Deanship of Graduate Studies

AL-Quds University

Synthesis, Characterization and *In Vitro* Kinetics of Amoxicillin and Cephalexin Antibacterial Prodrugs

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Preface

I have been intrigued by the topic of prodrug design for some time. As a pharmacist, bitter tastant drugs have created serious challenges to both pediatric and geriatric patients.

When I started to pursue my Master's degree of pharmaceutical sciences at AL-Quds University, I was fortunate to have the chance to work with Professor Dr. Rafik Karaman as a research assistant on a project in the medicinal field, especially prodrugs design and synthesis. The importance of improving the bitter taste, stability and bioavailability of many of the marketed drugs especially the antibiotics that became evident clinically and in the pharmaceutical industry. Fortunately, there are many published studies on this topic. Prof. Dr. Karaman guided me and many other students to the new technology "computational approach" of prodrugs design. This thesis worked on improving the pharmaceutical characteristics of amoxicillin and cephalexin in terms of masking bitter taste, improving solubility and stability.

Declaration

I certify that the thesis submitted for the degree of master is the result of my own research, except where otherwise acknowledged, and that this thesis (or any part of the same) has not be submitted for a higher degree to any other university or institution.

Signed: ……..

Ghadeer Abed Mattar Dukmak

Date: February 22, 2014

Dedication

I would like to express my sincere gratitude to the many people who have been involved and have contributed their time, effort, knowledge and expertise to this thesis. I am indebted to these people and wish to thank each one of them deeply.

I would like first to thank my academic supervisor Professor. Rafik Karaman for the helpful guidance, continuous support and supervision throughout the different stages of laboratory work of this research. Thank you also for the helpful assistance, feedback and advice you have provided me with your respective areas of expertise.

I owe my loving thanks, appreciation and respect especially to my parents, who encouraged and inspired me all through my educational journey. To my siblings, who stood by me all through my life and helped me to excel.

Special and loving thanks go out to my friends who assisted, advised and supported me spiritually throughout my research and writing efforts. Without their encouragement and understanding it would have been difficult to finish this thesis.

Ghadeer A. Dukmak

Abstract

Marketed antibacterial drugs suffer several problems, such as bitter taste and low stability which lead to patient incompliance. Prodrug technology for solving such problems is extremely exciting. Based on previously reported density functional theory calculations, amoxicillin **ProD 1-2** and cephalexin **ProD 1-2** were designed and synthesized. For the intraconversion of both antibacterial prodrugs the k_{obs} and $t_{1/2}$ values in different media were calculated from the linear regression equation obtained from the correlation of log concentration of the residual prodrug versus time. At constant temperature and pH the hydrolysis reaction for the above mentioned prodrugs displayed strict first order kinetics as the k_{obs} was quite constant and a straight line was obtained. Kinetic studies in 1N HCl, pH 2.5 and pH 5 were selected to examine the intraconversion of both prodrugs to their parent drugs. The acid-catalyzed hydrolysis of the prodrugs was found to be much higher in 1N HCl than in pH 2.5 and pH 5. The experimental $t_{1/2}$ values of amoxicillin **ProD 1** in 1N HCl, pH 2.5 and pH 5 were 2.5, 7 and 81 hours respectively and for cephalexin **ProD 1** in 1 N HCl and pH 2.5 were 2 and 14 hours respectively. In contrast, $t_{1/2}$ values of amoxicillin **ProD 2** in 1N HCl and pH 2.5 were 8 and 44 hours respectively and for cephalexin **ProD 2** in 1 N HCl was 6 hours. On the other hand, at pH 7.4, the four prodrugs were quite stable and no release of the parent drugs was observed. At pH 5 the hydrolysis of the prodrugs was too slow. The four antibacterial prodrugs were found to be bitterless. The bitter taste masking by the prodrugs is believed to be via altering the ability of the drug to interact with bitter taste receptors.

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List of Abbreviations

ADME	Absorption, Distribution, Metabolism and Excretion
AUC	Area under the curve
C_{max}	The maximum concentration
DAG	di-acyl glycerol
DFT	Density Functional Theory
FT-IR	Fourier Transform Infrared Spectrophotometer
h	Hour
hTAS2R	Human bitter taste receptor
HPLC	High-Performance Liquid Chromatography
IP_3	Inositol 1,4,5-trisphosphate
k_{obs}	The observed rate constant of hydrolysis
LC-MS	Liquid Chromatography- Mass Spectroscopy
m/z	Mass-to-Charge ratio
NMR	Nuclear magnetic resonance spectroscopy
NSAID	Non steroidal anti-inflammatory drug
OTC	Over the counter drug
PLC	Phospholipase C
ppm	Part per million
RT	Retention time
$t_{1/2}$	A time needed for 50% hydrolysis of the reactant to the desired product
TLC	Thin Layer Chromatography
TRPM5	Transient receptor potential cation channel subfamily M member 5
UV	UV Spectrophotometery
λ_{max}	The wavelength at which the maximum absorbance is observed
1N NaOH	Normalized sodium hydroxide
1N HCl	Normalized hydrochloric acid

Introduction

Chapter One

Introduction

1.1 Background

The palatability of the active ingredient of a drug is a significant obstacle in developing a patient friendly dosage form. Organoleptic properties, such as taste and odor, are an important factor when selecting a certain drug from the generic products available in the market that have the same active ingredient. It is a key issue for doctors and pharmacists administering the drugs and particularly for the pediatric and geriatric populations. Nowadays, pharmaceutical companies are recognizing the importance of taste masking and a significant number of techniques have been developed for concealing the objectionable taste [1].

Several marketed antibacterial drugs suffer several problems, among the various types of penicillins and cephalosporins antibiotic medications; we have chosen to study the most popular bitter taste antibacterial drugs, amoxicillin and cephalexin. The major drawbacks in these two antibacterial drugs their low stability in suspension formulation and mostly their bitter tastes which lead to patient incompliance and in acceptance. Bitter tastant molecules interact with taste receptors on the tongue to give bitter sensation. Thus, modification on their structural features might give a solution to overcome their bitterness.
Amoxicillin and cephalexin bitter taste sensation is the result of the hydrogen bonding between the free amino groups in both drugs with the active site of the bitter taste receptors on the tongue. Designing a prodrug promiety with a suitable linker could reduce or eliminate their bitterness by altering the ability of the drug to interact with their bitter taste receptors; this could be achieved by an appropriate modification of the structure and the size of the bitter compound. The new novel chemical approach involves the design of prodrugs for masking bitter taste of pharmaceuticals based on intramolecular processes using density functional theory (DFT) and *ab initio* methods and correlations of experimental and calculated reactions rates. In this approach no enzyme is needed to catalyze the interconversion of a prodrug to its corresponding drug. The rate of drug release is controlled by the nature of the linker bound to the bitter drug. The role of the

linker is to block the free amine group in the corresponding parental drug and to convert it into the more stable amide group, the former is believed to be responsible for the bitterness of the drug.

1.1.1 Introduction

Most of the therapeutic drugs have pharmacological and pharmacokinetic barriers in clinical drug applications, such as low oral drug absorption, lack of site specifity, chemical instability, toxicity and poor patient acceptance (unpleasant taste, odor, pain at injection site, etc). Among these various approaches that are used in order to minimize the undesirable properties of the drug while retaining the desirable therapeutic activity, the prodrug approach. This approach can be useful in the optimization of the clinical application of most of the drugs [2].

Prodrugs are bio-reversible pharmacologically inactive drug molecules that prior to exerting the desired pharmacological effect they undergo an enzymatic and or\chemical transformation *in vivo* to release the active parent drugs, to exert their desired pharmacological effects. The design of prodrugs is very challenging. Thus, modifying the absorption, distribution, metabolism and excretion (ADME) properties of the parent drug requires a comprehensive understanding of both biological and physiological properties of the drug. The prodrug strategy is more feasible and faster than searching for a new biologically active molecule with appropriate ADME properties. The prodrug approach is becoming more successful and popular nowadays. To date, prodrugs comprise around 10% of the world's marketed medications and 20% of all small molecular medications approved between 2000-2008 [3].

Recently, computer modeling techniques, which is often referred as computer aided drug design using computational chemistry has become increasingly useful in designing drugs for the purpose to enhance, study or discover drugs and related biologically active molecules [4]. The computational chemistry was also utilized to enhance the solubility, stability and bioavailability of drugs and to mask their bitter taste as well. Numerous novel prodrugs have been designed and synthesized by Karaman's group for the treatment of various diseases using DFT calculation methods. The design and synthesis of prodrugs were based on intramolecular processes utilizing molecular orbital methods and

correlations between experimental and calculated values. In this approach no enzyme is needed for the interconversion of the prodrug to its parent drug. The designed prodrugs have the potential to undergo cleavage reactions in physiological environments in rates that are completely dependent on the structural features of the inactive linker attached to the parental drug. The rate of drug release is dependent only on the rate limiting step for the conversion of its corresponding prodrug.

The most important factor in product performance and the development of different dosage are taste, smell and texture. Good flavor and texture are found to significantly increase sales of many products. Most oral medications have an unpleasant bitter taste which creates a serious challenge in pediatric and geriatric patients, which in turn affects their compliance and acceptance.

Several techniques that are based on physiological modifications have been investigated and resulted in the development of efficient approaches for masking unpleasant and bitter taste of many compound. These approaches include: (1) coating is one of the most efficient and commonly used taste masking techniques; (2) microencapsulation used are commonly based on the principle of solvent extraction or evaporation; (3) taste masking with flavors, sweeteners and amino acids**;** (4) taste masking with lipophilic vehicles such as lipids, lecithin and lecithin-like substances**;** (5) sweeteners are generally used in combination with other taste masking technologies; (6) taste suppressants and potentiators, such as Linguagen's bitter blockers (e.g., adenosine monophosphate), are used for masking the bitter taste of various compounds by competing with binding to the G-protein coupled receptor sites (GPCR); (7) pH modifiers; (8) adsorbates; (9) resins and (10) inclusion complexes [5].

Although the mentioned approaches have helped to improve the taste of some drug formulations, the problem of the bitter taste of drugs in pediatric and geriatric formulations still creates a serious challenge to pharmacists. Thus, different strategies should be developed in order to overcome this serious problem.

The prodrug approach can be the most effective and useful strategy in masking the bitterness in the clinical application of most of the drugs.

1.1.2 Bitter taste

The word “medicine” for a child is considered a bad thing to administer because of its aversive taste. Medicines dissolve in saliva and bind to taste receptors on the tongue giving a bitter, sweet, salty, sour, or umami sensation. Sweet and sour taste receptors are concentrated on the tip and lateral borders of the tongue respectively. Bitter taste is sensed by the receptors on the posterior part of the tongue and umami taste receptors are located all over the tongue. A short period after birth, infants reject bitter tastes and prefer sweet and umami tastes [1]. Children have larger number of taste buds than adults which are responsible for sensitivity toward taste. These taste buds regenerate every two weeks. Taste becomes altered as a function of the aging process, which explains why most children find certain flavors to be too strong when adults do not. The American Academy of Pediatrics estimates that compliance in children is as low as 53%, indicating that children frequently fail to take medications properly. Noncompliance can lead to: (1) persistent symptoms, (2) need for additional doctor visits or even hospitalizations, (3) worsening of condition, (4) need for additional medications, (5) increased healthcare costs and (6) development of drug-resistant organisms in cases of infectious diseases [6].

In mammals, taste buds are groups of 30-100 individual elongated "neuroepithelial" cells which are often embedded in special structure in the surrounding epithelium known as papillae. Just below the taste bud apex, taste cells are joined by tight junctional complexes that prevent gaps between cells. Food molecules cannot therefore squeeze between taste cells and get into the taste bud. Taste papillae located on the tongue appear as little red dots, or raised bumps, particularly at the front of the tongue called "fungiform" papillae. There are three other kinds of papillae, foliate, circumvallate and the non-gustatory filiform. In mammals taste buds are located throughout the oral cavity, in the pharynx, the laryngeal epiglottis and at the entrance of the esophagus. Taste perception fades with age; on average, people lose half their taste receptors by time they turn 20 [7]. The sensation of taste can be categorized into five basic tastes: sweetness, sourness, saltiness, bitterness, and umami. Taste buds are able to differentiate among different tastes through detecting interaction with different molecules or ions. Sweet, umami, and bitter tastes are triggered by the binding of molecules to G protein-coupled receptors on the cell membranes of taste buds. Saltiness and sourness are perceived when alkali metal or hydrogen ions enter taste buds, respectively [8]. As taste senses both harmful and beneficial things, all basic tastes

are classified as either aversive or appetitive, depending upon the effect the things they sense have on our bodies [9]. Sweetness helps to identify energy-rich foods, while bitterness serves as a warning sign of poisons [10].

For a long period, it was commonly accepted that there is a finite and small number of "basic tastes" of which all seemingly complex tastes are ultimately composed. As of the early twentieth century, physiologists and psychologists believed there were four basic tastes: sweetness, sourness, saltiness and bitterness. At that time umami was not proposed as a fifth taste but now a large number of authorities recognize it as the fifth taste [11]. In Asian countries within the sphere of mainly Chinese and Indian cultural influence, pungency (piquancy or hotness) had traditionally been considered a sixth basic taste.

Today, the consensus is that sweet, amino acid (umami), and bitter taste converge on a common transduction channel, the transient receptor potential channel TRPM5, *via* PLC. TRPM5 is a newly discovered TRP related to other channels in sensory signaling systems. It has been shown that PLC, a major signaling effector of G-protein coupled receptors (GPCRs), and TRPM5 are co expressed with T1Rs and T2Rs and are vital for sweet, amino acid, and bitter taste transduction. Activation of T1R or T2R receptors by their respective taste molecules would stimulate G proteins, and in turn PLC (PLC-ß2). The activation of PLC generates two intracellular messengers - IP3 and DAG - from the hydrolysis of phosphatidylinositol-4, 5-bisphosphate (PIP2) and opens the TRPM5 channel, resulting in the generation of a depolarizing receptor potential. Other additional pathways may modulate sweet, amino acid, or bitter taste reception but would not, themselves, trigger a taste response. It is not at present known how PLC activates TRPM5 or whether DAG is involved [12-22].

Bitter taste*:* Bitterness is the most sensitive of the tastes, and many perceive it as unpleasant, sharp, or disagreeable, but it is sometimes desirable and intentionally added *via* various bittering agents. Common bitter foods and beverages include coffee, unsweetened cocoa, south American mate, marmalade, bitter gourd, beer , olives, citrus peel, many plants in the Brassicaceae family, dandelion greens, wild chicory, and escarole. Bitterness is of interest to those who study evolution, as well as various health researchers [23, 24] since a large number of natural bitter compounds are known to be toxic. The ability to

detect bitter-tasting, toxic compounds at low thresholds is considered to provide an important protective function [23-25]. Plant leaves often contain toxic compounds, yet even amongst leaf-eating primates; there is a tendency to prefer immature leaves, which tend to be higher in protein and lower in fiber and poisons than mature leaves [26]. For humans, various food processing techniques are used worldwide to detoxify them in order to make them palatable, otherwise foods can be considered inedible for use [27]. The threshold for stimulation of bitter taste by quinine averages a concentration of 0.000008 M [23]. The taste thresholds of other bitter substances are rated relative to quinine, which is thus given a reference index of 1 [23, 28]. For example, Brucine has an index of 11, is thus perceived as intensely more bitter in taste than quinine, and is detected at a much lower solution threshold [20]. The most bitter in taste substance known is the synthetic chemical denatonium, which has an index of 1,000 [23]. It is used as an aversive agent (a bitterant) that is added to toxic substances to prevent accidental ingestion. This was discovered in 1958 during research on ligocaine, a local anesthetic, by MacFarlan Smith of Gorgie, Edinburgh, Scotland. Research has shown that TAS2Rs (taste receptors, type 2, also known as T2Rs) such as TAS2R38 coupled to the G protein gustducin are responsible for the human ability to taste bitter substances [12]. They are identified not only by their ability to taste for certain "bitter" ligands, but also by the morphology of the receptor itself (surface bound, monomeric) [13]. The TAS2R family in humans is thought to comprise about 25 different taste receptors, some of which can recognize a wide variety of bitter-tasting compounds. Over 550 bitter-tasting compounds have been identified, of which about 100 have been assigned to one or more specific receptors [16]. Recently it is speculated that the selective constraints on the TAS2R family have been weakened due to the relatively high rate of mutation and pseudogenization [29].

Researchers use two synthetic substances, phenylthiocarbamide (PTC) and 6-n-propylthiouracil (PROP) to study the genetics of bitter perception. These two substances taste bitter to some people, but are virtually tasteless to others. Among the tasters, some are so-called "supertasters" to whom phenylthiocarbamide (PTC) and 6-n-propylthiouracil (PROP) are extremely bitter. The variation in sensitivity is determined by two common alleles at the TAS2R38 locus [30]. This genetic variation in the ability to taste a substance has been a source of great interest to those who study genetics.

Bitter substances bind to the T2R receptors activating the G-protein and causing activation of PLC. The second messengers DAG and IP3 are produced (by hydrolysis of phosphatidylinositol-4, 5-bisphosphate) activating TRPM5 and mediating release of Ca^{2+} from internal stores. The elevated Ca^{2+} causes transmitter release and this increases the firing of the primary afferent nerve.

The sensation is the result of signal transduction from taste receptors located in areas known as taste buds. The taste buds contain very sensitive nerve endings, which are responsible for the production and transmission of electrical impulses *via* cranial nerves VII, IX, and X to certain areas in the brain that are devoted to the perception of taste [6]. Bitter taste receptors are believed to have evolved for organism protection against the ingestion of poisonous food products. Bitter tastants [31-35] are very diverse in their chemical structure and physicochemical properties [14, 22]. In humans, bitter taste perception is mediated by 25 G-protein coupled receptors of the hTAS2R gene family [21]. The structural basis for hTAS2R's unique ability to recognize a large number of chemically diverse and low-affinity agonists is not fully understood [15-20].

1.1.2.1 Challenges and criteria for pursuing masking bitter taste approaches

The most significant challenges that facing developers when pursuing masking bitter taste drugs approaches are: (i) Safety, tolerability and efficacy of the compound which are based on non-clinical testing, and physicochemical properties such as solubility, permeability and stability, (ii) lack of robust and reliable techniques for early taste screening of compounds with limited toxicity data, (iii) structure–taste relationships of pharmaceutically active molecules is limited, (iv) The perception of taste of pharmaceuticals has been shown to be different between adults and children and it might differ between healthy and patient children [8] and (v) ethical concerns to perform taste studies in healthy children unless the study is a 'swill and spit' one with drugs known to have a good safety profile [31, 34, 36-38].

There are numerous pharmaceutical and OTC preparations that contain active ingredients, which are bitter in taste; with respect to OTC preparations, such as cough and cold syrups. A variety of taste masking approaches has been used to address the patient compliance

problem. Conventional taste masking methods such as the use of sweeteners, amino acids and flavoring agents alone are often inadequate in masking the taste of highly bitter drugs.

1.1.3 Prodrug background

Physicochemical, pharmacokinetic and pharmacodynamic characteristics of a drug molecule have been modified in the past few decades extensively and successfully by the most important chemical tools known as prodrug. In 1958, Albert was the first that introduced the term of 'Prodrug' or 'Proagent' [39], which describes compounds that prior to exhibiting their pharmacological effects undergo biotransformation process by eliminating or minimizing the undesirable properties while retaining the desirable ones. The term 'Prodrug' signifies a pharmacologically inactive chemical derivative that could be used in order to convert the physicochemical properties of drugs in a temporary manner. Prodrug is also known as 'Proagent', 'Bioreversible derivative' or 'Latentiated drug'. The term of prodrug is the mostly used term. Also the term "Drug Latentiation" is referred to the prodrug design approach. The introduction of a new drug to the market is very expensive and is time consuming, and their use is restricted for many demerits reasons such as side-effects, improper organoleptic properties, difficulties in formulation, frequent requirements etc [40]. Thus, it is easier to modify the physicochemical parameters of most existing drugs, by the many approaches for prodrug design, which can enhance their usefulness, reduce their toxicity and alter their duration of action. Such approaches as the biological approach, is used to alter the route of administration to a route more acceptable to the patient. The physical approach, that involves modification of the design of the dosage form such as controlled delivery of drugs. The chemical approach where a biologically active compound forms a new compound that emphasizes to minimize toxicity in order to enhance the selectivity of most of these desired drugs, upon *in vivo* enzymatic attack will liberate the parent compound. Nowadays, prodrug remains as a promising and effective therapeutic tool in the future. Prodrug can be defined as pharmacologically inert chemical derivatives that can be converted *in vivo* either enzymatically or non-enzymatically to the active drug molecule in order to exert their therapeutic effect. Followed by the subsequent rapid elimination of the released derivatizing group as soon as the goal is achieved it should be converted to the original drug.

In order to optimize the drug therapeutics, various chemical means are required such as a design and development of [40]

1. New drugs with desirable properties: such approach requires screening of thousands of molecules for biological activity of which only one may become a clinically useful drug.

2. Hard and soft drugs: which are basically analogs of existing drugs with desirable characteristics.

a) A hard drug is known as a chemical entity resistant to biotransformation and has a long biological half-life; it is eliminated in unchanged form by the body through excretion. It also avoids generation of potentially harmful metabolites, decreases tendency for the possibility of drug-drug interaction and limits inter-subject variations. For example; Chlorpropamide, has too long half life which results in drug accumulation that leads to subsequent fluctuations in plasma drug concentration level on long term therapy. Metabolic stabilization is involved in hard drugs, whereas, the introduction of a functional group of predictable metabolic reactivity in a pharmacophore moiety is the concept of metabolic switching or metabolic promotion that is used in 'Soft Drug' and 'Prodrug' design.

b) A soft drug is known as a biologically active compound that is bio-transformed *in vivo* into nontoxic moieties in a rapid and predictable manner. In case of agents having very short duration of action such as insulin and adrenaline- natural endogenous agent. The design of synthetic soft drugs involves introduction of a group or a bond susceptible to rapid metabolic action. For example, the replacement of a part of the alkyl side chain of the drug with an ester group that can be readily hydrolyzed *in vivo*. The formation of relatively inert metabolites is the most important advantage of soft drugs design.

3. Prodrug

The purpose from the design in the latter two approaches, was to develop moieties in contrast to conventional new drug development methods by which having predictable biotransformation or excretion.

1.1.3.1 Prodrug classification

Prodrugs are classified into two categories: carrier linked and bioprecursor, both depend upon the constitution, lipophilicity, method of bioactivation and the catalyst involved in bioactivation [40].

1.1.3.1.1 Carrier linked prodrug (Simple Prodrug)

Carrier linked prodrug is a compound that contains an active drug linked with a carrier group that can be easily removed enzymatically (such as an ester or labile amide). It alters the physicochemical or pharmacokinetic properties of the parent drug. The most common reaction for activation of carrier linked prodrug is hydrolysis.

Hence, the major drawback of carrier linked prodrugs is that they are linked through covalent linkage with specialized nontoxic protective carriers or promoieties in a transient manner. This depends upon the nature of the carrier in order to alter or eliminate the undesirable properties of the parent molecule. Carrier-linked prodrugs can be further subdivided into (a) bipartite which is composed of one carrier (group) attached to the drug, (b) tripartite which is a carrier group that is attached via linker to drug and (c) mutual prodrugs consisting of two drugs linked together.

1.1.3.1.2 Bioprecursors or metabolic precursors

Bioprecursors are inert molecules that do not contain a carrier and are obtained by chemical modification of the active drug. As the parent drug such a moiety has almost the same lipophilicity and is bioactivated only enzymatically by redox biotransformation. For example, aryl acetic acid NSAID such as fenbufen from aryl propionic acid precursors.

1.1.3.2 Prerequisites of an ideal prodrug

An ideal prodrug should possess the following properties [40]:

1) Pharmacological inertness.
2) Rapid transformation into the active form at the target site, either chemically or enzymatically.
3) Non-toxic metabolic fragments followed by their rapid elimination.

1.1.3.3 Application of prodrug approach

The prodrug approach has been extensively studied not only for correction of pharmacokinetic behavior but also pharmaceutical, organoleptic, physical and chemical

properties of the parent to enhance the stability and improve the efficacy of therapy for more patient compliance and acceptance.

1.1.3.4 Pharmaceutical and pharmacokinetic applications

The undesirable organoleptic properties and physicochemical problems associated with drug formulation can be resolved by different strategies such as, taste and odour masking, change of the physical form of the drug, reduction of gastrointestinal irritation, reduction of pain on injection, enhancement of solubility and dissolution rate (hydrophilicity) of drug and enhancement of chemical stability. Pharmacokinetic properties which affect the bioavailability and mean residence time of a drug are very important for its pharmacodynamic efficacy. The prodrug approach can modulate many of the application drawbacks in the body. The most important applications are:

a) Taste masking

Bitterness, acidity or causticity of the drug are the major reasons for patient incompliance and in acceptance particularly pediatrics. In order to overcome the bad taste of drug, two approaches can be utilized:

1) Reducing drug stability in saliva.
2) Lowering the affinity of drug for taste receptors, thus making the bitterness or causticity imperceptible.

Nowadays, it is believed that bitter taste is the results of drug interaction with taste receptors on the tongue as mentioned earlier. Blocking the interaction ability could reduce or eliminate the bitter taste sensation, by designing and synthesizing prodrugs with a suitable linker.

Due to the large variation of structural features of bitter tasting molecules, it is difficult to generalize the molecular requirements for bitterness. Nevertheless, it was reported that a bitter tastant molecule requires a polar group and a hydrophobic moiety. A quantitative structure activity relationship (QSAR) model was developed and has been established for the prediction of bitterness of several tastant analogues. For example, it was reported that the addition of a pyridinium moiety to an amino acid chain of a variety of bitter amino acid compounds decreases bitterness, such as in the case of glycine. Other structural modifications, such as an increase in the number of amino groups/residues to more than 3

and a reduction in the poly-hydroxyl group/ COOH, have been proven to decrease bitterness significantly. Moreover, changing the configuration of a bitter tastant molecule by making isomer analogues was found to be important for binding affinity to enhance bitterness agonist activity (e.g. L-tryptophan is bitter while D-tryptophan is sweet) [41].

b) Odor masking

Liquids with low boiling point have a strong odor such as ethyl mercaptan. The odor of many compounds depends upon their vapor pressure. For example, the ester form of a prodrug can be used for odor masking.

c) Enhancement of solubility and dissolution rate (hydrophilicity) of drugs

When dissolution is the rate limiting step in the absorption of poorly aqueous soluble agents or when parental or ophthalmic formulation of such agents is desired, hydrophilicity or water solubility is required. As more than 30% of drug discovery compounds have poor aqueous solubility and most of them are hydrophobic in nature and possess poor bioavailability. Prodrugs can increase their aqueous solubility by improving dissolution rate via ionizable or polar neutral functions attachment such as phosphates, amino acids or sugar moieties [42].

d) Enhancement of chemical stability

Drugs may be destabilized during its shelf life stability. The prodrug approach can stabilize the drugs aqueous solution, for example, against degradation at acidic pH and also enhance their water solubility.

e) Enhancing permeability and absorption

Oral drug delivery is the preferred route of administration for the majority of the drugs but most common absorption routes are largely nonspecific, unfacilitated and transported by passive mechanism. Absorption and permeability have a significant effect on drug efficiency, improving the lipophilicity of the parent drug by masking polar ionized or nonionized functional groups will enhance either topical or oral absorption [42].

f) Changing the distribution profile

In order to achieve site-selective drug delivery as mentioned for many decades, many attempts have been made to harness different macromolecular strategies and

nanotechnologies, but these methods lack clinical success. Today, the prodrug approach is one of the most promising site-selective drug delivery strategies which exploit target cell- or tissue- specific endogenous enzymes and transporters. In this prodrug approach many great prodrugs have increased their efficacy and safety profiles.

g) Protecting from rapid metabolism and excretion

The beneficial effects of drugs can be impaired by extensive excretion and\or metabolic pathways. First-pass effect problem in the gastrointestinal tract and liver has been bypassed by sublingual or buccal administration or by modified or controlled release formulations. A prodrug structure by adding a lipophilic promoieties can decrease the solubility of many drugs and is one way to prolong the duration of action of very water-soluble drugs.

There are two major challenges facing the prodrug approach strategy:

1. Hydrolysis of prodrugs by esterases.
2. Bioactivation of the prodrug by cytochrome P450 enzymes.

Prodrug has been one of the classical and highly studied topics by researchers in pharmaceutical developments. Still, it remains the subject of interest due to the fact that the drugs in the developmental pipeline do possess some pharmaceutical or pharmacokinetic drawbacks.

1.2 Research problem

The major problems in the administration of amoxicillin and cephalexin antibacterial drugs are:

1) The low stability in suspension formulation. These medications are very labile molecules when are exposed to aqueous media. They might undergo hydrolysis when they are standing in solutions.

   The main cause of their degradation is the reactivity of the strained lactam ring particularly towards hydrolysis, the course of the hydrolysis and the nature of the degradation products are influenced by the pH of the solution. The 3-lactam carbonyl group in both drugs readily undergo nucleophilic attack by water or especially hydroxide ion to form the inactive penecilloic acid in case of amoxicillin which is reasonably stable in neutral to alkaline solutions but readily undergoes decarboxylation and further hydrolytic reactions in acidic solutions.

2) Their bitter tastes which lead to lack of patient compliance and might create a serious challenge to the pharmacist in pediatric and geriatric formulations.
3) The current suspensions of these antibacterial are given three or four times to a patient for achieving the desired effect. Synthesis of prodrugs which have the potential to release the parental drugs in a controlled manner have a good chance to overcome the frequent dosing problem.

1.3 Thesis objective

Based on DFT calculations by Karaman's group on amine drugs [43-45] four novel antibacterial prodrugs of amoxicillin and cephalexin were synthesized. The designed prodrugs have the potential to be chemically, and not enzymatically, intraconverted to the parent drug in a programmable manner upon exposure to physiological environments.

1.3.1 General objectives:

The main three goals of this research were: (1) increase solution stability of the antibacterial drugs (amoxicillin and cephalexin); (2) masking their bitterness, and (3) making a sustain release dosage form.

1.3.2 Specific objectives:

- To be relatively stable in aqueous media.
- To NOT have bitter taste.
- To release the parental drug in a sustain release manner.
- To be readily soluble and stable in a physiological environment
- To have a moderate hydrophilic-lipophilic balance (HLB) value
- To furnish upon cleavage a safe and non-toxic by-products.

1.4 Research question

This study will provide the answers to the following questions:-

- Does the prodrug possess superior stability in water with no bitter taste properties?

- Would the synthesized prodrugs be capable of releasing the parent drug (amoxicillin and cephalexin) in a sustained release manner via the *in vivo* route?
- Does the synthesized prodrug have physiochemical properties which could lead to a good pharmacokinetic properties and a high bioavailability?

In this study, two linkers were utilized in order to be linked to an amine drug via amide bonding. The synthesized amide prodrugs of amoxicillin and cephalexin that were synthesized **(Scheme 2.1** and **2.2)** show a carboxylic acid group as a hydrophilic moiety and a hydrocarbon skeleton as a lipophilic moiety. Due to a balanced hydrophilic-lipophilic value the prodrug entity should have the potential to penetrate tissues in a good manner. It was reported that a polar group and a hydrophilic moiety are required for bitter tasting molecules. QSAR model was developed and established for the prediction of bitterness of several tastant analogues. The role of the linker was to block the free amine group in the corresponding parental drug and to convert it into an amide group, the former is believed to be responsible for the bitterness of the drug [43, 46-48]. Our strategy was to prepare amoxicillin **ProD 1-2** and cephalexin **ProD 1-2** as sodium or potassium carboxylate due to their high stability in neutral aqueous medium.

Based on DFT calculation results obtained by Karaman's group studies on similar amine drugs, design and synthesis of four novel prodrugs for amoxicillin and cephalexin are studied and discussed in the course of this work.

Literature Review

Chapter Two

Literature Review

2.1 Introduction

Most of the antibacterial drugs used in the market suffer several problems, mostly characterized with unpleasant, bitter taste and low stability. Amoxicillin and cephalexin have extremely unpleasant and bitter taste which is difficult to mask. This creates a serious problem in pediatric and geriatric patients, especially if the patients cannot swallow whole tablets or when small doses are required. Even though, the strategies that were used for masking bitter taste by the use of sweeteners and flavors may cause a serious problem in diabetic pediatrics and geriatrics patients. It is believed that the extremely bitter and unpleasant taste of antibiotics is due to the intermolecular forces between these drugs and the active site of the bitter taste receptors, most likely either due the hydrogen or ionic bonds.

Using the novel prodrug approach based on intramolecular processes we will have a good chance to mask the bitter taste of the concerned antibacterial agents. In addition overcoming the frequent dosing will be achieved.

2.1.1 Enzyme models utilized for the design of potential bitterless prodrugs for bitter drugs such as atenolol, paracetamol, guaifenasin, amoxicillin and cephalexin.

Scholar studies of enzyme mechanisms by several chemists and biochemists, over the past five decades, have had a significant contribution for understanding the mode and scope of enzymes catalysis.

Nowadays, the scientific community has reached to the conclusion that enzyme catalysis is based on the combined effects of the catalysis by functional groups and the ability to reroute intermolecular reactions through alternative pathways by which substrates can bind to preorganized active sites. It is believed that rate accelerations by enzymes can be proceed by (i) covalently enforced proximity, as seen in the case of chymotrypsin, [49] (ii) non-covalently enforced proximity, as represented in the catalysis of metallo-enzymes,

[50] (iii) covalently enforced strain, [51], and (iv) non-covalently enforced strain, which has been extensively studied on models mimicking the lysozyme enzyme which is most closely associated with rate acceleration due to this kind of strain [52].

Rates for the majority of enzymatic reactions ranges between 10^{10} and 10^{18} -fold their non-enzymatic bimolecular counterparts. For instance, biochemical reactions involving the catalysis of the enzyme cyclophilin are enhanced by 10^5 and those by the enzyme orotidine monophosphate decarboxylase are accelerated by 10^{17} [53]. The significant enhancement in rate manifested by enzymes is a result of the substrate binding within the confines of the enzyme active site. The substrate-enzyme binding energy is the dominant driving force and the major contributor to catalysis. A consensus has been reached that in all enzymatic processes binding energy is used to overcome physical and thermodynamic factors that make barriers to the reaction (free energy). These factors are: (1) the change in entropy (ΔS°), in the form of the freedom of motions of the reactants in solution; (2) the hydrogen bonding net around bio-molecules in aqueous solution; (3) a proper alignment of catalytic functional groups on the enzyme; and (4) the distortion of a substrate that must occur before the reaction takes place [54, 55].

Scholarly studies have been done by Bruice, Cohen, Menger, Kirby and others to design enzyme models having the potential to reach rates comparable to rates of biochemical reactions catalyzed by enzymes. Examples for such models are those based on rate enhancements driven by covalently enforced proximity. The most cited example is the intramolecular cyclization of dicarboxylic semi esters to anhydrides advocated by Bruice et al. Bruice et al. has demonstrated that a relative rate of anhydride formation can reach 5 x 10^7 upon cyclization of a dicarboxylic semi ester when compared to a similar counterpart's bimolecular process [55].

Other examples of rate acceleration based on proximity orientation include: (a) acid-catalyzed lactonization of hydroxy-acids as studied by Cohen et al. and Menger, (b) intramolecular S_N2-based cyclization reactions as researched by Brown et al. and Mandolini's group, (c) proton transfer between two oxygens in Kirby's acetals, and proton transfer between nitrogen and oxygen in Kirby's enzyme models, (d) proton transfer between two oxygens in rigid systems as investigated by Menger, and (e) proton transfer from oxygen to carbon in some of Kirby's enol ethers. The conclusions emerged from

these studies are (1) the driving force for enhancements in rate for intramolecular processes are both entropy and enthalpy effects. In the cases by which enthalpy effects were predominant such as ring-closing and proton transfer reactions proximity or/and steric effects were the driving force for rate accelerations. (2) The nature of the reaction being intermolecular or intramolecular is determined on the distance between the two reacting centers. (3) In S_N2-based ring-closing reactions leading to three-, four- and five-membered rings the *gem*-dialkyl effect is more dominant in processes involving the formation of an unstrained five-membered ring, and the need for directional flexibility decreases as the size of the ring being formed increases. (4) Accelerations in the rate for intramolecular reactions are a result of both entropy and enthalpy factors. (5) An efficient proton transfer between two oxygens and between nitrogen and oxygen in Kirby's acetal systems were affordable when a strong hydrogen bonding was developed in the products and the transition states leading to them [55].

In the past few years some prodrugs based on the trimethyl lock system have been reported. Borchardt et al. has shown that the pro–prodrug 3-(2'-acetoxy-4', 6'-dimethyl dimethyl) - phenyl-3, 3-dimethylpropionamide is capable of releasing the biologically active amine drug upon acetate hydrolysis by enzyme triggering. Another successful example exploiting a stereopopulation control model is the prodrug Taxol which enhances the drug water solubility and hence affords it to be administered to the human body *via* intravenous injection. Taxol is the brand name for paclitaxel, a natural diterpene, approved in the USA for use to treat cancer [55].

2.1.2 Computational methods used in the design of bitterless prodrugs for bitter tastant drugs

Nearly 65 years ago, organic, bioorganic and medicinal chemists alike have started using computational methods for calculating molecular properties of ground and transition states. These computational methods use principles of computer science to aid in solving chemical problems. Theoretical results emerged from these methods, incorporated into efficient computer programs, for calculating the structures and physical and chemical properties of molecules.

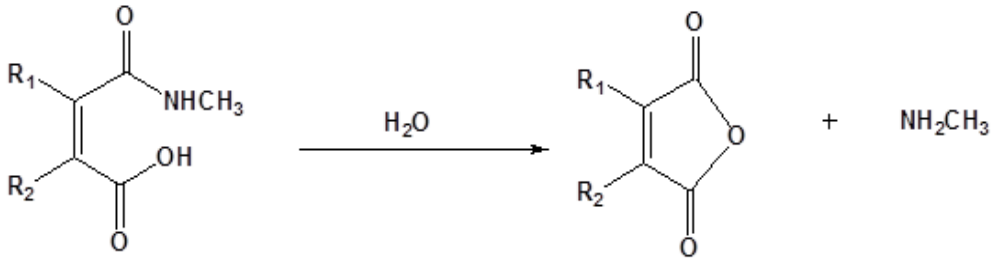
Equilibriums energy-based and reactions rates calculations for systems having medicinal interests are of a vast importance to the health community. Today, quantum mechanics (QM) such as *ab initio*, semi-empirical, DFT and molecular mechanics (MM) are commonly and increasingly being used and broadly accepted as precise tools for predicting structure-energy calculations for drugs and prodrugs alike [55].

2.1.3 Mechanistic study of the acid-catalyzed hydrolysis of maleamic acids 1-9 used for the design of atenolol, amoxicillin and cephalexin prodrugs

The acid-catalyzed hydrolysis of **1-9 (Figure 2.1)** was kinetically investigated by Kirby et al. The study demonstrated that the amide bond cleavage is due to intramolecular nucleophilic catalysis by the adjacent carboxylic acid group and the rate-limiting step is the tetrahedral intermediate breakdown **(Figure 2.2)** [56]. In 1996, the reaction was computationally investigated by Katagi using AM1 semiempirical calculations. In contrast to what was suggested by Kirby, Katagi's study demonstrated that the rate- limiting step is the formation of the tetrahedral intermediate and not its dissociation [57]. Later on Kluger and Chin have experimentally researched the mechanism of the intramolecular hydrolysis process utilizing several N-alkylmaleamic acids derived from aliphatic amines with a wide range of basicity [58]. The study findings demonstrated that the identity of the rate-limiting step is a function of both the basicity of the leaving group and the solution acidity [55].

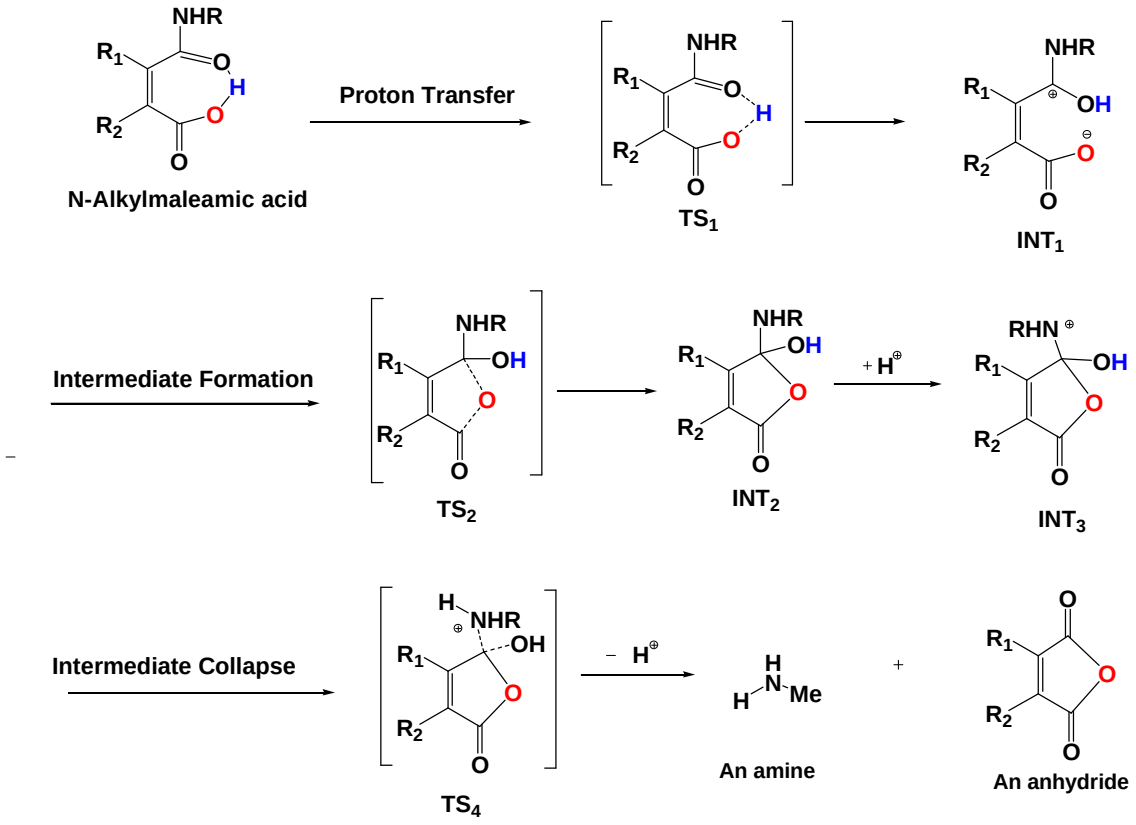
In order to utilize Kirby's enzyme model [56] for the design of prodrugs of the following drugs: atenolol, amoxicillin and cephalexin, a mechanistic study using DFT calculation methods at B3LYP/6-31G (d,p), B3LYP/311+G (d,p) levels and hybrid GGA (MPW1k) on an intramolecular acid catalyzed hydrolysis of maleamic (4-amino-4-oxo-2- butenoic) acids (Kirby's N-alkylmaleamic acids) **1-9** was conducted. The calculations confirmed that the reaction involves three steps: (1) proton transfer from the carboxylic group to the adjacent amide carbonyl oxygen, (2) nucleophilic attack of the carboxylate anion onto the protonated carbonyl carbon; and (3) dissociation of the tetrahedral intermediate to provide products **(Figure 2.2)**. Moreover, the calculations demonstrate that the rate-limiting step is dependent on the reaction medium. When the calculations were run in the gas phase the rate-limiting step was the tetrahedral intermediate formation, whereas when the calculations were conducted in the presence of a cluster of water the dissociation of the tetrahedral intermediate was the rate-limiting step. When the leaving group (methylamine)

in **1-9** was replaced with a group having a low pKa value the rate-limiting step of the hydrolysis in water was the formation of the tetrahedral intermediate. In addition, the calculations revealed that the efficiency of the intramolecular acid-catalyzed hydrolysis by the carboxyl group is remarkably sensitive to the pattern of substitution on the carbon–carbon double bond; 1) difference between strain energy between intermediate and product and strain energy between intermediate and reactant; 2) distance between hydroxyl oxygen of the carboxylic group and amide carbonyl carbon and 3) the attack angle. The rate of hydrolysis was found to be linearly correlated with the strain energy of the tetrahedral intermediate or the product. Systems having strained tetrahedral intermediates or products experience low rates and vice versa [45, 59-61]. This acid catalyzed hydrolysis occurs in pH ranges between 1-5 [56], as shown in **Figure 2.3**.



1 ; $R_1=R_2=H$
2 ; $R_1=R_2=Me$
3 ; $R_1=H$; $R_2=Me$
4 ; R_1,R_2 Cyclopent-l-ene-1,2-diyl
5 ; R_1, R_2 Cyclohex-l-ene-1,2-diyl
6 ; $R_1=H$; $R_2=Et$
7 ; $R_1=H$; $R_2=$n-Propyl
8 ; $R_1=H$; $R_2=$Trifluoromethyl
9 ; $R_1=R_2=$ Trifluoromethyl

Figure (2.1): Acid-catalyzed hydrolysis of maleamic acids **1-9**.



NHR = atenolol, acyclovir, cefuroxime, tranexamic acid or methyl
R1 and R2; H, methyl or trifluoromethyl

Figure (2.2): Proposed mechanism for the acid-catalyzed hydrolysis of maleamic acids.

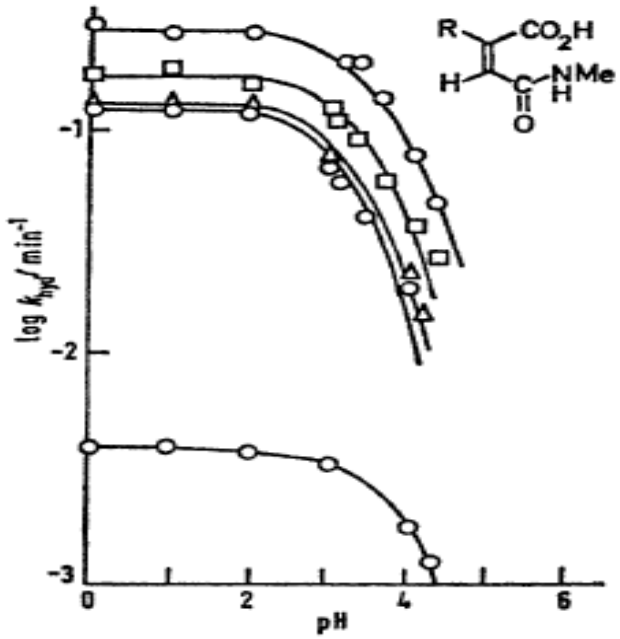


Figure (2.3): pH-Rate profiles for the hydrolysis of alkyl-N-methylmaleamic acids at 39 c and ionic strength 1-0. In increasing order of reactivity R = H, Me, Et, Pr, and Bu.

2.2 Bitterless amoxicillin and cephalexin prodrugs based on Kirby's maleamic acids enzyme model

As mentioned previously, most of the antibacterial agents that are commonly used suffer unpleasant taste and a respected number of them are characterized with bitter taste. For example, amoxicillin and cephalexin have an extremely unpleasant and bitter taste which is difficult to mask. This is a particular problem in geriatric patients who cannot swallow whole tablets or when small doses are required. Even the antibacterial suspension is difficult for pediatrics to administer due to its better and unpleasant taste [62-67]. It is widely assumed that the extremely bitter and unpleasant taste of these antibacterial drugs is due to a formation of intermolecular force/s between the drug and the active site of the bitter taste receptor/s. The intermolecular bond/s is/are most likely due to formation either *via* hydrogen bond of the amine (in amoxicillin and cephalexin) group to the active site of the bitter taste receptors.

Antimicrobial agents are classified according to their specific mode of action against bacterial cell. By which these agents may interfere with cell wall synthesis, inhibit protein synthesis, interfere with nucleic acid synthesis or inhibit a metabolic pathway. They have a broad spectrum of activity against both gram-positive and gram-negative bacteria. Among these agents, β-lactams – penicillins, cephalosporins, carbapenems and monobactams, which represent 60% of all antimicrobial use by weight. They are preferred because of their efficacy, safety, and because their activity can be extended or restored by chemical manipulation. Inevitably, however, their usage has been restricted because of their bacterial resistance.

2.2.1 Amoxicillin

Amoxicillin is an oral semi-synthetic penicillin, moderate-spectrum, bacteriolytic, β-lactam antibiotic used to treat bacterial infections caused by susceptible microorganisms by which it is susceptible to the action of the β-lactamases. Amoxicillin has a bactericidal action and acts against both Gram positive and Gram-negative microorganisms by inhibiting the biosynthesis and repair of the bacterial mucopeptide wall. It is usually the drug of choice within its class because it is well absorbed following oral administration. Amoxicillin

presents some outstanding advantages in comparison with other aminopenicillins, such as: a better absorption from the intestinal tract, better capacity for reaching effective concentrations at the sites of action and a more rapid capacity for penetrating the cellular wall of Gram-negative microorganisms. Aminopenicillins are frequently prescribed agents for the oral treatment of lower respiratory tract infections and are generally highly effective against S. pneumonia and non- β-lactamase-producing H. influenza. Amoxicillin is mostly common antibiotics prescribed for children. It has high absorption after oral administration which is not altered and affected by the presence of food. Amoxicillin dose reaches C_{max} about 2 hours after administration and is quickly distributed eliminated by excretion in urine (about 60%- 75%). The antibacterial effect of amoxicillin is extended by the presence of a benzyl ring in the side chain. Because amoxicillin is susceptible to degradation by β-lactamase-producing bacteria, which are resistant to a broad spectrum of β-lactam antibiotics, such as penicillin, for this reason, it is often combined with clavulanic acid, a β-lactamase inhibitor. This increases effectiveness by reducing its susceptibility to β-lactamase resistance. Amoxicillin has two ionizable groups in the physiological range (the amino group in α-position to the amide carbonyl group and the carboxyl group). Amoxicillin has a good pharmacokinetic profile with bioavailability of 95% if taken orally, its half-life is 61.3 minutes and it is excreted by the renal and less than 30% bio-transformed in the liver [68-71].

2.2.2 Cephalexin

Cephalexin is a first-generation cephalosporin antibiotic, which was chosen as the model drug candidate to obtain dosage with improved stability, palatability and attractive pediatric elegance, cost effective with ease of administration. Cephalosporins are the most widely used for treatment of skin infections because of their safety profile and their wide range of activity against both gram positive and gram negative microorganism. Cephalexin is also used for the treatment of articular infections as a rational first-line treatment for cellulitis, it is a useful alternative to penicillins hypersensitivity, and thought to be safe in a patient with penicillin allergy but caution should always be taken, that’s because cephalexin and other first-generation cephalosporins are known to have a modest cross-allergy in patients with penicillin hypersensitivity. In addition, cephalexin is also effective and used in the treatment of group A β-hemolytic streptococcal throat infections. Cephalexin works by interfering with the bacteria's cell wall formation, causing it to

rupture, and thus killing the bacteria. The compound is zwitterion by which it contains both a basic and an acidic group, the isoelectric point of cephalexin in water is approximately 4.5 to 5.0. Cephalexin has a good pharmacokinetic profile; it is well absorbed, 80% excreted unchanged in urine within 6 hours of administration. Cephalexin's half-life is 0.5-1.2 hours and it is excreted *via* the renal. It is used for the treatment of infections including otitis media, streptococcal pharyngitis, bone and joint infections, pneumonia, cellulitis and urinary tract infection, and so it may be used to prevent bacterial endocarditis [72-75].

2.3 Antibacterial drugs history

Amoxicillin and cephalexin as mentioned before suffer low stability and bitter taste sensation. In general, several attempts were made in order to enhance antibacterial drugs aqueous solubility and bioavailability. Among several research approaches, the prodrug approach has been widely used for an improvement of drugs delivery to their site of action by physicochemical modulation properties that affect absorption or by targeting to specific enzymes or membrane transporters [76, 77]. Generally, enzymatic catalysis is required for most of prodrugs that are in clinical use in order to be converted into the parent drug. This is mostly particular for those prodrugs designed to liberate the parent drug in the blood stream following gastro-intestinal absorption. These prodrugs are typically ester derivatives of drugs containing carboxyl or hydroxyl groups which are converted into the parent drug by esterase catalyzed hydrolysis. However, a high chemical reactivity that precludes either liquid or solid formulation of the prodrug (e.g. some phenol esters) or low chemical reactivity, resulting in reduced regeneration of the parent drug due to enzymatic activation for other functional groups. Thus, non-enzymatic pathways for some prodrugs that can regenerate the parent drug, have emerged as an alternative approach by which prodrug activation is not influenced by inter- and intra-individual variability that affects the enzymatic activity. In particular, since the middle-1980s, cyclization-activated prodrugs have been capturing the attention of medicinal chemists, and reached maturity in prodrug design in the late 1990s. Activation of prodrugs *via* a cyclization pathway allows a fine tuning of the rate of drug release through the appropriate choice of the functional groups involved in ring closure and stereoelectronic constraints in the course of the cyclization step. As noticed from the history of prodrugs mostly in preclinical and clinical consideration of prodrug bioconversion, the most common that several hydrolase-activated prodrugs of penicillins, cephalosporins, and angiotensin-converting enzyme inhibitors have

less than complete absorption which was observed and highlights yet another challenge with prodrugs susceptible to esterase hydrolysis. The oral bioavailability of these mentioned types of prodrugs is typically around 50% since these prodrugs undergo premature hydrolysis during the absorption process in the enterocytes of the gastrointestinal tract [78]. Another approach which has been utilized to enhance bioavailability of antibacterial drugs in general, is by making the corresponding prodrugs with optimum lipophilicity. Some drugs remain poorly absorbed from most of the administration routes due to their poor lipophilicity. Two approaches were utilized to enhance the bioavailability of antibacterial drugs by increasing their lipophilicity: (a) membrane/water partition coefficient of the lipophilic form of a drug has been enhanced as compared to the hydrophilic form, thus favoring passive diffusion such as in the cases of pivampicillin, bacampicillin and talamipicillin (prodrugs of ampicillin) which are more lipophilic and better absorbed than amoxicillin and are rapidly interconverted and (b) the lipophilic prodrugs have poor solubility in gastric fluids and thus greater stability and absorption example for such approach is erythromycin esters [79]. Some ampicillin esters were prepared for improving the bioavailability of ampicillin. For example, the pivaloyloxyethyl (pivampicillin), phthalidyl (talampicillin), and ethoxycarbonyloxyethyl (bacampicillin) were found to have two fold the oral bioavailability of their parent drug, ampicillin. Complete hydrolysis of these esters was occurred in the gastrointestinal mucosa, whereas methoxymethyl ester of ampicillin was partially hydrolyzed by gut and hepatic first-pass metabolism and appears in the systemic circulation and tissues as intact ester [80, 81].

Amoxicillin and cephalexin antibacterial drugs have good pharmacokinetic properties with a good bioavailability. However, to our knowledge there was no report on studies involving masking bitter taste of antibacterial agents using the prodrug approach. We believe that blocking the amine group in amoxicillin and cephalexin by making the proposed prodrugs will result in inhibition of the interaction between the amine group of the antibacterial agent and the bitter taste receptors. It is worth noting that bitter sensation is a result of either hydrogen bonding or ionic bonding between these substrate and its receptors [47].

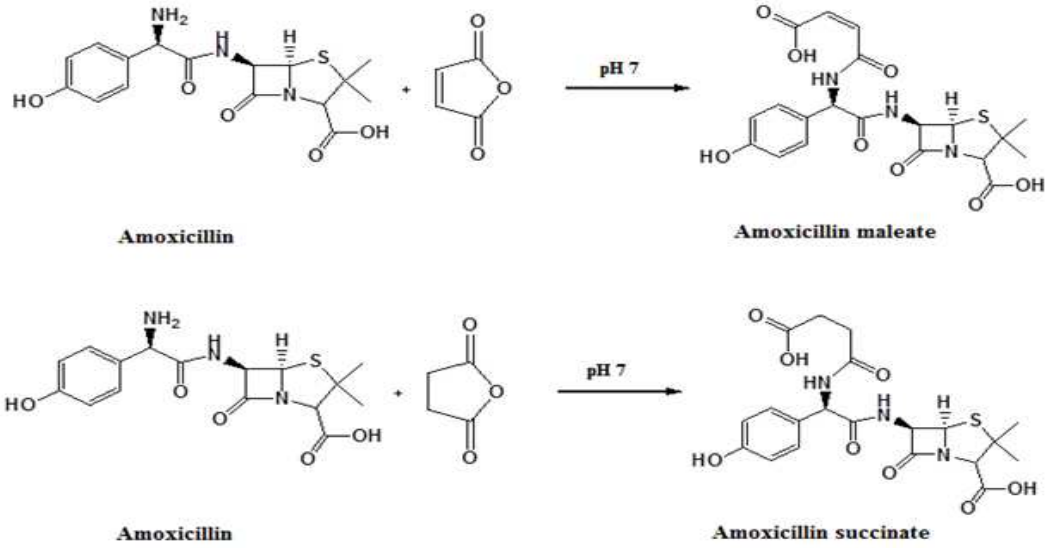
Computational chemistry methods could be useful for the design of innovative prodrugs for hydroxyl, phenol, or amine containing drugs. For instance, mechanisms of

intramolecular processes for a respected number of enzyme models that were previously investigated by others to understand enzyme catalysis have been recently explored by us and exploited for a design of some new novel prodrug [38]. Using the DFT, molecular mechanics and *ab initio* at different levels, numerous enzyme model processes were calculated for determining the factors governing the synthesized prodrugs rates. According to their demonstration, there is a need to further explore the mechanisms for the above mentioned processes for assigning the factors affecting the nature and the mode of the reaction. Unraveling the reaction mechanism would allow for an accurate design of an efficient chemical device to be utilized as a prodrug promoiety that can be covalently linked to a parent drug to provide chemically and not enzymatically the parent drug in a programmable manner upon exposure to physiological environments. For example, exploring the mechanism for proton transfer in Kirby's acetals has led to a design and synthesis of novel prodrugs of aza-nucleosides to treat myelodysplastic syndromes [48], statins to treat high cholesterol blood levels [59], paracetamol prodrugs with no bitter taste to be administered to children and elderly as antipyretic and pain killer and prodrugs of phenylephrine as decongestants [82]. The prodrug moiety was attached to the hydroxyl group of the active drug such that the drug promoiety (prodrug) has a potential to degrade upon exposure to physiological environment such as stomach, intestine, and/or blood circulation, with rates that are solely dependent on the structural features of the pharmacologically inactive promoiety (Kirby's enzyme model). Other different linkers such as Kirby's N-alkylmaleamic acids (enzyme model) were also investigated for the design of some prodrugs such as those of tranexamic acid to treat bleeding conditions and acyclovir (anti-viral drug) to treat Herpes Simplex. Further, prodrugs for masking the bitterness of antibacterial drugs such as cefuroxime were designed and made as well. The role of the promoiety in the antibacterial (cefuroxime) and paracetamol prodrugs was to block the free amine (cefuroxime) or phenol (paracetamol) which is believed to be responsible for the drug bitterness, and to enable the release of the drug in a programmable manner. Menger's Kemp acid enzyme model was also exploited for the design of dopamine prodrugs for the treatment of Parkinson's disease. In addition, dimethyl fumarate prodrugs to treat psoriasis have been designed, synthesized and currently under *in vitro* and *in vivo* kinetic studies [55].

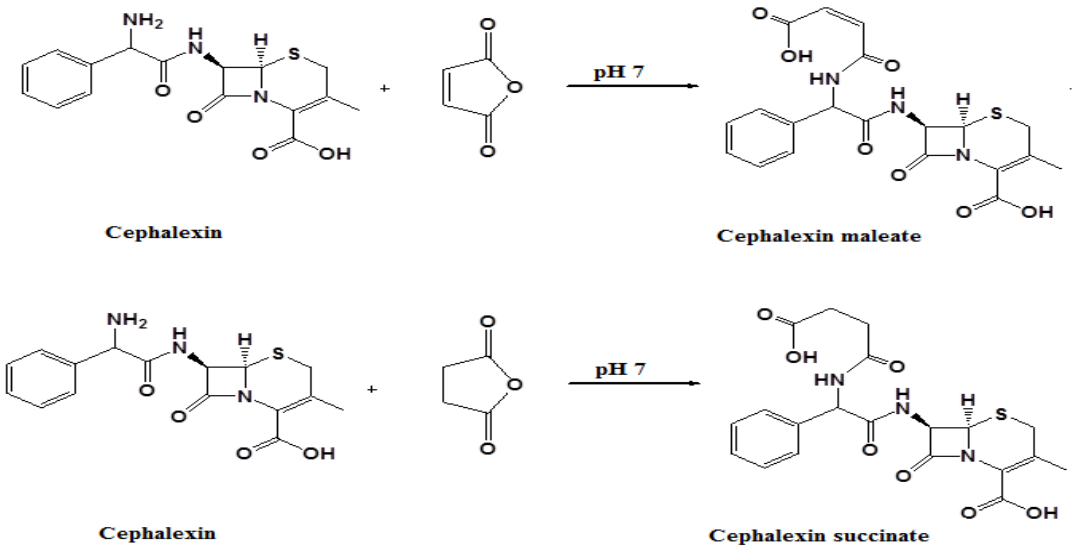
2.3.1 Proposed bitterless antibacterial prodrug

Amoxicillin and cephalexin are antibacterial drugs that have been developed and widely used for clinical purposes. They have a relatively narrow spectrum against pathogens and are hydrolyzed by various types of β-lactamases. These antibacterial drugs suffer several problem, they have a low stability and bitter taste sensation that leads to patient incompliance. There are limited studies to improve their clinical profiles and mask their bitter taste. Several attempts were made in order to enhance their aqueous solubility, potency and bioavailability. According to Karaman work and based on the previously reported DFT calculations and on experimental data for the acid catalyzed hydrolysis of amine acids **1**-**9** [56, 61], 4 antibiotic prodrugs were designed and synthesized **(Scheme 2.1** and **2.2)**: (1) to improve the stability and aqueous solubility of the parent drugs, (2) to make a chemical device that is capable for releasing the parent drug in a sustained release manner, and 3) to provide drugs without bitter taste. As shown in **Scheme 2.1** and **2.2**, amoxicillin and cephalexin prodrugs are composed of an amide acid linker containing a carboxylic acid group (hydrophilic moiety) and the rest of molecule is composed of a lipophilic moiety. The combination of both hydrophilic and lipophilic groups provides a prodrug moiety with a high permeability (a moderate HLB). This balance of the prodrug molecule will be dependent on the pH of the target physiological environment. In the stomach (pH 1-2), it is expected that prodrugs of amoxicillin and cephalexin will be in a free carboxylic acid form (a relatively high lipophilicity) whereas in the blood stream circulation (pH 7.4) a carboxylate anion form with a relatively low lipophilicity is expected to be predominant. Prodrugs of amoxicillin and cephalexin **(Scheme 2.1** and **2.2)** were synthesized in the form of sodium or potassium carboxylate due to their high stability in neutral aqueous medium. The only difference between the proposed prodrugs and the parent drugs is that the former has an amide moiety instead of the free amine group in the latter, which makes the prodrug more stable than its corresponding amine parent drug. In addition, kinetic studies on amoxicillin and cephalexin revealed that increasing the lipophilicity of the drug leads to an increase in the stability of its aqueous solutions**.** Based on the above, it is expected that the four antibacterial proposed prodrugs of amoxicillin and cephalexin will be more resistant to hydrolysis when standing in aqueous solutions, in addition...

In this study, we describe the synthesis, characterization and kinetic study of the interconversion of the four proposed antibacterial prodrugs, of amoxicillin **ProD 1-2** and cephalexin **ProD 1-2** in different media such as 1N HCl, buffer pH 2.5, buffer pH 5.0 and buffer pH 7.4. This study was performed in order to achieve desirable penicillin derivatives prodrugs of both amoxicillin and cephalexin antibacterial agents that are capable of being stable in aqueous solutions, more lipophilic, less bitter and have the potential for releasing the corresponding drugs in a slow release manner.



Scheme (2.1): Chemical structures for amoxicillin prodrugs.



Scheme (2.2): Chemical structures for cephalexin prodrugs.

Experimental Part

Chapter Three

Experimental Part

This chapter consists of three main parts. Part one is concerned with the identification and synthesis of the most popular antibacterial prodrugs that are used worldwide. Part two describes all instruments, chemicals and reagents used in this study. Part three describes the synthetic methods and analysis of the four antibacterial prodrugs of amoxicillin and cephalexin.

3.1 Part One

3.1.1 Identification of the most important and popular antibiotic prodrugs

Numerous novel prodrugs have been designed by Karaman and coworkers for the treatment of various diseases using DFT calculation methods. According to the results obtained from DFT calculation on similar amine drugs, design and synthesis of four novel amoxicillin and cephalexin prodrugs containing two different linkers were studied in the course of this work. The main goals of this work were: (1) increase solution stability of the two antibacterial drugs, amoxicillin and cephalexin; (2) masking their bitter taste sensation, and (3) attempting to make a sustain release dosage forms of the above mentioned antibacterial drugs.

3.2 Part Two

3.2.1 Instrumentations

3.2.1.1 pH meter

pH meter model HM-30G: TOA electronics™ was used to measure the pH values for all buffers and reaction media involved in this study.

3.2.1.2 UV-Spectrophotometer

The concentrations of each sample of the four antibacterial prodrugs and their parental drugs were determined spectrophotometerically (UV-spectrophotometer, Model: UV-1601, Shimadzu, Japan) by monitoring the absorbance at λ_{max} that was determined from both the standard and its prodrug.

3.2.1.3 Fourier Transform Infrared Spectrophotometer (FT-IR)

It is the most useful and preferred method of infrared spectroscopy. In infrared spectroscopy, IR radiation is passed through a sample. Some of the infrared radiation is transmitted or absorbed by the sample. This will create a molecular fingerprint of the sample from the resulting spectrum that corresponds to the frequencies of vibration between the bonds. The spectrum represents the molecular absorption and transmission. Infrared spectroscopy is useful for several types of analysis, as no two unique molecular structures produce the same infrared spectrum.

FT-IR can result in a positive identification of unknown samples that is known as quantitative analysis of every different kind of material and can determine the consistency or quality of a sample. In addition, the size of the peaks in the spectrum is a direct indication of the amount of components in the mixture.

All infrared spectra (FTIR) were obtained from KBr (potassium bromide) matrix (4000–400 cm^{-1}) using a PerkinElmer Precisely, Spectrum 100, FT-IR spectrometer.

3.2.1.4 Nuclear magnetic resonance spectroscopy (^{1}H -NMR)

^{1}H NMR is a technique that identifies the carbon-hydrogen framework of any organic compound. Its use is related to the other instrumental methods to determine the compound's unique structure and its purity. ^{1}H NMR works by generating a magnetic field from the atomic nucleus that is known as a spinning charged particle. The nuclear spins are random and spin in random directions when an external applied magnetic field is absent, otherwise, the nuclei align themselves either with or against the field of the external magnet when an external magnetic field is present.

For ^{1}H-NMR, chemical shifts are reported in parts per million (ppm, δ) downfield from tetramethylsilane (TMS). Spin multiplicities are described as s (singlet), brs (broad singlet), t (triplet), q (quartet), and m (multiplet).

3.2.1.5 High Performance Liquid Chromatography (HPLC)

HPLC is probably the most important and widely used analytical technique for quantitative analysis of organic and biomolecules. HPLC is a physical separation technique by which a sample of the drug or prodrug dissolved in a liquid and injected into a column packed with small particles separated into its constituent components.

All HPLC measurements were carried out using Shimadzu prominence high performance liquid chromatography system HPLC-PDA, (Shimadzu corp. Japan). Samples were shaken using Big Bill, (Banstaed/ Themolyne, USA). The high pressure liquid chromatography system consisted of a model 2695 HPLC from Waters (Israel) equipped with a Waters 2996 Photodiode array. Data acquisition and control were carried out using Empower ™ software (Waters: Israel). Analytes were separated on a 4.6 mm x150 mm C18 XBridge® column (5 μm particle size) used in conjunction with a 4.6 mm, 20 μm, XBridge® C18 guard column was used. Microfilters 0.45 μm porosity was normally used (Acrodisc® GHP, Waters). The C-18 (1 gm) cartridges 6cc single use for general laboratory use, were purchased form Waters Company (Milford, MA, USA).

3.2.1.6 Liquid Chromatography- Mass Spectroscopy (LC-MS)

LC-MS is a powerful, selective and sensitive technique used to separate a very wide range of organic compounds, from small molecule metabolites drug to peptides and proteins. This system technique is mostly used for fast and mass directed purification of many products and new molecular entities. It is used to detect the molecular weight of many products. HPLC–MS/MS measurements were performed employing a Shimadzu prominence HPLC system (Shimadzu corp. Japan).

3.2.2 Chemicals and reagents

Pure standards (>99%) of amoxicillin and cephalexin were available commercially from Sigma Aldrich. Inorganic salts were of analytical grade and were used without further purification. Organic buffer components were distilled or recrystallized. Distilled water was redistilled twice before use from all-glass apparatus. Maleic anhydride, anhydrous potassium dihydrogen phosphate, succinic anhydride and sodium bicarbonate were commercially available from Sigma Aldrich. High purity methanol, ethylacetate and water (>99%) were used for HPLC grade and purchased from Sigma Aldrich.
Prodrugs of amoxicillin and cephalexin antibiotic drugs were synthesized by a modification of published procedures as mentioned below.

3.3 Part three

3.3.1 Methods (amoxicillin and cephalexin extraction and purification)

3.3.1.1 Preparation of amoxicillin ProD 1-2 (Figure 3.1)

Amoxicillin **ProD 1** preparation**:** In a 250 ml round-bottom flask, 2.12 g of amoxicillin trihydrate (5 mmol) was dissolved in H_2O (100 ml), 0.45 g of sodium bicarbonate was added. The resulting solution was stirred for 30 minutes then 0.50 g of maleic anhydride (5 mmol) was slowly added to the reaction mixture. The pH of the prepared reaction was measured in order to verify that the reaction was neutral (pH = 7). The reaction mixture was left to be stirred for 2 hours at room temperature. The reaction was monitored by Thin Layer Chromatography (TLC) which was performed on regular basis to check the reactions completion. The solvent was evaporated and the resulting precipitate was washed with ethyl acetate and filtered. The precipitate was dissolved in methanol, filtered and evaporated. The white residue after evaporation was dried at 39 ˚C (2.6 g). The product was characterized by melting point (M.P), H-NMR, FTIR and LC-MS. M.P. 170 ˚C. ^{1}H-NMR δ (ppm) CD3OD: 1.5 (CH3-C-CH3, M), 4.2 (HN-CH-CH-S), D, J=20C MHZ), 4.7 (N-CH-COOH), S), 5 (HC-CH-S-C), D, J=4 MHZ), 5.4 (NH-CH-C, D. J=6.4 MHZ), 5.5 (NH-CH-(Ar), D, J=20 MHZ), 6.3 (HOOC-CH=CH-C=O, M), 6.7 (HC-CH=C-CH=C, D, J=10.4 MHZ), 6.8 (O=C-NH-C-Ar, D, J=8.8 MHZ), 7.3 (aromatic, M). IR (KBr/ν_{max} cm^{-1})

1763 (C=O), 1585 (C=C), 1650 (C=O), 1600-1700 (NH), 1369, 1246, 2753, 3355, *m*/*z* 486.1 (M+1).

Amoxicillin **ProD 2**: we followed the same procedure as for amoxicillin **ProD 1** but instead of using maleic anhydride linker, 0.50 g succinic anhydride was used (5 mmol) (product; 2.8 g as white product). M.P. 140-150 ˚C. ^{1}H-NMR δ (ppm) CD3OD: 1.5 (CH3-C-CH3, M), 2.5 (COOH-CH2-CH2-C=O, M), 3.4 (HC-CH-S-C, D, J=56 MHZ), 4.2 (N-CH-COOH), S), 4.9 (HN-(C=O)-CH-Ar), S), 5.4 (NH-CH-C=O, D. J=4 MHZ), 6.9 (aromatic ((OH)C-HC-HC-C-CH=CH-C(OH)OH), M), 7.3 (aromatic((OH)C-HC-HC-C-CH=CH-C(OH)OH), M). IR (KBr/ν_{max} cm^{-1}) 1769 (C=O), 1576 (C=C), 1676 (NH), 1576 amide C=O, 1514, 1402, from 1890-3305, *m*/*z* 488.1 (M+1).

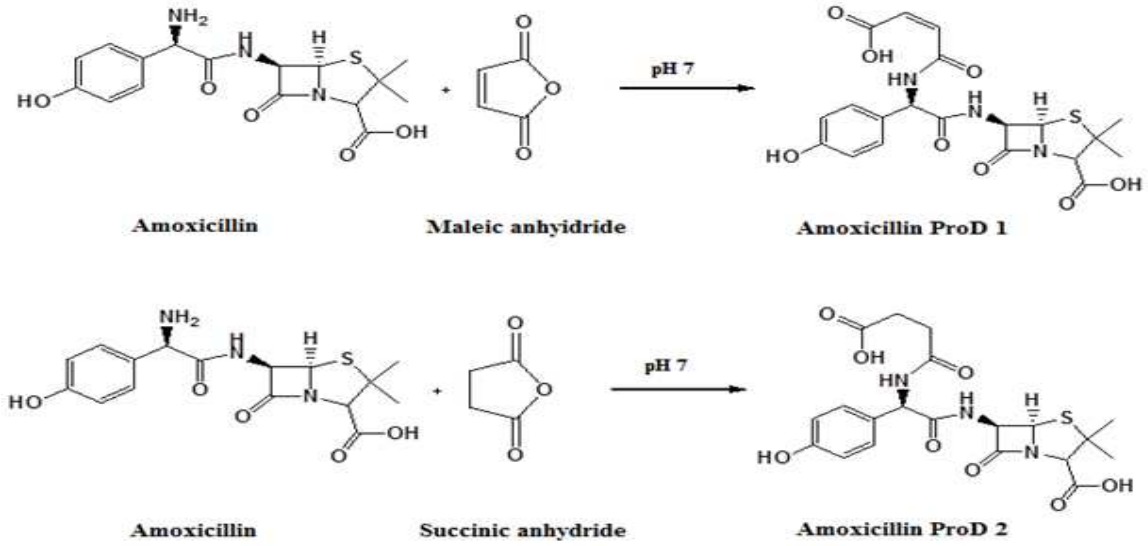


Figure (3.1): Synthesis of amoxicillin trihydrate **ProD 1-2** from its parent drug, amoxicillin trihydrate.

3.3.1.2 Preparation of cephalexin ProD 1-2 (Figure 3.2)

Cephalexin **ProD 1** preparation: In a 250 ml round-bottom flask, 1.75 g of pure cephalexin standard (5 mmol) was dissolved in H_2O (100 ml), 0.45 g of sodium bicarbonate was added, the resulting solution was stirred for 30 minutes, then 0.52 g of maleic anhydride (5 mmol) was slowly added to the reaction mixture. The pH of the reaction was maintained to pH=7. The reaction mixture was stirred for 2 hours at room temperature. The reaction was monitored by TLC which was done on a regular basis to check the reactions completion. The solvent was evaporated and the resulting precipitate was washed with ethyl acetate

then filtration. The solid residue was dissolved in methanol, dried over MgSO4, filtered and evaporated. The resulting white precipitate was collected and dried at 39 ˚C (2.9 gm). The product was characterized by M.P, H-NMR, FTIR, and LC-MS. M.P. 140-160 ˚C. ^{1}H-NMR δ (ppm) CD3OD: 1.5 (CH3-C=C, S), 3.3 (S-CH2-C=C, S), 4.9 (C-CH-C=O, S), 5.5 (S-CH-CH-NH, D, J= 12.8 MHZ), 5.6 (NH-CH-CH, D, J= 4.8 MHZ), 6.3 (O=C-CH=CH-COOH, M), 7.4 (aromatic, M). IR (KBr/ν_{max} cm^{-1}) 1758 (C=O), 1249 (C-O), 1578 (C=C), 1600-1700 (NH), 1674 amide C=O, 3222 shifts. *m*/*z* 468 (M+1).

Cephalexin **ProD 2**: we followed the same procedure of cephalexin **ProD 1** but instead of using maleic anhydride linker, we used 0.52 g succinic anhydride (5 mmol) (product; 2.0 gm). M.P. 240 ˚C. ^{1}H-NMR δ (ppm) CD3OD: 2 (CH3-C=C, S), 2.5 (COOH-CH2-CH2-C=O, M), 3 (NH-CH-CH-N-C, D, J=17.6 MHZ), 3.2 (NH-CH-CH-S), D, J=1.6 MHZ), 4.9 (NH-CH-C=O), S), 5.5 (AR-CH-C=O), S), 7.5 (aromatic, M). IR (KBr/ν_{max} cm^{-1}) 1755 (C=O), 1586 (C=C), 1643 (NH), 1665 amide C=O, 3627, 2879, 2933 shifts, *m*/*z* 470 (M+1).

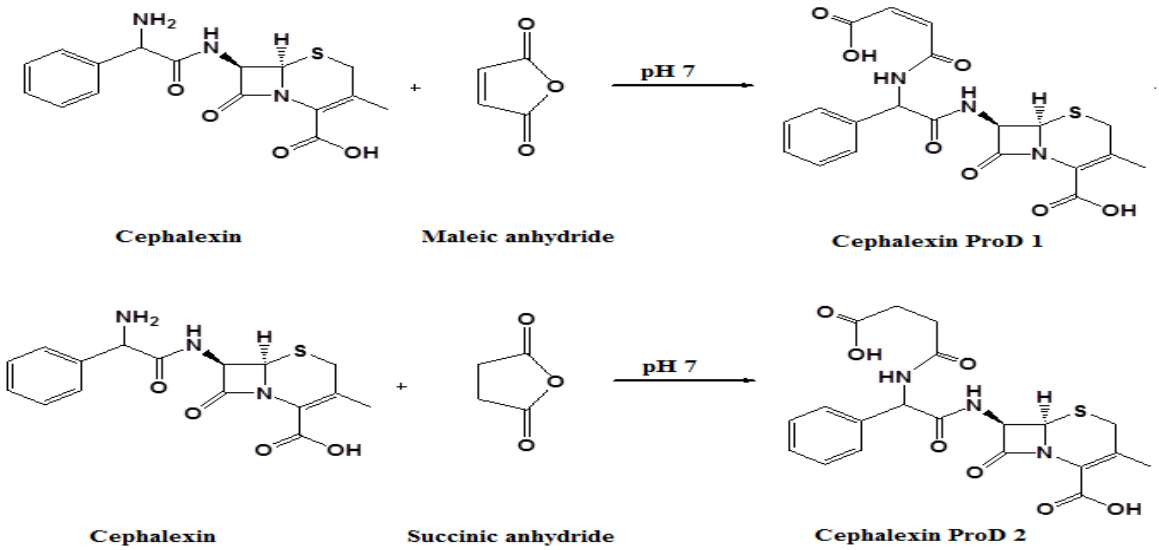


Figure (3.2): Synthesis of cephalexin ProD **1-2** from its parent drug, cephalexin.

3.4 Kinetic Methods

3.4.1 Preparation of samples and buffer solutions

6.8 gm potassium dihydrogen phosphate was dissolved in 900 ml water for HPLC. The pH of buffer 2.5 was adjusted by diluted o- phosphoric acid and water was added to a final volume of 1000 ml (0.05 M). The same procedure was repeated for the preparation of buffers pH 5.0 and 7.4, however, the required pH was adjusted using 1 N NaOH.

Interconversion of 500 ppm amoxicillin **ProD 1-2** solutions, in 1N HCl, buffer pH 2.5, buffer pH 5.0 and buffer pH 7.4, to their parent drug, amoxicillin was followed by HPLC at a wavelength of 254 for amoxicillin **ProD 1** and 230 nm for amoxicillin **ProD 2**. Conversion reactions were run at 37.0 ˚C.

Interconversion of 500 ppm cephalexin **ProD 1-2** solution, in 1N HCl, buffer pH 2.5, buffer pH 5.0 and buffer pH 7.4, to its parent drug, cephalexin, was followed by HPLC at a wavelength of 230 nm. Conversion reactions were run at 37.0 ˚C.

3.4.2 Calibration curve for amoxicillin trihydrate and amoxicillin trihydrate ProD1-2

To construct a calibration curve for amoxicillin trihydrate and amoxicillin **ProD1-2**, 6 calibrants (100, 200, 300, 400, 500 and 600 ppm) were prepared. 20 µl of each solution was injected into the HPLC and the peak for the pharmaceuticals was recorded using the following HPLC conditions: 6 mm x 250 mm, 5 µm C18 XBridge® column using mobile phase contains ACN: water (20:80 V\V), a flow rate of 1 ml min^{-1} and UV detection at a wavelength of 230 nm.
Peak area vs. concentration of the pharmaceutical (ppm) was then plotted, and R^2 of the plot was recorded.

3.4.2.1 Preparation of amoxicillin trihydrate standard and sample solution

Three samples of amoxicillin were prepared, a standard sample, a linker sample and a prodrug sample to detect the retention time for each.

1) Amoxicillin trihydrate standard (500 ppm) was prepared by dissolving 50 mg of drug in 100 ml of 1N HCl, buffer pH 2.5, buffer pH 5 and buffer pH 7.4, and then each sample was injected into HPLC to detect the retention time of amoxicillin trihydrate.
2) Maleic anhydride linker (500 ppm) was prepared by dissolving 50 mg of drug in 100 ml of 1N HCl, buffer pH 2.5, buffer pH 5 and buffer pH 7.4, and then each sample was injected into HPLC to detect the retention time of maleic anhydride.
3) Amoxicillin **ProD 1-2** (500 ppm) were prepared by dissolving 50 mg of the prodrug in 100 ml of 1NHCl, buffer pH 2.5, buffer pH 5 and buffer pH 7.4, and then each sample was injected into HPLC to detect the retention time.

The progression of reaction was followed by monitoring the disappearance of the prodrug and appearance of amoxicillin and the linker attached vs. time.

3.4.3 Calibration curve for cephalexin and cephalexin ProD 1-2

To construct a calibration curve for cephalexin and cephalexin **ProD 1-2**, 6 calibrants (100, 200, 300, 400, 500 and 600ppm) were prepared. 20 µl of each solution was injected into the HPLC and the peak for them was recorded using the following HPLC conditions: 6 mm x 250 mm, 5 µm C18 XBridge ® column using mobile phase contains ACN: water (20:80 V\V), a flow rate of 1 ml min^{-1} and UV detection at a wavelength of 230 nm.
Peak area vs. concentration of the pharmaceutical (ppm) was then plotted, and R^2 of the plot was recorded.

3.4.3.1 Preparation of cephalexin standard and sample solution

Three samples were prepared for cephalexin, a standard sample, a linker sample and a prodrug sample to detect the retention time.

1) Cephalexin standard (500 ppm) was prepared by dissolving 50 mg of drug in 100 ml of 1N HCl, buffer pH 3, buffer pH 5.5 and buffer pH 7.4. Each sample was injected into HPLC to detect the retention time of cephalexin.
2) Maleic anhydride linker (500 ppm) was prepared by dissolving 50 mg of drug in 100 ml of 1N HCl, buffer pH 3, buffer pH 5.5 and buffer pH 7.4, and then each sample was injected into HPLC to detect the retention time of maleic anhydride.

3) Cephalexin **ProD 1-2** (500 ppm) was prepared by dissolving 50 mg of the prodrug in 100 ml of 1N HCl, buffer pH 3, buffer pH 5.5 or buffer pH 7.4 then each sample was injected into HPLC to detect the retention time.

The progression of reaction was followed by monitoring the disappearance of the prodrug and appearance of cephalexin and the linker attached vs. time.

Amoxicillin **ProD 1-2** and cephalexin **ProD 1-2** were left to be monitored on the HPLC for several days to detect the interconversion of the four prodrugs to their corresponding parental drugs, to calculate the $t_{1/2}$ of each prodrug.

Results and Discussion

Chapter Four

Results and Discussion part

4.1 Results and discussion

We have successfully obtained four antibacterial prodrugs of amoxicillin and cephalexin with two different linkers. They were characterized by M.P, FT-IR, ^{1}H-NMR and LC-MS analytical techniques, to guarantee pure antibacterial prodrugs that are bitterless taste, with improved stability and solubility and are capable of releasing the parent drugs in a sustained release manner as proposed.

4.1.1 Prodrugs characterization using different analytical techniques

4.1.1.1 Melting point, FT-IR, NMR and LC-MS analysis of amoxicillin maleate ProD 1

1) Decomposition point of amoxicillin maleate **ProD 1** was 170 ˚C.
2) IR (KBr/ν_{max} cm^{-1}) 1763 (C=O), 1585 (C=C), 1650 (C=O), 1600-1700 (NH), 1369, 1246, 2753, 3355. The frequency of the reactant free amine group (NH2) from 3500-3600 was disappeared and the frequency of the more stable amide product was appeared in 1650. In addition carboxylic acid group frequency was changed and appeared in 1686 as shown in **Figures 4.1** and **4.2**.
3) ^{1}H-NMR δ (ppm) CD3OD: 1.5 (CH3-C-CH3, M), 4.2 (HN-CH-CH-S), D, J=20C MHZ), 4.7 (N-CH-COOH), S), 5 (HC-CH-S-C), D, J=4 MHZ), 5.4 (NH-CH-C, D. J=6.4 MHZ), 5.5 (NH-CH-(Ar), D, J=20 MHZ), 6.3 (HOOC-CH=CH-C=O, M), 6.7 (HC-CH=C-CH=C, D, J=10.4 MHZ), 6.8 (O=C-NH-C-Ar, D, J=8.8 MHZ), 7.3 (aromatic, M). ^{1}H-NMR analysis shows that the product has an additional signal in the region between 6-6.5 ppm as shown in **Figures 4.3** and **4.4**.
4) The product molecular formula is C20H21N3O8S (yield 85%). LC-MS (positive mode) *m*/*z* 486.1 (M+1) (Figure **4.5)**.

Figure (4.1): FT-IR spectrum of amoxicillin standard.

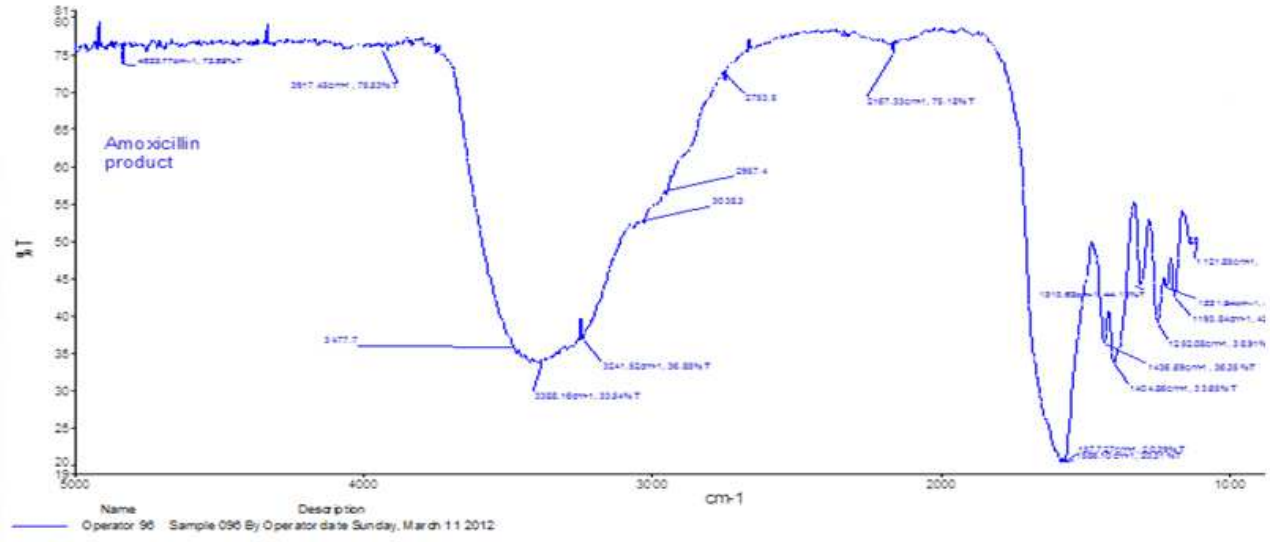


Figure (4.2): FT-IR spectrum of amoxicillin **ProD 1**.

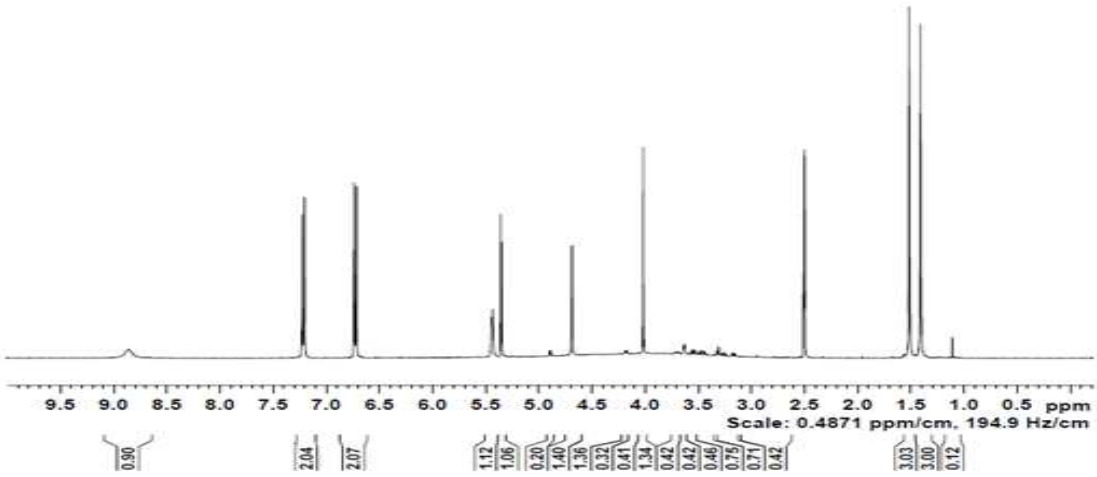


Figure (4.3): H-NMR spectrum of amoxicillin standard.

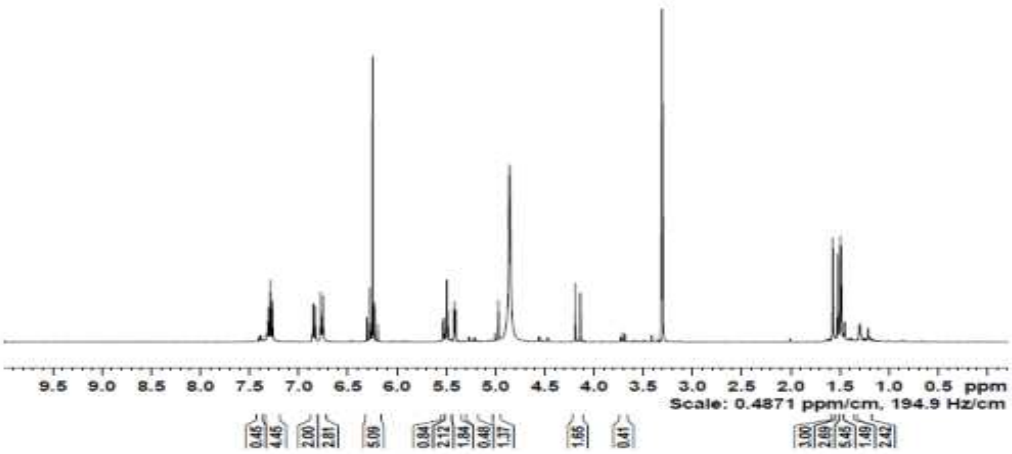
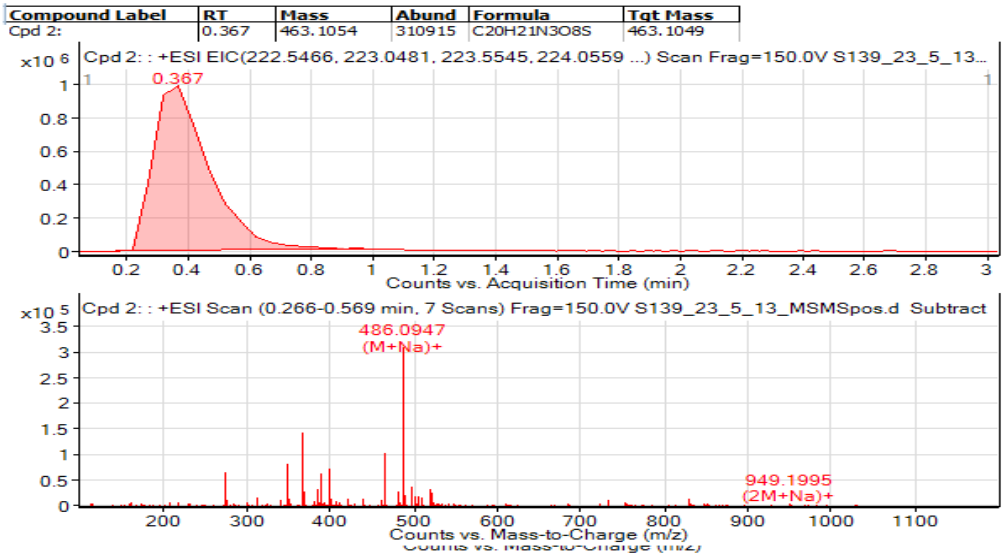


Figure (4.4): H-NMR spectrum of amoxicillin maleate **ProD 1**.



m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
446.1028	446.1016	2.65	1	2154.7	C20H20N3O7S	(M+H)+[-H2O]
464.1128	464.1122	1.31	1	103843.5	C20H22N3O8S	(M+H)+
468.0834	468.0836	-0.48	1	733.6	C20H19N3NaO7S	(M+Na)+[-H2O]
481.1391	481.1388	0.77	1	6318.7	C20H25N4O8S	(M+NH4)+
486.0947			1	310915.3		
486.0947	486.0942	1.05		310915.3	C20H21N3NaO8S	(M+Na)+
487.0975	487.0971	0.85	1	66930.2	C20H21N3NaO8S	(M+Na)+
931.1878	931.1885	-0.76	1	647.3	C40H40N6NaO15S2	(2M+Na)+[-H2O]
944.2426	944.2437	-1.14	1	111.6	C40H46N7O16S2	(2M+NH4)+
949.1995	949.1991	0.39	1	3512.9	C40H42N6NaO16S2	(2M+Na)+

Figure (4.5): LC-MS spectrum of amoxicillin maleate **ProD 1**.

4.1.1.2 Melting point, FT-IR, NMR and LC-MS analysis of amoxicillin succinate ProD 2

1. Decomposition point of amoxicillin succinate **ProD 2** was 140-150 ^{o}C.
2. IR (KBr/ν_{max} cm^{-1}) 1769 (C=O), 1576 (C=C), 1676 (NH), 1576 amide C=O, 1514, 1402, from 1890-3305. The frequency of the reactant free amine group (NH2) from 3400-3800 was disappeared and the frequency of the more stable amide product was appeared on 1676, in addition carboxylic acid group frequency changed and appeared on 1576 as shown in **Figure 4.6**.
3. ^{1}H-NMR δ (ppm) CD3OD: 1.5 (CH3-C-CH3, M), 2.5 (COOH-CH2-CH2-C=O, M), 3.4 (HC-CH-S-C, D, J=56 MHZ), 4.2 (N-CH-COOH), S), 4.9 (HN-(C=O)-CH-Ar), S), 5.4 (NH-CH-C=O, D. J=4 MHZ), 6.9 (aromatic ((OH)C-HC-HC-C-CH=CH-C(OH)OH), M), 7.3 (aromatic((OH)C-HC-HC-C-CH=CH-C(OH)OH), M). ^{1}H-NMR analysis shows that the product has an additional signal in the region between 2-2.5 ppm as shown in **Figure 4.7.**
4. The product molecular formula is C20H23N3O8S (yield 90%). LC-MS (positive mode) *m/z* 488.1 (M+\1) (**Figure 4.8)**.

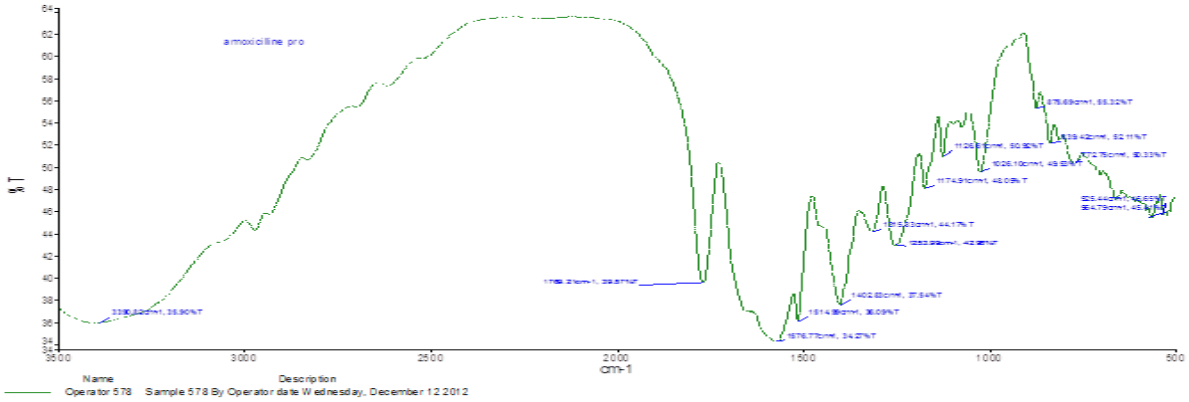


Figure (4.6): FT-IR spectrum of amoxicillin succinate **ProD 2**.

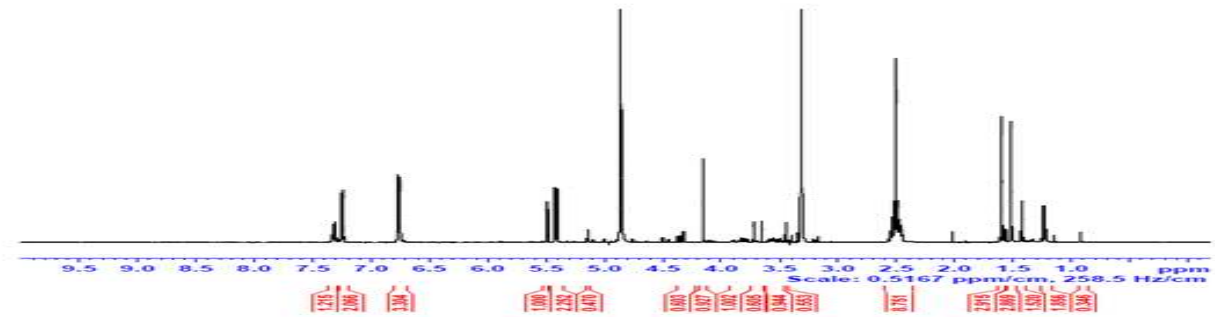
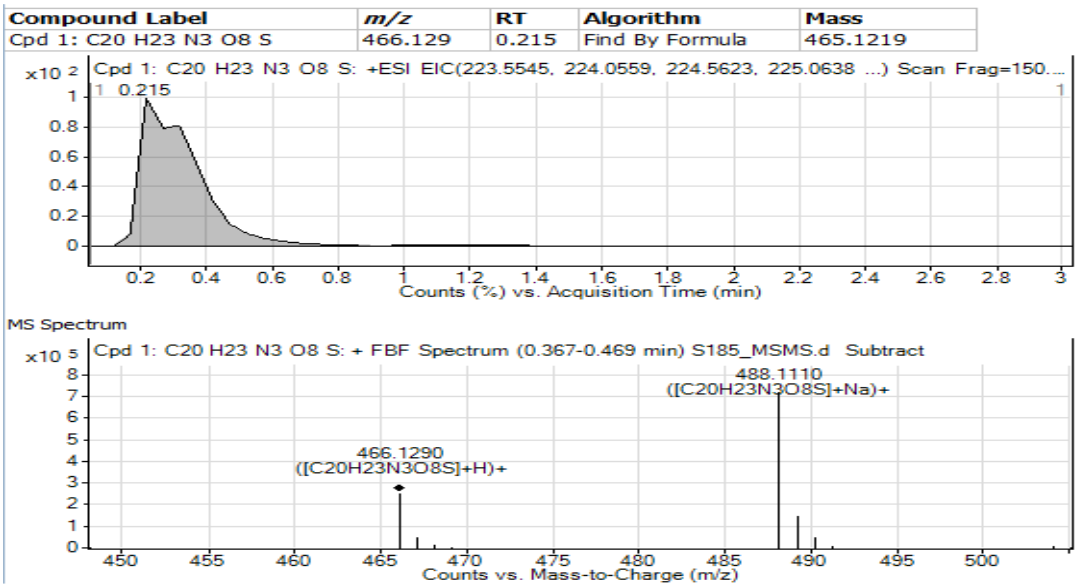


Figure (4.7): H-NMR spectrum of amoxicillin succinate **ProD 2**.



MS Spectrum Peak List

m/z	z	Abund	Formula	Ion
448.1182	1	5088.87	C20H23N3O8S	(M+H)+[-H2O]
466.129	1	253841.45	C20H23N3O8S	(M+H)+
467.1324	1	53805.59	C20H23N3O8S	(M+H)+
468.13	1	18140.13	C20H23N3O8S	(M+H)+
488.111	1	726763.94	C20H23N3O8S	(M+Na)+
489.1145	1	153816.75	C20H23N3O8S	(M+Na)+
490.1122	1	51110.46	C20H23N3O8S	(M+Na)+
491.1139	1	9093.1	C20H23N3O8S	(M+Na)+
504.0845	1	12743.78	C20H23N3O8S	(M+K)+
505.0923	1	4183.69	C20H23N3O8S	(M+K)+

Figure (4.8): LC-MS spectrum of amoxicillin succinate **ProD 2**.

4.1.1.3 Melting point, FT-IR and NMR analysis of cephalexin maleate ProD 1

1) Decomposition point of cephalexin maleate **ProD 1** was 140-160 ^{o}C.
2) IR (KBr/ν_{max} cm^{-1}) 1758 (C=O), 1249 (C-O), 1578 (C=C), 1600-1700 (NH), 1674 amide C=O, 3222. The reactant frequency of the free amine group (NH2) from 3500-3700 was disappeared and the frequency of the more stable amide product was appeared on 1660, in addition carboxylic acid group frequency changed and appeared on 1674 as shown on **Figure 4.9** and **4.10**.
3) ^{1}H-NMR δ (ppm) CD3OD: 1.5 (CH3-C=C, S), 3.3 (S-CH2-C=C, S), 4.9 (C-CH-C=O, S), 5.5 (S-CH-CH-NH, D, J= 12.8 MHZ), 5.6 (NH-CH-CH, D, J= 4.8 MHZ), 6.3 (O=C-CH=CH-COOH, M), 7.4 (aromatic, M). ^{1}H-NMR analysis shows that the product has an additional shift region between 6-6.5ppm as shown in **Figure 4.11** and **4.12**.
4) The product formula is C20H18N3NaO7S (yield 100%). LC-MS (positive mode) *m/z* 468 (M+\1) as shown in **Figure 4.13**.

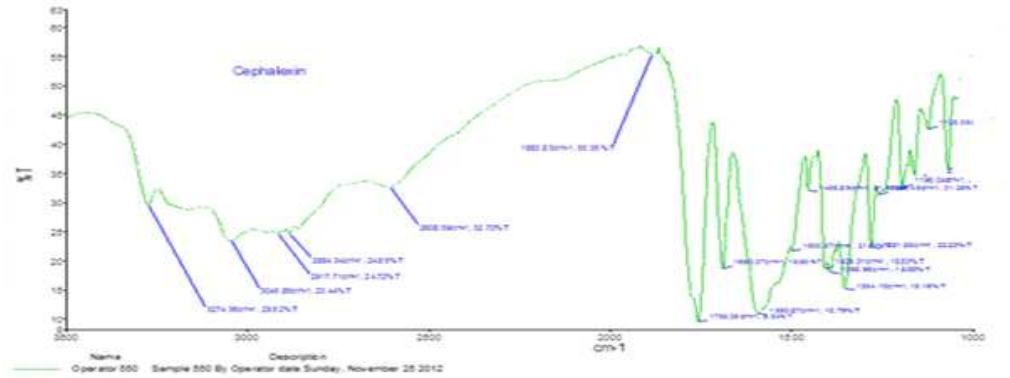


Figure (4.9): FT-IR spectrum of cephalexin standard.

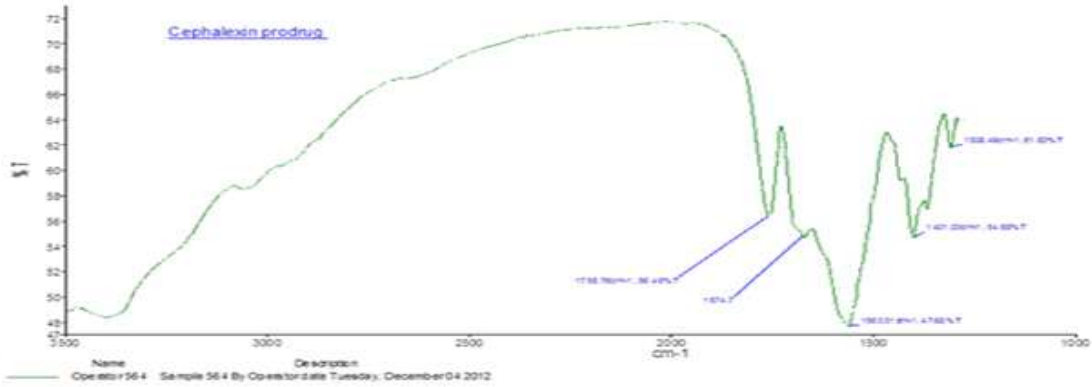


Figure (4.10): FT-IR spectrum of cephalexin **ProD 1**.

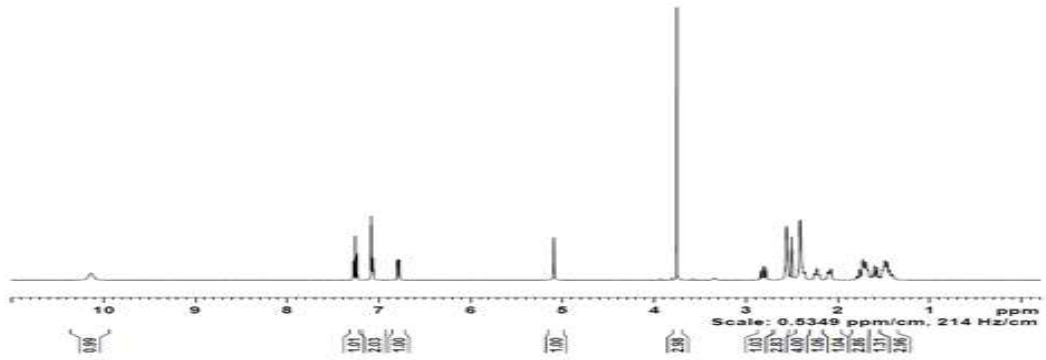


Figure (4.11): H-NMR spectrum of cephalexin standard.

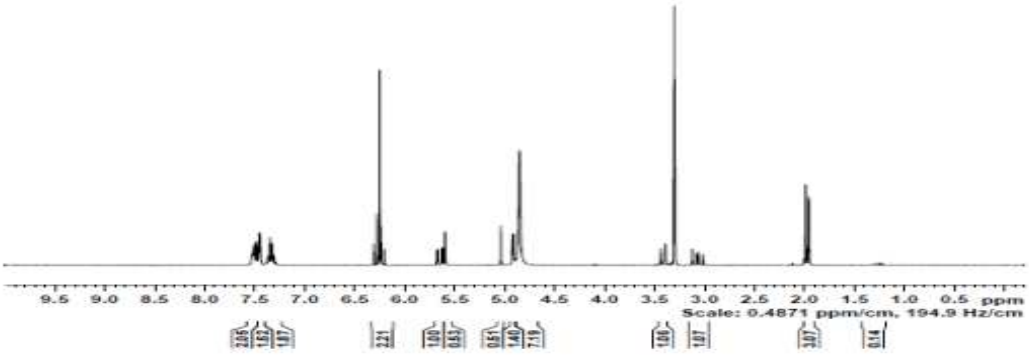
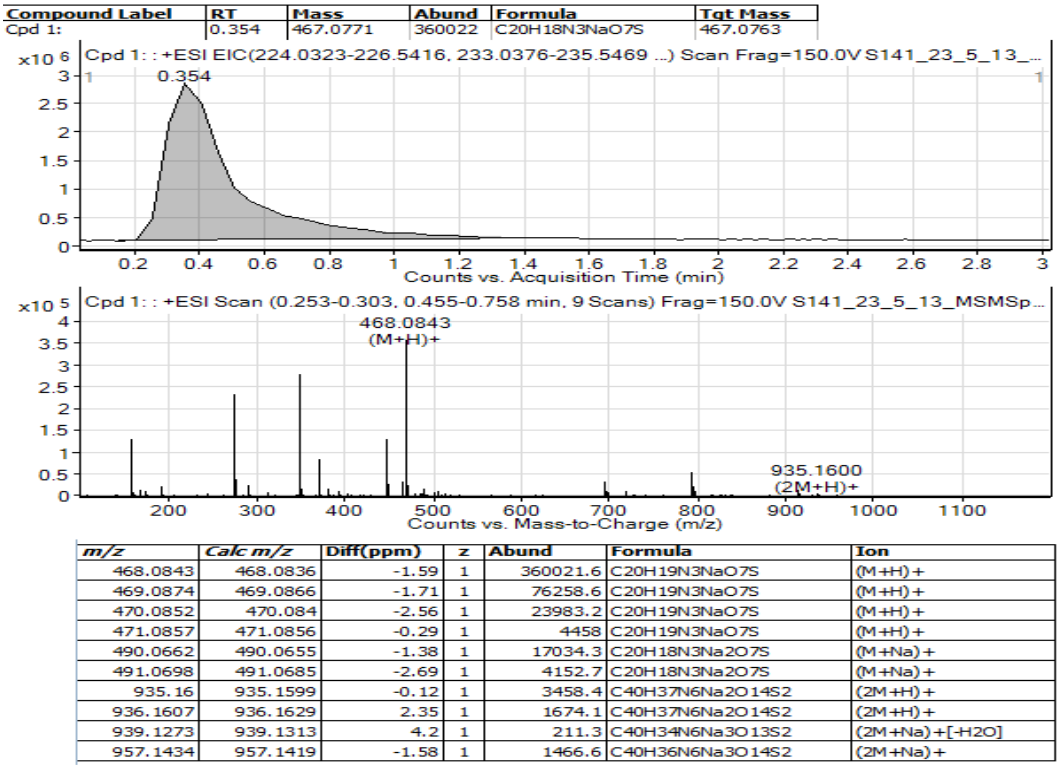


Figure (4.12): H-NMR spectrum of cephalexin maleate **ProD 1**.



Compound Label	RT	Mass	Abund	Formula	Tgt Mass
Cpd 1:	0.354	467.0771	360022	C20H18N3NaO7S	467.0763

m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
468.0843	468.0836	-1.59	1	360021.6	C20H19N3NaO7S	(M+H)+
469.0874	469.0866	-1.71	1	76258.6	C20H19N3NaO7S	(M+H)+
470.0852	470.084	-2.56	1	23983.2	C20H19N3NaO7S	(M+H)+
471.0857	471.0856	-0.29	1	4458	C20H19N3NaO7S	(M+H)+
490.0662	490.0655	-1.38	1	17034.3	C20H18N3Na2O7S	(M+Na)+
491.0698	491.0685	-2.69	1	4152.7	C20H18N3Na2O7S	(M+Na)+
935.16	935.1599	-0.12	1	3458.4	C40H37N6Na2O14S2	(2M+H)+
936.1607	936.1629	2.35	1	1674.1	C40H37N6Na2O14S2	(2M+H)+
939.1273	939.1313	4.2	1	211.3	C40H34N6Na3O13S2	(2M+Na)+[-H2O]
957.1434	957.1419	-1.58	1	1466.6	C40H36N6Na3O14S2	(2M+Na)+

Figure (4.13): LC-MS spectrum of cephalexin maleate **ProD 1.**

4.1.1.4 Melting point, FT-IR and NMR analysis results of cephalexin succinate ProD 2

1. Melting point of cephalexin succinate ProD 2 was 240 ^{o}C.
2. IR (KBr/ν_{max} cm^{-1}) 1755 (C=O), 1586 (C=C), 1643 (NH), 1665 amide C=O, 3627, 2879, 2933. The frequency of the reactant free amine group (NH2) from 3500-3700 was disappeared and the frequency of the more stable amide product was appeared on 1643, in addition carboxylic acid group frequency changed and appeared on 1665 as shown on **Figure 4.14.**
3. ^{1}H-NMR δ (ppm) CD3OD: 2 (CH3-C=C, S), 2.5 (COOH-CH2-CH2-C=O, M), 3 (NH-CH-CH-N-C, D, J=17.6 MHZ), 3.2 (NH-CH-CH-S), D, J=1.6 MHZ), 4.9 (NH-CH-C=O), S), 5.5 (AR-CH-C=O), S), 7.5 (aromatic, M). ^{1}H-NMR analysis shows the product has an additional signal in the region between 2-2.5 ppm as shown in **Figure 4.15**.
4. The product formula is C20H20N3NaO7S (yield 90%). LC-MS (positive mode) *m/z* 470.1 (M+\1) as shown in **Figure 4.16**.

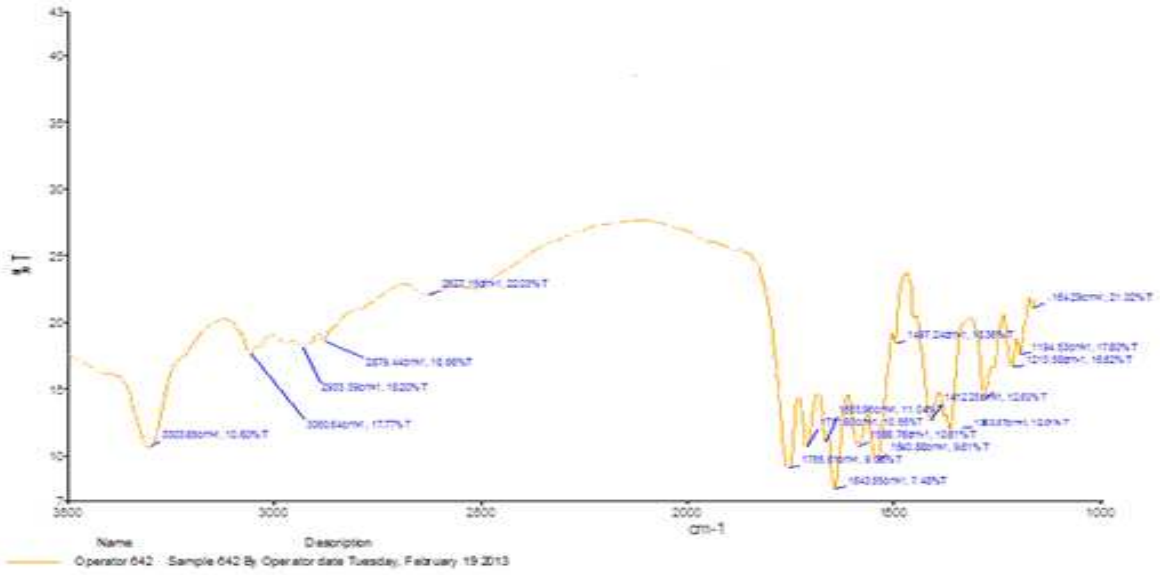


Figure (4.14): FT-IR spectrum of cephalexin succinate **ProD 2**.

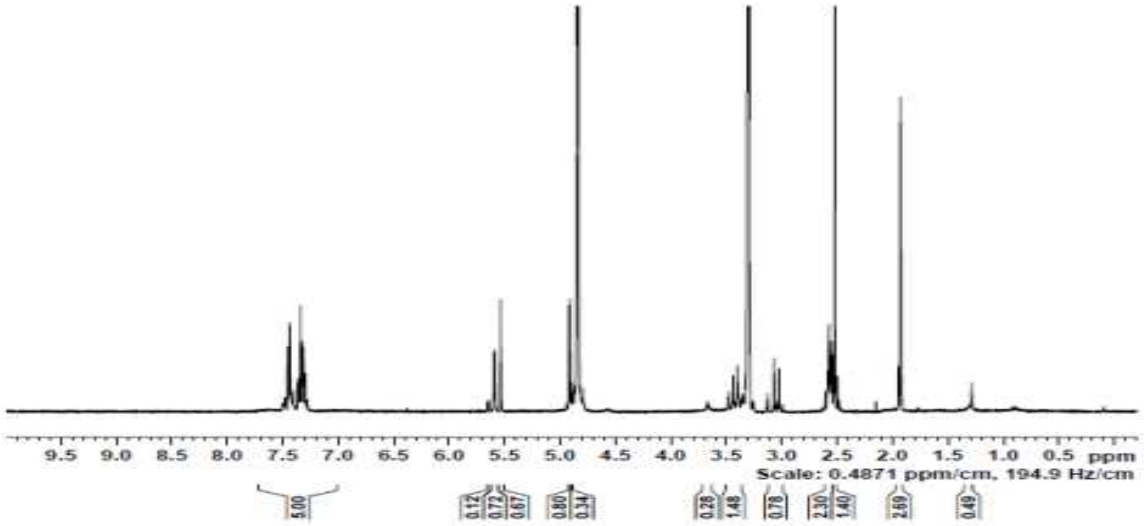
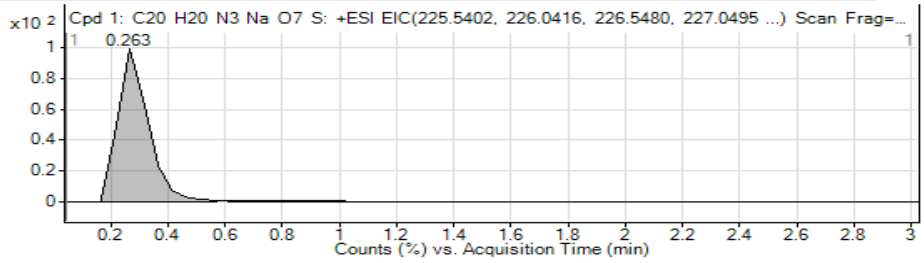


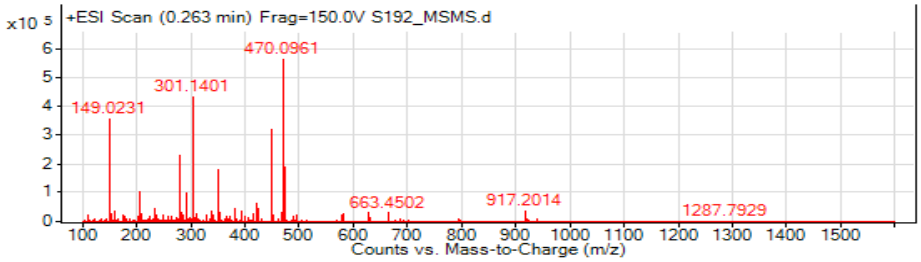
Figure (4.15): H-NMR spectrum of cephalexin succinate **ProD 2**.

Compound Label	m/z	RT	Algorithm	Mass
Cpd 1: C20 H20 N3 Na O7 S	470.1003	0.263	Find By Formula	469.093



User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
150	0	ESI



MS Spectrum Peak List

m/z	z	Abund	Formula	Ion
470.1003	1	307498.88	C20H20N3NaO7S	(M+H)+
471.1036	1	64422.98	C20H20N3NaO7S	(M+H)+
472.1006	1	21953.53	C20H20N3NaO7S	(M+H)+
473.1015	1	4097.1	C20H20N3NaO7S	(M+H)+
474.1031	1	829.43	C20H20N3NaO7S	(M+H)+
492.0815	1	14484.56	C20H20N3NaO7S	(M+Na)+
493.0834	1	3692.99	C20H20N3NaO7S	(M+Na)+

Figure (4.16): LC-MS spectrum of cephalexin succinate **ProD 2**.

The FT-IR, ^{1}H-NMR and LC-MS spectra mentioned before confirm that the predicted compounds are the desired ones.

4.2 Hydrolysis studies:

In this part of study, stability of amoxicillin prodrugs and cephalexin prodrugs was investigated using high performance liquid chromatography (HPLC). Peaks of standards and degradation products were monitored to determine the rate of cleavage of the four antibacterial prodrugs. Kinetic studies were performed at constant temperature (37 ^{o}C) and at ambient pressure in different buffers such as 1N HCl, pH 2.5 (stomach), pH 5 (intestine), and pH 7.4 (blood) which correspond to the physiological environments in the human body.

Calibration curves were made for the four antibacterial prodrugs. The results show that R^2 values were above 0.95 for all of the prodrugs as indicated in **Figure 4.17**. The hydrolysis

monitoring for the four prodrugs in 1N HCl, pH 2.5, pH 5, and pH 7.4 was conducted and the results of the study are summarized in **Figures 4.18-4.35**.

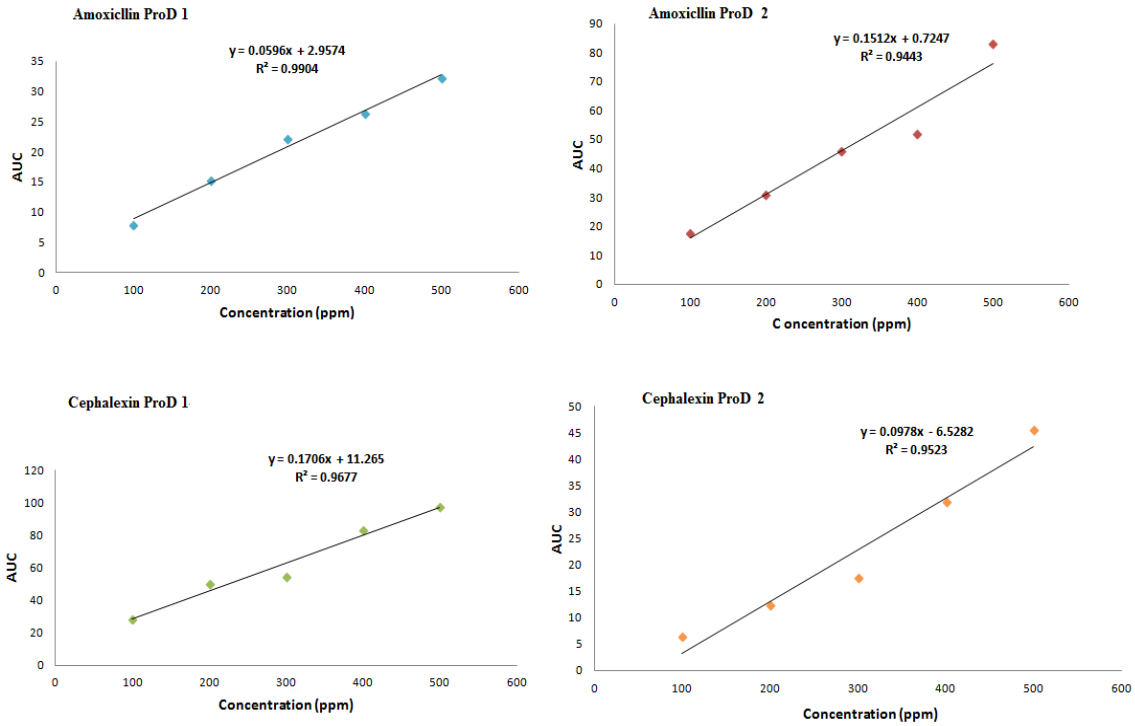


Figure (4.17): Calibration curves for amoxicillin **ProD 1-2** and Cephalexin **ProD 1-2**.

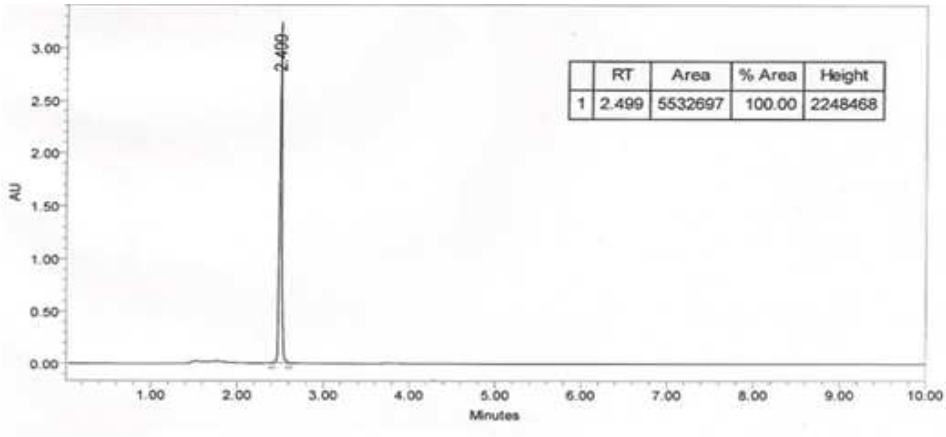


Figure (4.18): Amoxicillin standard.

1) Amoxicillin maleate **ProD 1** kinetic studies at 1N HCl, pH 2.5, 5.0 and 7.4**.**

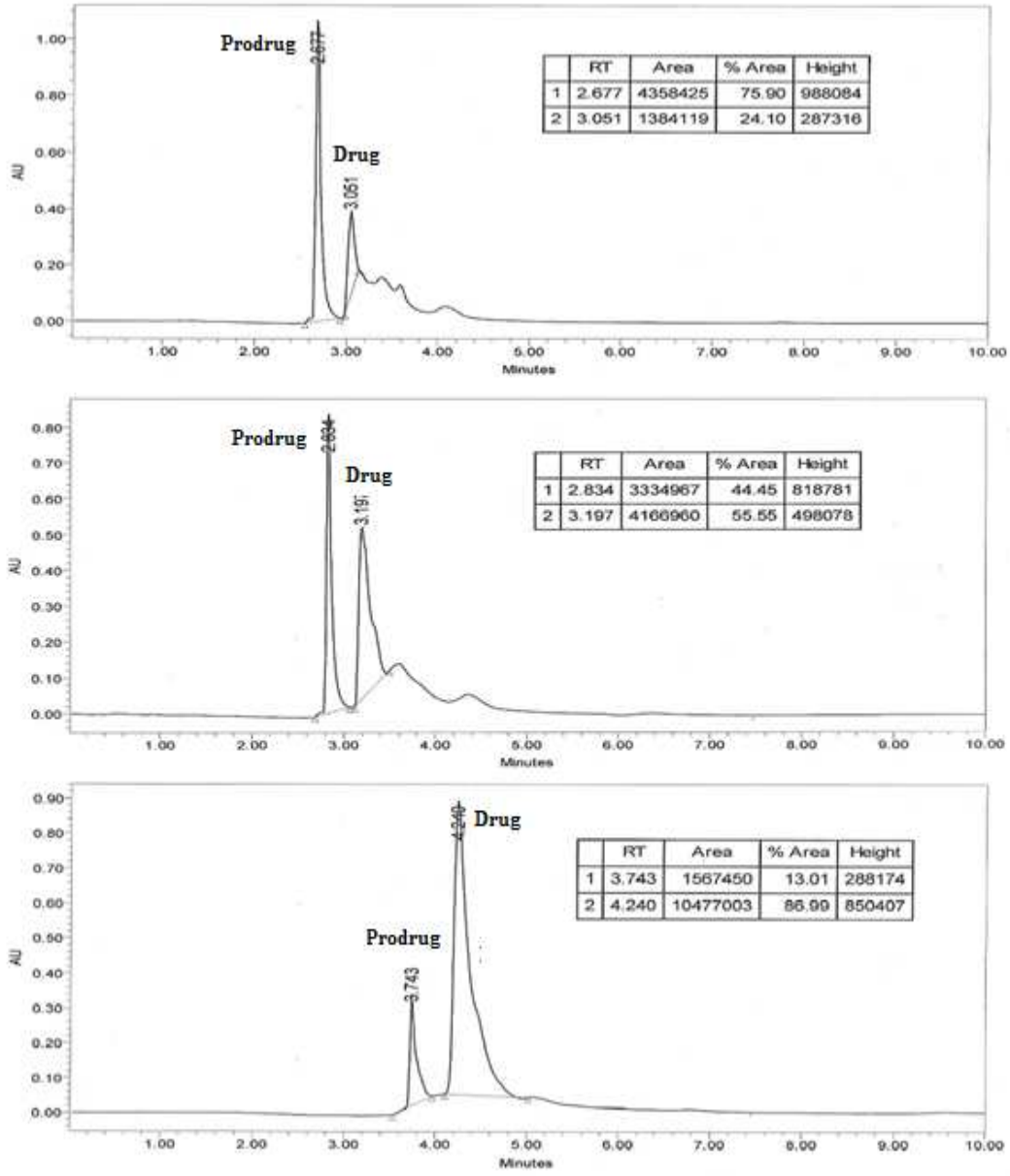
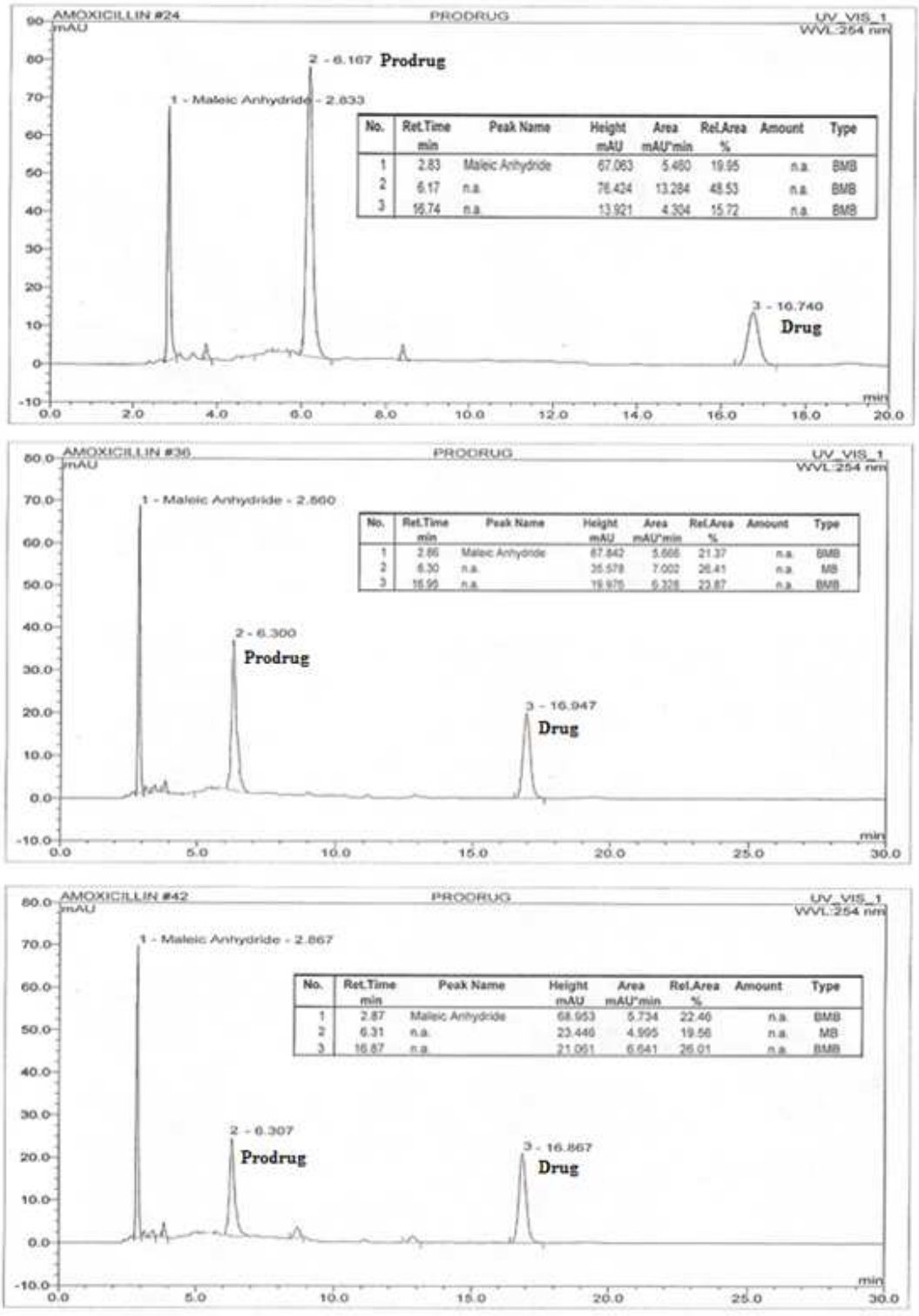


Figure (4.19): Amoxicillin maleate prodrug at 1N HCl at zero time, after 5 and 10 hours, respectively.



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	2.83	Maleic Anhydride	67.063	5.460	19.95	n.a.	BMB
2	6.17	n.a.	76.424	13.284	48.53	n.a.	BMB
3	16.74	n.a.	13.921	4.304	15.72	n.a.	BMB

No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	2.86	Maleic Anhydride	67.842	5.668	21.37	n.a.	BMB
2	6.30	n.a.	35.579	7.002	26.41	n.a.	MB
3	16.95	n.a.	19.975	6.328	22.87	n.a.	BMB

No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	2.87	Maleic Anhydride	68.953	5.734	22.46	n.a.	BMB
2	6.31	n.a.	23.446	4.995	19.56	n.a.	MB
3	16.87	n.a.	21.061	6.641	26.01	n.a.	BMB

Figure (4.20): Amoxicillin maleate prodrug at pH 2.5 at t = 0, after 5 hr and 10 hr, respectively.

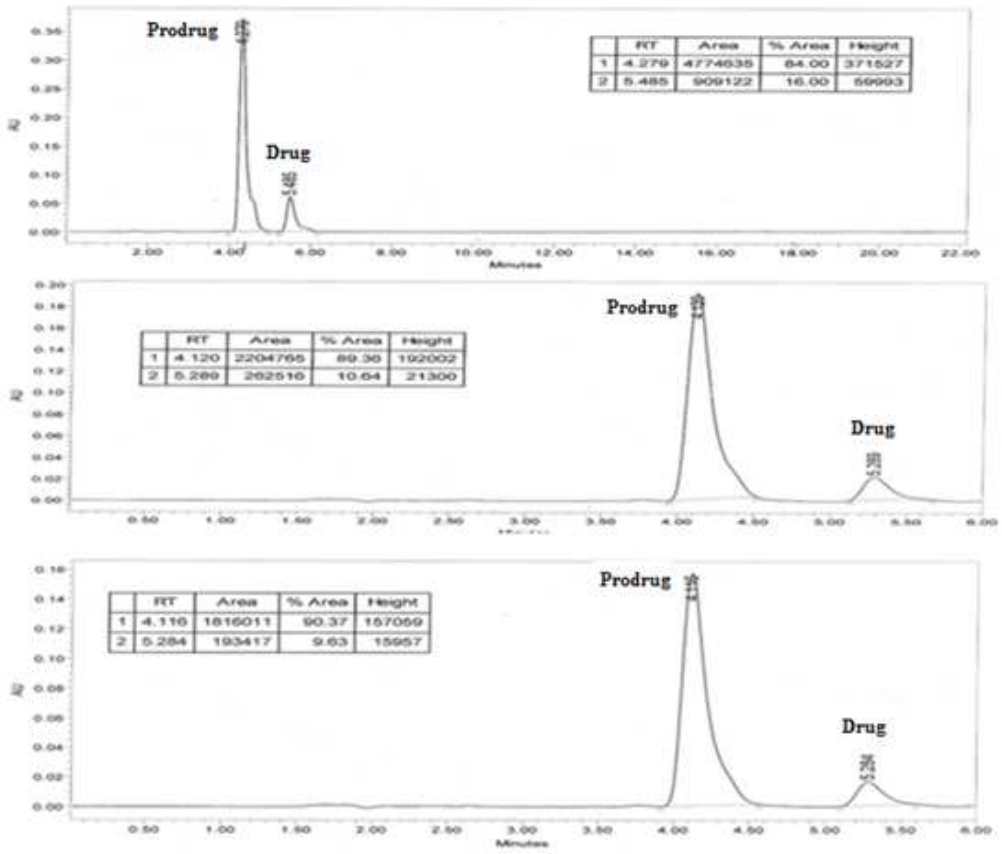


Figure (4.21): Amoxicillin maleate prodrug at pH 5.0 at t = 0, after 2 days and 4 days, respectively.

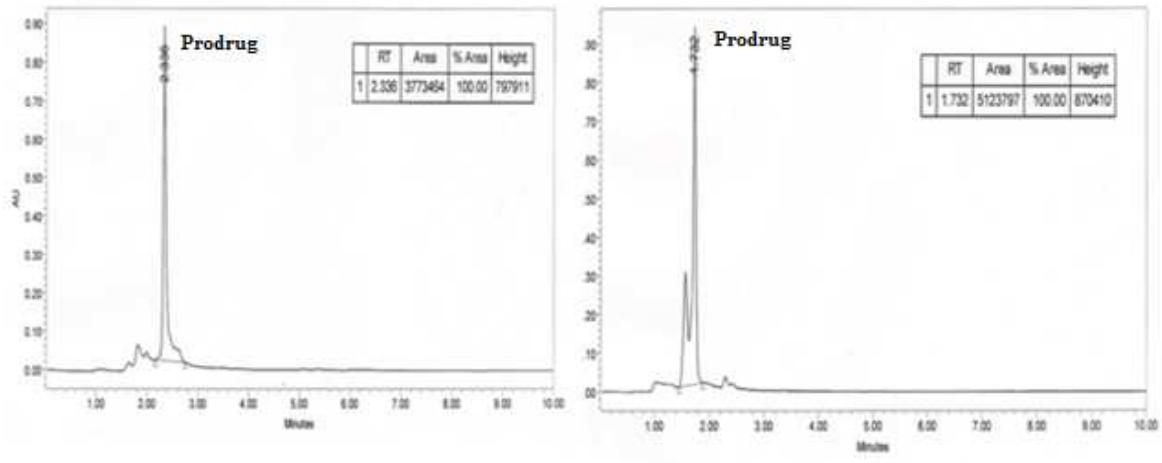


Figure (4.22): Amoxicillin maleate prodrug at pH 7.4 at t = 0 and after 3 months, respectively.

2) Amoxicillin succinate **ProD 2** kinetic studies at 1N HCl, pH 2.5, 5 and 7.4.

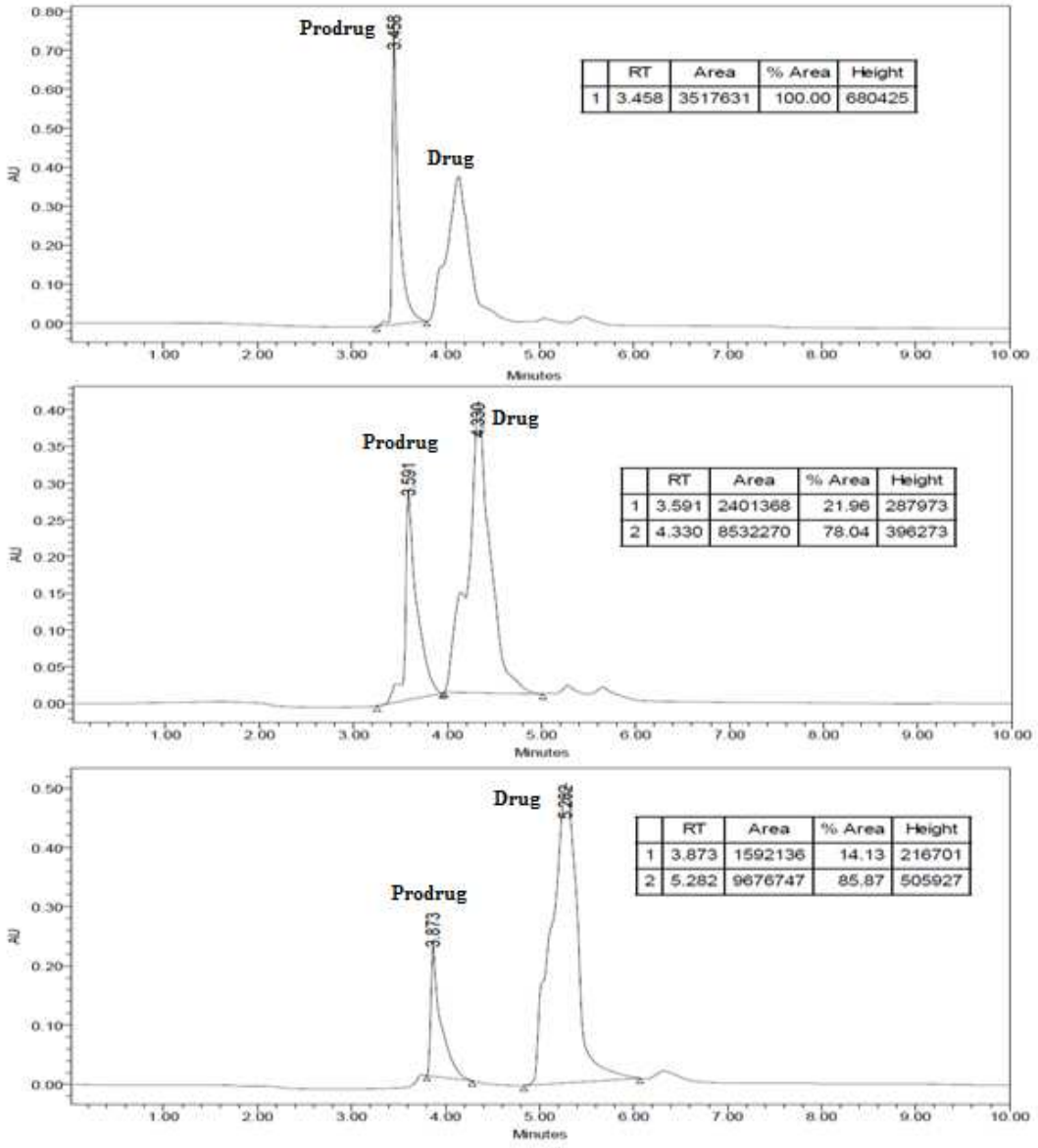


Figure (4.23): Amoxicillin succinate prodrug at 1 N HCl at t = 0, after 5 hour**s** and after 11 hours, respectively.

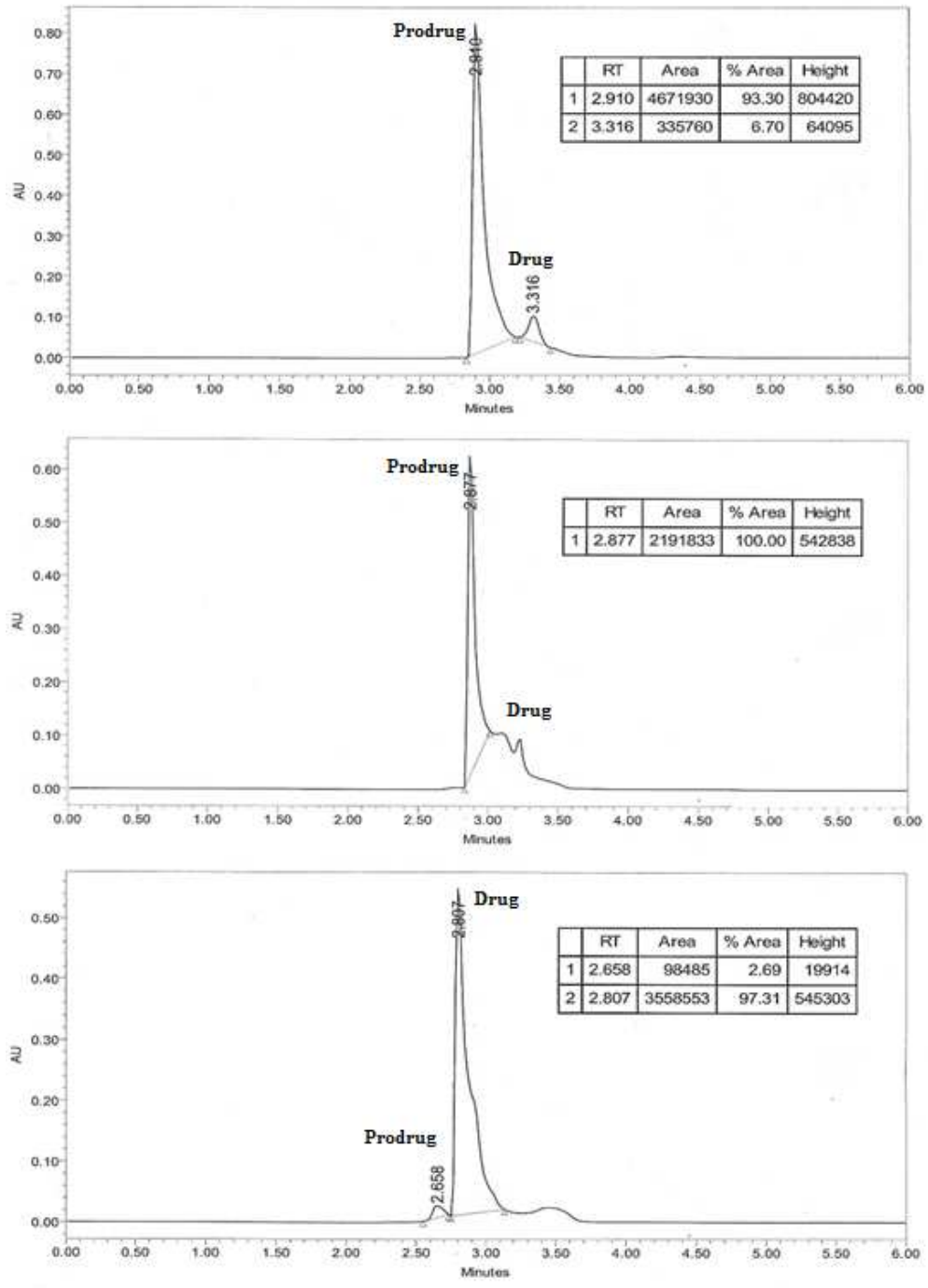


Figure (4.24): Amoxicillin succinate prodrug at pH 2.5 at t = 0, after 2 days and after 4 days, respectively.

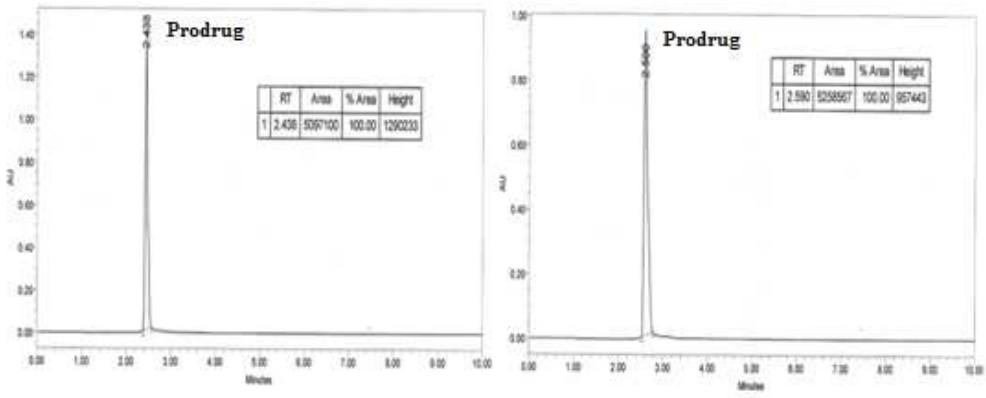


Figure (4.25): Amoxicillin succinate prodrug at pH 5.0 at t = 0 and after 7 days, respectively.

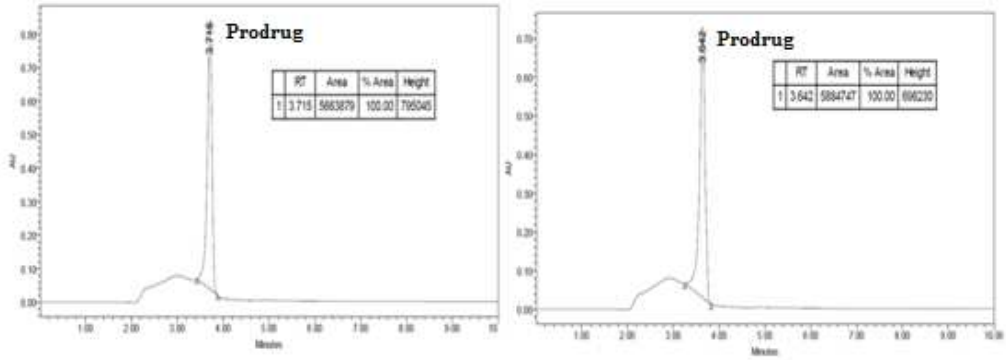


Figure (4.26): Amoxicillin succinate prodrug at pH 7.4 at t = 0 and after 7 days, respectively.

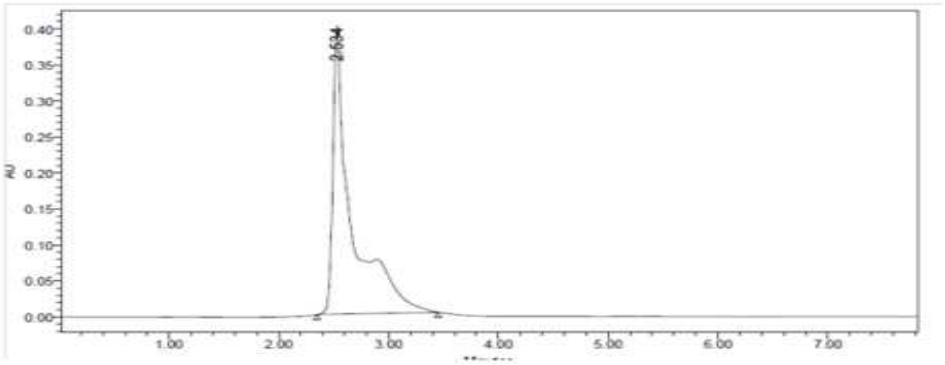


Figure (4.27): Cephalexin standard.

3) Cephalexin maleate **ProD 1** kinetic studies at 1N HCl, pH 2.5, 5.0 and 7.4.

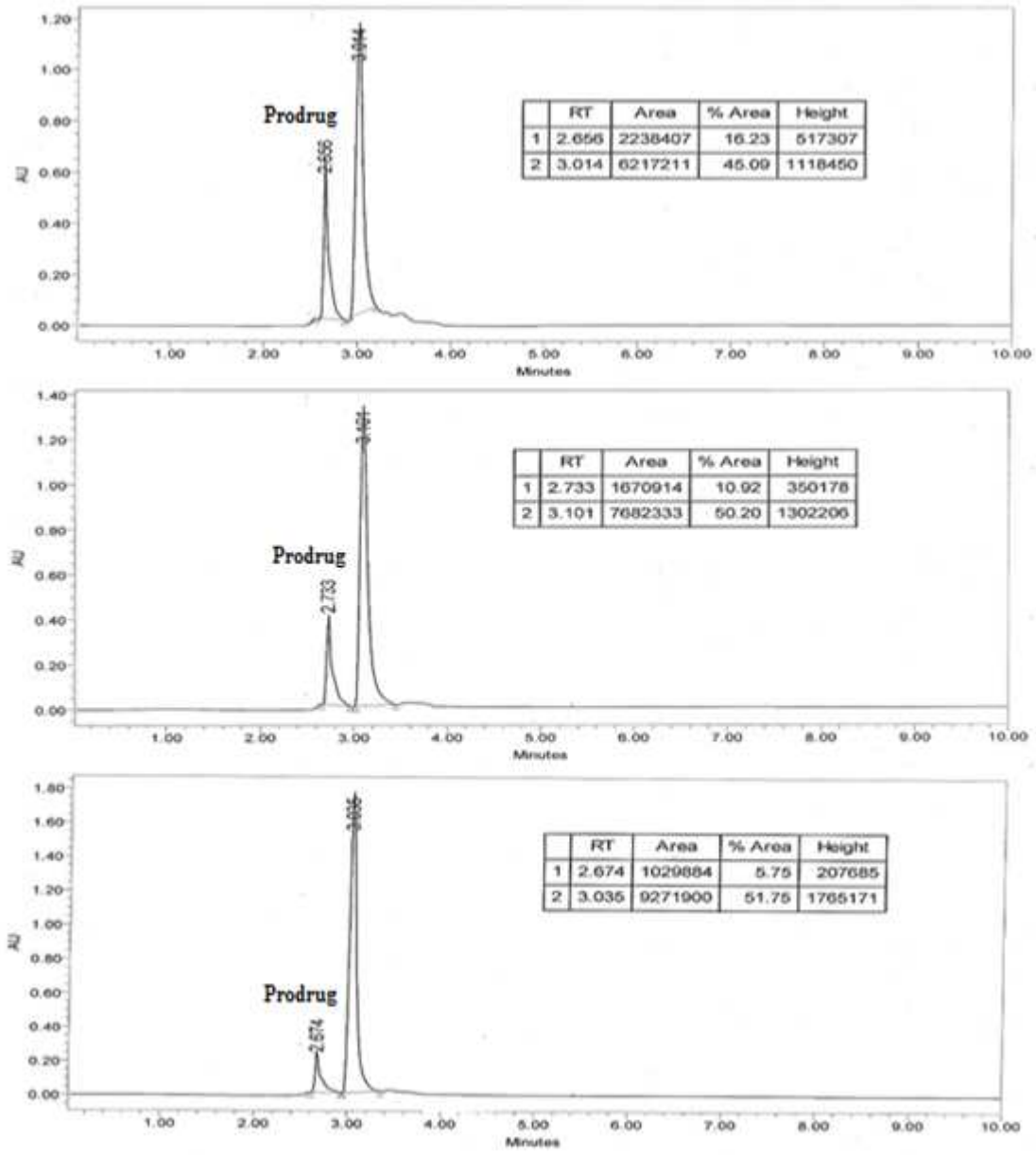


Figure (4.28): Cephalexin maleate prodrug at 1N HCl at t = 0, after 5 and 10 hours, respectively.

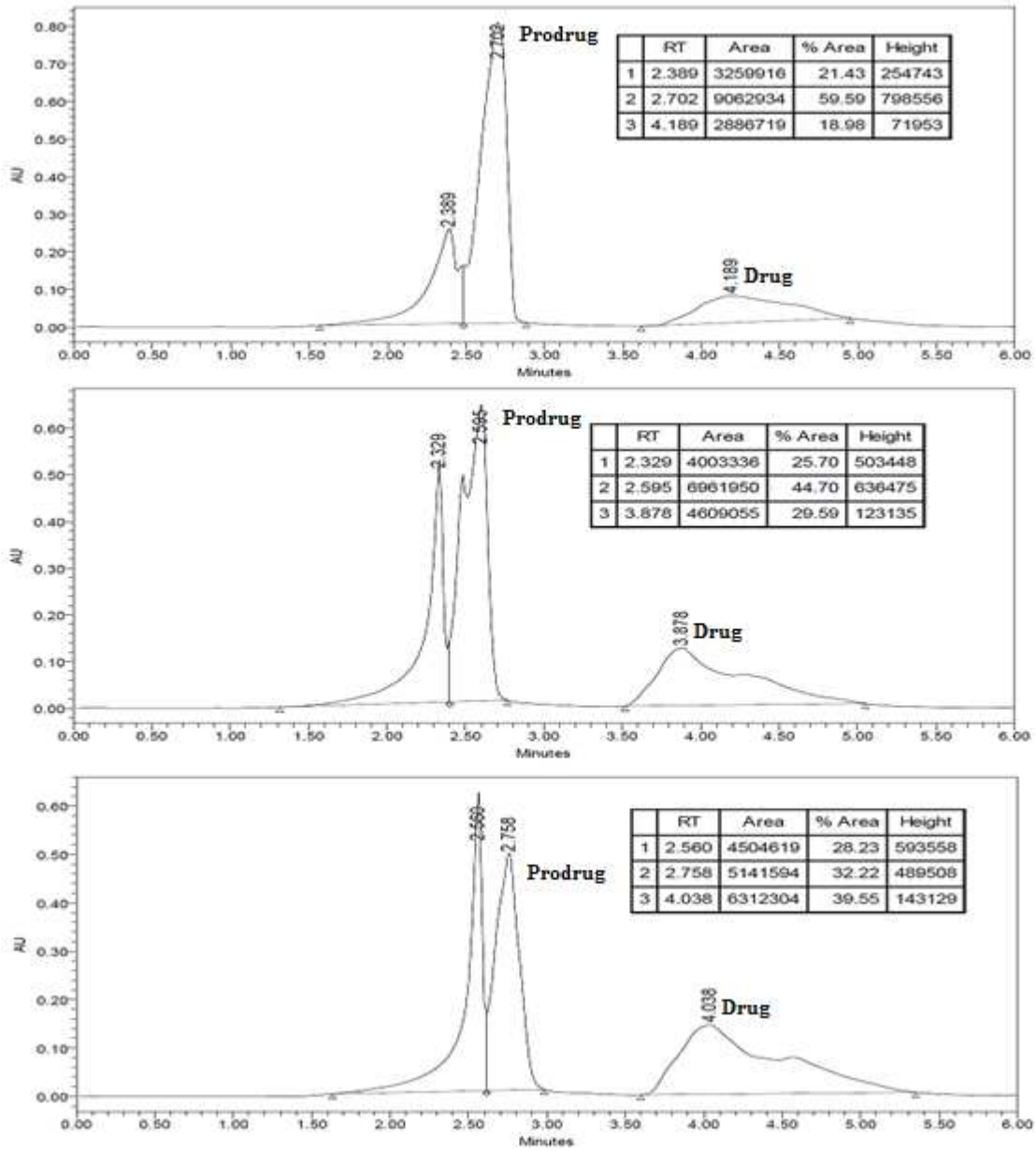


Figure (4.29): Cephalexin maleate prodrug at pH 2.5 at t0, after 11hr and 14 hr, respectively.

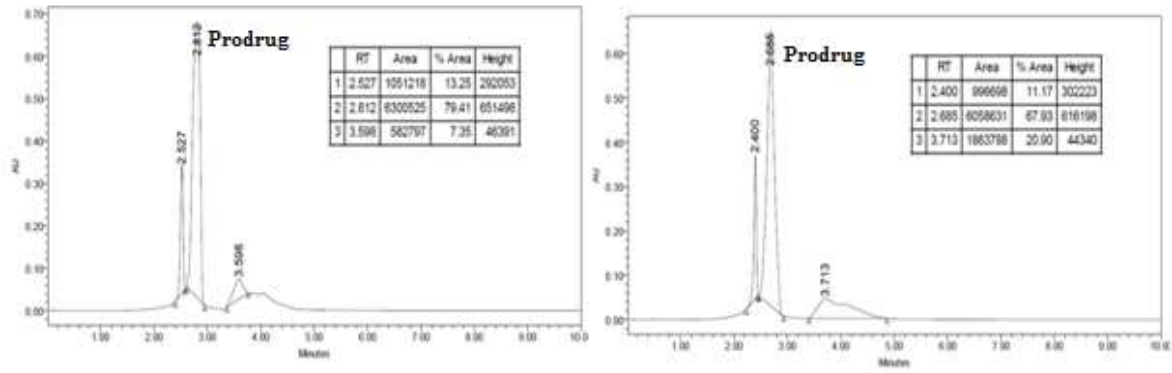


Figure (4.30): Cephalexin prodrug at pH 5.0 at t = 0 and after 4 days respectively.

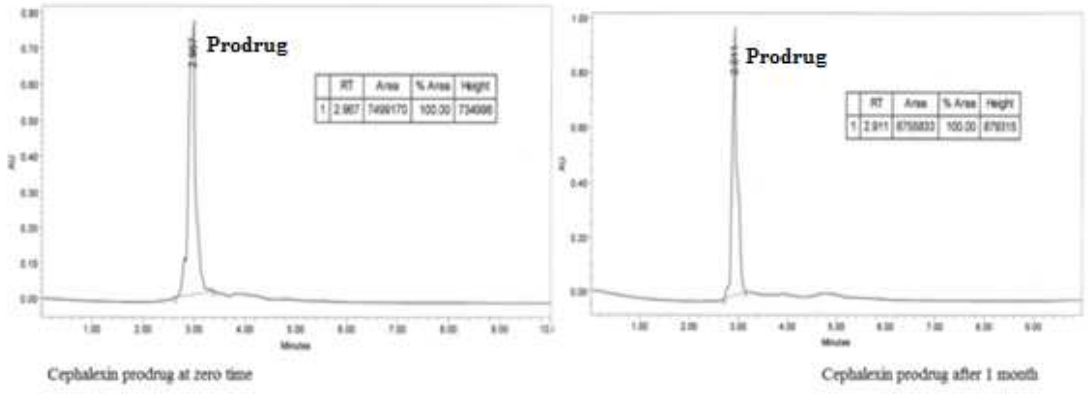


Figure (4.31): Cephalexin prodrug at pH 7.4 at t = 0 and after 1 month, respectively.

4) Cephalexin succinate **ProD 2** kinetics at 1N HCl, pH 2.5, 5.0 and 7.4.

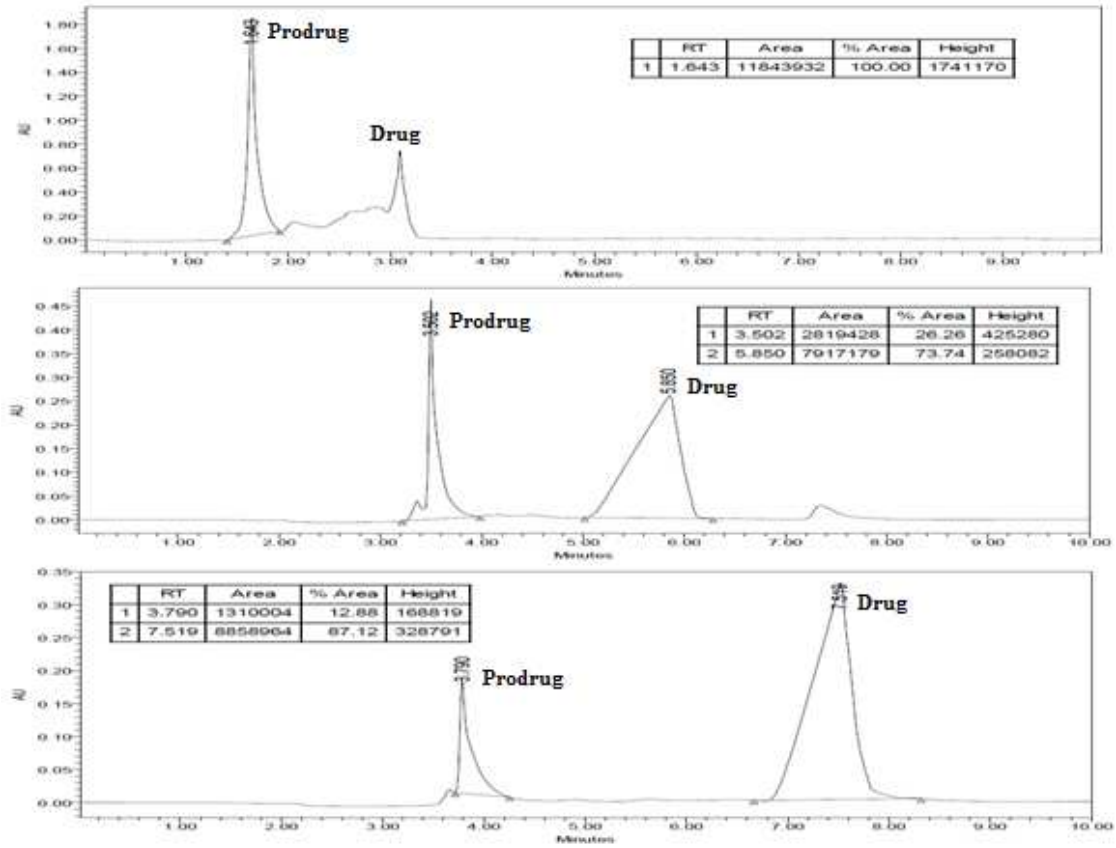


Figure (4.32): Cephalexin succinate prodrug at 1N HCl at t = 0, after 4 hours and after 8 hours, respectively.

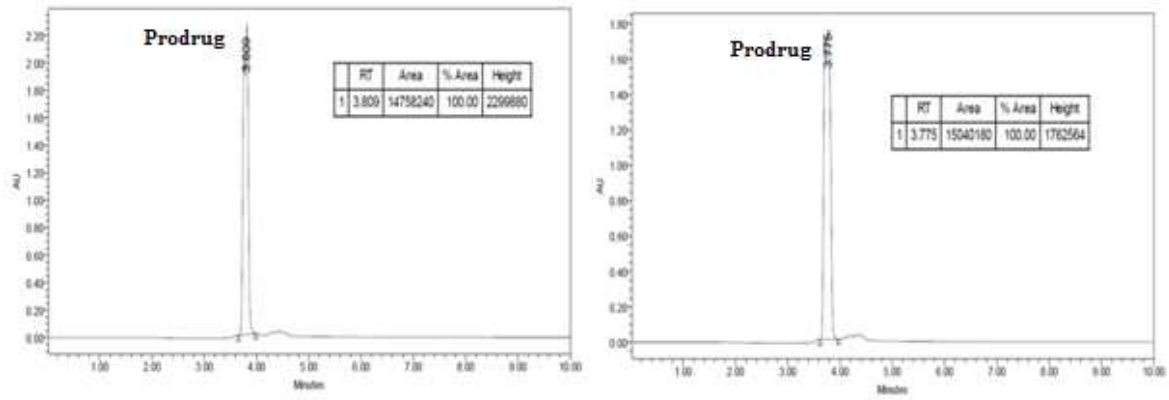


Figure (4.33): Cephalexin succinate prodrug at pH 2.5 at t = 0 and after 7 days respectively.

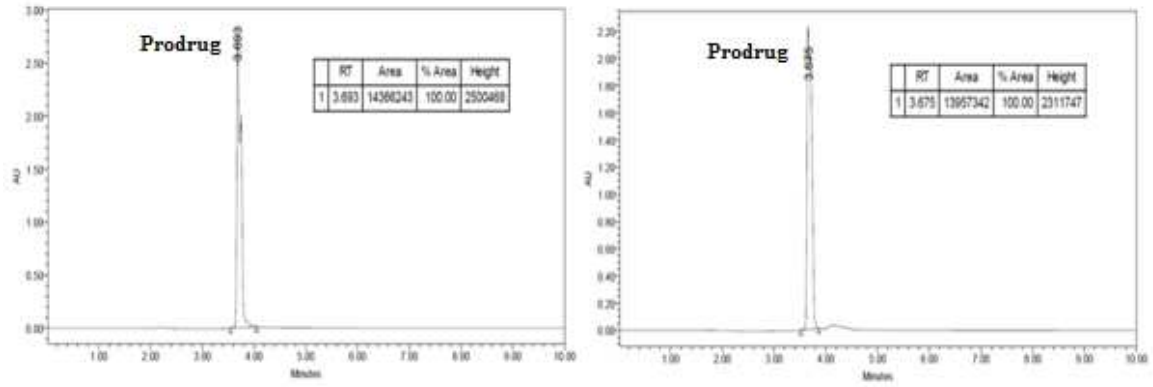


Figure (4.34): Cephalexin succinate prodrug at pH 5.0 at t = 0 and after 7 days, respectively.

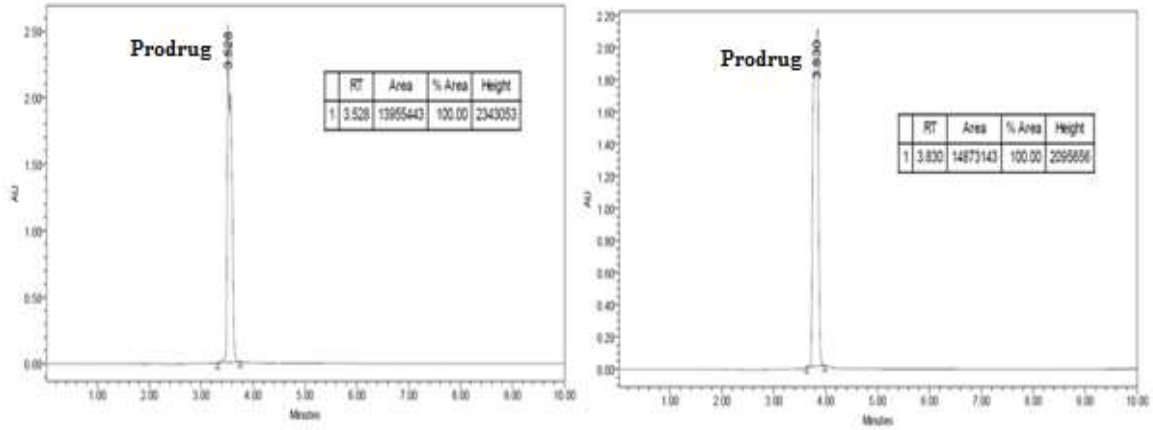


Figure (4.35): Cephalexin succinate prodrug at pH 7.4 at t = 0 and after 7 days, respectively.

4.3 *In vitro* intraconversion of amoxicillin (ProD 1-2) and cephalexin (ProD 1-2) to their parent drugs.

Based on previously reported DFT calculations and on experimental data for the acid-catalyzed hydrolysis of amide acids **1-9 (Figure 2.1)** [56, 61], four amoxicillin and cephalexin prodrugs were proposed utilizing two different linker **(Figures 4.36** and **4.37**, respectively**)**. As shown in **Figures 4.36** and **4.37**, the antibacterial prodrugs, amoxicillin **ProD 1-2** and cephalexin **ProD 1-2** are composed of a promoiety containing a carboxylic acid group (hydrophilic moiety) and the rest of the antibacterial prodrug molecule (a lipophilic moiety).

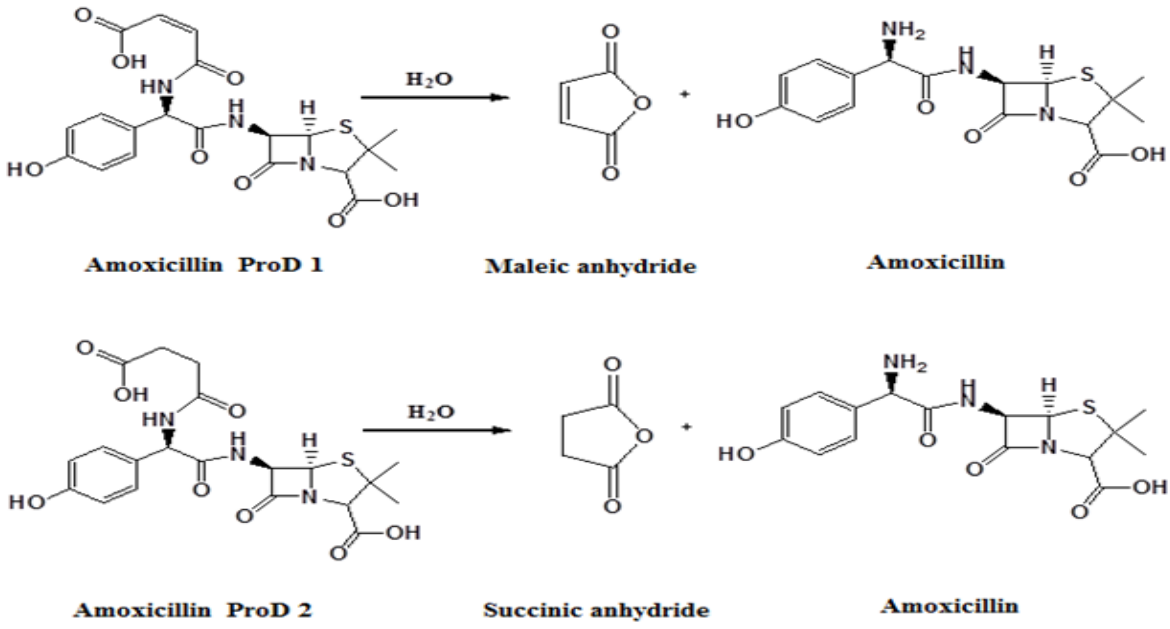


Figure (4.36): Acid-catalyzed hydrolysis of amoxicillin **ProD 1-2**.

The combination of both, the hydrophilic and lipophilic groups provides a prodrug entity with a potential to be with a high permeability (a moderate HLB). It should be emphasized, that the HLB value of the prodrug entity will be determined upon the pH of the target physiological environment. In the stomach where the pH is in the range 1-2, it is expected that prodrugs, amoxicillin **ProD 1-2** and cephalexin **ProD 1-2** will be in a free carboxylic acid form (a relatively high hydrophobicity) whereas in the blood stream circulation where the pH is 7.4 a carboxylate anion (a relatively low hydrophobicity) is expected to be predominant form. Our strategy was to prepare amoxicillin **ProD 1-2** and cephalexin **ProD 1-2** as sodium or potassium carboxylates due to their high stability in neutral aqueous

medium. It should be indicated that compounds **1-9** undergo a relatively fast hydrolysis in acidic aqueous medium whereas they are quite stable at neutral pH.

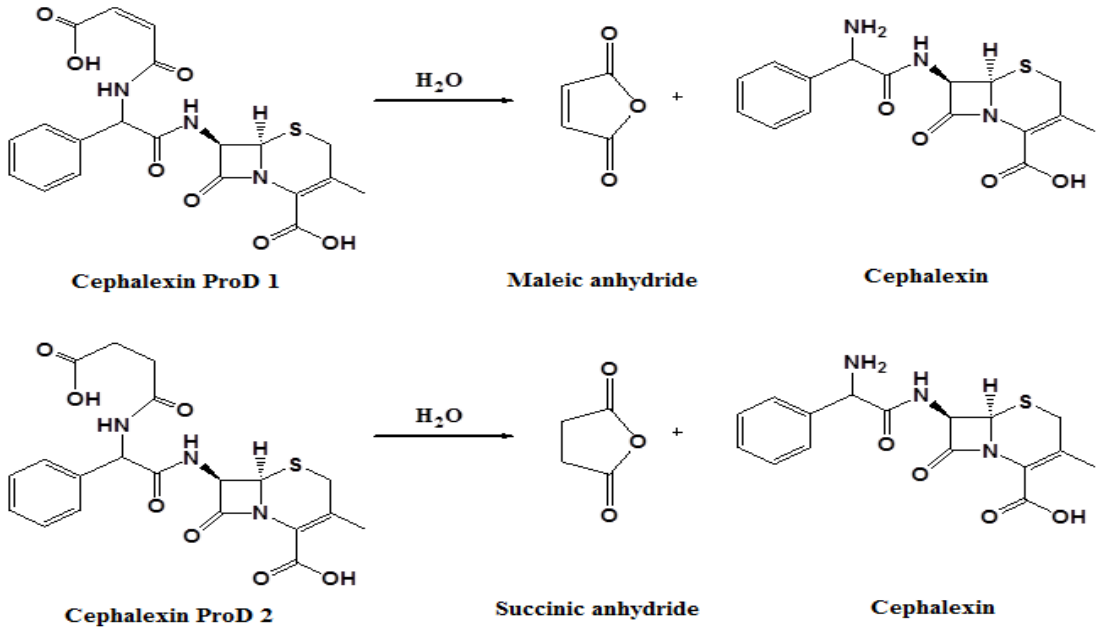


Figure (4.37): Acid-catalyzed hydrolysis of cephalexin **ProD 1-2**.

The hydrolysis kinetic studies for amoxicillin **ProD 1-2** and cephalexin **ProD 1-2** were carried out in aqueous buffers in the same manner to that executed by Kirby *et al.* on maleamic acids **1-9**. This is to investigate whether the antibacterial prodrugs undergo hydrolysis in aqueous medium and to what extent or not, suggesting the fate of the prodrugs in the system. The kinetics for the acid-catalyzed hydrolysis of the synthesized amoxicillin **ProD 1-2** and cephalexin **ProD 1-2** were carried out in four different aqueous media: 1 N HCl, buffer pH 2.5, buffer pH 5 and buffer pH 7.4. Under the experimental conditions the four antibacterial prodrugs intraconverted to release the parent drugs **(Figures 4.38-4.41)** as was determined by HPLC analysis. For amoxicillin and cephalexin prodrugs, at constant temperature and pH the hydrolysis reaction displayed strict first order kinetics as the k_{obs} was quite constant and a straight line was obtained on plotting log concentration of residual prodrug verves time. k_{obs} and the corresponding $t_{1/2}$ for amoxicillin **ProD 1-2** and cephalexin **ProD 1-2** in the different media were calculated from the linear regression equation obtained from the correlation of log concentration of the residual prodrug verses time, log concentration versus time for the four prodrugs were obtained from plotting the AUC of each product versus time. The kinetic data for amoxicillin **ProD 1-2** and cephalexin **ProD 1-2** are listed from **Tables 4.1**, **4.2**, **4.3** and **4.4**. It is worth noting that 1N HCl and pH 2.5 were selected to examine the

intraconversion of amoxicillin **ProD 1-2** and cephalexin **ProD 1-2** in the pH as of stomach, since the mean fasting stomach pH of adult is approximately 1-2.5. Furthermore, environment of buffer pH 5 mimics that of beginning small intestine route, whereas pH 7.4 was selected to determine the intraconversion of the tested prodrugs in blood circulation system. Acid-catalyzed hydrolysis of amoxicillin **ProD 1-2** and cephalexin **ProD 1-2** was found to be much higher in 1N HCl than at pH 2.5 and 5 **(Figures 4.38-4.41)**. At 1N HCl the $t_{1/2}$ values for the intraconversion of amoxicillin **ProD 1** and cephalexin **ProD 1** were about 2.5 hours and that of amoxicillin **ProD 2** and cephalexin **ProD 2** were about 7 and 6 hours, respectively. On the other hand, at pH 7.4, both prodrugs amoxicillin **ProD 1-2** and cephalexin **ProD 1-2** were quite stable and no release of the parent drugs was observed. At pH 5 the hydrolysis of prodrugs amoxicillin **ProD 1-2** and cephalexin **ProD 1-2** was too slow**.** This is because the pK_a of amoxicillin **ProD 1-2** and cephalexin **ProD 1-2** is in the range of 3-4, it is expected that at pH 5 the anionic form of the prodrug will be dominant and the percentage of the free acidic form that undergoes an acid-catalyzed hydrolysis will be relatively low. In 1N HCl and pH 2.5 most of the prodrug will exist as the free acid form and while at pH 7.4 most of the prodrug will be in the anionic form. Thus, the discrepancy in rates between amoxicillin **ProD 1** and amoxicillin **ProD 2** at the different pH buffers is attributed to the strained effects imposed in the case of amoxicillin **ProD 1**, which upon cleavage gives maleic anhydride while in the case of amoxicillin **ProD 2**, the byproduct is the less-strained succinic anhydride. The same picture is also applied for the discrepancy between cephalexin **ProD 1** and cephalexin **ProD 2**. It is worth noting that previous DFT calculations [61] and experimental data [56] on the acid catalyzed hydrolysis of **1-9** revealed that the efficiency of the intramolecular acid-catalyzed hydrolysis by the carboxyl group is remarkably sensitive to the pattern of substitution on the carbon–carbon double bond. The rate of hydrolysis was found to be linearly correlated with the strain energy of the tetrahedral intermediate or the product. Systems having strained tetrahedral intermediates or products experience low rates and vice versa. In addition, the difference in the rates between amoxicillin **ProD 1-2** and cephalexin **ProD 1-2** is due to their conformational structures, in case of amoxicillin **ProD 1-2** the distance between the electrophile and nucelophile is less than cephalexin **ProD 1-2** hence the higher in rates.

Table (4.1). The observed *k* value and $t_{1/2}$ of amoxicillin **ProD 1** in 1N HCl, pH 2.5, 5.0 and 7.4

Medium	k_{obs} (h^{-1})	$t_{1/2}$ (h)
1 N HCl	2.33×10^{-4}	2.5
Buffer pH 2.5	9.60×10^{-5}	7
Buffer pH 5.0	7.55×10^{-6}	81
Buffer pH 7.4	No reaction	----

Table (4.2). The observed *k* value and $t_{1/2}$ of amoxicillin **ProD 2** in 1N HCl, pH 2.5, 5.0 and 7.4

Medium	k_{obs} (h^{-1})	$t_{1/2}$ (h)
1 N HCl	8.37×10^{-5}	8.2
Buffer pH 2.5	1.54×10^{-5}	44
Buffer pH 5.0	No reaction	----
Buffer pH 7.4	No reaction	----

Table (4.3). The observed *k* value and $t_{1/2}$ of cephalexin **ProD 1** in 1N HCl, pH 2.5, 5.0 and 7.4

Medium	k_{obs} (h^{-1})	$t_{1/2}$ (h)
1 N HCl	2.41×10^{-4}	2.4
Buffer pH 2.5	4.17×10^{-5}	14
Buffer pH 5.0	No reaction	----
Buffer pH 7.4	No reaction	----

Table (4.4). The observed *k* value and $t_{1/2}$ of cephalexin **ProD 2** in 1N HCl, pH 2.5, 5.0 and 7.4

Medium	k_{obs} (h^{-1})	$t_{1/2}$ (h)
1 N HCl	11.38×10^{-5}	6
Buffer pH 2.5	No reaction	---
Buffer pH 5.0	No reaction	---
Buffer pH 7.4	No reaction	----

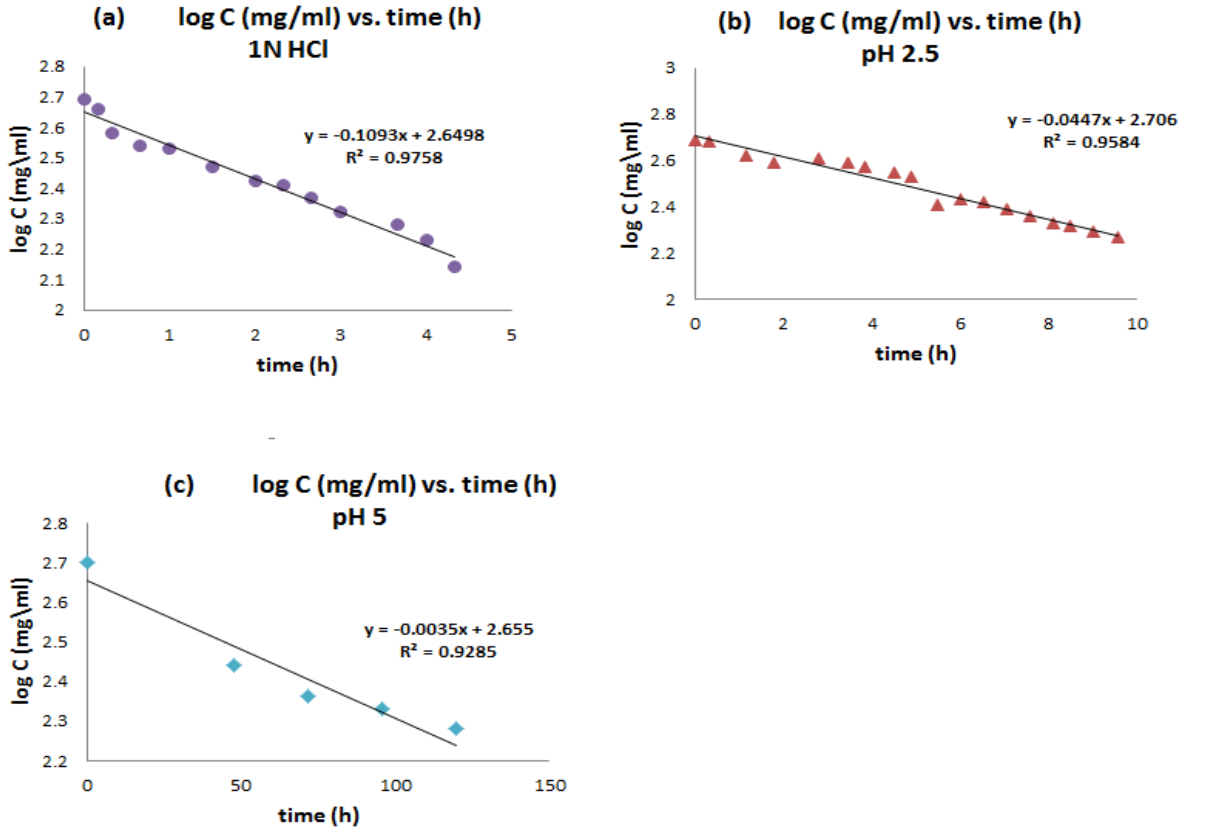


Figure (4.38): First order hydrolysis plot of amoxicillin **ProD 1** in (a) 1N HCl, (b) buffer pH 2.5 and (c) buffer pH 5.0.

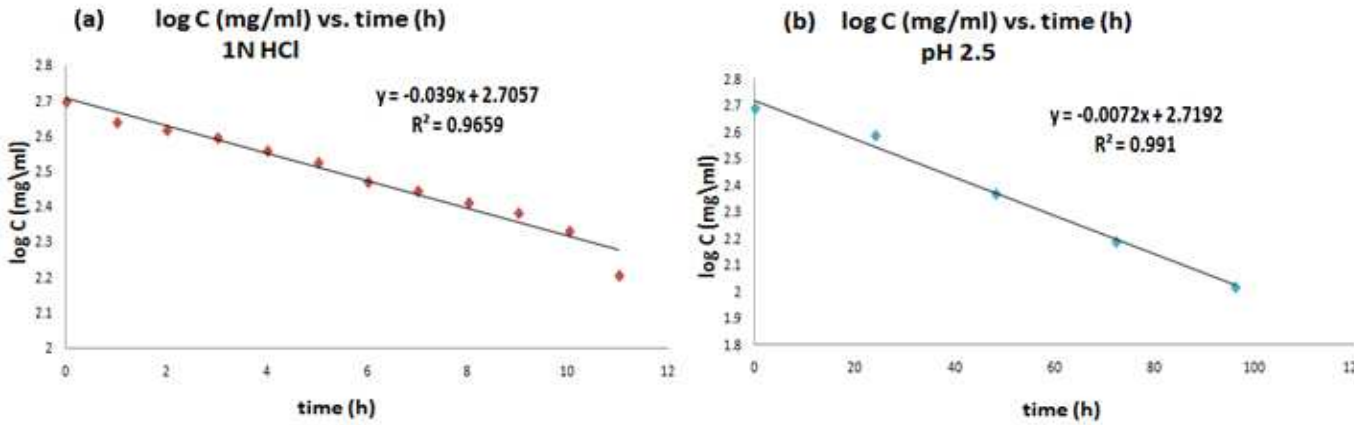


Figure (4.39): First order hydrolysis plot of amoxicillin **ProD 2** in (a) 1N HCl, (b) buffer pH 2.5 and (c) buffer pH 5.0.

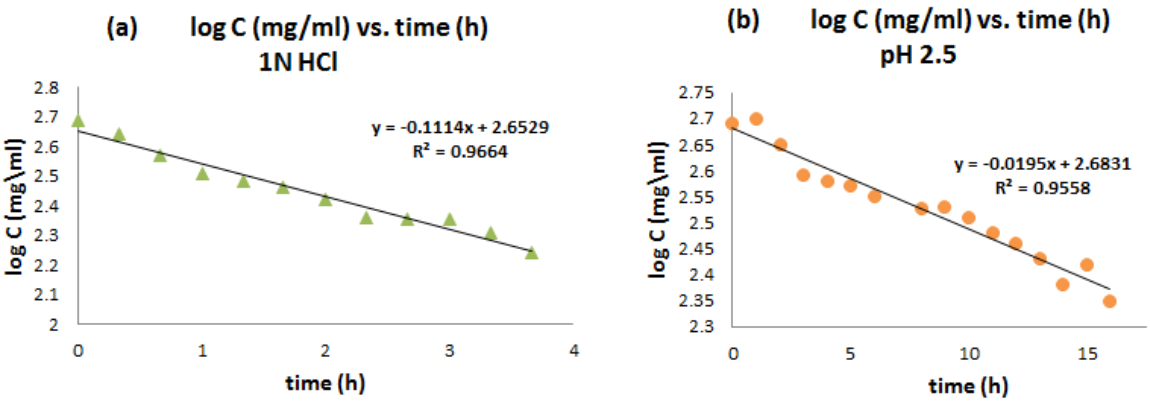


Figure (4.40): First order hydrolysis plot of cephalexin **ProD 1** in (a) 1N HCl, (b) buffer pH 2.5 and (c) buffer pH 5.0.

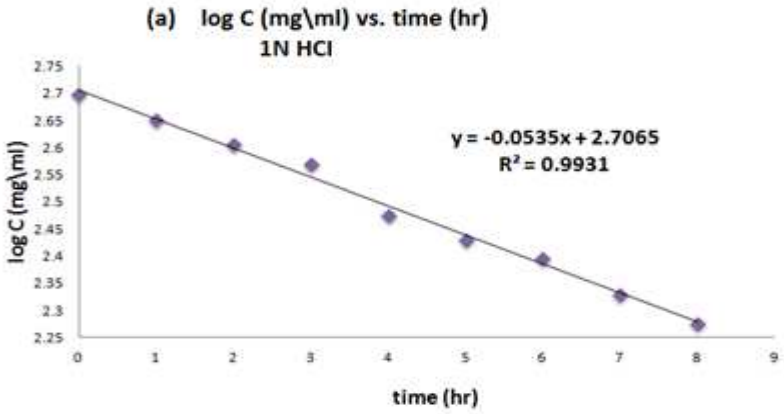


Figure (4.41): First order hydrolysis plot of cephalexin **ProD 2** in 1N HCl.

Conclusions and Future directions

Chapter five

Conclusions and Future directions

5.1 Conclusions

The future of prodrug design is forthcoming yet extremely challenging. Progresses must be made in better understanding the chemistry of many organic mechanisms that can be effectively exploited to push forward the development and advances of even more types of prodrugs. The understanding of the organic reactions mechanisms of intramolecular processes will be the next major milestone in this field. It is envisioned that the future of prodrug design holds the ability to produce safe and efficacious delivery of a wide range of active small molecule and biotherapeutics.

Based on Kirby's enzyme model, we utilized two linkers for making novel prodrugs of both antibacterials, amoxicillin and cephalexin with the expectation to have prodrugs lacking the bitter sensation of their parent drugs as well as to be cleaved in different rates. The quantum mechanics (QM) calculations using different methods revealed that the acid-catalyzed hydrolysis efficiency of processes **1-9**, amoxicillin **ProD 1-2** and cephalexin **ProD 1-2** is significantly sensitive to the pattern of substitution on the carbon-carbon double bond and nature of the amine leaving group. According to DFT calculations, the four antibacterial prodrugs will exist as a free carboxylic acid form (a relatively high lipophilicity) in the stomach, whereas in the blood circulation system, the carboxylate anion form (a relatively low lipophilicity) will be predominant. The synthesized amide prodrugs of amoxicillin **ProD 1-2** and cephalexin **ProD 1-2** were found to undergo hydrolysis in acidic aqueous medium, whereas they were stable at pH 7.4. The predicted $t_{1/2}$ and k_{obs} of amoxicillin **ProD 1-2** and cephalexin **ProD 1-2** were calculated. Kinetics studies on the interconversion of the newly synthesized amoxicillin and cephalexin prodrugs revealed that the $t_{1/2}$ was largely affected by the pH medium as predicted.

In vitro binding test to bitter taste receptors for the four antibacterial prodrugs, amoxicillin **ProD1**-**ProD2** and cephalexin **ProD1**-**ProD2**, were found to be bitterless. The bitter taste masking is believed to be via altering the ability of the drug to interact with bitter taste

receptors. The strategy of the synthesis and kinetic studies of these antibacterial drugs was to achieve desirable amoxicillin and cephalexin prodrugs capable of releasing amoxicillin and cephalexin parental drugs in a controlled release manner and enhancing their stability and solubility with masking their bitter taste sensation.

5.2 Future directions:

In vivo pharmacokinetic studies and in vitro binding to bitter taste receptors for amoxicillin **ProD 1-2** and cephalexin **ProD 1-2** will be done. In vivo pharmacokinetic studies will be done in order to determine the bioavailability and the duration of action of the tested prodrugs.

References:-

1. Sohi, H., Sultana, Y., & Khar, R. K. (2004). Taste masking technologies in oral pharmaceuticals: recent developments and approaches. *Drug development and industrial pharmacy*, *30*(5), 429-448.
2. Rooseboom, M., Commandeur, J. N., & Vermeulen, N. P. (2004). Enzyme-catalyzed activation of anticancer prodrugs. *Pharmacological reviews*, *56*(1), 53-102.
3. Dahan, A., Khamis, M., Agbaria, R., & Karaman, R. (2012). Targeted prodrugs in oral drug delivery: the modern molecular biopharmaceutical approach. *Expert Opinion on Drug Delivery*, *9*(8), 1001-1013.
4. Claude N. Cohen (Ed.). (1996). *Guidebook on molecular modeling in drug design*. Access Online via Elsevier.
5. Karaman, R. (2013). Prodrugs for masking bitter taste of antibacterial drugs—a computational approach. *Journal of molecular modeling*, 1-14.
6. Reilly WJ. (2002). Pharmaceutical necessities in Remington: The science and practice of pharmacy, Mack Publishing Company; pp1018-1020.
7. Gowan Jr. Walter G., Richard D. Bruce. (2003). Aliphatic esters as a solventless coating for pharmaceuticals. No. CA2082137. 19 Aug.
8. Gowthamarajan, K., Kulkarni, G. T., & Kumar, M. N. (2004). Pop the pills without bitterness. *resonance*, *9*(12), 25-32.
9. Davis JD. (2000). Drug Cosmet. India: Encyclopedia of pharmaceutical technology, 2: 1-5.
10. Bakan JA. (1986). Microencapsulation, Theory and practice of Industrial Pharmacy, Third Edition; pp 412-429.
11. Ikeda, K. (2002). New seasonings. *Chemical senses*, *27*(9), 847-849.
12. Maehashi, K., Matano, M., Wang, H., Vo, L. A., Yamamoto, Y., & Huang, L. (2008). Bitter peptides activate hTAS2Rs, the human bitter receptors. *Biochemical and biophysical research communications*, *365*(4), 851-855.
13. Lindemann, B. (2001). *Receptors and transduction in taste*. Nature, **413**(6852): p. 219-225.
14. Behrens, M., & Meyerhof, W. (2006). Bitter taste receptors and human bitter taste perception. *Cell Mol Life Sci*, *63*(13), 1501-1509.

15. Meyerhof, W., Batram, C., Kuhn, C., Brockhoff, A., Chudoba, E., Bufe, B., ... & Behrens, M. (2010). The molecular receptive ranges of human TAS2R bitter taste receptors. *Chemical senses*, *35*(2), 157-170.
16. Wiener, A., Shudler, M., Levit, A., & Niv, M. Y. (2012). *BitterDB: a database of bitter compounds.* Nucleic acids research, 2012. **40**(D1): p. D413-D419.
17. Bufe, B., Hofmann, T., Krautwurst, D., Raguse, J. D., & Meyerhof, W. (2002). The human TAS2R16 receptor mediates bitter taste in response to β-glucopyranosides. *Nature genetics*, *32*(3), 397-401.
18. Sakurai T., Misaka T., Ishiguro M., Masuda K., Sugawara T., Ito K., Kobayashi T., Matsuo S., Ishimaru Y., Asakura T. et al. (2010). Characterization of the beta-D-glucopyranoside binding site of the human bitter taste receptor hTAS2R16. J. Biol. Chem;285: 28373–28378.
19. Sainz, E., Cavenagh, M., Gutierrez, J., Battey, J., Northup, J., & Sullivan, S. (2007). Functional characterization of human bitter taste receptors. *Biochem. J*,*403*, 537-543.
20. Brockhoff, A., Behrens, M., Massarotti, A., Appendino, G., & Meyerhof, W. (2007). Broad tuning of the human bitter taste receptor hTAS2R46 to various sesquiterpene lactones, clerodane and labdane diterpenoids, strychnine, and denatonium. *Journal of agricultural and food chemistry*, *55*(15), 6236-6243.
21. Behrens, M., & Meyerhof, W. (2009). Mammalian bitter taste perception. In *Chemosensory Systems in Mammals, Fishes, and Insects* (pp. 77-96). Springer Berlin Heidelberg.
22. Meyerhof, W., Born, S., Brockhoff, A., & Behrens, M. (2011). Molecular biology of mammalian bitter taste receptors. A review. *Flavour and Fragrance Journal*,*26*(4), 260-268.
23. Guyton AC. (1991). Textbook of Medical Physiology. (8th ed). Philadelphia: W.B. Saunders.
24. Logue, A. W. (2004). *The psychology of eating and drinking*. Psychology Press.
25. Glendinning, J. I. (1994). Is the bitter rejection response always adaptive?.*Physiology & Behavior*, *56*(6), 1217-1227.
26. Jones, S., & Martin, R. D. (1992). *The Cambridge encyclopedia of human evolution*. Cambridge Univ Pr.
27. Johns, T. (1990). *With bitter herbs they shall eat it: chemical ecology and the origins of human diet and medicine*. University of Arizona Press.

28. McLaughlin, S., & Margolskee, R. F. (1994). The sense of taste. *American Scientist*, *82*(6), 538-545.
29. Wang, X., Thomas, S. D., & Zhang, J. (2004). Relaxation of selective constraint and loss of function in the evolution of human bitter taste receptor genes.*Human molecular genetics*, *13*(21), 2671-2678.
30. Wooding, S., Kim, U. K., Bamshad, M. J., Larsen, J., Jorde, L. B., & Drayna, D. (2004). Natural Selection and Molecular Evolution in< i> PTC,</i> a Bitter-Taste Receptor Gene. *The American Journal of Human Genetics*, *74*(4), 637-646.
31. Drewnowski, A., & Gomez-Carneros, C. (2000). Bitter taste, phytonutrients, and the consumer: a review. *The American Journal of Clinical Nutrition*, *72*(6), 1424-1435.
32. Hofmann, T. (2009). Identification of the key bitter compounds in our daily diet is a prerequisite for the understanding of the hTAS2R gene polymorphisms affecting food choice. *Annals of the New York Academy of Sciences*, *1170*(1), 116-125.
33. Rodgers, S., Busch, J., Peters, H., & Christ-Hazelhof, E. (2005). Building a tree of knowledge: analysis of bitter molecules. *Chemical senses*, *30*(7), 547-557.
34. Rodgers, S., Glen, R. C., & Bender, A. (2006). Characterizing bitterness: Identification of key structural features and development of a classification model. *Journal of chemical information and modeling*, *46*(2), 569-576.
35. Maehashi, K., & Huang, L. (2009). Bitter peptides and bitter taste receptors.*Cellular and molecular life sciences*, *66*(10), 1661-1671.
36. Karaman, R. (2012), *Computationally Designed Prodrugs for Masking the Bitter Taste of Drugs.*.
37. Karaman, R. (2013). A Solution to Aversive Tasting Drugs for Pediatric and Geriatric Patients. *Drug Des*, *2*, e116.
38. Karaman, R., Fattash, B., & Qtait, A. (2013). The future of prodrugs-design by quantum mechanics methods. *Expert opinion on drug delivery*, *10*(5), 713-729.
39. Albert, A. (1958). Chemical aspects of selective toxicity. *Nature*, *182*(4633), 421.
40. Tegeli, V. S., Thorat, Y. S., Chougule, G. K., Shivsharan, U. S., Gajeli, G. B., & Kumbhar, S. T. (2010). Review on Concepts and Advances in Prodrug Technology. *International Journal*, *1*.
41. Scotti, L., Scotti, M. T., Ishiki, H. M., Ferreira, M. J., Emerenciano, V. P., de S Menezes, C. M., & Ferreira, E. I. (2007). Quantitative elucidation of the structure–bitterness relationship of cynaropicrin and grosheimin derivatives.*Food chemistry*, *105*(1), 77-83.

42. Karaman, R. (2013). Prodrug Design by Computation Methods: A New Era.*Drug Des*, *1*, e113.
43. Karaman, R. (2012). The future of prodrugs designed by computational chemistry. *Drug Des*, *1*, e103.
44. Karaman, R. (2011). Computational-Aided Design for Dopamine Prodrugs Based on Novel Chemical Approach. *Chemical biology & drug design*, *78*(5), 853-863.
45. Karaman, R., Dajani, K. K., Qtait, A., & Khamis, M. (2012). Prodrugs of Acyclovir–A Computational Approach. *Chemical biology & drug design*, *79*(5), 819-834.
46. Karaman, R. (2010). The efficiency of proton transfer in Kirby's enzyme model, a computational approach. *Tetrahedron Letters*, *51*(16), 2130-2135.
47. Karaman, R. (2010). Prodrugs of aza nucleosides based on proton transfer reaction. *Journal of computer-aided molecular design*, *24*(12), 961-970.
48. Hejaz, H., Karaman, R., & Khamis, M. (2012). Computer-assisted design for paracetamol masking bitter taste prodrugs. *Journal of molecular modeling*,*18*(1), 103-114.
49. Blow, D. M., Birktoft, J. J., & Hartley, B. S. (1969). Role of a buried acid group in the mechanism of action of chymotrypsin. *Nature*, *221*, 337-340.
50. Page, M. I. (1983). *The chemistry of enzyme action* (Vol. 6). Elsevier.
51. Gandour, R. D., & Schowen, R. L. (Eds.). (1978). *Transition states of biochemical processes*. New York: Plenum Press.
52. Kirby, A. J. (1996). Enzyme mechanisms, models, and mimics. *Angewandte Chemie International Edition in English*, *35*(7), 706-724.
53. Nelson, D. L., Lehninger, A. L., & Cox, M. M. (2008). *Lehninger principles of biochemistry*. Macmillan.
54. Fersht, A. (1999). *Structure and mechanism in protein science: a guide to enzyme catalysis and protein folding*. Macmillan.
55. Karaman, R. (2013). Prodrugs Design Based on Inter-and Intramolecular Chemical Processes. *Chemical biology & drug design*, *82*(6), 643-668.
56. Kirby, A. J., & Lancaster, P. W. (1972). Structure and efficiency in intramolecular and enzymic catalysis. Catalysis of amide hydrolysis by the carboxy-group of substituted maleamic acids. *J. Chem. Soc., Perkin Trans. 2*, (9), 1206-1214.
57. Katagi, T. (1990). AM1 study of acid-catalyzed hydrolysis of maleamic (4-amino-4-oxo-2-butenoic) acids. *Journal of Computational Chemistry*, *11*(9), 1094-1100.

58. Kluger, R., & Chin, J. (1982). Carboxylic acid participation in amide hydrolysis. Evidence that separation of a nonbonded complex can be rate determining.*Journal of the American Chemical Society*, *104*(10), 2891-2897.
59. Karaman, R., Amly, W., Scrano, L., Mecca, G., & Bufo, S. A. (2013). Computationally designed prodrugs of statins based on Kirby's enzyme model.*Journal of molecular modeling*, *19*(9), 3969-3982.
60. Kirby, A. J., & Hollfelder, F. (2009). *From enzyme models to model enzymes*. Royal Society of Chemistry.
61. Karaman, R. (2011). Analyzing the efficiency in intramolecular amide hydrolysis of Kirby's N-alkylmaleamic acids–A computational approach. *Computational and Theoretical Chemistry*, *974*(1), 133-142.
62. Finn, A., Straughn, A., Meyer, M., & Chubb, J. (1987). Effect of dose and food on the bioavailability of cefuroxime axetil. *Biopharmaceutics & drug disposition*,*8*(6), 519-526.
63. Kees, F. K., Lukassek, U., Naber, K. G., & Grobecker, H. (1991). Comparative investigations on the bioavailability of cefuroxime axetil. *Arzneimittel-Forschung*,*41*(8), 843-846.
64. Bora, D., Borude, P., & Bhise, K. (2008). Taste masking by spray-drying technique. *AAPS PharmSciTech*, *9*(4), 1159-1164.
65. Yajima, T., Nogata, A., Demachi, M., Umeki, N., Itai, S., Yunoki, N., & Nemoto, M. (1996). Particle design for taste-masking using a spray-congealing technique. *Chemical and pharmaceutical bulletin*, *44*(1), 187-191.
66. Al-Omran, M. F., Al-Suwayeh, S. A., El-Helw, A. M., & Saleh, S. I. (2002). Taste masking of diclofenac sodium using microencapsulation. *Journal of microencapsulation*, *19*(1), 45-52.
67. Shidhaye, S., Malke, S., & Kadam, V. (2008). Taste masked, orally disintegrating tablet containing microspheres for immediate release. *J Pharm Res*, *1*, 225-9.
68. Parr, T. R., & Bryan, L. E. (1984). Mechanism of resistance of an ampicillin-resistant, beta-lactamase-negative clinical isolate of Haemophilus influenzae type b to beta-lactam antibiotics. *Antimicrobial agents and chemotherapy*,*25*(6), 747-753.
69. Waxman, D. J., & Strominger, J. L. (1983). Penicillin-binding proteins and the mechanism of action of beta-lactam antibiotics1. *Annual review of biochemistry*,*52*(1), 825-869.

70. Brogden, R. N., Speight, T. M., & Avery, G. S. (1975). Amoxicillin: A review of its antibacterial and pharmacokinetic properties and therapeutic use. *Drugs*,*9*(2), 88-140.
71. Neu, H. C., & Winshell, E. B. (1970). Pharmacological studies of 6 (D (-)-amino-p-hydroxyphenylacetamido) penicillanic acid in humans. *Antimicrobial agents and chemotherapy*, *10*, 423.
72. Tack, K. J., Keyserling, C. H., McCarty, J., & Hedrick, J. A. (1997). Study of use of cefdinir versus cephalexin for treatment of skin infections in pediatric patients. The Cefdinir Pediatric Skin Infection Study Group. *Antimicrobial agents and chemotherapy*, *41*(4), 739-742.
73. Jacobs, M. R., Jones, R. N., & Giordano, P. A. (2007). Oral β-lactams applied to uncomplicated infections of skin and skin structures. *Diagnostic microbiology and infectious disease*, *57*(3), S55-S65.
74. Disney, F. A., Dillon, H., Blumer, J. L., Dudding, B. A., McLinn, S. E., Nelson, D. B., & Selbst, S. M. (1992). Cephalexin and Penicillin in the Treatment of Group A {beta}-Hemolytic Streptococcal Throat Infections. *Archives of Pediatrics & Adolescent Medicine*, *146*(11), 1324.
75. Davis, J. L., Salmon, J. H., & Papich, M. G. (2005). Pharmacokinetics and tissue fluid distribution of cephalexin in the horse after oral and iv administration.*Journal of veterinary pharmacology and therapeutics*, *28*(5), 425-431.
76. Han, H. K., & Amidon, G. L. (2000). Targeted prodrug design to optimize drug delivery. *AAPS PharmSci*, *2*(1), 48-58.
77. Sinkula, A. A., & Yalkowsky, S. H. (1975). Rationale for design of biologically reversible drug derivatives: prodrugs. *Journal of pharmaceutical sciences*, *64*(2), 181-210.
78. Beaumont, K., Webster, R., Gardner, I., & Dack, K. (2003). Design of ester prodrugs to enhance oral absorption of poorly permeable compounds: challenges to the discovery scientist. *Current drug metabolism*, *4*(6), 461-485.
79. Amidon, G. L., Leesman, G. D., & Elliott, R. L. (1980). Improving intestinal absorption of water-insoluble compounds: A membrane metabolism strategy.*Journal of pharmaceutical sciences*, *69*(12), 1363-1368.
80. Ehrnebo, M., Nilsson, S. O., & Boréus, L. O. (1979). Pharmacokinetics of ampicillin and its prodrugs bacampicillin and pivampicillin in man. *Journal of pharmacokinetics and biopharmaceutics*, *7*(5), 429-451.

81. Jones, K. H. (1977). Bioavailability of talampicillin. *British medical journal*,*2*(6081), 232.
82. Karaman, R., Karaman, D., & Zeiadeh, I. (2013). Computationally-designed phenylephrine prodrugs–a model for enhancing bioavailability. *Molecular Physics*, (ahead-of-print), 1-16.

التركيب الكيميائي والخصائص والقوى المحركة المخبرية لدوائيْن أوليّيْن جديديْن مبتكريْن من مضادات البكتيريا؛ الأموكسيلين والسيفالكسين

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ملخص

تواجه العقاقير والأدوية المضادة للبكتيريا التي يتم تسويقها العديد من المشاكل مثل: المذاق المرّ وقلة الثبات مما يؤدي إلى عدم امتثال المريض للعلاج. وقد تم إيجاد تكنولوجيا الأدوية الأولية التي تساهم في حلّ مثل تلك المشاكل. وبناء على حسابات نظرية الكثافة الوظيفية التي تم تقديمها سابقاً، فقد تم تصميم وتركيب كل من أموكسيلين **ProD 1-2** وسيفاليكسين **ProD 1-2**. كما تم احتساب قيمة عمر النصف (t ½) وقيمة القوى المحركة والنسبة الثابتة التي تتم ملاحظتها (k_{obs}) لكلا هذين الدوائين المضادين للبكتيريا بوسائل مختلفة من المعادلة الانحدارية الخطية التي تم الحصول عليها من علاقة تركيز الدواء الأولي المتبقي مع الوقت. وعند درجة حرارة وحموضة ثابتة، أظهر تفاعل التحليل المائي للأدوية الأولية المذكورة أعلاه وجود قوى محركة دقيقة من الرتبة الأولى حيث كانت قيمة القوى المحركة والنسبة الثابتة التي تتم ملاحظتها (k_{obs}) ثابتة نوعاً ما وقد تم الحصول على خط مستقيم. كما تم اختيار الدراسات المتعلقة بالقوى المحركة في حمض الهيدروكلوريك (1N HCl) ودرجة الحموضة 2.5 ودرجة الحموضة 5 وذلك بهدف البحث في التحويل الداخلي لكلا الدوائين الأوليين إلى الأدوية الأصلية لهما.

وقد تبين بأن التحليل المائي لكلا الدوائين يتفكك بشكل اسرع في حمض الهيدروكلوريك (1N HCl) مقارنة بدرجة الحموضة 2.5 ودرجة الحموضة 5. كما أن القيم التجريبية لعمر النصف ($t_{1/2}$) للأموكسيلين **ProD 1** في حمض الهيدروكلوريك (1N HCl) ودرجة الحموضة 2.5 ودرجة الحموضة 5 قد بلغت 2.5 و 7 و81 ساعة على التوالي، أما بالنسبة للسيفالكسين **ProD 1** في حمض الهيدروكلوريك (1N HCl) ودرجة الحموضة 2.5 فقد بلغت القيم التجريبية له 2 و 14 ساعة على التوالي. وبالمقابل، فإن قيم $t_{1/2}$ للأموكسيلين في **ProD 2** في حمض الهيدروكلوريك (1N HCl) ودرجة الحموضة 2.5 قد بلغت 8 و 44 ساعة على التوالي، وبالنسبة للسيفالكسين ProD 2 في حمض الهيدروكلوريك (1N HCl)، فقد بلغت القيم التجريبية له 6 ساعات.

ومن جهة أخرى، عند درجة الحموضة 7.4، كانت الأدوية الأولية الأربعة مستقرة وثابتة نوعاً ما ولم تتم ملاحظة أي إطلاق للأدوية الأصلية. أما عند درجة الحموضة 5 فقد كان التحليل المائي بطيئاً جداً. وقد وُجد بأن الأدوية الأولية الأربعة المضادة للبكتيريا لا تتسم بوجود مذاق مرّ لها. ومن المُعتقد بأن ازالة المذاق المُرّ الذي تتسم به الأدوية الأولية ينتج عن تبديل قدرة الدواء على التفاعل مع مستقبلات المذاق المرّ.

عمادة الدراسات العليا

جامعة القدس

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رسالة ماجستير

فلسطين ـ القدس

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التركيب الكيميائي والخصائص والقوى المحركة المخبرية لدوائيْن أوليّيْن جديديْن مبتكريْن من مضادات البكتيريا؛ الأموكسيلين والسيفالكسين

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قدمت هذه الأطروحة استكمالا لمتطلبات درجة الماجستير في العلوم الصيدلانية من كلية الدراسات العليا جامعة القدس-فلسطين.

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