivudine is highly soluble, but its permeability characteristics are not well-defined. Reported BA values in adults ranged from 82% to 88%. Therefore, lamivudine was assigned to the biopharmaceutics classification system (BCS) class III, noting that its permeability characteristics are near the border of BCS class I. Lamivudine is not a narrow therapeutic index drug. Provided that (a) the test product contains only excipients present in lamivudine IR solid oral drug products approved in the International Conference on Harmonization or associated countries in usual amounts and (b) the test product as well as the comparator product fulfills the BCS dissolution criteria for very rapidly dissolving; a biowaiver can be recommended for new lamivudine multisource IR products and major post-approval changes of marketed drug products.

24. Strauch et al evaluated the possibility of granting a biowaiver decision for Levetiracetam[74]. Literature data relevant to the decision to allow a waiver of *in vivo* bioequivalence (BE) testing for the approval of immediate release (IR) solid oral dosage forms containing lamivudine as the only active pharmaceutical ingredient were reviewed. The solubility and permeability data of lamivudine as well as its therapeutic index, its pharmacokinetic properties, data indicating

excipient interactions, and reported BE/bioavailability (BA) studies were taken into consideration. Lamivudine is highly soluble, but its permeability characteristics arenot well-defined. Reported BA values in adults ranged from 82% to 88%. Therefore, lamivudineis assigned to the biopharmaceutics classification system (BCS) class III, noting that its permeability characteristics are near the border of BCS class I. Lamivudine is not a narrow therapeutic index drug. Provided that (a) the test product contains only excipients present inlamivudine IR solid oral drug products approved in the International Conference on Harmonization or associated countries in usual amounts and (b) the test product as well as the comparator product fulfills the BCS dissolution criteria for very rapidly dissolving; a biowaiver can be recommendedfor new lamivudine multisource IR products and major post-approval changes of marketed drug products.

25. Koeppe et al evaluated the possibility of granting a biowaiver decision for Levofloxacin [75]. Literature data relevant to the decision to allow a waiver of *in vivo* bioequivalence (BE) testing for the approval of immediate release (IR) solid oral dosage forms containing levofloxacin as the only active pharmaceutical ingredient (API) were reviewed. According to the current Biopharmaceutics Classification System, levofloxacin can be assigned to Class I. No problems with BE of IR levofloxacin formulations containing different excipients and produced by different manufacturing methods have been reported and hence the risk of bioinequivalence caused by these factors appears to

be low. In addition, levofloxacin has a wide therapeutic index. On the basis of this evidence, a biowaiverwas recommended for IR solid oral dosage forms containing levofloxacin as the single API provided that (a) the test product contains only excipients present in IR levofloxacin drug products that have been approved in International Conference on Harmonization (ICH) or associated countries and which have the same dosage form; (b) both the test and comparator dosage form are "very rapidly dissolving" or "rapidly dissolving" with similarity of the dissolution profiles demonstrated at pH 1.2, 4.5, and 6.8; and (c) if the test product contains polysorbates, it should be both qualitatively and quantitatively identical to its comparator in terms of polysorbate content.

26. Strauch et al evaluated the possibility of granting a biowaiver decision for Mefloquine Hydrochloride [76]. Literature data relevant to the decision to allow a waiver of *in vivo* bioequivalence (BE) testing for the approval of immediate release solid oral dosage forms containing mefloquine hydrochloride as the only active pharmaceutical ingredient (API) were reviewed. The solubility and permeability data of mefloquine hydrochloride as well as its therapeutic use and therapeutic index, its pharmacokinetic properties, data related to the possibility of excipient interactions and reported BE/bioavailability studies were taken into consideration. Mefloquine hydrochloride is not a highly soluble API. Since no data on permeability are available, it cannot be classified according to the Biopharmaceutics Classification System

with certainty. Additionally, several studies in the literature failed to demonstrate BE of existing products. For these reasons, the biowaiver cannot be justified for the approval of new multisource drug products containing mefloquine hydrochloride. However, scale-up and post approval changes (HHS-FDA SUPAC) levels 1 and 2 and most EU type I variations may be approvable without *in vivo*BE, using the dissolution tests described in these regulatory documents.

27. Stosik et al evaluated the possibility of granting a biowaiver decision for Metoclopramide Hydrochloride [77]. Literature data were reviewed relevant to the decision for a biowaiver of immediate release (IR) solid oral dosage forms containing metoclopramide hydrochloride. In addition, new solubility data, obtained under Biopharmaceutics Classification System (BCS) conditions were presented. MetoclopramideHClis conservatively assigned to BCS Class III. Taken also into consideration excipient interactions reported in metoclopramide drug products, its pharmacokinetic properties and therapeutic use and therapeutic index, a biowaiver can be recommended when: (a) the test product contains only excipients present also in metoclopramide HCl containing IR solid oral drug products approved in ICH or associated countries, for instance as presented in this paper, (b) in amounts in normal use in IR solid oral dosage forms, and (c) the test product and the comparator both comply with the criteria for very rapidly dissolving.

28. Rediguieri et al evaluated the possibility of granting a biowaiver decision for Metronidazole [78]. Metronidazole can be assigned to Biopharmaceutics Classification System Class I. Most BE studies that were identified reported the investigated formulations to be bioequivalent, indicating the risk of bioinequivalence to be low. Formulations showing differences in bioavailability showed dissimilarities *in vitro* dissolution profiles. Furthermore, metronidazole has a wide therapeutic index. It was concluded that a biowaiver for solid IR formulations is justified, provided: (a) the test product and its comparator are both rapidly dissolving; (b) meet similarity of the dissolution profiles at pH 1.2, 4.5, and 6.8; (c) the test product contains only excipients present in IR drug products approved in International Conference on Harmonisation (ICH) or associated countries in the same dosage form; and (d) if the test product contains sorbitol, sodium laurilsulfate, or propylene glycol. The test product needs to be qualitatively and quantitatively identical to its comparator with respect to these excipients

29. Shohin et al evaluated the possibility of granting a biowaiver decision for piroxicam as free acid form [79]. Literature and experimental data relevant to the decision to allow a waiver of *in vivo* bioequivalence (BE) testing for the approval of immediate release (IR) solid oral dosage forms containing piroxicam in the free acid form were reviewed. Piroxicam solubility and permeability, its therapeutic use and therapeutic index, pharmacokinetic properties, data related to the

possibility of excipient interactions and reported BE/bioavailability (BA), and corresponding dissolution data were taken into consideration. The available data suggest that according to the current biopharmaceutics classification system (BCS) and all current guidances, piroxicamwould be assigned to BCS Class II. The extent of piroxicam absorption seems not to depend on manufacturing conditions or excipients, so the risk of bioinequivalence in terms of area under the curve (AUC) is very low, but the rate of absorption (i.e., BE in terms of Cmax) can be affected by the formulation. Current *in vitro* dissolution methods may not always reflect differences in terms of Cmax for BCS Class II weak acids; however, minor differences in absorption rate of piroxicam would not subject the patient to unacceptable risks: as piroxicam products may be taken before or after meals, the rate of absorption cannot be considered crucial to drug action. Therefore, a biowaiver for IR piroxicam solid oral dosage form is considered feasible, provided that (a) the test product contains only excipients, which are also present in IR solid oral drug products containing piroxicam, which have been approved in ICH or associated countries(b) both the test and comparator drug products dissolve 85% in 30 min or less at pH 1.2, 4.5, and 6.8; and (c) the test product and comparator show dissolution profile similarity in pH 1.2, 4.5, and 6.8. When not all of these conditions can be fulfilled, BE of the products should be established *in vivo.*

30. Vogt et al evaluated the possibility of granting a biowaiver decision for prednisolone [80]. Literature data relevant to the decision to allow a waiver of *in vivo* bioequivalence (BE) testing for the approval of immediate release (IR) solid oral dosage forms containing prednisolone were reviewed. Data on its solubility, oral absorption, and permeability were not totally conclusive, but strongly suggested a BCS Class 1 classification. Prednisolone's therapeutic indications and therapeutic index, pharmacokinetics, and the possibility of excipient interactions were also taken into consideration. Available evidence indicates that a biowaiver for IR solid oral dosage forms formulated with the excipients tabulated in this article would be unlikely to expose patients to undue risks.
31. Nair et al evaluated the possibility of granting a biowaiver decision for primaquine phosphate [58]. Solubility studies of the API using the standard shake-flask method in water and compendial buffers of pH 1.0, 1.2, 4.5, 6.8, and 7.5 at 37°C revealed that primaquine phosphate was "highly soluble". Dissolution experiments were also conducted on IR dosage forms containing primaquinephosphate as API. oral solid dosage forms showed "very rapid dissolution", i.e. more than 85% released in less than 15 min. Literature reported BA of primaquine phosphate was well over 90%, which indicates that primaquine phosphate was "highly permeable". The study concluded that a biowaiver-procedure-based approval for IR solid oral dosage forms of primaquine phosphate can be justified.

32. Becker et al evaluated the possibility of granting a biowaiver decision for Pyrazinamide [81]. Literature data relevant to the decision to allow a waiver of *in vivo* bioequivalence (BE) testing for the approval of immediate release (IR) solid oral dosage forms containing pyrazinamide as the only active pharmaceutical ingredient (API) were reviewed. Pyrazinamide is BCS Class III, with linear absorption over a wide dosing range. The risk of bioinequivalenceis estimated to be low. Depending on the definition used, pyrazinamide can be classified as a narrow therapeutic index (NTI) drug, which is usually a caveat to biowaiving but may be deemed acceptable if the Summary of Product Characteristics (SmPCs) of the test product stipulates the need for regular monitoring of liver function. It is concluded that a biowaiver can be recommended for IR solid oral dosage only when the test product (a) contains only excipients present in Pyrazinamide IR solid oral drug products approved in ICH or associated countries, (b) these excipients are present in amounts normally used in IR solid oral dosage forms, (c) the test product is very rapidly dissolving, (d) the SmPC of the test product indicates the need for monitoring of the patient's liver function.

33. Grube et al evaluated the possibility of granting a biowaiver decision for Quinidine Sulfate [82]. Literature data were reviewed relevant to the decision to allow a waiver of *in vivo* bioequivalence (BE) testing for the approval of new multisource and reformulated immediate release (IR) solid oral dosage forms containing quinidine sulfate.

Quinidine sulfate’s solubility and permeability, its therapeutic use and index, pharmacokinetics, excipient interactions and reported BE/bioavailability (BA) problems were taken into consideration. The available data were not fully conclusive, but do suggest that quinidine sulfate is highly soluble and moderately to highly permeable and would likely be assigned to BCS Class I (or at worst BCS III). In view of the inconclusiveness of the data and, more important, quinidine’s narrow therapeutic window and critical indication, a biowaiver based approval of quinidine containing dosage forms cannot be recommended for either new multisource drug products or for major postapproval changes (variations) to existing drug products.

34. Strauch et al evaluated the possibility of granting a biowaiver decision for Quinine sulfate [83]. The biowaiver approach permits evaluation of bioequivalence (BE) using a set of laboratory tests, obviating the need for expensive and time-consuming pharmacokinetic BE studies provided that both the active pharmaceutical ingredient and the formulations can meet the specified criteria. In the present monograph, the biowaiver-relevant data including solubility and permeability data, therapeutic use and therapeutic index, pharmacokinetic properties, reported excipient interactions, and BE/bioavailability studies of quinine sulfate were itemized and discussed. Quinine sulfate has borderline solubility characteristics and, on the whole, is highly permeable. Thus, depending on the jurisdiction, it was assigned to Biopharmaceutics Classification System class I or II. Although these

characteristics would suggest a low risk of bioinequivalence among oral quinine products, a recent pharmacokinetic study showed bioinequivalence of two products. Even though quinine does not, strictly speaking, fit the definition of a narrow therapeutic index drug, it shows dose-related and, in some cases, irreversible side effects and toxicities at concentrations not far above the therapeutic concentration range. Taking all relevant aspects into consideration, a biowaiver cannot be recommended for new quinine immediate-release multisource products or major post-approval changes of already marketed quinine products, and in such cases, BE should be evaluated using an *in vivo* BE study.

35. Becker et al evaluate the possibility of granting a biowaiver decision for Rifampicin [72]. Literature data relevant to the decision to allow a waiver of *in vivo* bioequivalence (BE) testing for the approval of new multisource and reformulated immediate release (IR) solid oral dosage forms containing rifampicin as the only Active Pharmaceutical Ingredient (API) were reviewed. Rifampicin's solubility and permeability, its therapeutic use and index, pharmacokinetics, excipient interactions and reported BE/bioavailability (BA) problems were taken into consideration. Solubility and absolute BA data indicate that rifampicin is a BCS Class II drug. Of special concern for biowaiving is that many reports of failure of IR solid oral dosage forms of rifampicin to meet BE have been published and the reasons for these failures are yet insufficiently understood. Moreover, no

reports were identified in which *in vitro* dissolution was shown to be predictive of nonequivalence among products. Therefore, a biowaiver based approval of rifampicin containing IR solid oral dosage forms cannot be recommended for either new multisource drug products or for major scale-up and post approval changes (variations) to existing drug products.

36. Silva et al evaluate the possibility of granting a biowaiver decision for Stavudine[84]. Literature data relevant to the decision to allow a waiver of *in vivo* bioequivalence (BE) testing for the approval of immediate-release (IR) solid oral dosage forms containing stavudine (d4T) were reviewed. According to Biopharmaceutics Classification System (BCS), d4T can be assigned to BCS class I. No problems with BE of IR d4T formulations containing different excipients and produced by different manufacturing methods have been reported and, hence, the risk of bioinequivalence caused by these factors appears to be low. Furthermore, d4T has a wide therapeutic index. It is concluded that a biowaiver is appropriate for IR solid oral dosage forms containing d4T as the single active pharmaceutical ingredient (API) provided that (a) the test product contains only excipients present in the IR d4T drug products that have been approved in a number of countries for the same dosage form, and (b) both test product and its comparator are either “very rapidly dissolving” or “rapidly dissolving” with similarity of dissolution profiles demonstrated at pH 1.2, 4.5, and 6.8.

37. Soares et al evaluate the possibility of granting a biowaiver decision for Zidovudine[85]. Literature data on the properties of zidovudine relevant to waiver of *in vivo* bioequivalence (BE) testing requirements for the approval of immediate-release (IR) solid oral dosage forms containing zidovudine alone or in combination with other active pharmaceutical ingredients (APIs) were reviewed. Solubility, dissolution, and permeability data for zidovudine, along with its dosing schedule, therapeutic index and pharmacokinetic properties, and reports related to BE/bioavailability were all taken into consideration. Data for solubility and permeability suggest that zidovudine belongs to Class I according to the Biopharmaceutics Classification System. Also, zidovudine is not a narrow therapeutic index drug. Although five out of 13 formulations tested *in vivo* (mostly of unreported composition) failed to show BE, it appears that *in vitro* studies performed according to biowaiver methods could predict *in vivo* behavior. Nevertheless, it is highly recommended that if a biowaiver is to be applied, excipient choices be limited to those found in IR drug products approved in International Conference on Harmonisation (ICH) or associated countries in the same dosage form, in their usual amounts. These conclusions apply to products containing zidovudine as the only API and also to fixed combination products containing zidovudine with respect to the zidovudine component of the formulation.

3 CHAPTER THREE: PROBLEM STATEMENT, OBJECTIVES, AND SIGNIFICANCE

Problem statement

Since 1960s, BE studies have emerged as pivotal in the development of generic versions of originator products. The BCS-based biowaiver offers considerable economic and technical advantages for the pharmaceutical industry.

Regulatory agencies like the FDA and the EMA allow the replacement of *in vivo*BE studies by *in vitro* dissolution testing for the approval of new and/or reformulated IR containing BCS class I and more recently class III APIs. The risks of granting a biowaiver for IR dosage forms containing ascorbic acid were not evaluated

The importance of BE studies is increasing due to the large growth of the production and consumption of generic products[86]. Any solid oral generic drug product needs a proof of similar efficacy and safety compared to the innovator productto obtaining a MA. BE studies are expensive and time consuming in vivo method.

Biowaiver means that in vivo BA and/or BE studies may be waived, and instead, in vitro dissolution testingcan be adopted as a surrogate for the decision as to whether the twopharmaceutical productsare equivalents.

A major advantage of the biowaiver procedure is the simplification and reduction of time required for product approval, thus reducing the cost of bringing new products to the market place.

This study is carried out on one of the WHO’s EML drugs, which is ascorbic acid. This study is needed to assign a correct BCS class for ascorbic acid based on its aqueous solubility in the pH range specified by

the regulatory agencies.Ascorbic acid is among the list adopted by the State of Palestine. In 2006, the WHO launched an initiative to waive *in vivo* BE studies requirements for solid oral dosage forms on the list. This initiative aims to reduce production costs of high quality and affordable generic versions of these medicines in developing and poor nations. The focus group on BCS and Biowaiver of the FIP has adopted the WHO initiative and invited researchers around the world to prepare biowaiver monographs for the orally administered solid dosage forms on the WHO essential medicines list [61]. As stated earlier, this monograph is a part of the biowaiver monographs series by the FIP.

1.5 Objectives

This study will be conducted with the following objectives:

- To gather and organize all relevant data on ascorbic acid IRsolid oral dosage forms, which includes solubility, pharmacokinetics (especially with respect to absorption and bioavailability) and permeability of ascorbic acid, and the dissolution of dosage forms as per current BCS rules; the therapeutic use and therapeutic window of ascorbic acid; any history of problems with BA/BE and, if it exists, data on excipient interactions, Which have to be taken into consideration when a decision is to be made as to whether a new formulation of ascorbic acid (either a reformulation or a new, multi-source product) needs to be tested in an *in vivo* bioequivalence study, or whether a biowaiver is appropriate and can be recommended.

- To assess the validity of the present BCS Guidance on the basis of the gathered data. To illustrate some of the possibilities: the results may show that there is a need to re-define the present BCS Classes (e.g. relax permeability requirements or D: S limits), to invoke other dissolution test conditions than those currently recommended or to change the specifications used for dissolution test results.
- To assess the risks associated with an inappropriate biowaiver decision. Risks are defined not only as the probability of reaching an incorrect decision with respect to applicability of the biowaiver but also with respect to the ramifications of this decision in terms of public health and risks to individual patients.
- To assess the release profiles of comparator and a generic IR oral dosage forms containing ascorbic acid as the only API using compendial dissolution method.

3.1 Significance of the study

This study may give foundations for the pharmaceutical industry to formulate IR solid oral dosage forms containing ascorbic acid as an API and use dissolution testing as a standard quality control test, similarly, this study may be beneficial for the regulatory authorities to whether grant a biowaiver for IR dosage forms containing ascorbic acid as an API.

The significance of the present study can be summarized in the following bullets:

- ✓ Correctly classifying ascorbic acid into BCS

- ✓ Economical, avoiding subjecting health volunteers to risk in BE studies
- ✓ This study may give foundations for the pharmaceutical industry to formulate IR solid oral dosage forms containing ascorbic acid as an API and use dissolution testing as a standard quality control test, similarly, this study may be beneficial for the regulatory authorities to whether grant a biowaiver for IR dosage forms containing ascorbic acid as an API.

4 CHAPTER FOUR: MATERIALS AND METHODS

4.1 Methods

4.1.1 Chemical and Reagents

0.1 N HCl (pH 1.2), phosphate buffer (pH 4.5), and phosphate buffer (pH 6.8). Monobasic potassium phosphate, deionized water, hydrochloric acid, and sodium hydroxide were all obtained from the central chemical store of An-Najah National University.

4.1.2 Products tested

The tested products were comparator ascorbic acid 500 mg tablets[(batch No.: 205478, manufacturing date: 05/2012, expiry date: 05/2015)]andGeneric ascorbic acid 500 mg[(batch No.: 212027, manufacturing date: 12/2012, expiry date: 12/2015)]. Both products were IRsolid oral dosage form tablets.

4.1.3 Instruments

The following instruments were used in this study: a) dissolution apparatus paddle type (DT-6, China), b) analytical balance, pH meter (Jenway 3510, Bibby Scientific, UK) and d) UV spectrophotometer (jenway 7315, bibby scientific ltd, UK).

4.1.4 Literature search

Literature data were obtained from PubMed, Micromedex, Scopus, Martindale, the Merck Index, Drug Bank, and Goodman and Gilman's "The pharmacological basis of therapeutics" (11th edition). The keywords used for searching were: ascorbic acid, indication, therapeutic index,

toxicity, intestinal absorption, permeability, distribution, metabolism, excretion, first pass effect, pharmacokinetics (PK), bioavailability (BA), bioequivalence (BE), mass balance, radio labeled studies, polymorphism, log *P*, solubility, and dissolution. Information was also obtained from regulatory documents published by the WHO, FDA, and EMA.

4.1.5 Solubility experiments

Solubility studies were conducted to determine the aqueous solubility of ascorbic acid at different pH points in the range of 1-7.5 at 37 ºC and to assign a correct solubility class for ascorbic acid. According to the BCS, and the FDA guidelines, a drug substance is considered highly soluble when the highest dosestrength is soluble in 250 ml or less of aqueous media over the pH range of 1–7.5 [87, 88].

This can be demonstrated through the calculation of the dose number (D_0) according to the equation [89, 90]:

$$Do = \frac{\left(\frac{Mo}{Vo}\right)}{Cs}$$

Where, M_o is the highest dose strength (in milligrams), C_s is the solubility (milligrams per milliliter), and V_o = 250 ml. A D_0 value of ≤ 1 indicates that the highest dose strength is soluble in 250 ml of the investigated aqueous media, and hence indicates “high solubility” class.

Solubility studies were performed at the Department of Pharmacy, An-Najah National University Nablus, Palestine, using analytical grade ascorbic acid (purchased from Alfa Aesar, Johnson Matthey Company). A

standard shake-flask method was applied using three different aqueous media with pH values of1.2, 4.5, 6.8, and 7.5 PH points

4.1.5.1 Buffer preparation:

- 1.2 buffer was prepared by placing 50ml of KCL solution in 200 ml volumetric flask then adding 85 ml of 0.2M HCL then adding deionized water to 1000ml.
- 4.5 (Phosphate buffer) was prepared by dissolving0.46 g of NaOH in 30 ml deionized water then adding few drops of concentrated HCL.
- 6.8 buffer was prepared by dissolving 6.8 g of KH_2PO_4 in 500 ml of deionized water and then adding of 0.94 g of NaOH and fill the flask to 1000 ml of deionized water.
- 7.5 PH was achieved by adding KCL to deionized water at 37 °C.

  The establishment of equilibrium was confirmed by comparing the solubility at 24 h and 48h. The pH was measured both before and after the experiment to ensure that the solubility was indeed registered at the correct pH. Drug levels in the samples were analyzed using UV spectrophotometric assay of ascorbic acid at 260nm[91]. Unknown concentrations were determined against calibration curve in the appropriate media.

  The calibration curve was done by Preparing known samples of ascorbic acid covering a range of concentrations expected for unknowns.Then measuring the response of the UV spectrophotometer at 260 nm wavelength for these standards.

Measurements were obtained in triplicates in each pH condition, then we make a graph of corrected versus concentration of standard, then find the best straight line through the linear portion of the data.

Table 5: calibration curve data of ascorbic acid at 260 nm wave length

Absorbance	Conc.\microgram
0.400462	10
0.811734	20
1.288152	30
1.802088	40
2.300075	50
2.639165	60

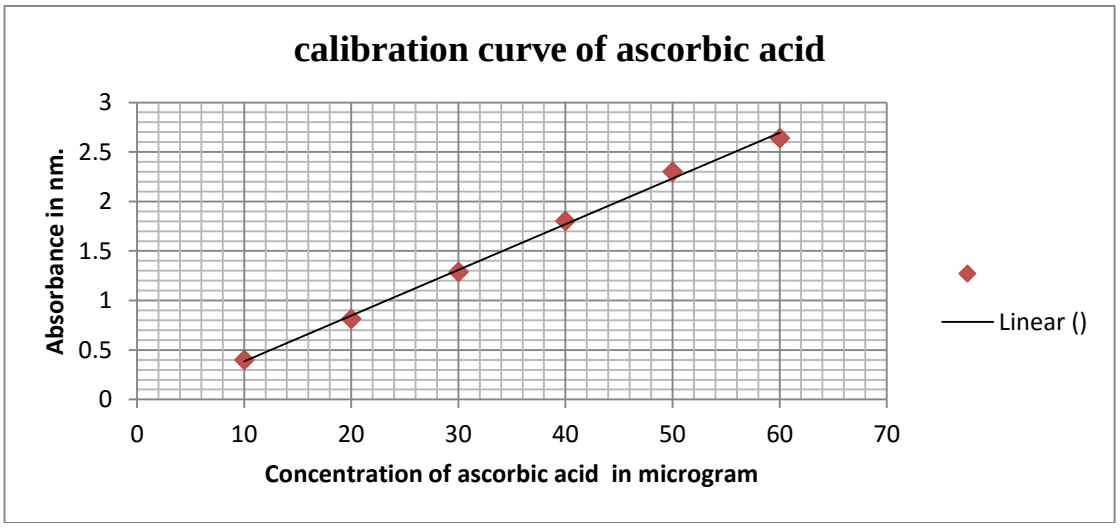

Figure 4: calibration curve of ascorbic acid immediate release oral dosage form

4.1.6 Dissolution testing

Dissolution experiments on IR tablets containing ascorbic acid 500 mg were carried out at the Department of Pharmacy, An-Najah National University, Nablus, Palestine. Dissolution profiles of ascorbic acid 500 mg tabletswere generated in 900 ml of deionized water at pH points of 1.2, 4.5

and 6.8 adjusted using 0.1 N HCl or NaOHsolutions. A paddle type dissolution apparatus was used and dissolution was tested in according to the USP type-II method [92]. Paddles rotated at 75 rpm and the temperature of the dissolution media was maintained at 37 ± 0.5 ºC. Aliquots of 5 mL were withdrawn at predetermined time intervals of 5, 10, 15, 20, 25, 30, 45 and60 min. Withdrawn aliquots were replaced with the fresh medium of the appropriate buffer. Samples were filtered through 0.45 mm membrane filter. Samples were suitably diluted and analyzed at260 nm using UV spectrophotometer[91]. Release of the ascorbic acid from different formulations was determined against standard calibration curves. Measurements were obtained in triplicates in each pH condition.

4.1.7 Excipients used in different formulations

Excipients present in ascorbic acid IR solid oral formulations with a MA in Canada (CA), Czech Republic (CZ), Spain (ES), Ireland (IE), The Netherlands (NL), Slovakia (SK), Germany (DE), Denmark (DK), France (FR), United Kingdome (UK), Romania (RO), Sweden (SE), were searched using the databases on the websites of Health Canada, Czech State Institute for Drug Control, Spanish Agency of Medicines and Health Products, Irish Health Products Regulatory Authority, Netherland's Medicines Evaluation Board, Swedish Medical Products Agency, and Slovakian State Institute for Drug Control, German drug database websites, France High Authority of Health (HAS),United Kingdome Medicines and Healthcare products Regulatory Agency, and the Romanian National Agency For Medicines. The amount range of each excipient in a solid oral IR dosage forms with a

MA in the US in mg was taken from the FDA's inactive ingredient database.

4.1.8 Molecular descriptors

Molecular descriptors like polar surface area (PSA), *n*-octanol/water partition coefficient (log *P*), distribution-coefficient at pH 7.4 (log $D_{7.4}$), number of hydrogen bond acceptors, number of hydrogen bond donors and p*K*a of ascorbic acid were calculated using ACD/Labs (ACD/Labs, Advanced Chemistry Development: Toronto, Canada), ChemAxon (ChemAxon, Budapest, Hungary), ALOGPS (The Virtual Computational Chemistry Laboratory, VCCLAB, Germany), and ChemBioDraw Ultra, Cambridge Soft Corporation, USA) software packages.

4.1.9 Dosage form performance

The BE and dissolution data of solid oral formulations containing ascorbic acid as an API were searched using the above mentioned databases. Data pertaining to bio*in*equivalence of ascorbic acid formulations were also searched.

5 CHAPTER FIVE: RESULTS, DISCUSSION AND CONCLUSION

5.1 Results

5.1.1 Solubility measurement

Solubility of ascorbic acid in aqueous media over the pH range of 1-7.5 was determined at 37 ± 0.5 °C. The solubility measurements show that the maximum dose listed on the WHO's EML list was soluble in less than 250 mL of water over the pH range specified by the regulatory agencies at 37 °C. The calculated D_0 values are shown in Table 1.

Table 1: Solubility measurements in aqueous media at various pH points in the range of 1-7.5 at 37°C.

pH	Medium	Solubility (mg/mL)	Dose number (D_0)	Acceptance criterion ($D_0 \leq 1$) for "highly soluble" drugs (Yes/No)
1.2	0.1M HCL	1900	0.00011	Yes
4.5	Phosphate buffer	900	0.00022	yes
6.8	Phosphate buffer	1400	0.00014	Yes
7.5	Phosphate buffer	700	0.00029	Yes

5.1.2 Solubility reported in the literature

Ascorbic acid was reported to be "freely soluble in water" in Martindale and the Merck Index[93, 94].The Drug Bank database reported an experimental solubility of 400 g/L of water measured at 40 °C [95]. ALOGPS predicted a solubility of 245 mg/mL in water.

5.1.1 Molecular descriptors

Ascorbic acid is a hydrophilic compound with a log *P* of -1.85 (octanol–water) (Drug Bank, 2015). At the small intestinal pH 6.8, ascorbic acid has

a negative log *D* value of -4.90 [96].Applying the Lipinski's rule of five, ascorbic acid passes and would be preferably formulated as oral dosage form[97]. The Lipinski's rule of five can be summarized as below:

- No more than 5 hydrogen bond donors
- No more than 10 hydrogen bond acceptors
- A molecular mass less than 500 Dalton
- A log *P* value not greater than 5

The molecular descriptors of ascorbic acid calculated using different software packages are shown in Table 2.

Table 2: Predicted molecular descriptors of ascorbicacid using different software packages

	Software			
Property	**ChemBioDraw**	**ACD/Labs**	**ChemAxon**	**ALOGPS**
Log *P*	-1.75	-2.41	-1.91	-1.6
Log *D* at pH 6.8D (log $D_{6.8}$)	-	-4.99	-4.90	-
Number of hydrogen bond acceptors	-	6	5	-
Number of hydrogen bond donors	-	4	4	-
PSA (Å)	107.22	107	107.22	-
P*K*a	-	-	4.36 (Strongest Acidic), -3 (Strongest Basic)	-
Mol. wt.	176.12			

5.1.2 Permeability and bioavailability

Transport of ascorbic acid was found to be Na^+-dependent which is saturable[23]. Studies suggested that some transporters like GLUT1, GLUT3, SVCT1 and SVCT2 might be implicated in the transport of dehydroascorbic acid transport[98].Using intestinal perfusion to measure

uptake rates, absorption of ascorbic acid was calculated as 50 mg/cm-hr [99]. The BA of a liquid solution given orally at fasting state was shown to be 90% for doses less or equal to 200 mg [100, 101]. BA was shown to decrease with increasing doses. In a study conducted by Levine et al, ascorbic acid showed dose dependent BA[101]. The study compared the pharmacokinetics after doses ranging from 30 mg to 2500 mg administered orally and intravenously. Interestingly, the BA was 100% after a single oral dose of 200 mg while decreased to about 33% after a single dose of 1250 mg. The pharmacokinetics of ascorbic acid was shown to be nonlinear, especially at lower doses.

5.1.3 Dissolution

Dissolution experiments showed that comparator and generic ascorbic acid formulations (C-Tamin and Vitamin C)released different quantities of ascorbic acid in function of time in different dissolution media. The difference (f_1) and similarity (f_2) factors are shown in Table3.

Table 3: Difference (f_1) and similarity (f_2) factors

pH	Difference (f_1) factors	Similarity (f_2) factors
1.2	21.7	40.4
4.5	11.3	57.2
6.8	23.6	45.1

Similarity factor (f2) equation is :

$$f2=50*Log\{1+1/N[\sum(Rt-Tt)2]\}-0.5*100$$

And the difference factor (f1)equation is:

$$f1= \{[\sum_{t=1}^{n} |R_t-T_t|] / [\sum_{t=1}^{n} R_t]\} x100$$

where, Rt and Tt are the cumulative percentage dissolved at each of the selected n time points of the reference and test product, respectively.

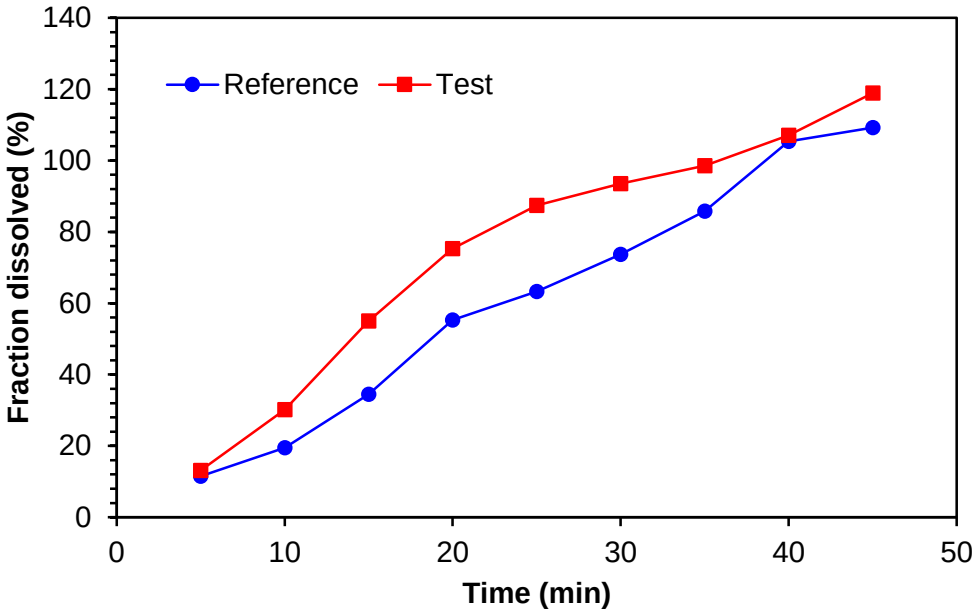

Figure 2: Dissolution profile of comparator (reference) and generic (test)ascorbic acid 500 mg tablets at pH 1.2.

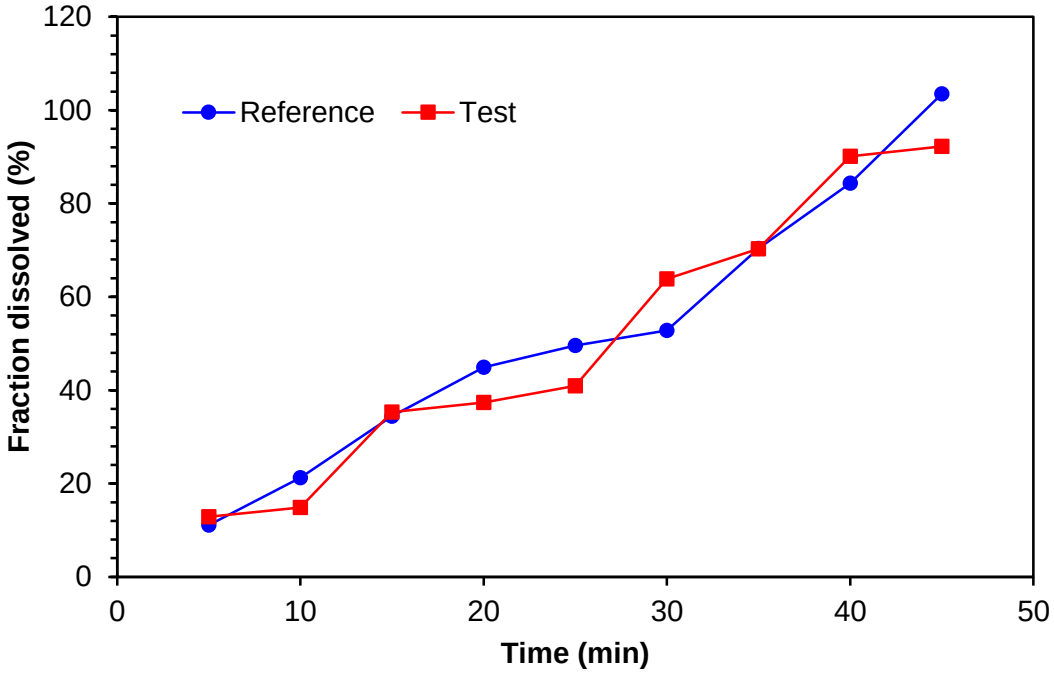

Figure 3: Dissolution profile of comparator (reference) and generic (test)ascorbic acid 500 mg tablets at pH 4.5.

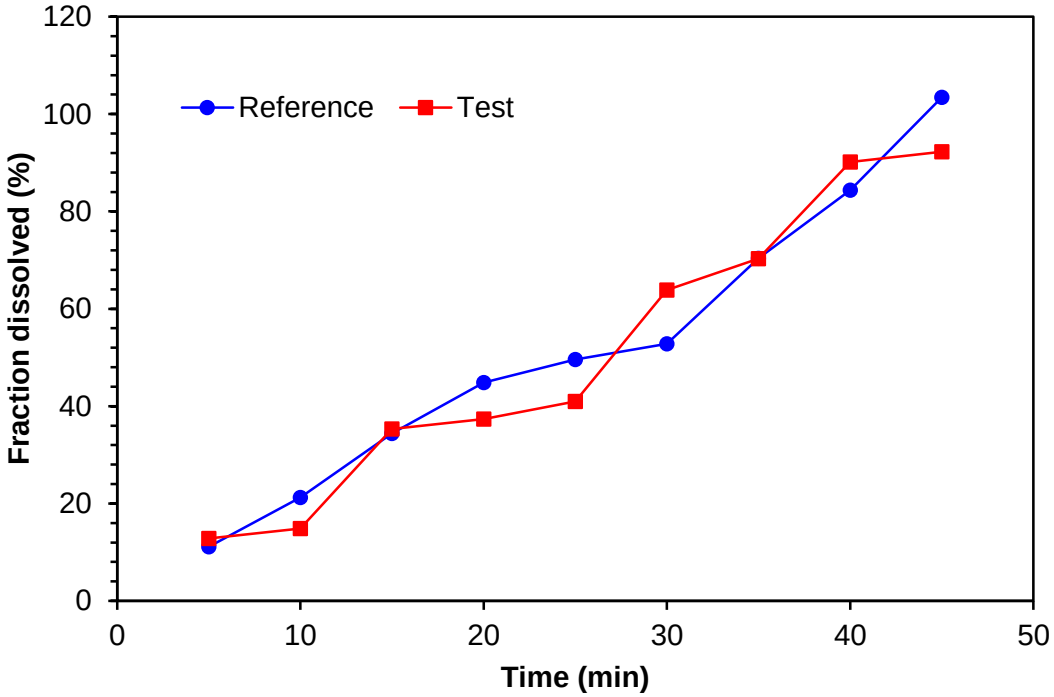

Figure 4: Dissolution profile of comparator (reference) and generic (test)ascorbic acid 500 mg tablets at pH 6.8.

5.1.4 Excipients used in different dosage forms

Excipients present in ascorbic acid IR solid oral drug products with a MA in ICH and associated countries are shown in Table 4. It can be assumed that these drug products successfully passed an in vivo BE study, clinical trial or were judged by other appropriate means by the regulatory authority to provide adequately similar clinical safety and efficacy. Excipients present in these drug products seem, therefore, to be safe .

Table 4: Excipients Present in ascorbic acid IR Solid Oral Drug Products with a Marketing Authorization (MA) in Canada (CA), Czech Republic (CZ), Spain (ES), Ireland (IE), The Netherlands (NL), Slovakia (SK),Germany (DE),Denmark (DK),France (FR),United Kingdome (UK),Romania (RO), and Sweden (SE).

Excipient	Drug Products Containing That Excipient with a MA Granted by the Named Country	Range Present in Solid Oral Dosage Forms with a MA in the US (mg)
Acesulfame Potassium	CA(1),CZ(6)	0.12-117
Alcohol Phenylethylalcohol	CA(2)	0.25-0.5
Allura Red Ac.	CA(4),CZ(6)	0.0005-50
Ammonium Hydroxide	CA(4)	0.02-39.75
Anhydrous Silica	CA(5),ES(12),SE(19)	2.25-7.2
Aspartam	CZ(6),DK(9),ES(11),FR(14)	0.01-65
Benzoic Aldehyde	DE(7),FR(14)	0-2
Bitter Almond Essencemaltose Dextrin	DE(8),FR(14)	0.158-295
Capsule-Gelatin	DK(9),RO(18)	0.13-1000
Carnauba Wax	CA(3,4),DK(10)	0.046-300
Carrageenan	CA(1),ES(11)	0.1534-33
Cellulose	CA(2,3,4,5),SE(17,19),UK(20)	4.5-1120
Citric Acid	CA(1,2),ES(12),FR(13)	0.0002-500
Corn Starch	DE(7),FR(14)	0.337-1135
Compressible Sugar	IE(15)	49-623
Copolymere Methacrylate Acrylate Methyle	FR(13),NL(16)	6.7-93.3
Crospovidone	DE(8) ,SE(17)	0.02-722
Dextrose	CA(5),RO(18)	4.4-903

D&C Red #27	SE(19)	0.0007-48.75
Fd&C Blue #1	CA(4),UK(20)	0.002-3.7
Fd&C Red #40	CA(4)	0.0006-40
Fd&C Blue #2	CA(4)	0.008-24.12
Fd&C Yellow #6	CA(4)	0.0006-6.97
Fructose	CA(1,2)	0.6667-438
Gelatin	DE(7)	0.26-1000
Glucose Monohydrate	SE(17)	0.025-5
Gum Arabic	FR(14)	0-9
Hydroxypropyl Cellulose	SE(19)	0.0004-187
Hydroxypropyl Methylcellulose	CA(3)	0.25-100.4
Hypromellose.	IE(15)	0.125-320
Isopropyl Alcohol	CA(4)	2-398
Lactose	CZ(6),DE(8),NL(16)	0.45-1013
Lemon Flavoring	DK(9)	0.1-340
Macrogol 6000th	SE(19)	0.06-128
Macrogol 8000	FR(13)	0.18-190
Macrogol/Peg 3350	CA(4)	0.5-1425
Magnesium Stearate	CA(1,3,4,5), SE(19)	0.0015-250
Microcrystalline Cellulose	CA(3,4),SE(17,19),UK(20)	0.05-1385.3
Malic Acid	CA(1)	0.01-4
Mannitol	CA(1),DK(9),ES(11),FR(14)	0.05-606
N-Butyl Alcohol	CA(4)	0.0786-2
Orange Flavor Powder	CZ(6)	0.01-50
Paraffin Oil	DK(9)	0.06-50
Pectin	IE(15)	25.5-1400
Polyvinyl Alcohol	CA(4)	0.05-119
Povidone	DE(8),FR(13)	0.9-240
Propylene Glycol	CA(4)	0.44-252
Saccharin Sodium	DK(9)	0.05-60
Shellac	CA(4),DE(7),RO(18)	3.3-87
Silica	CA(1,5),DE(7),DK(10),ES(12),SE(19)	2.25-7.2
Silicon Dioxide	CA(2,3)	0.65-100
Sodium Bicarbonate	CZ(6)	0.005-267
Sodium Dodecyl Sulfate	DE(7)	0.02-20
Sodium Metabisulphate(E223)	UK(20)	0.003-27.5
Sorbitol	CA(2),CZ(6),ES(12)	0.25-337
Stearic Acid	CA(1,2,5),DE(7),SE(17)	0.2-187
Sucrose	CA(1),DE(7),ES(12)	0.02-1200
Talc	DE(7),DK(10),FR(14),SE(17),RO(18)	0.1-110
Tartaric Acid	DE(7),RO(18)	10-215
Titanium Dioxide (E 171).	RO(18)	0.10-35.7
Xanthan Gum	CA(1)	0.15-75

Sources of data: CA, www.hc-sc.gc.ca (accessed on 02-7-2015); CZ, www.sukl.cz/ (accessed n 02-7-2015); ES, www.aemps.es (accessed on 04-7-2015); IE, www.imb.ie/ (accessed on 04-7-2015); NL, www.cbg-meb.nl (accessed on 09-7-2015); SE, www.lakemedelsverket.se (accessed on 09-7-2015); SK, www.sukl.sk (accessed on 14-7-2015),DE www.rote-liste.de(accessed on 14-7-2015),DK www.dkma.dk(accessed on 14-7-2015),FR www.theriaque.org (accessed on 14-7-2015),RO www.anm.ro(accessed on 14-7-2015),UK www.medicines.org.uk/emc (accessed on 014-17-2015),SE www.lakemedelsverket (accessed on 014-17-2015).

1 Kanga Vites C

2 Life C

3 formula hh

4 PREGVIT

5 Jamieson™ Vitamin C

6 Celaskon 500 mg

7 Cetebe® Vitamin C Retard 500

8 VITAMIN C MP 500 Tabletten

9 APOVIT C-vitamin ekstrastærk

10 Bio-C-Vitamin ”Pharma Nord” syreneutral

11 REDOXC 500 mg COMPRIMIDOS MASTICABLES

12 Cebión 500 mg

13 FERO GRAD VITAMINE C 105MG/500MG CPR

14 VITAMINE C UPSA 500mg CPR

15 VITAMIN C Tablets.

16 Vitamine C Apotex 500 mg tablet

17 CitroVit Vitamin C tab

18 SICOVIT C 500mg tablet

19 Ido-C 0.5 g tablet

20 Ascorbic Acid Tablets 500mg

5.1.5 Dosage form performance

A study investigated theBE of three formulations containing ascorbic acid as a single API. The formulations were of 1000 mg strength. The study was open with a randomized 3-period crossover designon 17 volunteers [102].Tested formulations were well tolerated and no adverse effects were reported. Plasma pharmacokinetic parameters like areas under the curves (AUC) from 0 to 12h and from 0 to 48h, maximum achieved concentration (C_{max}) and the time at which it occurred (T_{max}) were compared and were within the accepted BE range of 80-125%[102]. The three formulations were deemed bioequivalents.

6.1 Discussion

6.1.1 Solubility

Ascorbic acid was reported to be freely soluble in water in different databases. However, solubility was not measured in accordance with the BCS principles. In order to assign an API to either high or low BCS solubility class, it is a prerequisite to measure solubility in aqueous media

at 37 ± 0.5 °C in the pH range of 1-7.5 in accordance to the BCS principles set by the FDA and 1.2-6.8 according to the criteria specified by the EMA, WHO and recent FDA drafted guidelines [6-8, 103]. Solubility data for ascorbic acid reported in the literature was not suitable to assign a solubility class for the API. Therefore, we had to measure the solubility in accordance with the guidelines. Solubility measured in buffered aqueous media showed that the highest dose strength of ascorbic acid would be soluble in less than 250 mL of water. The D_0 values were in the range of 0.00011 to 0.00029 as shown in Table 1. These results, unequivocally indicate that ascorbic acid belongs to “high solubility” BCS class drugs.

6.1.2 Permeability

Permeability of ascorbic acid was not intensively investigated.However, reports of complete absorption at doses less than 200 mg were published. Despite the implication of active transport, it is thought that ascorbic acid would have “high” permeability, especially at low doses. As the dose specified by the WHO on the EML and the lists adopted by various countries including Palestine, it is believed that the dose of 50 mg would be completely absorbed.Previous studies showed that transporters like GLUT1, GLUT3, SVCT1 and SVTC2 are implicated in the transport of ascorbic acid[98]. This active transport is saturable which might explain the nonlinear pharmacokinetics of ascorbic acid. In fact, Nelson et al. showed that 50 mg would be absorbed in the upper intestine within 1 h [99]. Therefore, based on these data, ascorbic acid would be assigned “high” permeability BCS class.

6.1.3 Dissolution of tested comparator and generic version

Comparing the release profiles of the comparator and generic version showed that the two profiles were not similar as shown in Table 3 and Figures 2-4. This could be interesting in the context of biowaiver and conducting in vitro dissolution testing to ensure similarity between innovator products and their generic versions. As the tested generic product failed to demonstrate similar release profile it is highly likely that this product will fail a pivotal BE study. Therefore, it is believed that conducting in vitro dissolution testing can reveal the quality of the tested products. In a commentary published in the AAPS journal, Polli discussed that in vitro dissolution testing are sometimes better than conventional human in vivo BE studies for IR solid oral dosage forms[104].

6.1.4 Risks of bio*in*equivalence caused by excipients and/or manufacturing parameters

No information was found in the literature concerning potential influence of excipients or manufacturing process on the performance of ascorbic acid formulations. Generally, ascorbic acid containing IR oral formulations tended to perform well in vivo and in vitro.

6.1.5 Patient's risks associated with bio*in*equivalence

In general, the safety margin of ascorbic acid is thought to be high as the upper limit for ascorbic acid intake is 2000 mg/day [105]. The main risk associated with bio*in*equivalence of generic ascorbic acid formulations is ascorbic acid toxicity. Up to 10 g/day of ascorbic acid are sometimes taken

for unproven health benefits, such as preventing or shortening the duration of viral infections or slowing or reversing the progression of cancer or atherosclerosis Such doses may acidify the urine, because nausea and diarrhea interfere with the healthy antioxidant-prooxidant balance in the body [106], and in patients with thalassemia or hemochromatosis, promote iron overload [107]. Ascorbic acid is generally non-toxic but at high doses (2–6 g/day) it can cause gastrointestinal disturbances or diarrhea [47, 108]. It has been reported that there is no evidence of carcinogenicity. Ascorbic acid overdose typically results in diarrhea after oral dosage of 1 g or more daily or greater. Doses of 8 g daily decrease serum uric acid.

5.1.6 Conclusions

Ascorbic acid is a high solubility and high permeability drug, and therefore is classified as a BCS class 1 compound. The risk of bio*in*equivalence is manageable as long as the use of ascorbic acid is safe. For these reasons, we consider ascorbic acid to be a good candidate for waiver of *in vivo* BE studies.

Granting a biowaiver for IR solid oral dosage forms containing ascorbic acid is scientifically justified, subjected to the following conditions:

1) The test product contains only excipients that are well known and used in normal amounts, for example, those tabulated for products with MA in ICH-associated countries .
2) Both the test and comparator dosage forms show very rapid dissolution of ascorbic acid or, rapid dissolution without similarity of

the dissolution profiles demonstrated at least at pH 1.2, and 6.8 for ascorbic acid.

For products containing other APIs in addition to ascorbic acid, the possibility of a biowaiver for each API should be separately considered.

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جامعة النجاح الوطنية

كلية الدراسات العليا

دراسة الاعفاء الحيوي للأشكال الصيدلانية ذات التحرير الفوري المحتوية على مادة حمض الاسكوربيك

إعداد

هيلين نجدي المصري

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د.أمجد حسين

قدمت هذه الأطروحة استكمالا لمتطلبات الحصول على درجة الماجستير في تكنلوجيا الصيدلية بكلية الدراسات العليا في جامعة النجاح الوطنية، نابلس- فلسطين.

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الملخص

خلفية: إظهارأوجه التشابه من حيث السلامة والفعالية بين المنتجات الدوائية المبتكرة وإصداراتها العامة هي خطوة حاسمة في منح تراخيص التسويق (MAs). و ذلك من خلال إجراء دراسات التكافؤ الحيوي في الجسم الحي (BE) على متطوعين أصحاء.وبسبب الحاجة الى معايير واضحة في اثبات التكافؤ و والتشابه بين المنتجات المتكافئةحيويا,برزت أهمية دراسات التكافؤ الحيوي(BE)و بديل دراسات التكافؤ الحيوي(Biowaiver).ان دراسات التكافؤ الحيوي(BE) دراسات مكلفة ومستهلكة للوقت وهي محفوفة بالمخاطر بسبب إجراؤها على متطوعين أصحاء,من هنا ظهر نظام التصنيف البيولوجي الصيدلاني (BCS) الذي عرضهAmidon والذي تم تبنيه من قبل السلطات الرقابية والمنظمات المختلفة مثل منظمة الغذاء والدواء الأمريكية (FDA)، والوكالة الأوروبية للأدوية (EMA) ومنظمة الصحة العالمية (WHO)والذيغير بشكل كبير من عمليات تطوير الأدوية والموافقة عليها.بحيث تسمح الهيئات التنظيمية الآن بالتنازل عن إجراءدراسات التكافؤ الحيوي(BE)واستخدام دراسات بديل التكافؤ الحيوي في اختبار الأدوية الصلبة ذات التحرير الفوري التي تعطى عن طريق الفم(IR) بحيث يكون الدواء عالي الذوبان وعالي النفاذية وبالتالي يصنف باعتباره فئة درجة أولى حسب نظام التصنيف البيولوجي الصيدلاني(BCS).

وكانهدف هذه الأطروحة التقييمالمنهجي لإمكانية منح بديل التكافؤ الحيوي(biowaiver) للتركيبات التي تحتوي على حمض الاسكوربيككمادة فعالة (API) ذات التحرير الفوري(IR) والتي تعطى عن طريق الفم. وتقييم خصائص الافراج عن اثنين من الصيغ التي تحتوي على حمض الاسكوربيك.

الأساليب: أجريت دراسات الذوبان لتحديد الذوبان المائي من حمض الاسكوربيك في درجات حموضة مختلفة: من(7.5–1) في درجة37 مئوية, وتعيين فئة الذوبان الصحيحة لحمض الاسكوربيك وفقا لنظام التصنيف البيولوجي الصيدلاني (BCS),والمبادئ التوجيهية لمنظمة الغذاء و الدواء الامريكية(FDA)، ويعتبر الدواءعالي الذائبية عندما تكون أعلى جرعة دوائية قابلة للذوبان في 250 مل أو أقل في وسط مائيبدرجات حموضة مختلفة من (1–7.5).

تم تطبيق الطريقة التقليدية(shake–flask)باستخدامثلاث أوساط مائيةبدرجات حموضة مختلفةمن(1– 7.5):

1.0(maleate buffer), 4.5 (acetate buffer) 7.5 (phosphate buffer).

تمتحقيق التوازن بمقارنة الذوبان في 24 ساعة و48 ساعة . وقد تم تحليل مستويات الدواء في العينات باستخدام الأشعة فوق البنفسجية للفحص الطيفي لحمض الاسكوربيك في 260 نانومتر. وأجريت تجارب الذائبية على صنفين تجاريينمنالأقراص ذات التحرير الفوري التي تعطى عن طريق الفم التي تحتوي على حمض الاسكوربيك بتركيز 500 مل في 900 مل من الماء منزوع الأيونات فيبدرجات حموضة مختلفة 1.2، 4.5 و 6.8 ومعايرة درجة حموضة الوسط المائي باستخدام 0.1 N حمض الهيدروكلوريك و هيدروكسيد الصوديوم. تم اختبار الذوبان فيه وفقا لطريقة (USP) النوع الثاني(باستخدام المجداف) على 75 دورة في الدقيقة والحفاظ على درجة الحرارة الوسط المائيعلى 37 ± 0.5 درجة مئوية. تم سحب 5 مل من المحلول في فترات زمنية محددة سلفا من 5، 10، 15، 20، 25، 30، 45 و 60 دقيقة.و تم أخذ عينات مخففة بشكل مناسب وتحليلها في 260 نانومتر باستخدام مطياف الأشعة فوق البنفسجية. والمواصفات الجزيئية مثل المساحة السطحية القطبية (PSA)،معامل الفصلبين الاوكتانول و الماء(log P)، معاملتوزيع درجة الحموضة 7.4 (log D7.4)، وعدد الروابط الهيدروجينة المستقبلة,وعدد الروابط

الهيدروجينية المانحة ودرجة pKaباستخدام نظام البرمجيات. تم البحث في قواعد البيانات عن الذوبان والنفاذية والتذويب ذات الصلة بحمض الاسكوربيك.

النتائج: أظهرت القياسات الذوبان أن الحد الأقصى للجرعة المدرجة على قائمة EML لمنظمة الصحة العالمية كانت قابلة للذوبان في أقل من 250 مل من الماء على درجة حموضة محددة من قبل الهيئات التنظيمية على درجة حرارة 37 °. كان dose number محسوبة في نطاق 0.00011 من 0.00029 في نطاق درجة الحموضة من 1،2-5،7. وتشير هذه النتائج إلى أن حمض الاسكوربيك يجب بشكل لا لبس فيه أن يسند إلى الفئة الاولى كدواء عالي الذائبية حسب نظام التصنيف البيولوجيالصيدلاني(BCS). استنادا إلى الخصائص الفيزيائية و السلوكيةالملاحظةفي الجسم الحي، حمض الاسكوربيك ينطبق عليه متطلبات الدواء عالي النفاذيةحسب نظام التصنيف البيولوجي الصيدلاني(BCS). ولذلك، فإننا نقترح أن حمض الاسكوربيك يجب أن تسند إلى أدوية الفئة الأولى حسبنظام التصنيف البيولوجي الصيدلاني(BCS).

أظهرت المقارنات بين اثنين من التركيبات التي تحتوي علىحمض الاسكوربيك كمادة فعالة وحيدة ذات التحرير الفوري عن طريق الفم (IR) ان تحرير حمض الاسكوربيك كان بشكل مختلف في اختبار الذوبان.وكذلك عواملالتشابه ($f2$) والاختلاف ($f1$).

الاستنتاجات: حمض الاسكوربيك هو دواء عالي الذوبان وعالي النفاذية وبالتالي يصنف باعتباره فئة درجة أولى حسب(BCS). خطر عدم التكافؤ الحيوي) (bioinequivalenceتم تجاوزه طالما أن استخدام حمض الاسكوربيك آمن.ولهذه الأسباب، تم إعتبار حمض الاسكوربيك مرشحا جيدا للتنازل عن إجراء تكافؤ حيويBioequivalence)(ومنح بديل التكافؤ الحيوي (biowaiver) للأشكال الصيدلانية الصلبة ذات التحرير الفوري التي تعطى عن طريق الفم التي تحوي حمض الأسكوربيك كمادة فعالة .

الكلمات الرئيسية:الاستيعاب ؛التوافر البيولوجي؛ التكافؤ الحيوي؛نظام التصنيف البيولوجي الصيدلانيحمض الاسكوربيك؛النفاذية؛ الذوبانيةالدوائية.