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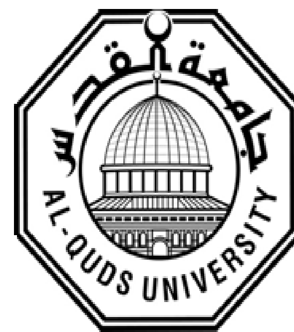

Synthesis, Characterization, and Evaluation of Potentially Useful Quinine Prodrugs

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M .Sc. Thesis

Jerusalem – Palestine

1444/2022

Synthesis, Characterization, and Evaluation of Potentially Useful Quinine Prodrug

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A thesis submitted in partial fulfillment of requirements for the degree of Master of Pharmaceutical Sciences in the Faculty of Pharmacy, Al-Quds University.

1444/2022

Al-Quds University

Deanship of Graduate Studies

Pharmaceutical Sciences Program

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Synthesis, Characterization and Evaluation of Potentially Useful Quinine Prodrugs

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Jerusalem–Palestine

1444\2022

Dedication

I dedicate this thesis to the soul of my dear father, whom I believe so much is very proud of me because he taught me strength and determination. To the land of love and goodness, Palestine, which I believe strongly, will return to us someday.

I dedicate it to my backbone, my dear mother, brothers, and sisters, who supported me throughout my educational journey. My God protects them.

Special thanks from the bottom of my heart go out to all my friends and to all who loved them, who supported me spiritually and psychologically throughout my research.

Tasneem Abudayyah

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 been submitted for a higher degree to any other university or institution.

Signed: ------------------------------------

Tasneem Mohammad Mahmoud Abudayyah

Date: 17/8/2022

Acknowledgment

Since he was my first supporter in the success and attainment of this tremendous feat, I would want to thank God for my success in attaining it from the beginning. Worship is directed primarily towards you, God.

After that, I want to express my gratitude to the supervisor of this essay, my dear Professor Rafik Karaman, for his encouragement and support of my research as well as for assisting me during all the research-related processes. To you, my love and gratitude. I sincerely appreciate it.

In particular, I would like to thank Miss. Fatima Haddad and Mr. Ibrahim Ayyad from the College of Pharmacy for providing me with all the resources and guidance I required for her postgraduate studies in the pharmaceutical sciences program at my beloved university, Al-Quds University in general. Because they had the endurance and tolerance to wait for this triumph on this unique and special day, I am appreciative of and lucky for their support.

I want to thank my entire family, especially my loving mother, for her patience and for giving me the courage and tenacity to pursue my goals and succeed.

I send you all my love, respect, and gratitude. I hope you are all fortunate and good.

Abstract

A prodrug is an active drug that has been disguised and is intended to be activated through an enzymatic or chemical reaction in the body as a pharmacologically inactive component that then goes through biotransformation to become its therapeutic activity. The prodrug method has also been utilized to improve the medications' target-specific selectivity. The prodrug can get past obstacles like low bioavailability, poor aqueous solubility, chemical instability, insufficient oral absorption, quick pre-systemic metabolism, insufficient brain penetration, toxicity, unpleasant taste and odor, local irritation, and change the physical form of the drug by chemically altering the active agent.

Quinine is a drug with a strong bitter taste that has a negative effect on its acceptance by patients, whether for the treatment of malaria or muscle spasms and others. In this study, we aimed to mask the intensely bitter taste of quinine by synthesizing prodrugs with suitable linkers that can release the parent drug (quinine) when exposed to a physiological environment. The prodrugs were synthesized by esterification of their free hydroxyl groups using different linkers (1, 2-cyclohexanedicarboxylic anhydride, succinic anhydride, maleic anhydride) instructions through (HPLC, Melting Point, LC-MS, FT-IR, H-NMR) to check the purity of the manufactured compounds.

In *vitro* kinetic studies for the prodrugs were tested and analyzed in the laboratory using the HPLC apparatus at a constant temperature of 37°C and various pHs such as 0.1 N HCl, pH 2.2, pH 5.5, and pH 7.4, which are similar to the pH of the human body. Unfortunately, the results of the hydrolyzing of the prodrugs at all pH levels were stable and did not release the parent drug (Quinine). This could be a result of the quinine anion's (R-O-) poor leaving group properties. The ester link could be broken by blood enzymes such esterase to furnish the parent drug in the near future.

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List of Abbreviations

ADME	Absorption, Distribution, Metabolism and Excretion
DFT	Density Functional Theory
DMF	Dimethylformamide
ESI	Electro Spectroscopy Ionization
D.W	Deionized Water
HCl	Hydrochloric acid
HPLC	High-Performance Liquid Chromatography
hr	hour
FDA	Food and drug administration
FT-IR	Fourier Transform Infrared Spectrophotometer
GI	Gastrointestinal tract
LC-MS	Liquid Chromatography- Mass Spectroscopy
m/z	Mass-to-Charge ratio
NMR	Nuclear magnetic resonance spectroscopy
NaOH	sodium hydroxide
NaH	Sodium Hydride
OTC	Over the counter drug
ppm	Part per million
TLC	Thin Layer Chromatography
THF	Tetrahydrofuran
$t_{1/2}$	Half Life
UV	Ultraviolet Spectrophotometery
UTI	Urinary Tract Infection

Chapter one

Introduction

Chapter one

Introduction

1.1. Background

A prodrug is an active drug that has been disguised and is intended to be activated through an enzymatic or chemical reaction in the body as a pharmacologically inactive component that then goes through biotransformation to become its therapeutic activity. [1] For the first time, Albert has utilized this phrase to successfully change a compound's physicochemical, pharmacokinetic, and biopharmaceutical characteristics. [2, 3]

The use of prodrugs is typically justified by the need to maximize their so-called (drug-like) qualities because if they are not favorable, they can significantly hinder subsequent drug development. [1]

The prodrug method has also been utilized to improve the medications' target-specific selectivity. [4] The prodrug can get past obstacles like low bioavailability, poor aqueous solubility, chemical instability, insufficient oral absorption, quick pre-systemic metabolism, insufficient brain penetration, toxicity, unpleasant taste and odor, local irritation, and changing the physical form of the drug by chemically altering the active agent. [1, 5]

This can lessen systemic and undesirable tissue- or organ-specific toxicity while also increasing the drug's efficacy. The creation of a prodrug with enhanced qualities might also present a chance for life-cycle management. The parent drug is produced using this method, which uses carrier-linked prodrugs that have a group that can be quickly removed enzymatically, like an ester or labile amide. [1, 3]

A mutual prodrug, which consists of two pharmacologically active substances joined together to form a single molecule, is a subtype of a carrier-linked prodrug. These medications all serve as carriers for one another. [6] Given that prodrugs have the potential to change the tissue distribution, efficacy, and even toxicity of the parent drug,

this alternative should be taken into account in the early stages of preclinical development. As a result, altering the parent medication's ADME characteristics necessitates a thorough comprehension of the physicochemical and biological behavior of the drug candidate. Prodrug design can nevertheless be more practical and quicker than looking for a whole novel therapeutically active compound with the right ADME qualities, notwithstanding how difficult it is. [5, 6]

Another misconception about prodrugs is that they are only of academic curiosity and have no practical uses in attempts to solve bioavailability and toxicity issues. Analyzing the market's abundance of prodrugs will give you a very good idea of how effective the prodrug strategy is. [1]

Despite these remarkable figures, there are still many innovative prodrug inventions that have yet to be discovered. We have only just begun to realize the full potential of the prodrug strategy in contemporary drug development. The linker is often removed from prodrugs by an enzymatic or chemical reaction, while some prodrugs also undergo molecular change, such as an oxidation or reduction reaction, to release their active ingredients. [1]

The design of prodrugs based on intramolecular processes using density functional theory (DFT), ab-initio methods, and correlations of experimental and calculated reaction rates is a novel chemical approach in this area of research. [7] No enzyme is required to catalyze the interconversion of a prodrug to its corresponding drug. The type of linker attached to the medicine regulates how quickly the drug is released. [3]

By using prodrug technology, a drug molecule's clinical utility may be increased without affecting the original drug's pharmacological action.[5] Nevertheless, it is ideal to take into account the creation of a suitable prodrug molecule early on in preclinical research, keeping in mind that prodrugs, although uncommon, may affect the tissue distribution, efficacy, and toxicity of the parent drug. Additionally, the optimum promoieties would be fast and safe body excretion. [8] **Figure 1.1** displays a schematic illustration of the prodrug concept.

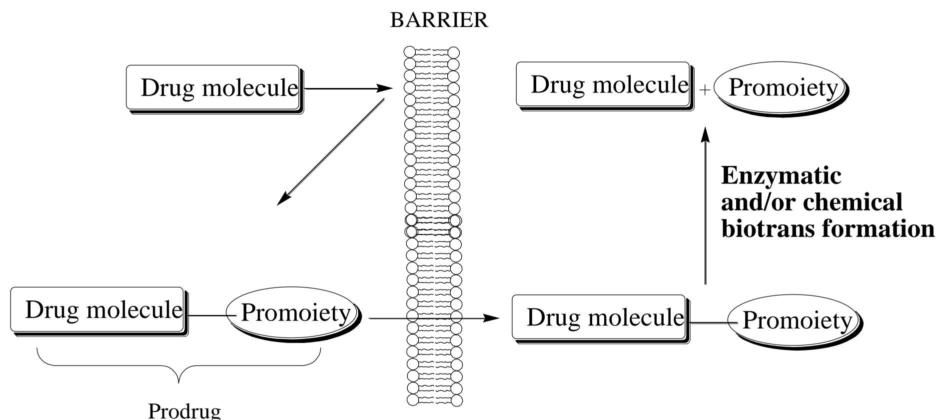


Figure 1.1 Schematic representation of the prodrug concept

Regarding the disease state, dosage, and length of therapy, the choice of promoiety should be taken into account. The prodrug technique can be utilized for practically all administration routes and dosage forms, and it can be used to develop novel drug molecules in the lead optimization stage early in the drug discovery process as well as a wide range of currently available medications. [5, 6]

Although just 5-7 percent of all medications licensed worldwide may be categorized as prodrugs, using a prodrug method in the early stages of drug development is becoming more and more popular. [7, 8]

1.2. Pharmaceutical application of the prodrug approach

The phase of growth encompassing the discovery of a new chemical entity with determined or hypothesized therapeutic potential and its inclusion into a drug delivery system is known as the pharmaceutical application. [9] One of the traditional types of delivery (injections, capsules, tablets, creams, or ointments) or a novel method of drug

delivery, such as liposome's or implants, may be used. [10, 11] In the process of creating economically viable drug items, two obstacles were found.

(a) Problems with drug formulation, including heightened permeability and absorption as well as unfavorable physicochemical characteristics including solubility and polarity.

(b) Aesthetic qualities, including taste and odor (especially when intended for pediatric use or oral administration).

1.2.1 Enhancing permeability and absorption

Increased lipophilicity of poorly permeable drugs allows the drug to be attached to more lipophilic groups, such as amino groups, and these prodrugs are easily hydrolyzed to parent drugs. It can also be achieved by masking polarly ionized or non-ionized functional groups, which increases either oral or topical absorption. The prodrug strategy can help with this. [12] Additionally, prodrugs with the same structural characteristics as substrates that are absorbed by carrier-mediated transport can be created to increase oral absorption. [13, 14]

1.2.2 Improvement of solubility and dissolution rate of drugs in a physiological environment

The water solubility of almost over 30% of drug development compounds is poor. This issue can be resolved by making the parent drug molecules more soluble in water by speeding up the dissolving process through attachment to polar or ionizable groups like phosphate, sugar, or amino acid moieties. These prodrugs can be used to increase the bioavailability of injectable medications, oral medications, and parenteral medication. [15,16]

1.2.3 Masking taste

When taste receptors are chemically stimulated, a sensory reaction called flavor results. Through saliva, these receptors bind chemicals, and these interactions cause electrical impulses in taste cells to be transmitted by cranial nerves to the brain, where they are converted into the impression of taste. [17] Chemicals dissolve in saliva and bind to taste receptors on the tongue, producing an unpleasant flavor that can be bitter, sweet, salty, or sour. The receptors on the back of the tongue detect the bitter flavor. The most complicated way to perceive a taste is said to be bitter. [8]

The active components in many medicinal medications have a bitter taste. Patients do not accept them, which is one of the bitterness and sourness of medications' most significant drawbacks. This is known as "patient noncompliance," particularly in settings with young patients, like pediatrics. [18] One of the main reasons the oral dosing regimen failed was the harsh taste. Commonly used bitter-tasting medications include pseudoephedrine, amphetamine, and dextromethorphan, which are antitussive (cough suppressant) medications. Many over-the-counter cold and cough medications have these as some of their active ingredients. [8, 19]

Reducing the drug's solubility in saliva and masking functional groups that may be in charge of the drug's attachment to taste receptors on the tongue are two strategies that can be used to get rid of the drug's unpleasant taste. These methods enhance the bioavailability and effectiveness of the medicine dosage form while also masking the bitter taste of the medication. [8]

1.3. Taste according to the chemical structure

The strength of a flavor is determined by how soluble it is in saliva; the more soluble a chemical is the higher the concentration of that item in saliva at the receptor site and, consequently, the stronger the taste. [20, 21]

Bitter: Phenolic or alcoholic hydroxyl groups, nitro or amino groups, esters of aromatic acids, lactones, and sulfur-containing aliphatic molecules all have the ability to produce bitterness in substances. [21] Ondansetron, quinine, guaifenesin, acetaminophen,

ibuprofen, naproxen, chlorpheniramine, astemizole, loperamide, famotidine, ranitidine, cimetidine, and pseudoephedrine are only a few examples of bitter-tasting pharmaceutical active components. [20]

1.4. Masking bitter taste

Technology for taste masking has two components: **1.** Choosing appropriate flavor-masking ingredients, such as polymers, sweeteners, flavors, amino acids, etc. **2.** Choosing the right taste-masking methods the efficacy of the process and the quality of the flavor masking can both be considerably impacted by a good taste masking technique. Many methods have been devised to cover up the unpleasant taste of medications. [17, 22] These are listed below: using tastes and sweeteners to hide a flavor, this method of taste masking is the most straightforward. However, this strategy does not work well with really bitter medications. [17, 18]

1.5. Microencapsulation's technique

By encapsulating the drug particles with an appropriate polymer, microencapsulation has significant applications in the taste masking of bitter medications. When dosage forms are administered orally to patients, the drug has no contact with the mouth's taste buds. For instance, the taste of diclofenac sodium was concealed using the micro-encapsulation method without affecting the rate of drug release. [23] The technique of coating incredibly tiny droplets or particles of liquid or solid material with a film or polymeric material is known as microencapsulation. [8]

1.6. Coating polymers

By creating a barrier around the drug particles, it is a method for hiding the bad taste. The interaction between the medicine and taste receptors can be reduced by utilizing the proper kind of coating material. The taste should be carefully considered when choosing a coating for polymers to mask with microencapsulation.[8] These polymers allow substances to enter the area where absorption is anticipated while preventing their release

in the mouth cavity following oral administration (stomach or small intestine). Polymers are a suitable choice for taste muffling since they dissolve at a pH of 1.2 in stomach fluids but not at a pH of 6.8 in saliva. The following should be taken into account before coating materials: **1-** The size of the drug's particle. **2-** The features of the drug's flow. **3-** Long-term steadiness. **4-** Sensitivity to moisture. **5-** The processing temperature is the most crucial element. **6-** A technique for administering an active drug molecule. [22, 24]

1.7. Modification of the distribution profile

The medicine must get through a number of pharmacological and pharmacokinetic obstacles before it can exert the desired effect at the target site. Many efforts must be made to combine different macromolecular tactics and nanotechnologies in order to do this, but these approaches have not been shown clinically effective. The prodrug strategy, on the other hand, is a useful site-selective drug liberation technique that makes use of the natural enzymes and transporters that the target cell and tissue already produce. [8, 16]

1.8. Rapid metabolism

The liver is the main organ for drug metabolism. First-pass metabolism, a phenomenon of drug metabolism that diminishes a medication before it reaches systemic circulation, especially after oral administration, considerably lowers the oral bioavailability of many pharmacological compounds. The rate of drug metabolism varies amongst patients. [25, 26] For certain people, the medication is metabolized so quickly that therapeutically important blood and tissue concentrations are not reached. This issue can be resolved by adopting a prodrug method to boost oral bioavailability by concealing functional groups that are biologically vulnerable. [25, 27]

1.9. Enzymatically versus chemically bioactivation

The prodrug strategy is a multifaceted method for maximizing the value of biologically active substances. Prodrugs often contain a promoiety that is removed to activate the prodrug by a chemical reaction to susceptible individual variation in response to

pharmacologic therapies or an enzymatic reaction, such as esterases and amidases. [28] The portion I eliminated is harmless and pharmacologically inactive. The drug-promotion relationship must be able to function well in *vivo*. [29]

In order to understand the mechanism by which enzymes might exert their powerful catalysis, the prodrugs were developed based on enzyme models using a computational approach that included calculations using molecular orbital and molecular mechanics methods as well as correlations between experimental and calculated activation energies for two intramolecular processes. [29, 30]

The prodrug strategy has been applied to improve the clinical profile and get around a number of undesirable drug features.

2. Quinine

Quinine is a cinchona alkaloid and a member of the pharmacological class known as aryl amino alcohols (see **Figure 2.1**). For more than 400 years, Plasmodium falciparum, a live organism, has been the cause of malaria in the west. In the 17th century, quinine was originally used for therapeutic purposes. [31]

Until the introduction of newer medications, such as chloroquine, in 1940, quinine was the most effective treatment for malaria. These medicines took the place of quinine and had fewer negative effects. [32, 33]

In the wild, eucalyptus tree bark contains quinine. It is one of the most popular treatments for simple malaria. Quinine is administered orally or intravenously in its salt form. It is a crystalline white alkaline substance with a bitter taste, analgesic, anti-inflammatory, and antipyretic characteristics. It also treats malaria as mentioned. [34]

Quinine is offered in a variety of generic formulations in low doses as an over-the-counter drug and as 324 mg tablets for the treatment of malaria. Higher doses are needed for treatment and prevention of malaria than for treating leg cramps. Quinine is

additionally used to treat arthritis and lupus. [35] It was also prescribed in the United States for applications that the Food and Drug Administration had not allowed or permitted, such as the treatment of muscle spasms that occurred at night, but this use was less frequent because of an FDA warning against this abuse. [34]

The patient needs to be watched when getting quinine intravenously. He needs to have his blood sugar and heart monitored since quinine can trigger a condition called cinchonism, which can be fatal. [36] Because quinine has the undesirable property that patients do not accept, due to its extremely bitter taste, there is a risk that cinchonism does not occur or may occur in a tiny percentage when quinine is administered orally to the patient.

The only medication that the World Health Organization advises using as the first line of treatment for uncomplicated malaria during pregnancy is quinine.[37]

Quinine is administered parenterally to treat life-threatening infections brought on by Plasmodium falciparum malaria, which is chloroquine-resistant. Quinine exhibits gametocytocidal effect against P. vivax and P. malaria in addition to acting as a blood schizonticide. It is abundant in P. falciparum feeding vacuoles because it is a weak base. [38] It is believed to work by preventing heme polymerase from producing its cytotoxic substrate, heme, allowing heme to build up instead. It is less effective and more dangerous than chloroquine as a schizonticidal medication. It does, however, play a unique role in the treatment of severe falciparum malaria in regions where chloroquine resistance is well-known. [38, 39]

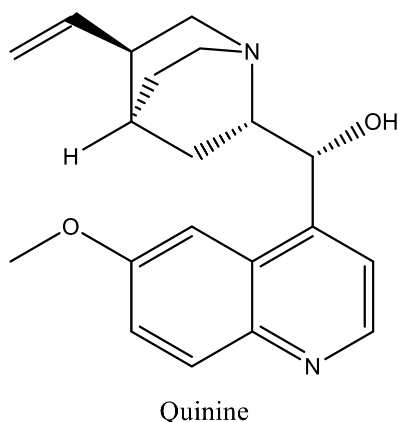


Figure (2.1) : Chemical Structure of Quinine

3. Problem statement

The 1600s saw the discovery of quinine. Since the bitterness of pharmaceutical medications is crucial for patient compliance when prescribing orally administered treatments, it is a substance that is unsuitable for juvenile and geriatric patients. [40]

The absolute bioavailability of quinine taken orally is a subject of little research. However, when given orally, the medication is absorbed more than 70% of the time, the maximum systemic concentration is attained between 1 and 3 hours, and over 80% of the administered dose is excreted through the liver. Due to its limited therapeutic index, the danger of adverse responses rises in the event that its disposition is significantly altered. [34, 41]

The presence of hydroxyl groups that have connected with bitter taste receptors to generate intermolecular connections through hydrogen bonding is the primary contributor to quinine's bitter flavor. As was observed for paracetamol, quinine's hydroxyl groups are blocked in order to mask its bitterness by reducing its solubility in saliva. The painkiller medicine paracetamol has a strong bitter taste, but its derivatives, in which the

paracetamol phenolic group was blocked by an alkyl group, lacked the bitterness that makes paracetamol distinctive. [40]

By altering the original molecule's molecular conformation and its contact with taste receptors, the prodrug method can be utilized to hide the bitter taste of active pharmacological components. In order to deliver an initial drug without the harshness of parental medication, a connection that masks this major issue with a bitter taste must be created. [18, 34]

By creating prodrugs with appropriate linkers that can release the parent drug (quinine) when exposed to a physiological environment and be able to chemically and enzymatically liberate the active agent in a controlled manner, we hope to achieve sustained release and mask the intensely bitter taste of quinine.

Specific objectives of our work are:

- To make quinine prodrugs synthetically by esterifying its free hydroxyl groups with various linkers.
- To characterize the proposed prodrug using several characterization techniques.
- To conduct kinetic experiments for the intraconversion of the novel prodrugs that were synthesized to their parent drug, quinine, and to measure the intraconversion rates and half-lives ($t_{1/2}$) at various buffers (1N HCl, pH 3.3, pH 5.5, and pH 7.4).

Chapter Two

Literature review

Chapter two

Literature review

2.1 Masking the Bitter taste of Paracetamol.

A common painkiller medicine called paracetamol is used to lower fever-stricken patients' temperatures. Analgesics and antipyretics are drugs that work in these ways.

Because paracetamol may form a hydrogen bond with the bitter taste receptor's active site *via* its phenolic hydroxyl group, it has the characteristics of a bitter taste. Three paracetamol prodrugs were created using DFT theoretical calculations for the acid-catalyzed hydrolysis of ten Kirby's enzyme models. [42] Because the form of ProD 1-3 was anticipated to be resistant to cleavage by a proton transfer procedure, it was intended that it would be obtained as sodium or potassium carboxylate salts**. Figure (2.1.1).**

The quantum chemistry software Gaussian was used to do the DFT calculations at the B3LYP/6-31 G (d, p) level. All of the compounds reported in this study had their initial geometries determined using the Argus lab program. According to the computational findings, the reaction rate is linearly proportional to both the hydrogen bonding generated along the reaction route and the separation between the two reactive centers (rGM). [40]

The development of three brand new flavorless paracetamol prodrugs with intramolecular proton transfer kinetics. The experiment also revealed that for processes 1 through 10, $t_{1/2}$ (the amount of time required for converting 50% of the reactants to products) and EM (effective molarity) values were comparable. According to the calculations, the $t_{1/2}$ values for ProD 1-3 are 21.3 hours, 4.7 hours, and 8 minutes, respectively.

Kirby's enzyme model (1–10) can be used to determine the rate of conversion of the three ProD to the parenteral drug paracetamol based on the characteristics of the linkers in the Prodrugs. Furthermore, it is thought that a linker moiety blocks the phenolic hydroxyl group to reduce the bitterness of paracetamol. [40]

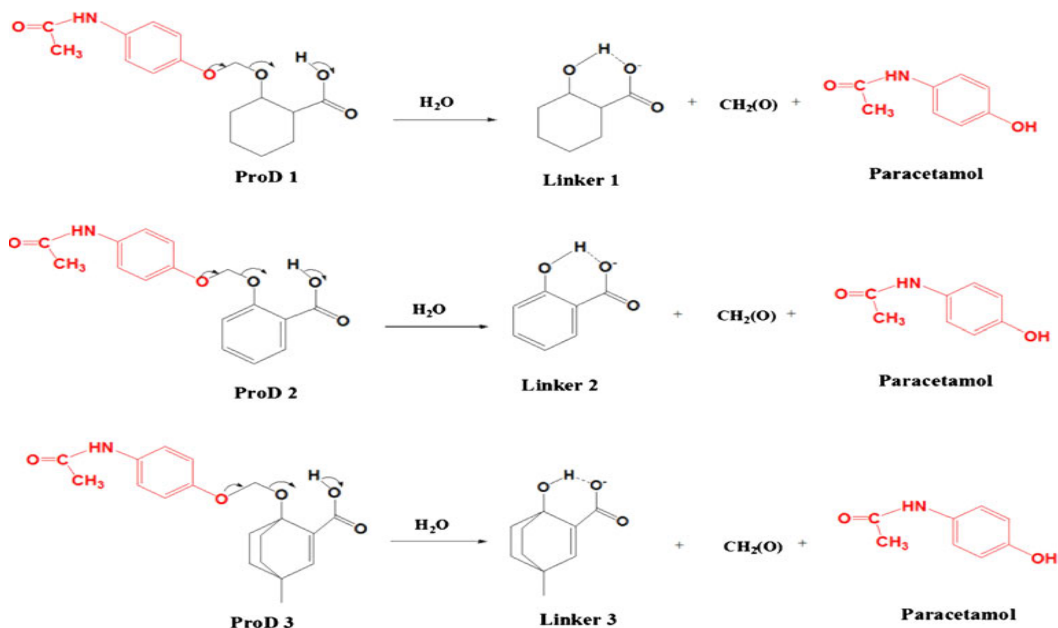


Figure (2.1.1): Paracetamol prodrugs hydrolysis to parent drug (paracetamol).

2.2 Chemical Modification of Clindamycin

A semi-synthetic antibiotic known as clindamycin has a higher level of antibacterial activity against gram-positive pathogens and a lower level of activity against gram-negative species. It is typically used to treat anaerobic bacterial infections, although it can also be used to treat some protozoa-related illnesses, such as malaria. It is a typical topical treatment for acne and is effective against some infections that are resistant to methicillin. [43]

The dosage is often administered in the form of coated tablets or capsules since it may taste highly bitter; it is inappropriate for use as a pediatric suspension or chewable tablet dosage form. The GI tract efficiently absorbs clindamycin. [44]

A number of Clindamycin 2 and 3 monoesters as well as certain Clindamycin 2, 3-dicarbonate esters were created to enhance the drug's taste qualities. The bitter flavor that distinguishes long-chain Clindamycin (palmitate and hexadecyl carbonate) is essentially absent. [43, 45]

Clindamycin's 3, 4 hydroxyl group was protected with acidic anisaldehyde to create 3,4-anisylidene Clindamycin, which was then used to make bitter-tasting Clindamycin prodrugs. As shown in **Figure 2.2.1**, the generated prodrugs were next subjected to the esterification of hydroxyl group number 2 with acid to form pure Clindamycin 2-monoester ester.

As shown in the scheme in **Figure 2.2.2**, a low-temperature reaction using pyridine as the solvent under -25 °C and alkyl chloroformate to create Clindamycin 3-monoester ester is used to selectively esterify the three hydroxyl groups.

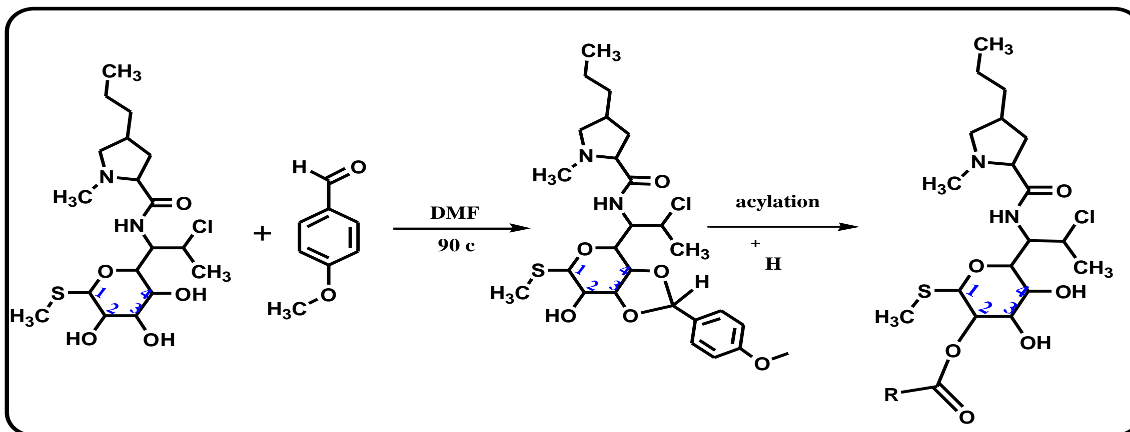


Figure (2.2.1): Clindamycin-2-monoesters

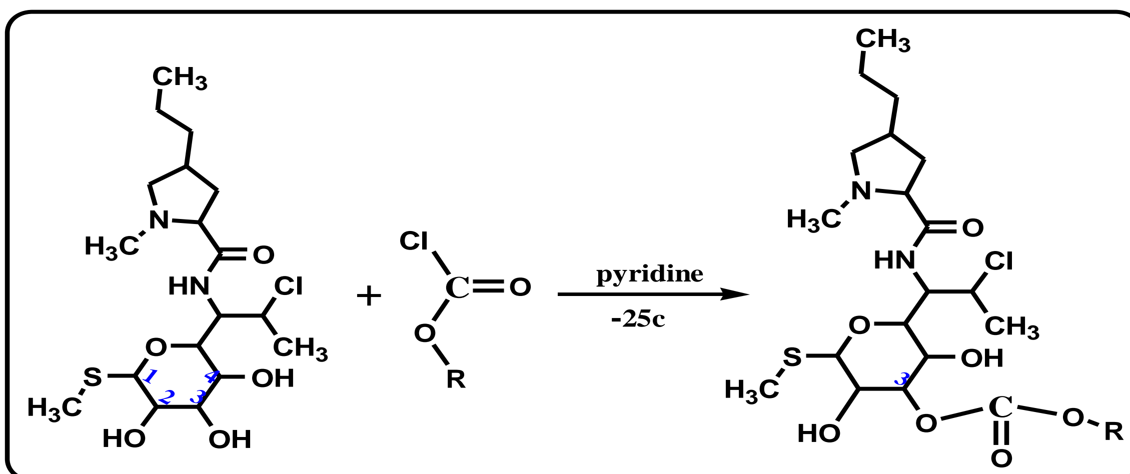


Figure (2.2.2): Clindamycin-3-monoesters

Taste studies:

Clindamycin 2-hexylcarbonate HCl, Clindamycin 2-laurate HCl, Clindamycin 2-palmitate HCl, and Clindamycin 2-diphenylacetate were four derivatives of two monoesters that were synthesized, then dissolved in 30 percent sucrose solutions and administered to a taste panel of 26 persons. A one-hour gap separated each sample as per the customary protocol. A similar methodology was used for 3 monoester derivatives, with separate tests conducted on the groups of 2 and 3 monoester derivatives.

The average ratings displayed in **Figure (2.2.3)** suggest a linear improvement in taste with longer chains. At the 5 levels of confidence, the palmitate ester is significantly superior to the laurate. Comparatively speaking, the laurate outperforms the hexanoate and acetate. Due to their extremely bitter flavors, the last two compounds were graded similarly poorly. [45]

Due to the fact that clindamycin 2 and 3 -palmitate hydrochloride has virtually no taste, it is currently undergoing comprehensive testing in humans as a flavorless pediatric formulation.

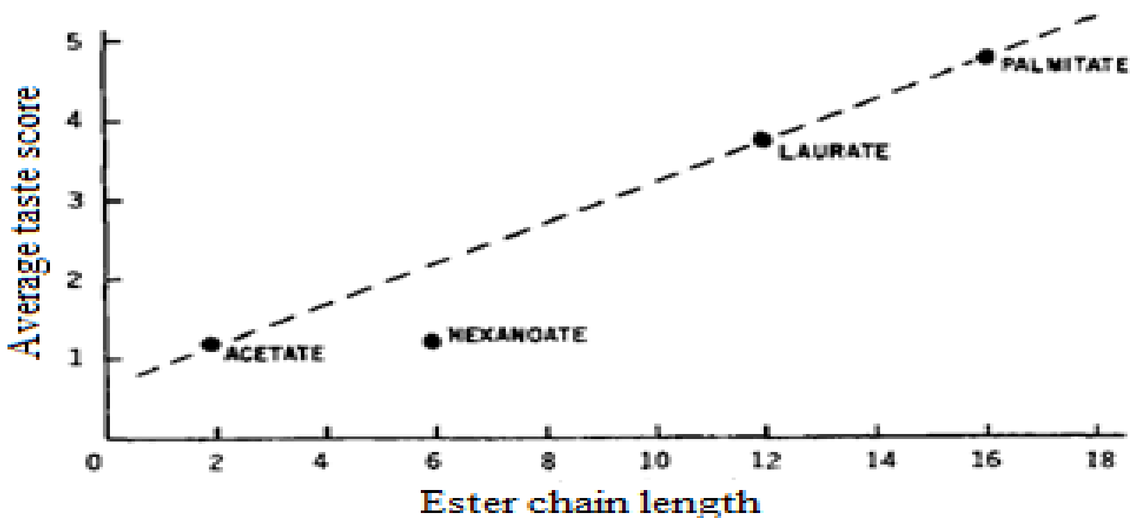


Figure (2.2.3): Effect of Clindamycin ester chain length on taste of ester in syrup.

2.3 Masking the Bitter taste of atenolol.[45, 46]

A cardioselective beta1-adrenergic receptor antagonist known as atenolol is used to treat arrhythmia, lower high blood pressure (hypertension), avoid angina pectoris, lower the risk of heart problems after a heart attack, and lessen the signs of alcohol withdrawal. It is a hydrophilic, lipid-insoluble substance that only passes through the kidneys and has a limited level of brain penetration.

Atenolol is sold as an oral formulation available in 25 mg, 50 mg, and 100 mg tablets as well as an injectable formulation available in 10 mL ampoules with a 5 mg/mL concentration. Atenolol has a harsh taste, which prevents people from taking it orally. This issue can be resolved by either decreasing the drug's oral solubility upon consumption or by using the prodrugs developed techniques to eliminate the interaction of drug particles with taste receptors.

Two atenolol prodrugs were created, and the atenolol prodrugs were synthesized using DFT, MP2, and the density functional from the Truhlar group (hybrid GGA: MPW1k) calculations for acid-catalyzed hydrolysis of nine Kirby's N-alkylmaleamic acids. The rates for the chemical intraconversion of atenolol ProD 1 and ProD 2 could be accurately predicted by DFT calculations of the kinetic and thermodynamic parameters for processes 1–9 **Figure (2.3.1).** The calculations showed that the nearby carboxylic acid group catalyzes the amide bond cleavage through intramolecular nucleophilic catalysis, and the rate-limiting step is identified based on the characteristics of the amine leaving group **Figure (2.3.2).** For instance, the projected values for the atenolol prodrugs ProD 1-ProD 2 at pH 2 were 65.3 hours (compared to 6.3 hours as determined by GGA: MPW1K) and 11.8 minutes, respectively, based on the computed B3LYP/6-31 G (d, p) rates. Atenolol prodrug ProD 1's in *vitro* kinetic investigation showed that the medium's pH had a significant impact on the $t_{1/2}$. The $t_{1/2}$ times in 1N HCl, buffer pH 2, and buffer pH 5 were respectively 2.53, 3.82, and 133 hours.

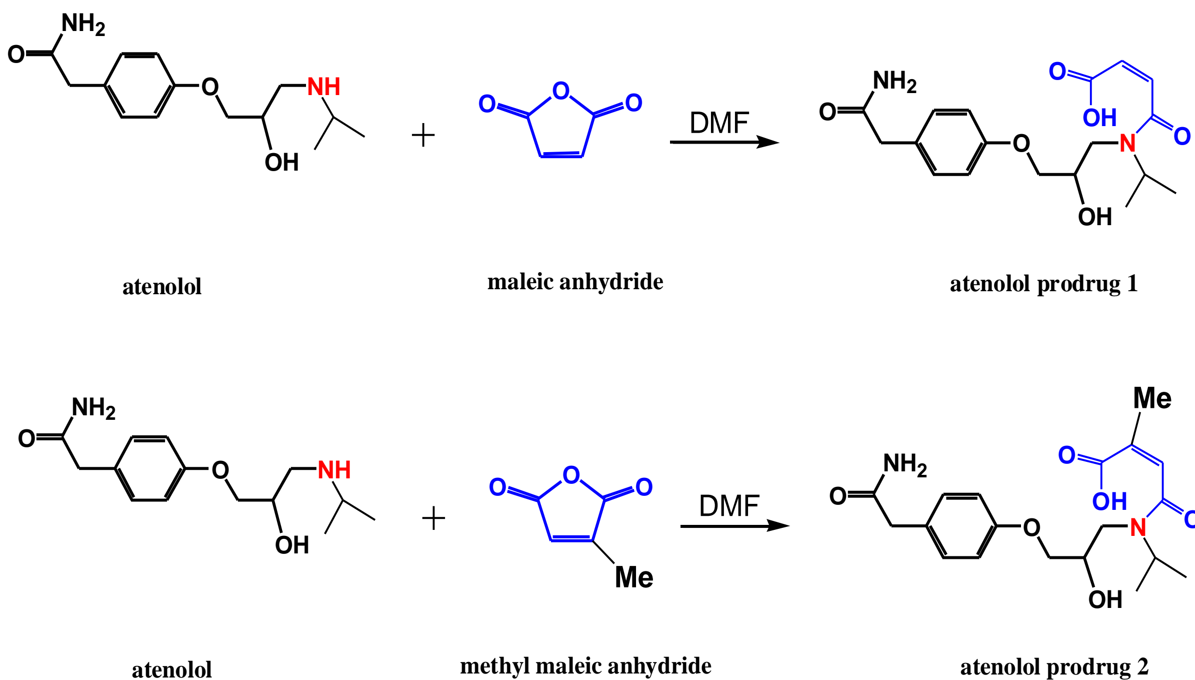


Figure (2.3.1): Atenolol prodrugs synthesis

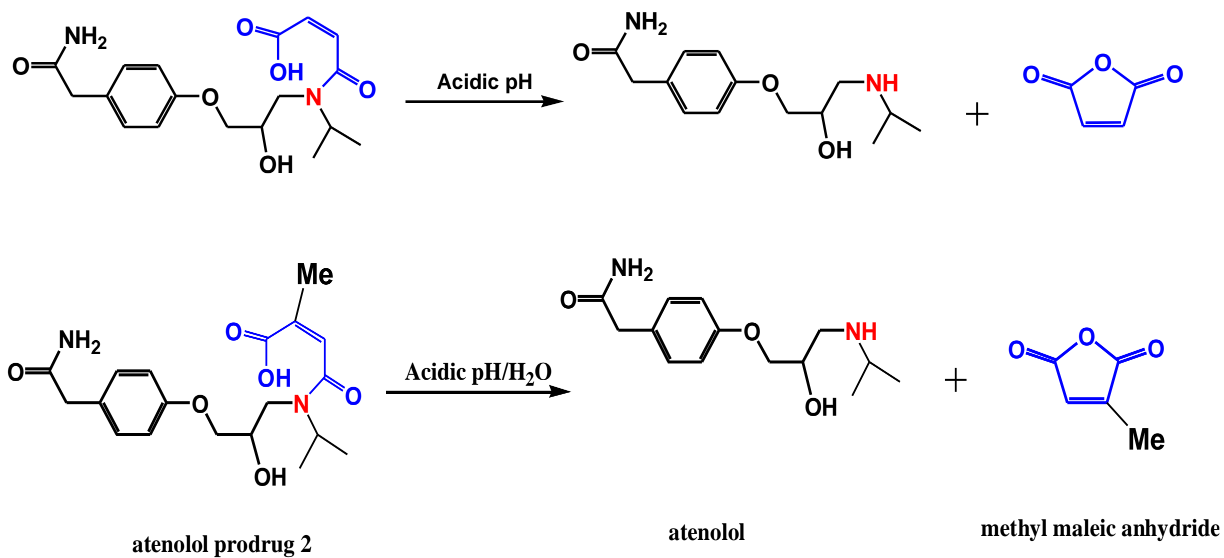


Figure (2.3.2): Atenolol prodrugs hydrolysis to parent drug

2.4 Masking the bitter taste of amoxicillin and cephalexin.[32, 33, 46-49]

Amoxicillin and cephalexin are two regularly used antibacterial medications that have a disagreeable taste. The bitter and disagreeable taste of these antibacterial medications is an issue brought on by intermolecular interactions that develop between the antibiotic and the bitter taste receptors' active site.

Amoxicillin is a moderate-spectrum, beta-lactam, and oral semi-synthetic penicillin antibiotic used to treat bacterial infections. It has a bactericidal effect and is effective against both gram-positive and gram-negative microbes by preventing the bacterial mucopeptide wall from being reconstructed and biosynthesized.

A first-generation cephalosporin antibiotic is a cephalexin. Both gram-positive and gram-negative microbes are susceptible to its effects. Since cephalexin is used to treat a variety of infections, such as otitis media, streptococcal pharyngitis, bone and joint infections, pneumonia, cellulitis, and urinary tract infections (UTI), it may also be used to prevent bacterial endocarditis. It is a helpful substitute for the hypersensitivity of penicillin.

Both amoxicillin and cephalexin have a harsh taste. They created a prodrug utilizing theoretical DFT calculations to solve this issue. Cephalexin ProD1 and amoxicillin ProD1 were created and manufactured **Figure (2.4.1).**

An amide acid moiety with a carboxylic acid group (hydrophilic moiety) and the remaining antibacterial prodrug molecules make up both antibacterial prodrug molecules (a lipophilic moiety).

The kinetics for these prodrugs was tested in four distinct aqueous environments. Amoxicillin ProD1 and cephalexin ProD1 were created using acid-catalyzed hydrolysis. We chose 1 N HCl, buffers with pH 2.5, 5, and 7.4 to study the intraconversion of both prodrugs to their parent drugs **Figure (2.4.2).** It was discovered that 1N HCl had stronger acid-catalyzed hydrolysis of both prodrugs than pH 2.5 and pH 5.

Approximately 2.5 hours and 7 hours, respectively, passed before amoxicillin ProD1 and cephalexin ProD1 intraconverted in 1N HCl, at pH 2.5. However, 81 hours at pH 5 was too slow, and both prodrugs were quite stable at pH 7.4 with no release of the parent drugs.

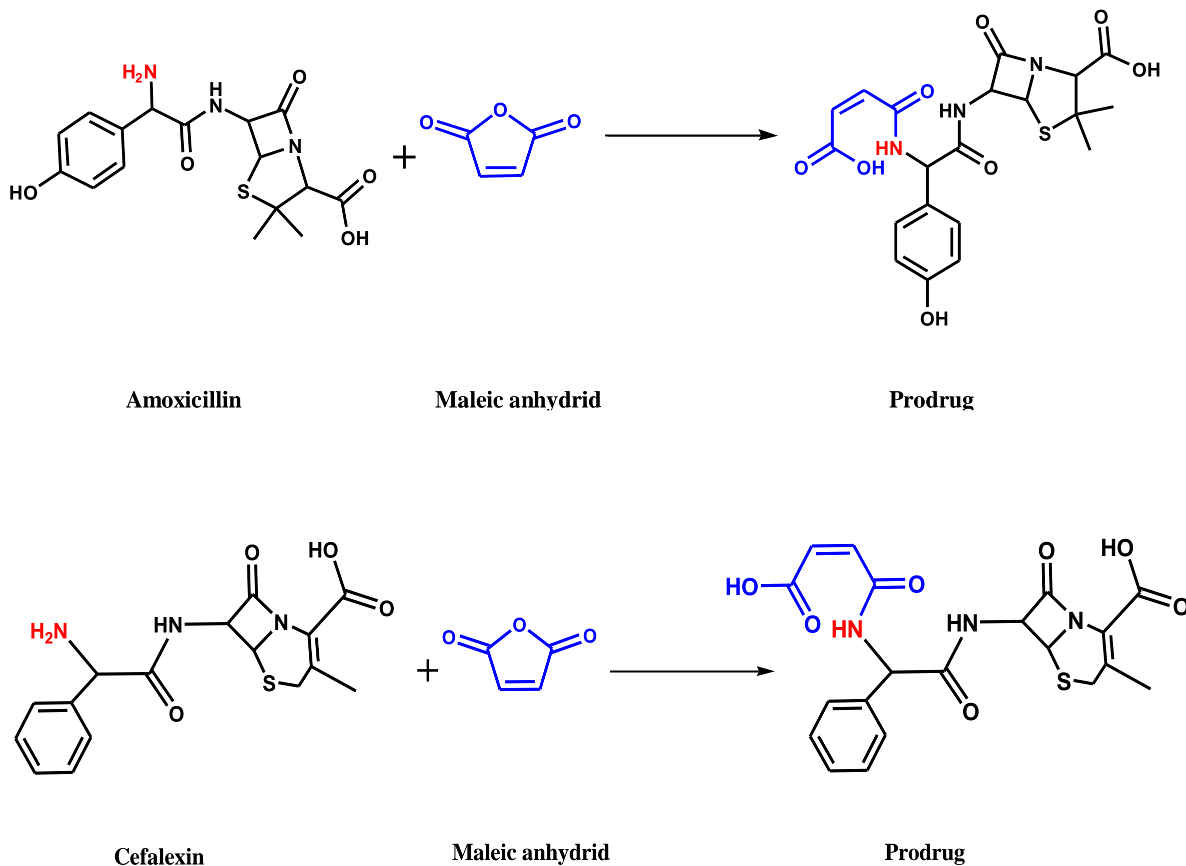


Figure (2.4.1): Amoxicillin and cephalexin prodrug synthesis

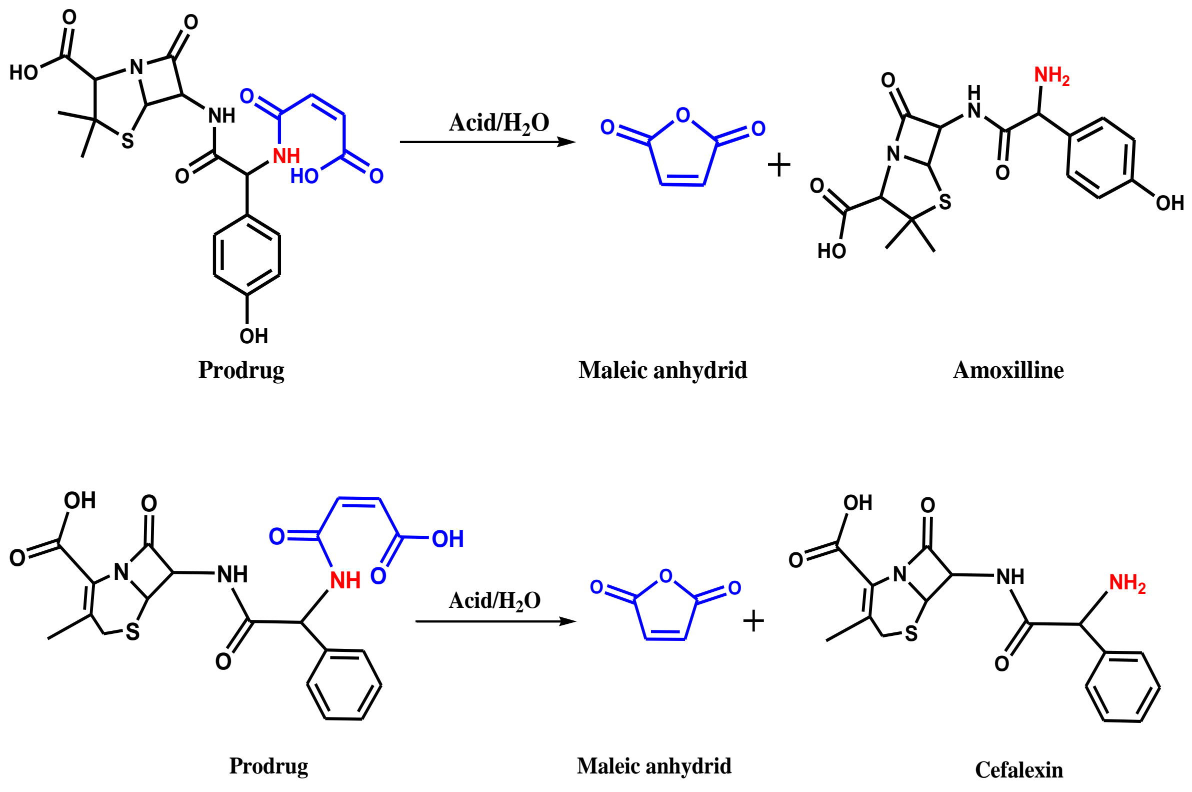


Figure (2.4.2) Amoxicillin and cephalexin prodrug hydrolysis to parent drug

2.5 Masking the Bitter taste of Guaifenesin.[50-52]

(RS)-3-(2-methoxyphenoxy)-propane-1, 2-diol is guaifenesin. It is a crystalline powder that ranges in color from white to slightly gray and is made from the resin of guaiacum trees. It is used as an expectorant in a variety of cough and cold remedies that are sold all over the world. Most frequently, this drug is used to break up mucus and phlegm, which then helps to relieve the symptoms of congestion brought on by a cold or allergies.

In addition to being freely soluble in alcohol, chloroform, and propylene glycol, guaifenesin is also soluble in water. It leaves the GI system quickly, is metabolized, and is eliminated in the urine.

For elderly and pediatric patients, its strong bitter flavor made it intolerable. The hydroxyl group of guaifenesin, which is connected to the formation of intermolecular connections by the bitter taste receptors, is what gives something its bitter flavor. (Bonding of hydrogen)

Guaifenesin hydroxyl groups are blocked by employing the linker technique to mask the bitter taste; it is available for oral administration as tablets of 200 mg or 400 mg and in liquid form (syrup). Guaifenesin 100 mg is contained in each 5ml. Three guaifenesin prodrugs were created and thoroughly described by ^{1}H-NMR, LC-MS, and FT-IR. These prodrugs are guaifenesin maleate, guaifenesin succinate, and guaifenesin glutarate **(Figures 2.5a, 2.5b, and 2.5c).**

In 1N HCl, the $t_{1/2}$ values for guaifenesin maleate, succinate, and glutarate are 2.01 hours, 7.03 hours, and 7.17 hours, respectively. However, all three of the prodrugs were completely stable at pH values of 3.3, 5.5, and 7.4, and there was no intraconversion of the prodrugs to the parent drug noted. Guaifenesin maleate had the highest rate constant (k_{obs}), which was 7.2X10-4, whereas glutarate prodrug had the lowest rate constant (k_{obs}), which was 2.36X10-4. This is because, in contrast to the succinate and glutarate prodrugs, the maleic prodrug has a double bond that exerts more strain and shortens the distance between the nucleophile and electrophile.

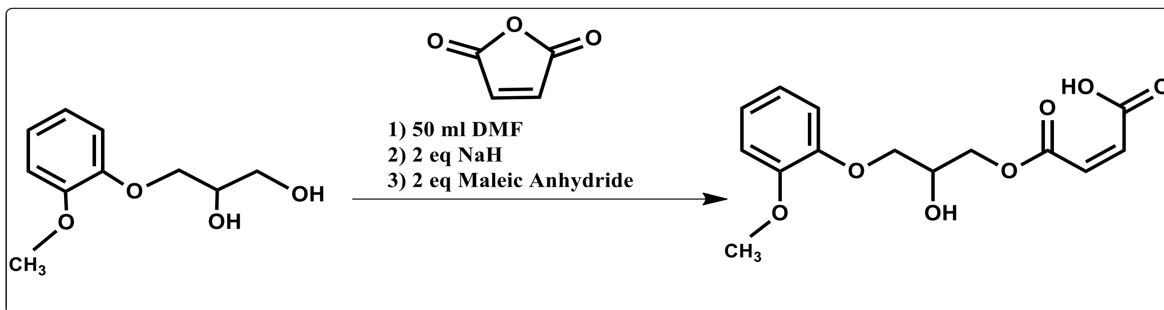


Figure (2.5a): Synthesis scheme for the preparation of guaifenesin maleate prodrug.

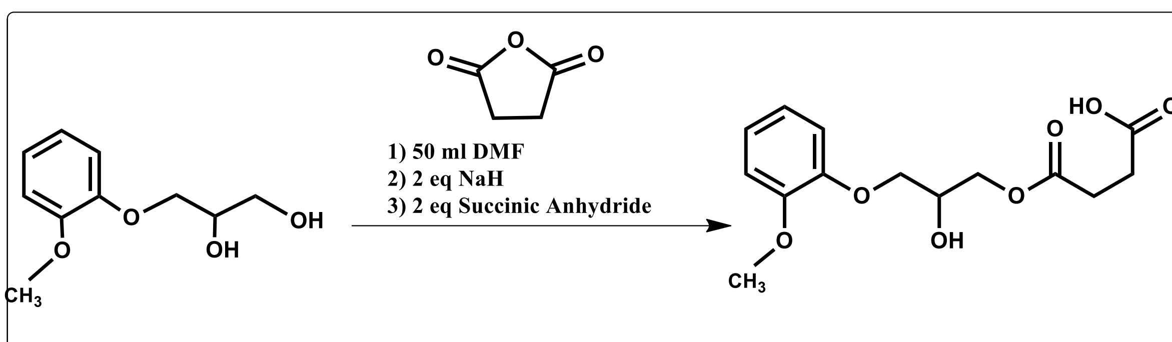


Figure (2.5b): Synthesis scheme for the preparation of guaifenesin succinate prodrug.

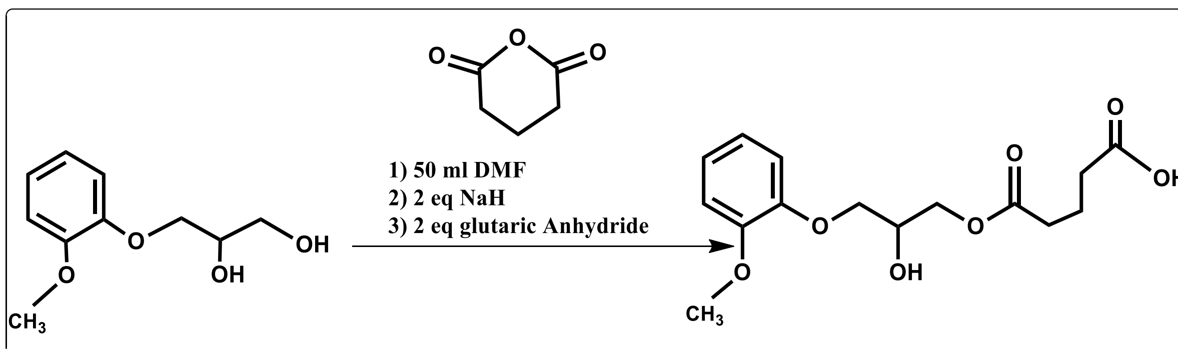


Figure (2.5c): Synthesis scheme for the preparation of guaifenesin glutarate prodrug.

Chapter three

Materials and Methods

Experimental part:

This chapter explains the three main parts of the study:

Part one describes all the reagents, materials, chemicals, and instruments used in this research.

The part two is the synthetic methods, which concern the identification of the synthesized prodrugs.

The part three includes kinetic studies that explain the specific preparations and analysis used to study quinine prodrugs intraconversion in buffer solutions by high-performance liquid chromatography (HPLC).

Chapter three

Materials and Methods

Part one

3.1.1 Chemicals and Reagents

Pure standards of quinine, maleic anhydride, succinic anhydride, Cis (1, 2)-cyclohexanedicarboxylic anhydride, potassium dihydrogen phosphate anhydrous, sodium hydroxide, concentrated hydrochloric acid (36%), sodium hydride (60%), sodium metals were obtained from Sigma Aldrich Co.

3.1.2 Solvents

Tetrahydrofuran (THF), Distilled water was obtained from a distillatory device available at Karaman‘s lab; hexane, ethyl acetate, high purity chloroform, and acetone. HPLC grade solvents of methanol, acetonitrile, and water were purchased from J.T. Paker.

3.1.3 Instrumentation and substance identification

The melting point and FT-IR were done at the pharmacy lab at Al-Quds University. HPLC was also done at the pharmacy lab at Al-Quds University. Proton nuclear magnetic resonance spectroscopy (^{1}H-NMR) was done at Hebrew University. Liquid chromatography-mass spectroscopy (LC-MS) was done at AlA'braj in Al-Quds University. Chemical hazard fuming hood, hotplates, pH meter, and the rotary evaporator are available at Karaman‘s Lab in the Faculty of Pharmacy, Al-Quds University.

3.1.3.1 FTIR

Infrared spectra (FT-IR) were obtained from a potassium bromide matrix (4000 – 400 cm^{-1}) using a Perkin-Elmer Precisely Spectrum 100 FT-IR spectrometer.

3.1.3.2 HPLC and NMR devices

The high-pressure liquid chromatography (HPLC) system at AlQuds University consisted of an Alliance 2695 module equipped with a 2996 Photodiode array detector from Waters (Germany). Empower 2 ™ software (Waters, Germany) was used to accomplish Data acquisition and control. Analyses were separated with a 4.6 mm x 250 mm XBridge® C18 column (5 µm particle size) used in conjunction with a 4.6 x 20 mm XBridge® C18 guard column. Microfilters with a porosity of 0.45m were typically used (Acrodisc® GHP, Waters). ^{1}H-NMR experiments were performed with a Bruker AvanceII 400 spectrometer equipped with a 5 mm BBO probe at Hebrew University.

3.1.3.4 LC MS device

The synthesized prodrugs were subjected to LCMS analysis in the electrospray ionization mode (ESI) using the LC-MSMS instrument by Thermo Fisher Scientific at Al-Quds University. (400-500) Dalton (Da) full scan, 3500-volt positive ion voltage, 325 ^{o}C ion transfer tube temperature, 275 ^{o}C vaporizer temperature.

3.1.3.5 PH meter and TLC

In this study, pH values for all buffers and reactions were measured using a pH meter model HM-30G: TOA electronicsTM. Thin-layer chromatography (TLC) was performed using TLC plastic sheets of silica gel, 20x20 cm, a layer thickness of 0.2 mm, and UV light to localize the spots on the chromatograms.

Part two

3.2.1 Synthesis of Quinine Prodrugs:

Quinine ProD 1 preparation: **(Figure 3.2.1a)** 3 millimoles (0.9725g) of quinine in a 250 milliliter (ml) round-bottom flask, was dissolved in 100 ml of THF after filtration from sodium, then added 0.65 g of NaH slowly to the same flask. The resulting solution was stirred for 30 minutes, then 3 mmol (0.345g) of the linker of Cis (1, 2)-cyclohexanedicarboxylic anhydride was added to the reaction mixture and stirred and heated at a temperature of 70 °C for 5 days. The reaction was monitored by thin-layer chromatography, which was performed to detect the completion of the reactions using a methanol and chloroform (1:2) system as an eluent. Reaction after completion: 1ml of 0.1 N HCL was added to the reaction. The solvent was evaporated by the rotary evaporator and the resulting precipitate was washed with 50 ml of ethyl acetate and hexane, respectively. The white precipitate was dried in an oven at 37 °C.

Quinine ProD 2: **(Figure 3.2.1b)** the same procedure was followed for quinine ProD 1, but instead of using cis-1, 2-cyclohexanedicarboxylic anhydride linker, succinic anhydride was used at 3 mmol (0.30021g).

Quinine ProD 3: **(Figure 3.2.1c)** the same procedure was followed for quinine ProD 1 and ProD 2, but the linker was maleic anhydride, which was used at 3 mmol (0.294g).

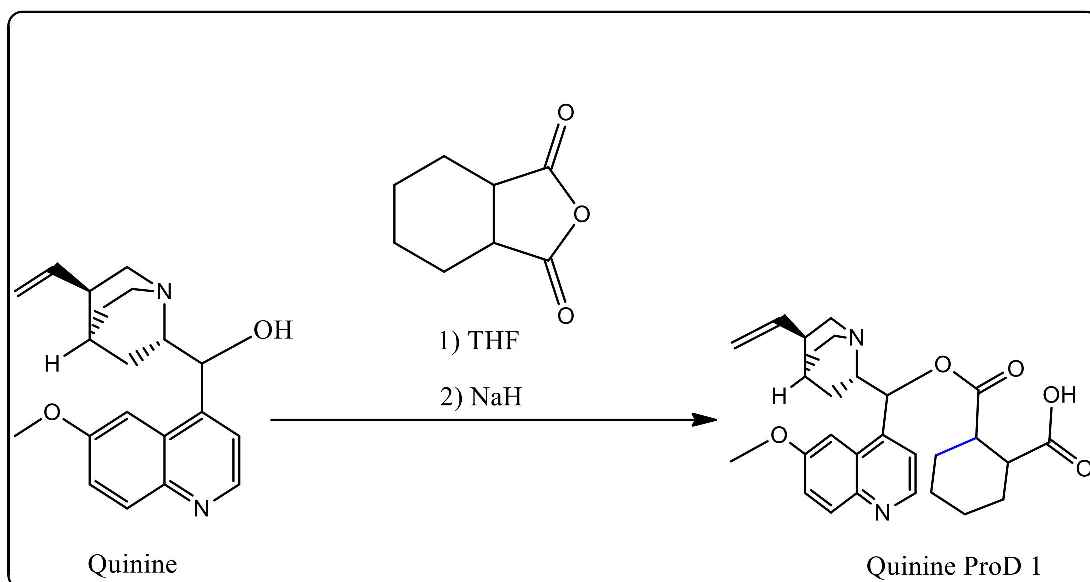


Figure (3.2.1a): Synthesis scheme for the preparation of Quinine ProD 1.

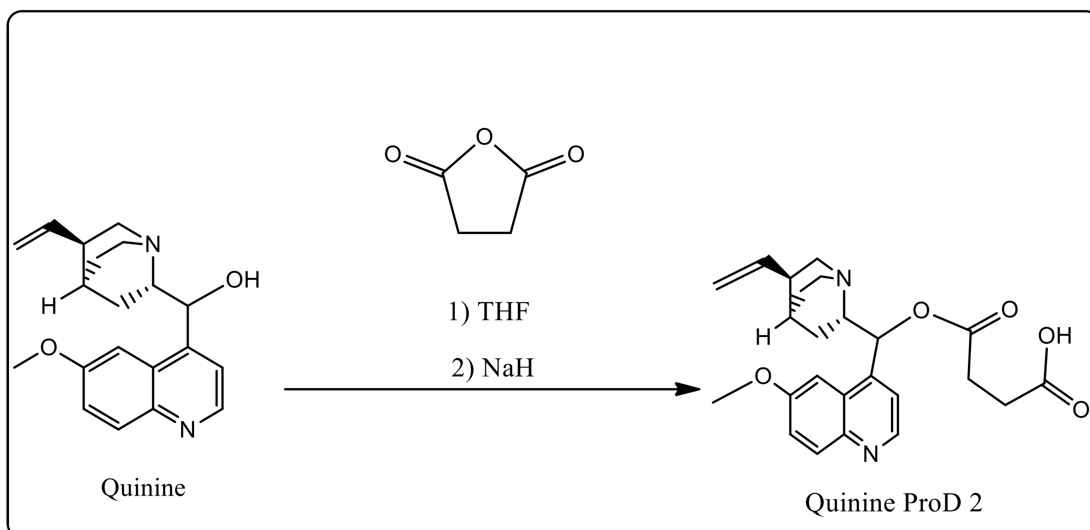


Figure (3.2.1b): Synthesis scheme for the preparation of Quinine ProD 2.

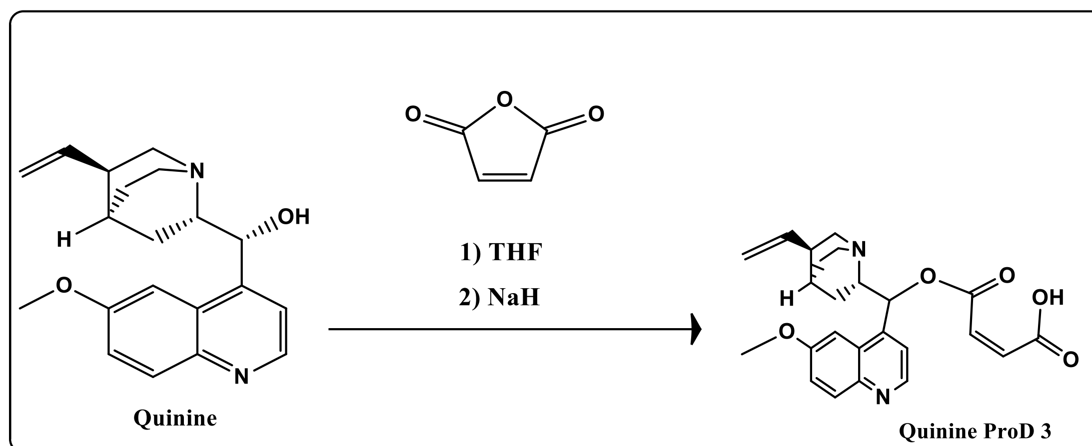


Figure (3.2.1c): Synthesis scheme for the preparation of Quinine ProD 3.

Melting point of quinine ProD 1 was 280 ºC, ^{1}H-NMR (500 MHz) (ppm, δ) of the ProD1 D_2O 5.29 (d, 1H,CH-O), 3.19 (t,1H,-N-CH-), 1.35-2.58 (tt, 4H,-CH_2-CH_2-) 1.37 (d,2H-CH-CH_2-CH-), 7.47-7.98 (dd,2H,-CH=CH-CN-), 7.54,8.47 (dd,2H, -CH=CH-),1.44 (m,1H,-CH-CH2-), 1.42 (m,4H,-CH_2-CH_2-) , 3.97 (s, O-CH_3) , 2.65-3.20 (qqq,6H, (-CH_2-CH_2-CH_2-COOH). IR (KBr/ν $_{max}$ cm $^{-1}$) 1559 (C=O), 2923 (CH), 1403 (C=C), 3392 (OH). LC-MS (+ESI mode) m/z 464.18 [M+H] + [-H_2O]. Chemical formula is $C_{28}H_{34}N_2O_2$ (% yield = 95%).

Melting point of quinine ProD 2 was 293 ºC, ^{1}H-NMR (500 MHz) (ppm, δ) of the ProD 2 D_2O 5.47-5.49 (d, 1H,CH-CH-N), 3.04-3.21 (q,1H,CH-N-CH_2), 2.67-2.69 (t,2H, N-CH_2-CH_2), 1.70-1.75 (q, 2H, N-CH_2-CH_2-CH), 2.14-2.15 (d, 2H, CH_2-CH-CH=CH_2), 2.08-2.12 (q,1H, CH_2-CH-CH=CH_2), 1.55-1.65 (m,1H, CH_2-CH_2-CH), 1.66-1.7(t, 2H, CH_2-CH_2-CH-CH_2), 7.40-7.42 (dd, 2H, CH= CO-CH), 7.46-8.63 (ddd, 3H, CH-C-N-CH=CH), 3.91 (s, O-CH_3) , 5.92-5.99 (ddd,3H,CH=CH_2), 5.15-5.21(dd,2H,C=O-CH_2-CH_2-COOH). IR (KBr/ν $_{max}$ cm $^{-1}$) 1548 (C=O), 2923 (CH), 1397 (C=C), 3352 (OH). LC-MS (-ESI mode) m/z 423.31 (M-H). Chemical formula is $C_{24}H_{28}N_2O_2$ (% yield = 91%).

Melting point of quinine ProD 3 was 279 °C, ^{1}H-NMR (500 MHz) (ppm, δ) of the ProD 3 D_2O 5.47-5.49 (d, 1H,CH-CH-N), 3.04-3.21 (q,1H,CH-N-CH_2), 2.67-2.69 (t,2H, N-CH_2-CH_2), 1.71-1.75 (q, 2H, N-CH_2-CH_2-CH), 2.231-2.236 (d, 2H, CH_2-CH-CH=CH_2), 2.13- 2.19 (q,1H, CH_2-CH-CH=CH_2), 1.75-1.8 (m,1H, CH_2-CH_2-CH), 1.66-1.7(t, 2H, CH_2-CH_2-CH-CH_2), 7.46-7.48 (dd, 2H, CH=CO-CH), 7.50-8.71 (ddd, 3H, CH-C-N-CH=CH), 3.96 (s, O-CH_3) , 5.92-5.99 (ddd,3H,CH=CH_2), 5.23-5.29 (dd,2H,C=O-CH=CH-COOH). IR (KBr/ν_{max} cm $^{-1}$) 1563 (C=O), 2924 (CH), 1401 (C=C), 3458 (OH).LC-MS (+ESI mode) m/z 471.35 (M+2Na). Chemical formula is $C_{24}H_{26}N_2O_2$ (% yield = 87%).

Part Three

3.3.1 Kinetic methods:

3.3.1.1 Buffer preparation

Potassium dihydrogen phosphate (6.8 g) was dissolved in 1000 ml of deionized water for HPLC, and the 1000 ml of buffer was divided into four equal volumes, each volume differing in pH, pH 2.2, pH 5.5, pH 7.4, and 0.1 N HCl. The pH of buffer pH 2.2 was prepared by diluting O-Phosphoric acid in a 250 ml volumetric flask and deionized water was added to the final volume of 250 ml. The same procedure was done for the preparation of buffers pH 5.5 and pH 7.4. The pH was adjusted by adding 1N NaOH. 0.1 N HCl was prepared by diluting 1ml of HCl in 100 ml of D.W.

Intra-conversion of 500 ppm quinine prodrug 1, quinine prodrug 2, and quinine prodrug 3 samples were prepared from each pH 2.2, pH 5.5, pH 7.4, and 0.1 N HCl was followed by HPLC at a wavelength of 250 nm. Conversion reactions were run mostly at 37°C.

3.3.1.2 Calibration Curve

A stock solution of Quinine prodrugs 1, 2, and 3 (50ml) was prepared by dissolving 25 mg of each prodrug in 50 ml of D.W. The following diluted solutions were prepared from the stock solution: 25 mg, 12.5 mg, and 6.25 mg, 3.125 mg. Then 20 microliter of each solution was injected into the HPLC apparatus using a 10 mm c18 column.

Acetonitrile, Methanol, and buffer (pH 3) with a ratio of (20%: 40%: 40%) were used as mobile phase with a flow rate of 1 ml/min and UV detection at a wavelength of 250 nm.

The peak area versus concentration of the pharmaceutical was then plotted, and the R^2 of the plot was recorded.

3.3.1.3 Preparation sample solution

A 500 ppm of quinine prodrugs 1, 2, and 3 was prepared by dissolving 25 mg of Quinine prodrug 1, 2 and 3 in 50 ml of 0.1 N HCl, pH 2.2, pH 5.5, and pH 7.4 then each sample was injected into HPLC apparatus to detect the retention time of quinine prodrugs 1, 2 and 3.

And we noticed that the peak of prodrugs disappears and the drugs appear with time.

Chapter Four

Results and Discussion

Chapter four

Results and discussion

We prepared three novel prodrugs of quinine by using three different linkers in order to overcome the bitter taste of quinine , the three prodrugs were prepared by esterification in high yield, which were characterized by melting point, FT-IR, ^{1}H-NMR, and LC-MS analytical techniques.

4.1 Quinine prodrugs characterization using different analytical techniques:

4.1.1 Melting point:

The melting point of quinine was 177 ^{o}C. But the melting point of quinine ProD 1 was 280 ^{o}C, quinine ProD 2 was 293 ^{o}C and quinine ProD 3 was 279 ^{o}C.

4.1.2 Fourier transforms infrared spectroscopy (FTIR)

The FTIR spectra for the synthesized prodrugs (quinine ProD 1, quinine ProD 2, and quinine ProD 3), **Figure (4.1.2b)**, **Figure (4.1.2c)**, and **Figure (4.1.2d)**, respectively, showed prominent peaks that were analyzed and compared to the quinine spectra, **Figure (4.1.2a)**.

The peaks observed in quinine ProD 1 at 3392 cm^{-1}, 3352 cm^{-1} in quinine ProD 2, and at 3458 cm^{-1} in quinine ProD 3 are characteristic of the O-H stretching seen in carboxylic acids, compared to the peak produced in the quinine standard at 3150 cm^{-1}, which in fact comes from the O-H stretch seen in alcohol. The prodrugs peak produced at (2924-2923) cm^{-1} is characteristic of the C-H stretching. But the peak pattern for the band produced by the quinine standard at 2930 cm^{-1} and the peaks produced at (1563–1548) cm^{-1} in quinine prodrugs is characteristic of the (C=O)

carbonyl group. The peaks produced at 1403 cm^{-1} in quinine ProD 1, 1397 cm^{-1} in quinine ProD 2, and 1401 cm^{-1} in quinine ProD 3, are characteristic of the C=C stretching of the benzene ring in the prodrugs compared with the peak produced in the quinine standard at 1429 cm^{-1}.

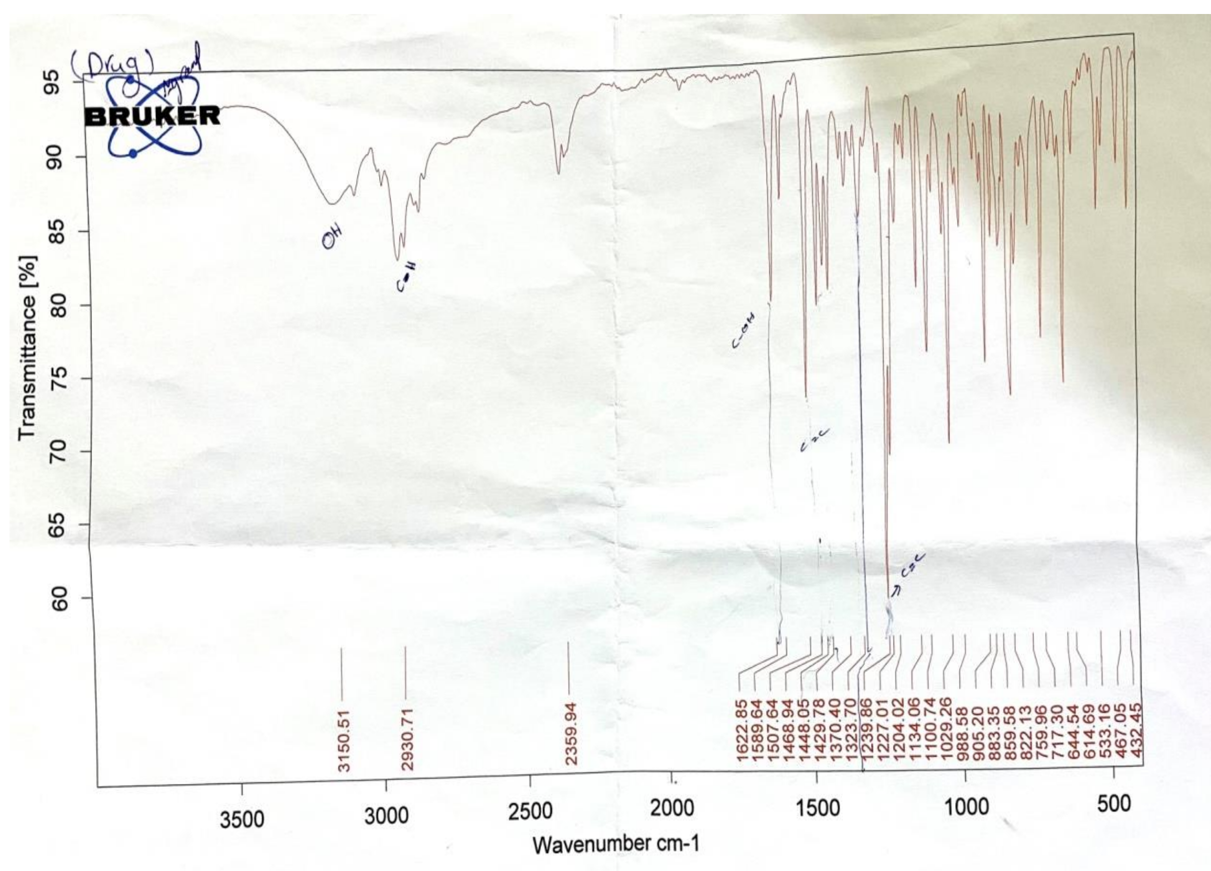


Figure (4.1.2a) FT-IR spectrum of quinine standard.

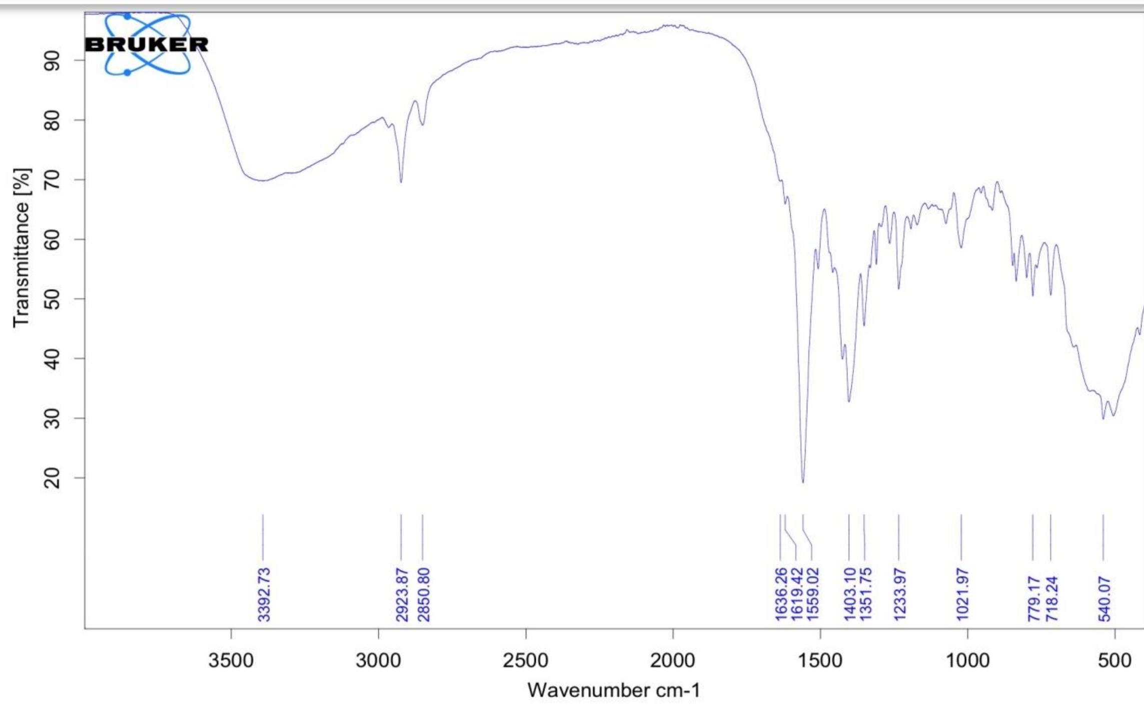


Figure (4.1.2b) FT-IR spectrum of quinine ProD 1.

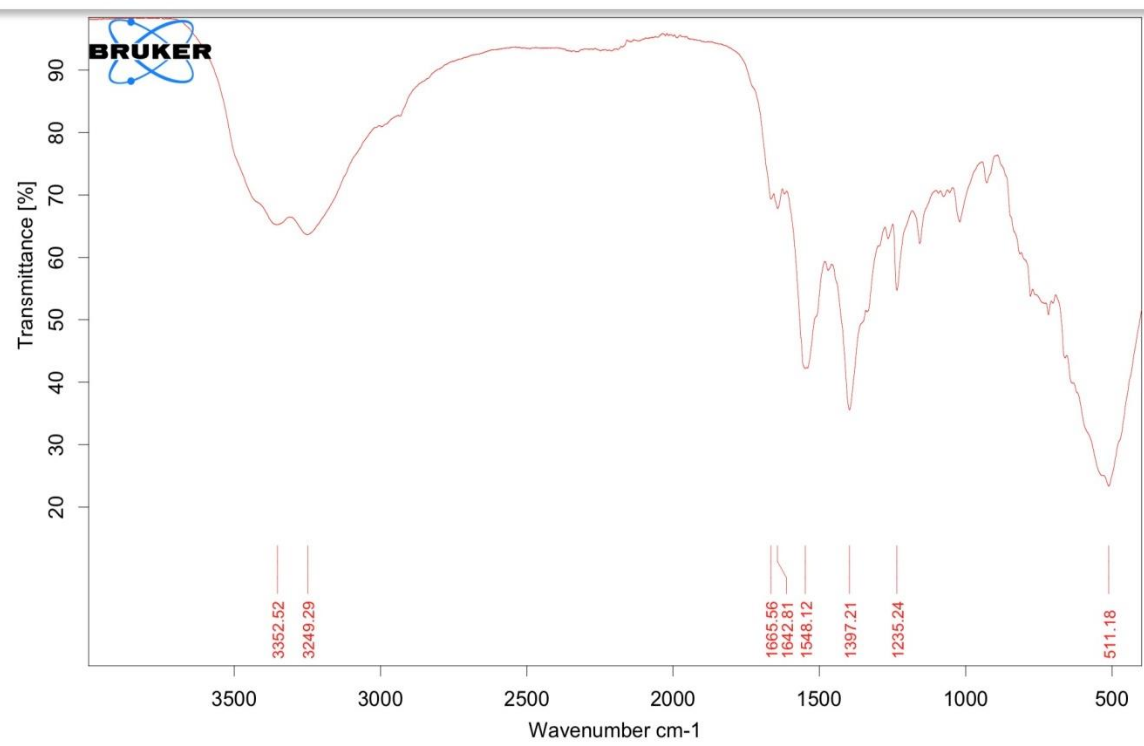


Figure (4.1.2c) FT-IR spectrum of quinine ProD 2.

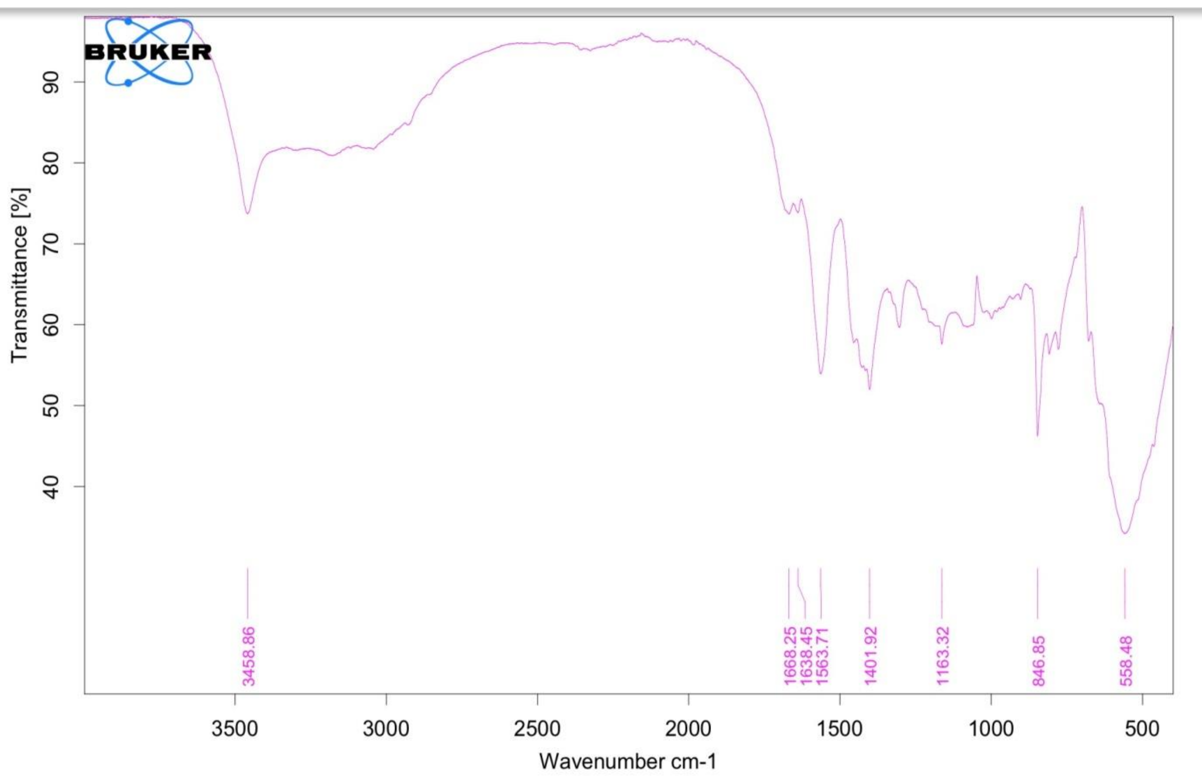
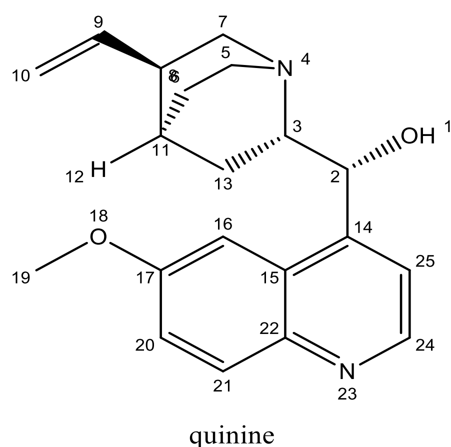


Figure (4.1.2d) FT-IR spectrum of quinine ProD 3.

4.1.3 ¹H-NMR

The ¹H-NMR spectrum of quinine prodrugs 1-3, **Figure (4.1.3b)**, **Figure (4.1.3c)**, and **Figure (4.1.3d)**, respectively, showed characteristic proton chemical shifts when compared with that of the quinine spectrum, **Figure (4.1.3a)**.

4.1.3.1¹H-NMR of Quinine:



quinine

24Hs, M.W=324 g/mol

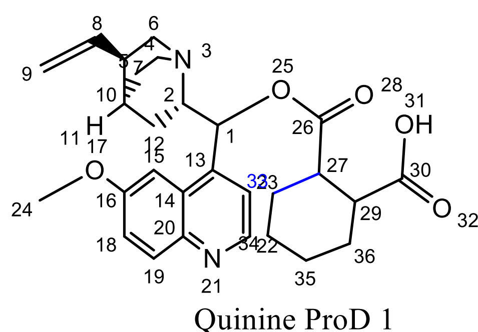
The chemical formula is $C_{20}H_{24}N_2O_2$

The doublet signals at 5.6 ppm are characteristic of methine (-CH) in the quinine. The quartet at 3.49 ppm of the piperidine from methine (-CH) proton. The triplet signals at 3.01 ppm are typical of piperidine (-$\underline{CH_2}$-CH_2-N-) proton and at 1.75 ppm is a proton from (-CH_2-$\underline{CH_2}$-N-). The singlet at 2.5 ppm is typical of an (-N-$\underline{CH2}$-CH-) proton and the triplet at 2.7 ppm is a proton of methine (-N-CH2-$\underline{CH}$-) from piperidine. The singlet at 2.00 ppm is a proton (H) at position no. (11) and the doublet at 1.68 ppm from (-CH_2-) at position no. (13). The singlet at 3.35 ppm is a proton from methyl (-CH_3).

The doublet at 7.55 ppm is typical of the aromatic benzene ring from the ethylene (-$\underline{HC}$=C-) proton at position (16), and the same shift at position (20) from the benzene

ring (-HC=C-CH-). The doublet at 8.75 ppm and 7.65 ppm are typical of the quinoline ring (-N-CH=CH-) at positions (24, 25) in the quinine standard. **Figure (4.1.3a).**

4.1.3.2 ^{1}H-NMR of Quinine ProD 1



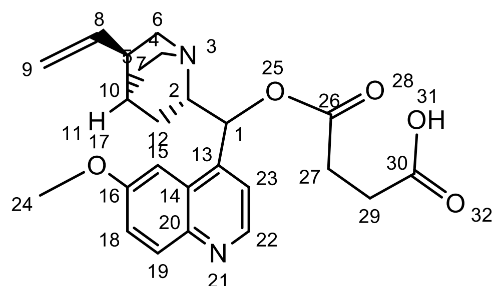
Quinine ProD 1

34Hs, MW= 478 g/mol

The chemical formula is $C_{28}H_{34}N_2O_2$ (95%).

The doublet at 5.29 ppm is a signal of methine from (-CH-O-). The chemical shift of proton for methine of piperidine (-NCH-) as a triplet signal at 3.19 ppm. The triplet signals at 2.58 ppm and 1.35 ppm are typical of (-CH_2-CH_2-), (-CH_2-CH_2-). The doublet signals at 1.37 ppm , 7.47 ppm and 7.98 ppm were observed of protons for piperidine ring of (-CH- CH_2 -CH-) , and quinoline ring of (-CH = CH- CN-) , (-CH= CH- CN-). The doublet signals at 8.72 ppm, and 7.54 ppm are chemical shifts of protons from methylene of quinoline (-CH=CH-), (-CH=CH-). The singlet at 3.97 ppm is a proton of a methyl group (-O-CH_3). The multiplet signals at 1.44 ppm are characteristic of the proton of the methine of the piperidine ring (—CH-CH_2—). The multiplet signals at 1.42 ppm are characteristic of the protons of cyclohexane (-CH_2-CH_2) the quartet at 2.65 ppm, 3.2 ppm, 1.94 ppm, 1.67 ppm are signals of protons of cyclohexane (-CH-CH-CH_2-) **(Figure 4.1.3b)**.

4.1.3.3 ^{1}H-NMR of Quinine ProD 2



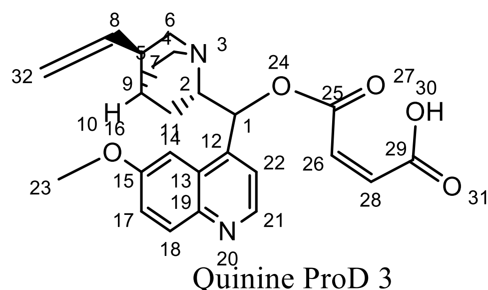
Quinine ProD 2

28Hs, MW= 424 g/mol

The chemical formula is $C_{24}H_{28}N_2O_2$ (91%).

^{1}H-NMR (500 MHz) (ppm, δ) of the ProD 2 D_2O 5.47-5.49 (d, 1H,CH-CH-N), 3.04-3.21 (q,1H,CH-N-CH_2), 2.67-2.69 (t,2H, N-CH_2-CH_2), 1.70-1.75 (q, 2H, N-CH_2-CH_2-CH), 2.14-2.15 (d, 2H, CH_2-CH-CH=CH_2), 2.08-2.12 (q,1H, CH_2-CH-CH=CH_2), 1.55-1.65 (m,1H, CH_2-CH_2-CH), 1.66-1.7(t, 2H, CH_2-CH_2-CH-CH_2), 7.40-7.42 (dd, 2H, CH=CO-CH), 7.46-8.63 (ddd, 3H, CH-C-N-CH=CH), 3.91 (s, O-CH_3) , 5.92-5.99 (ddd,3H,CH=CH_2), 5.15-5.21(dd,2H,C=O-CH_2-CH_2-COOH) **(Figure 4.1.3c).**

4.1.3.4 ^{1}H-NMR of Quinine ProD 3



Quinine ProD 3

26Hs, MW= 423 g/mol

The chemical formula is $C_{24}H_{26}N_2O_2$ (87%).

^{1}H-NMR (500 MHz) (ppm, δ) of the ProD 3 D_2O 5.47-5.49 (d, 1H,CH-CH-N), 3.04-3.21 (q,1H,CH-N-CH_2), 2.67-2.69 (t,2H, N-CH_2-CH_2), 1.71-1.75 (q, 2H, N-CH_2-CH_2-CH), 2.231-2.236 (d, 2H, CH_2-CH-CH=CH_2), 2.13- 2.19 (q,1H, CH_2-CH-CH=CH_2), 1.75-1.8 (q,1H, CH_2-CH_2-CH), 1.66-1.7(t, 2H, CH_2-CH_2-CH-CH_2), 7.46-7.48 (dd, 2H, CH=CO-CH), 7.50-8.71 (ddd, 3H, CH-C-N-CH=CH), 3.96 (s, O-CH_3) , 5.92-5.99 (ddd,3H,CH=CH_2), 5.23-5.29 (dd,2H,C=O-CH=CH-COOH) **(Figure 4.1.3d).**

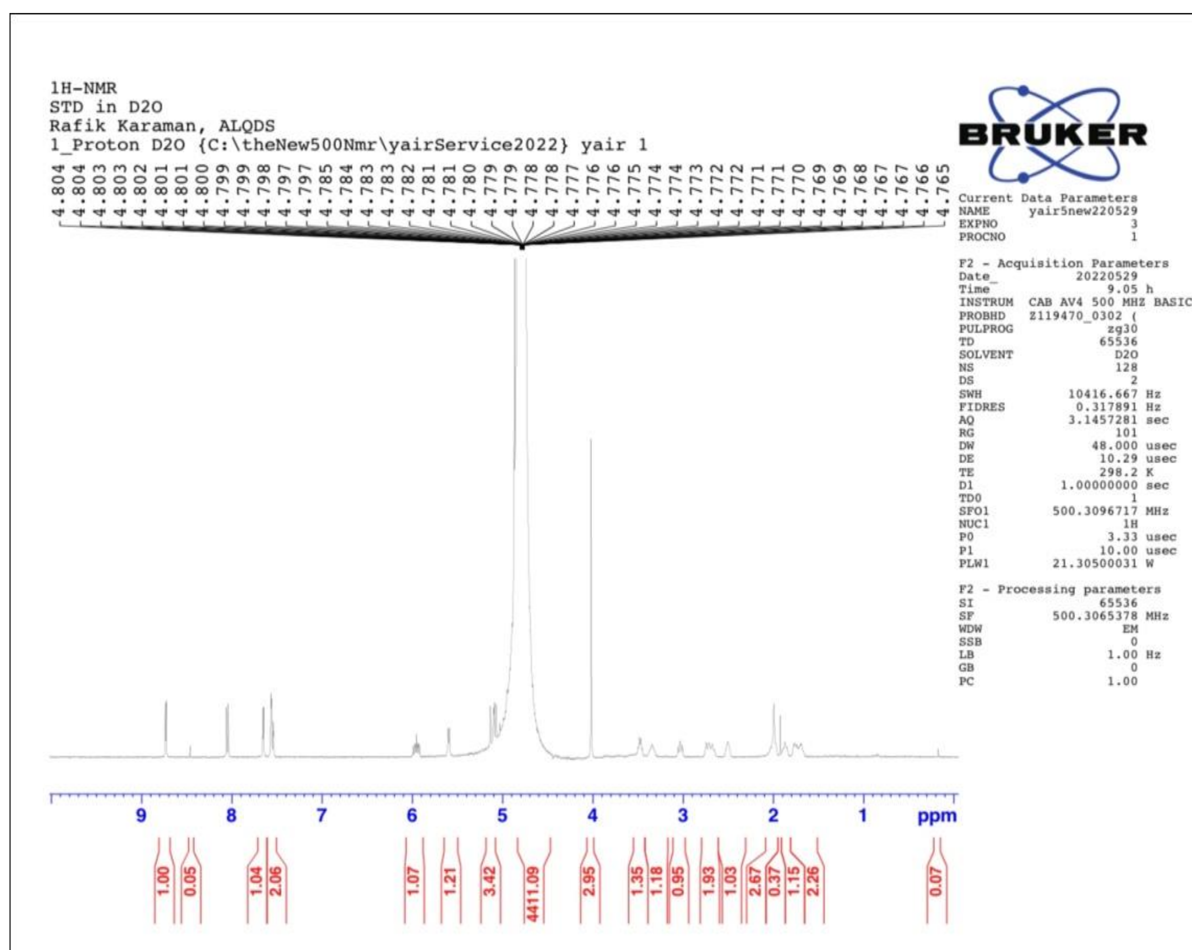


Figure (4.1.3a): Whole-spectrum ^{1}H-NMR of Quinine.

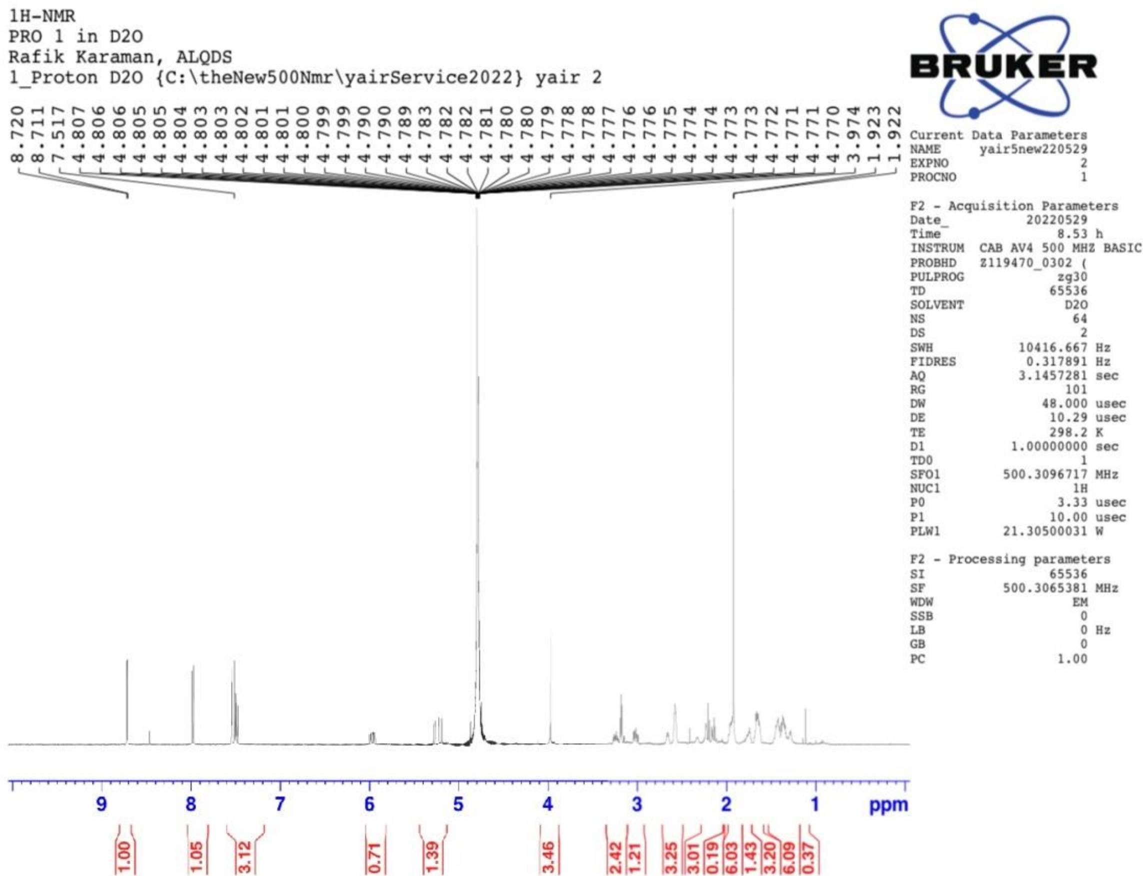


Figure (4.1.3b): ¹H-NMR spectrum of Quinine ProD 1

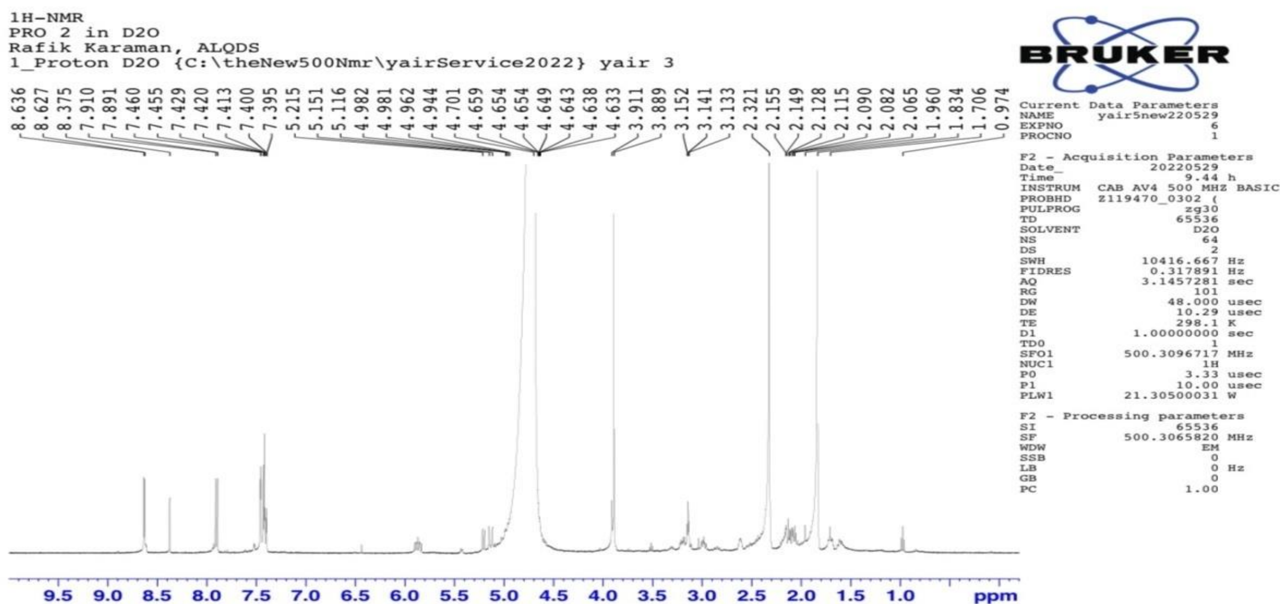


Figure (4.1.3c): ^{1}H-NMR spectrum of Quinine ProD 2

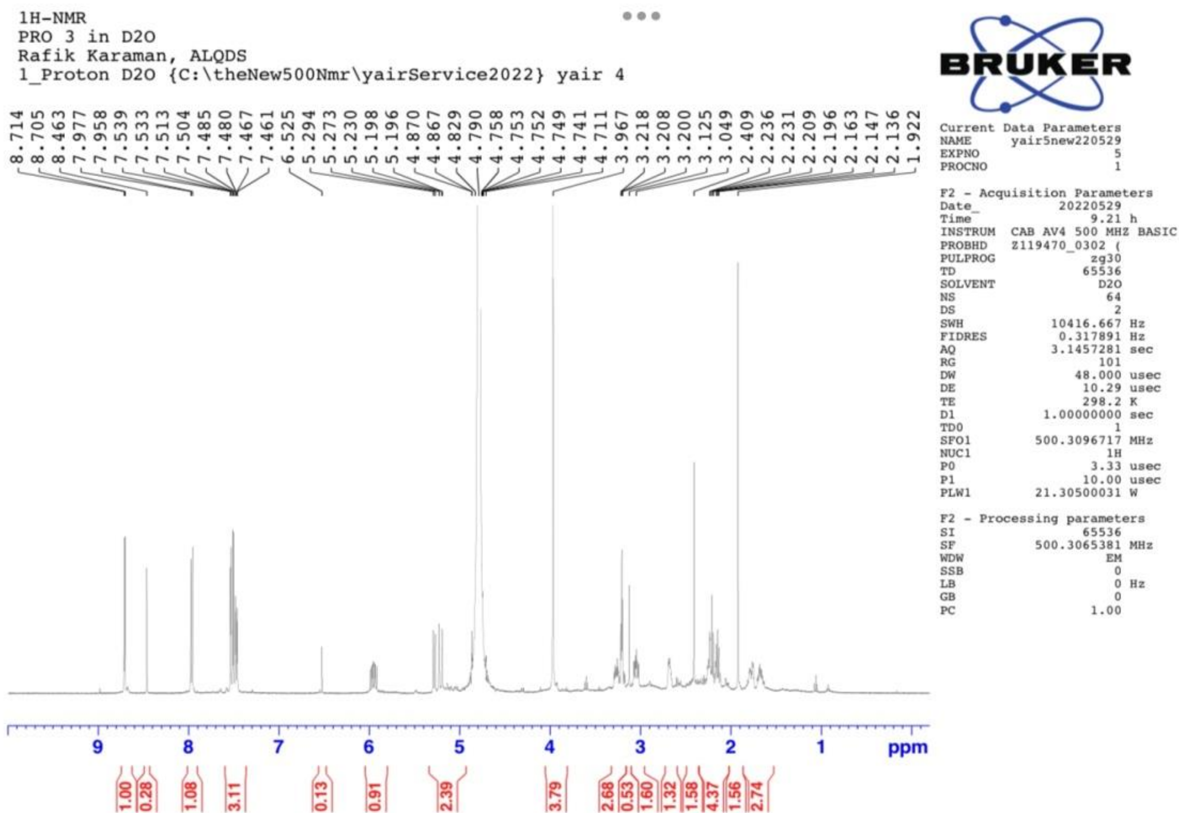


Figure (4.1.3d): ^{1}H-NMR spectrum of Quinine ProD 3

4.1.4 Liquid chromatography-mass spectrometry (LC-MS)

To characterize the synthesized prodrugs (quinine ProD 1, quinine ProD 2, and quinine ProD 3), samples were analyzed by liquid chromatography coupled with a mass quadruple-time of flight mass spectrometer. The analysis was performed in the electrospray ionization mode. The corresponding molecular masses were obtained (**Figures (4.1.4a)**, **(4.1.4b)**, and **(4.1.4c)**).

1) LC-MS (+ESI mode) m/z 464.18 Da [M+H] + [-H_2O]: the molecular mass of ProD 1 is 478.

2) LC-MS (-ESI mode) m/z 423.31 Da [M-1]: the molecular mass of ProD 2 is 424.

3) LC-MS (+ESI mode) m/z 471.35 Da [M+2Na]: the molecular mass of ProD 1 is 423.

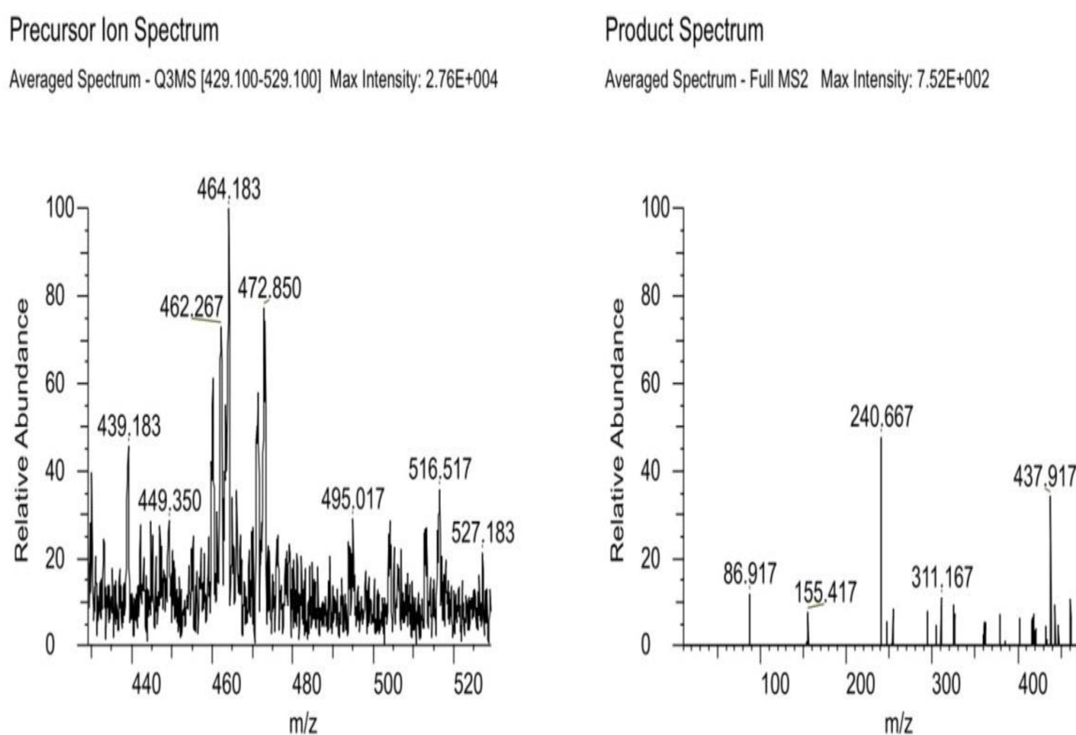
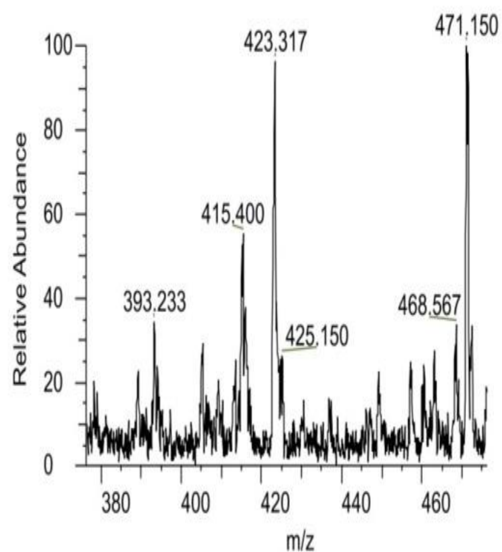


Figure (4.1.4a) LC-MS spectrum of quinine ProD 1.



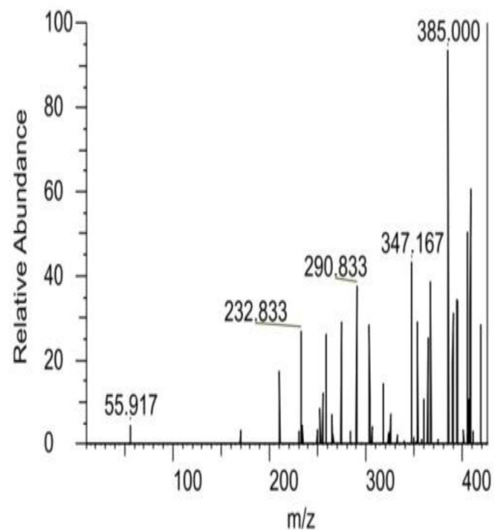
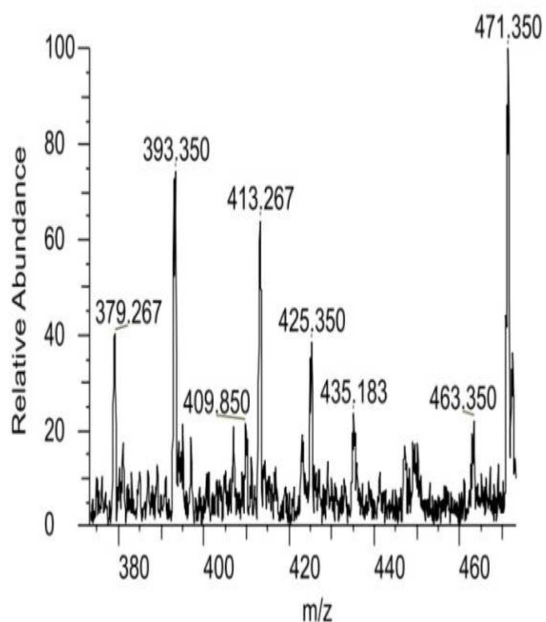


Figure (4.1.4b) LC-MS spectrum of quinine ProD 2.



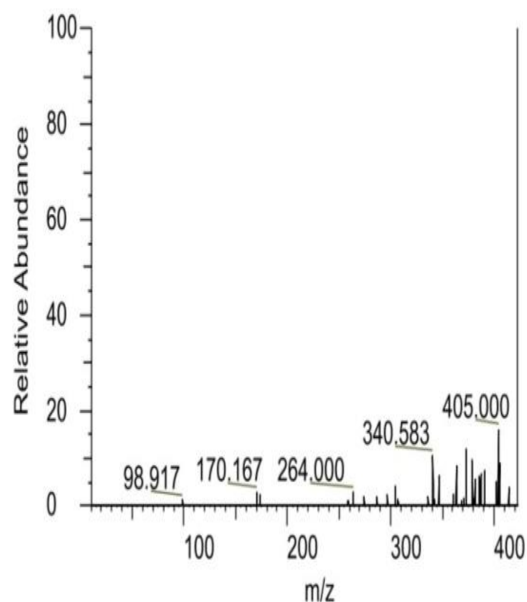


Figure (4.1.4c) LC-MS spectrum of quinine ProD 3.

4.2 Hydrolysis studies

The hydrolysis of quinine prodrugs was investigated using HPLC. Peaks of standard and degradation products were monitored to determine the rate of cleavage of the three synthesized quinine prodrugs. Kinetic studies were carried out at 37°C and at various pH in buffers such as 0.1N HCl, pH 2.2, pH 5.5, and pH 7.4 (which mimic physiological environments in the human body). The 0.1N HCl and pH 2.2 were chosen to study the intra-conversion of the quinine prodrugs in the pH of the stomach because the mean stomach pH of the adult is approximately 1-3, whereas buffer pH 5.5 represents the small intestine pathway. The pH 7.4 was selected to study the intra-conversion of the tested prodrugs in the pH of the blood circulation system.

Calibration curves were obtained by plotting the peak areas of the prodrugs versus concentration. As shown in **Figure (4.2.1)**, linearity with R^2 of 0.98 for both quinine ProD 1 quinine ProD 2 and 0.997 for ProD 3 were obtained.

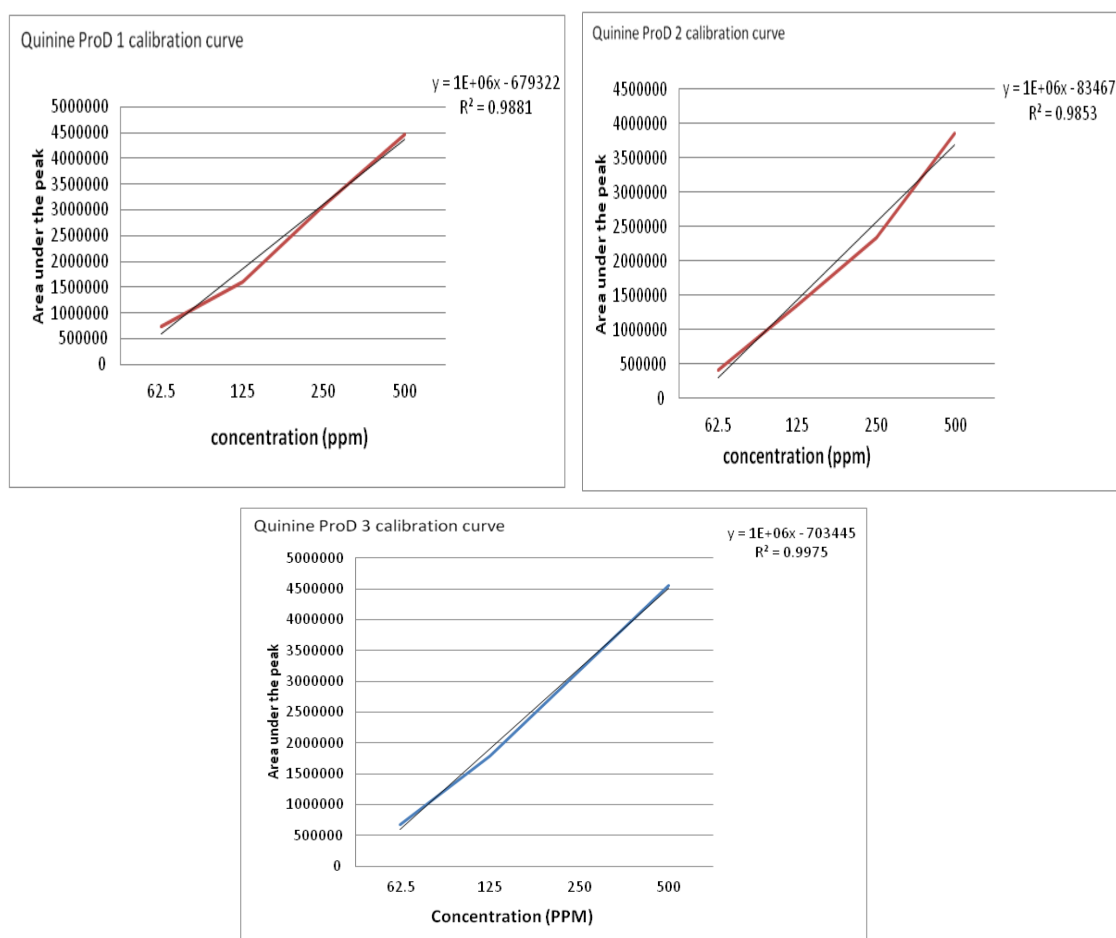


Figure (4.2.1): Calibration curves of ProD 1 (a), ProD 2 (b), and ProD 3 (c).

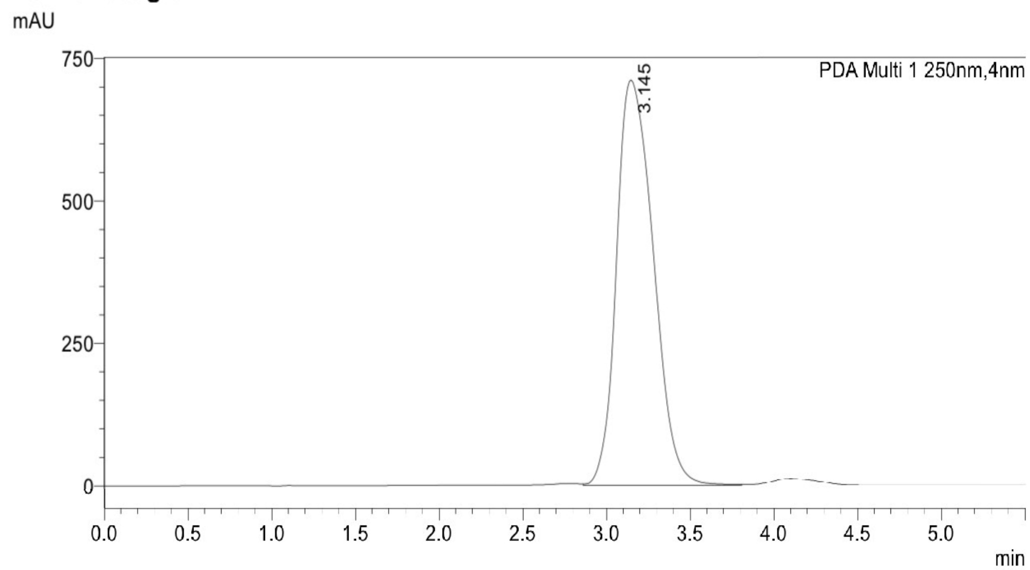
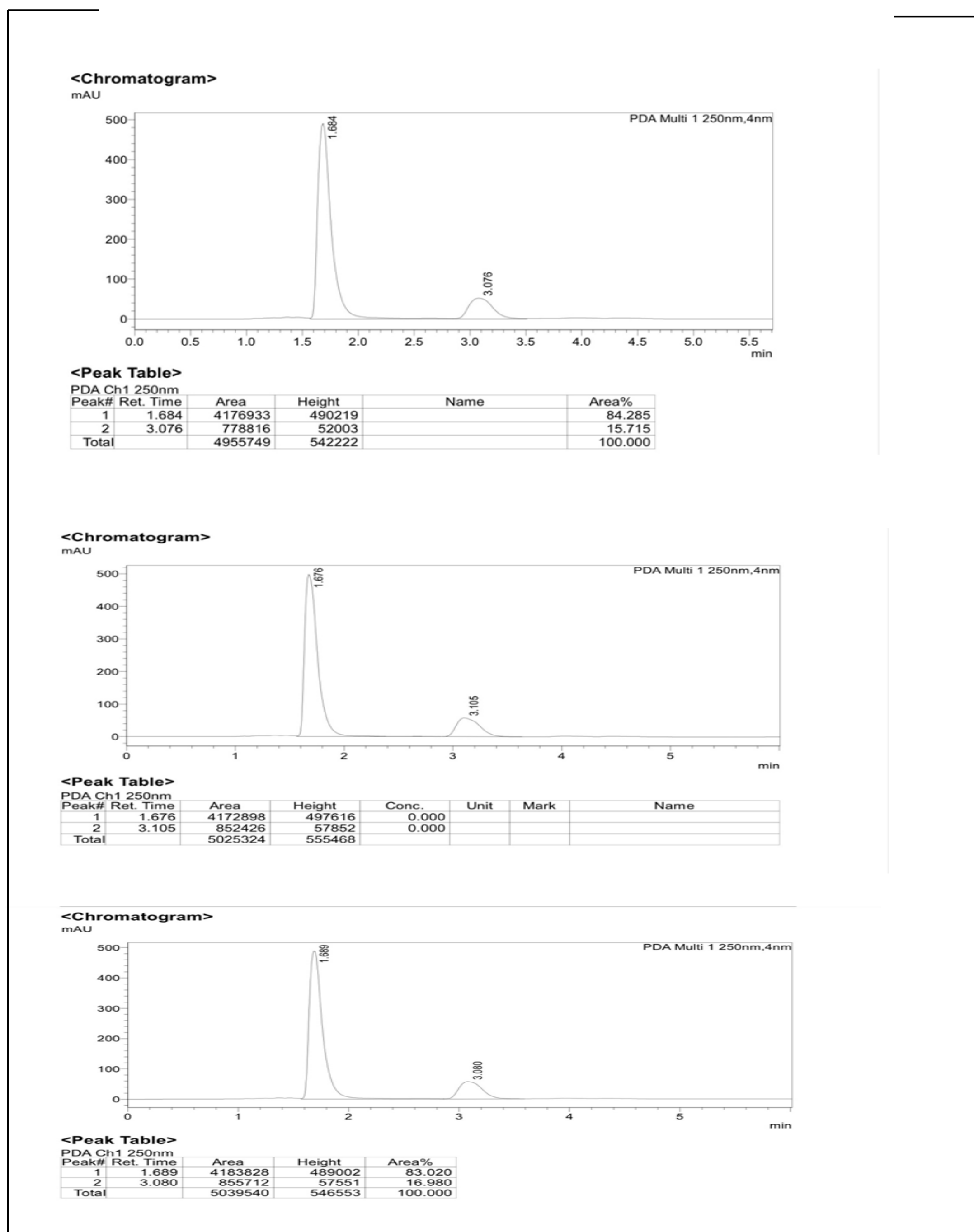


Figure (4.2.2): Spectrum of Quinine STD.

Quinine ProD 1 kinetic study at 0.1N HCl, pH 2.2, pH 5.5, and pH 7.4.

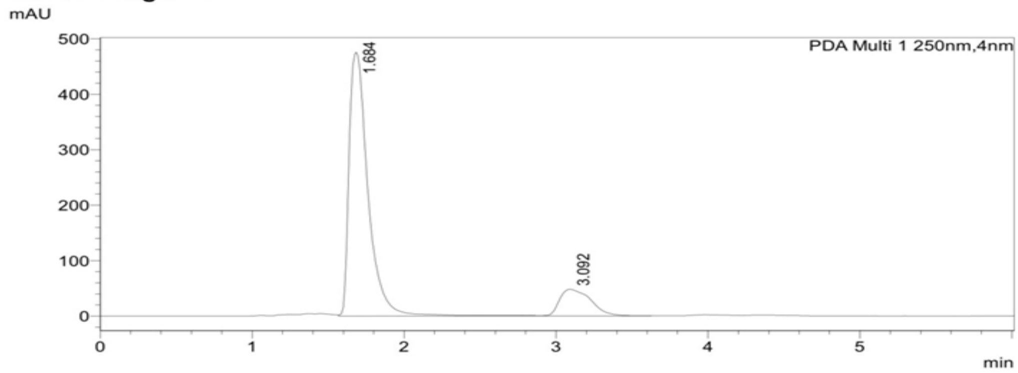


Peak#	Ret. Time	Area	Height	Name	Area%
1	1.684	4176933	490219		84.285
2	3.076	778816	52003		15.715
Total		4955749	542222		100.000

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	1.676	4172898	497616	0.000			
2	3.105	852426	57852	0.000			
Total		5025324	555468				

Peak#	Ret. Time	Area	Height	Area%
1	1.689	4183828	489002	83.020
2	3.080	855712	57551	16.980
Total		5039540	546553	100.000

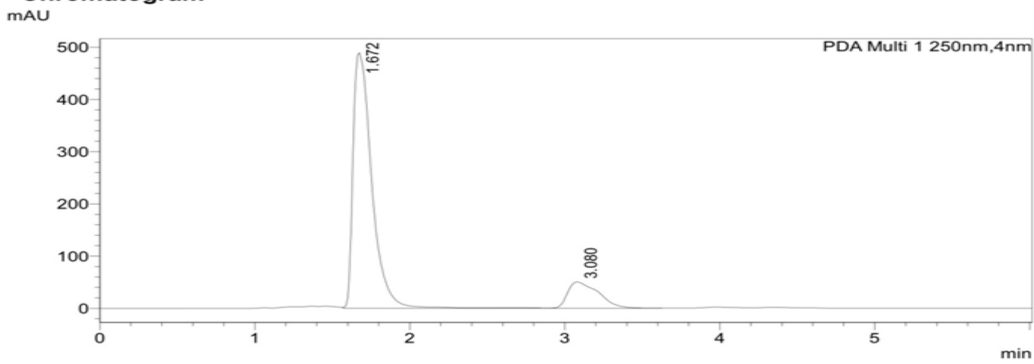
Figure (4.2.3): Quinine ProD 1 at 0.1N HCl at t=0 (A), after 24 (B) and 168 hours (C) respectively.



<Peak Table>

PDA Ch1 250nm

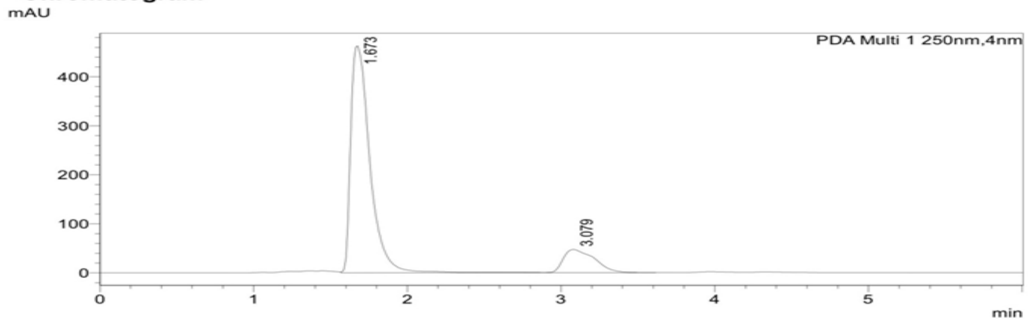
Peak#	Ret. Time	Area	Height	Area%
1	1.684	4122260	475473	85.630
2	3.092	691798	48261	14.370
Total		4814058	523734	100.000



<Peak Table>

PDA Ch1 250nm

Peak#	Ret. Time	Area	Height	Area%
1	1.672	4132823	489079	85.525
2	3.080	699483	50368	14.475
Total		4832306	539447	100.000

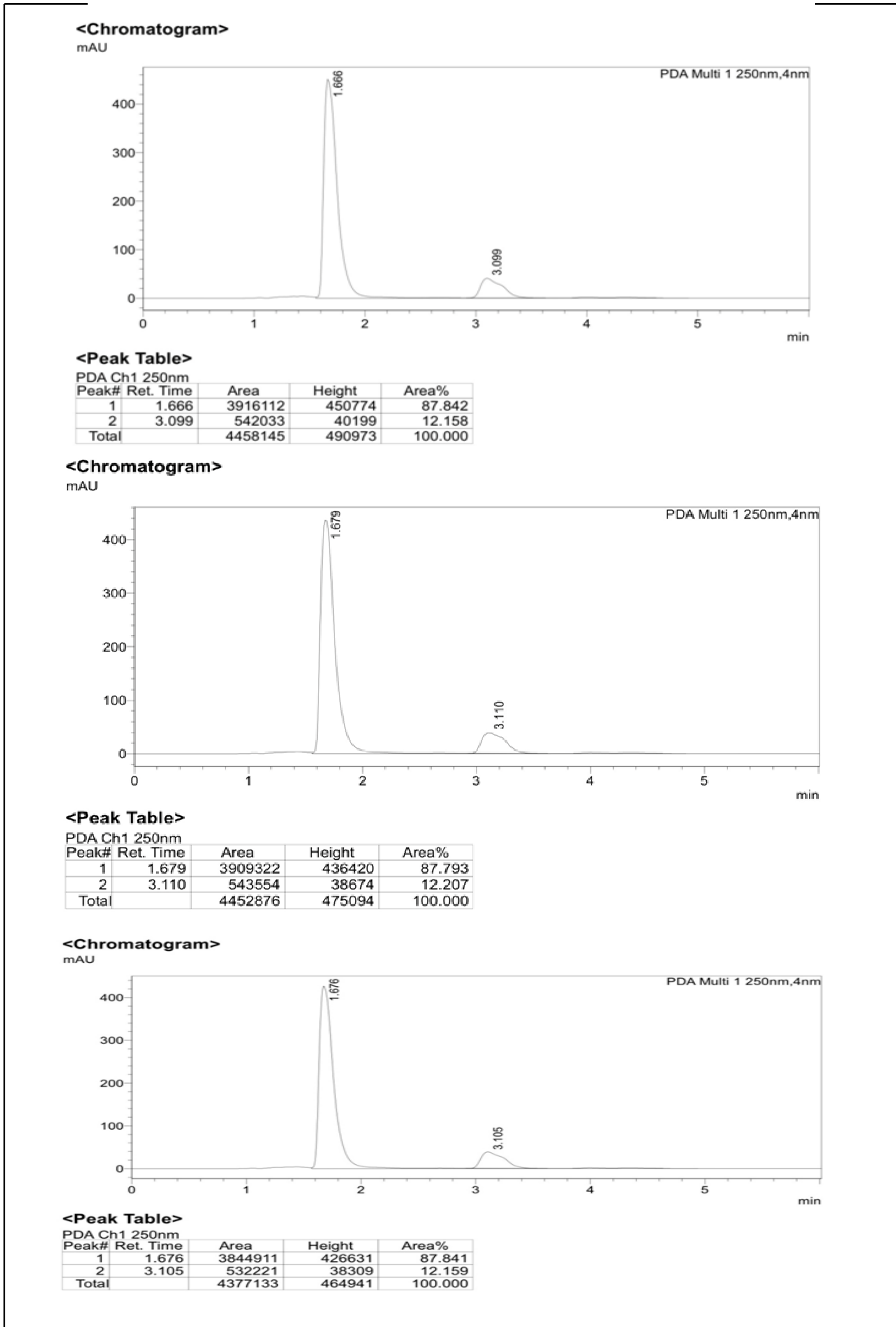


<Peak Table>

PDA Ch1 250nm

Peak#	Ret. Time	Area	Height	Area%
1	1.673	4050915	462980	85.631
2	3.079	679726	47511	14.369
Total		4730640	510491	100.000

Figure (4.2.4): Quinine ProD 1 at pH 2.2 at t=0 (A), after 24 (B) and 168 hours (C), respectively.



<Peak Table>

PDA Ch1 250nm

Peak#	Ret. Time	Area	Height	Area%
1	1.666	3916112	450774	87.842
2	3.099	542033	40199	12.158
Total		4458145	490973	100.000

<Peak Table>

PDA Ch1 250nm

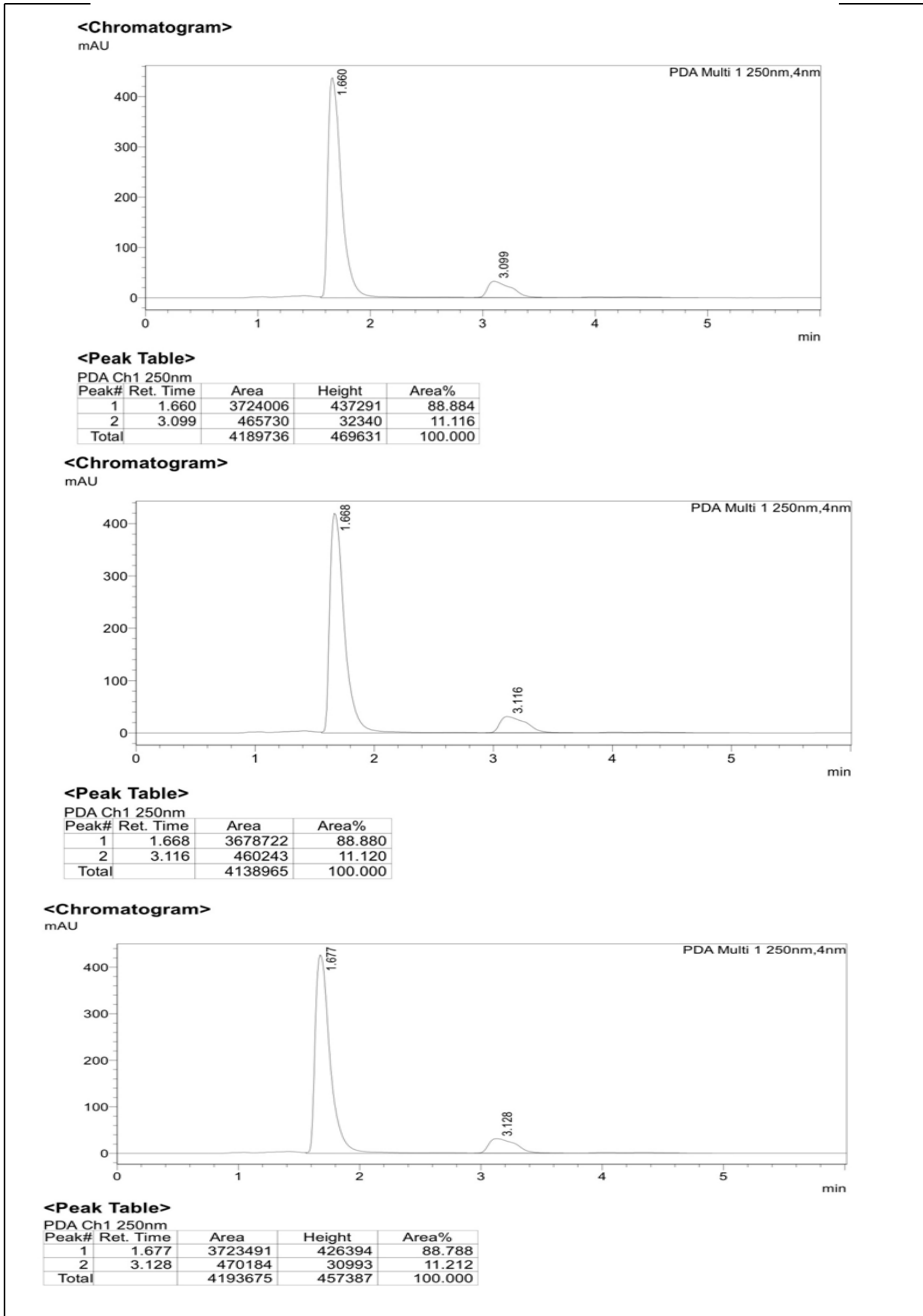
Peak#	Ret. Time	Area	Height	Area%
1	1.679	3909322	436420	87.793
2	3.110	543554	38674	12.207
Total		4452876	475094	100.000

<Peak Table>

PDA Ch1 250nm

Peak#	Ret. Time	Area	Height	Area%
1	1.676	3844911	426631	87.841
2	3.105	532221	38309	12.159
Total		4377133	464941	100.000

Figure (4.2.5): Quinine ProD 1 at pH 5.5 at t=0 (A), after 24 (B) and 168 hours (C) respectively.



<Peak Table>

PDA Ch1 250nm

Peak#	Ret. Time	Area	Height	Area%
1	1.660	3724006	437291	88.884
2	3.099	465730	32340	11.116
Total		4189736	469631	100.000

<Peak Table>

PDA Ch1 250nm

Peak#	Ret. Time	Area	Area%
1	1.668	3678722	88.880
2	3.116	460243	11.120
Total		4138965	100.000

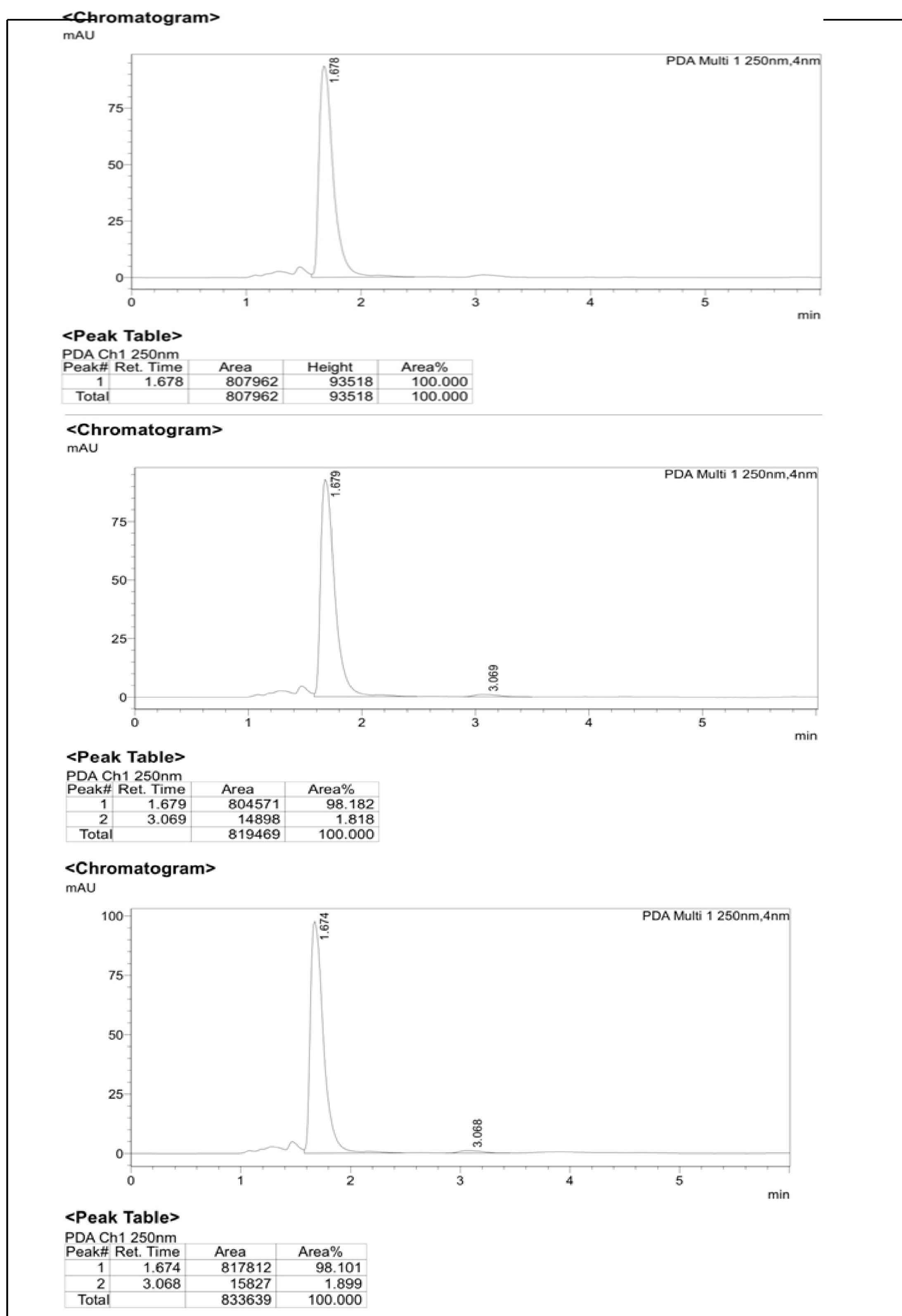
<Peak Table>

PDA Ch1 250nm

Peak#	Ret. Time	Area	Height	Area%
1	1.677	3723491	426394	88.788
2	3.128	470184	30993	11.212
Total		4193675	457387	100.000

Figure (4.2.6): Quinine ProD 1 at pH 7.4 1t t=0 (A), after 24 (B) and 168 hours (C) respectively.

Quinine ProD 2 kinetic study at 0.1N HCl, pH 2.2, pH 5.5, and pH 7.4.



<Peak Table>
PDA Ch1 250nm

Peak#	Ret. Time	Area	Height	Area%
1	1.678	807962	93518	100.000
Total		807962	93518	100.000

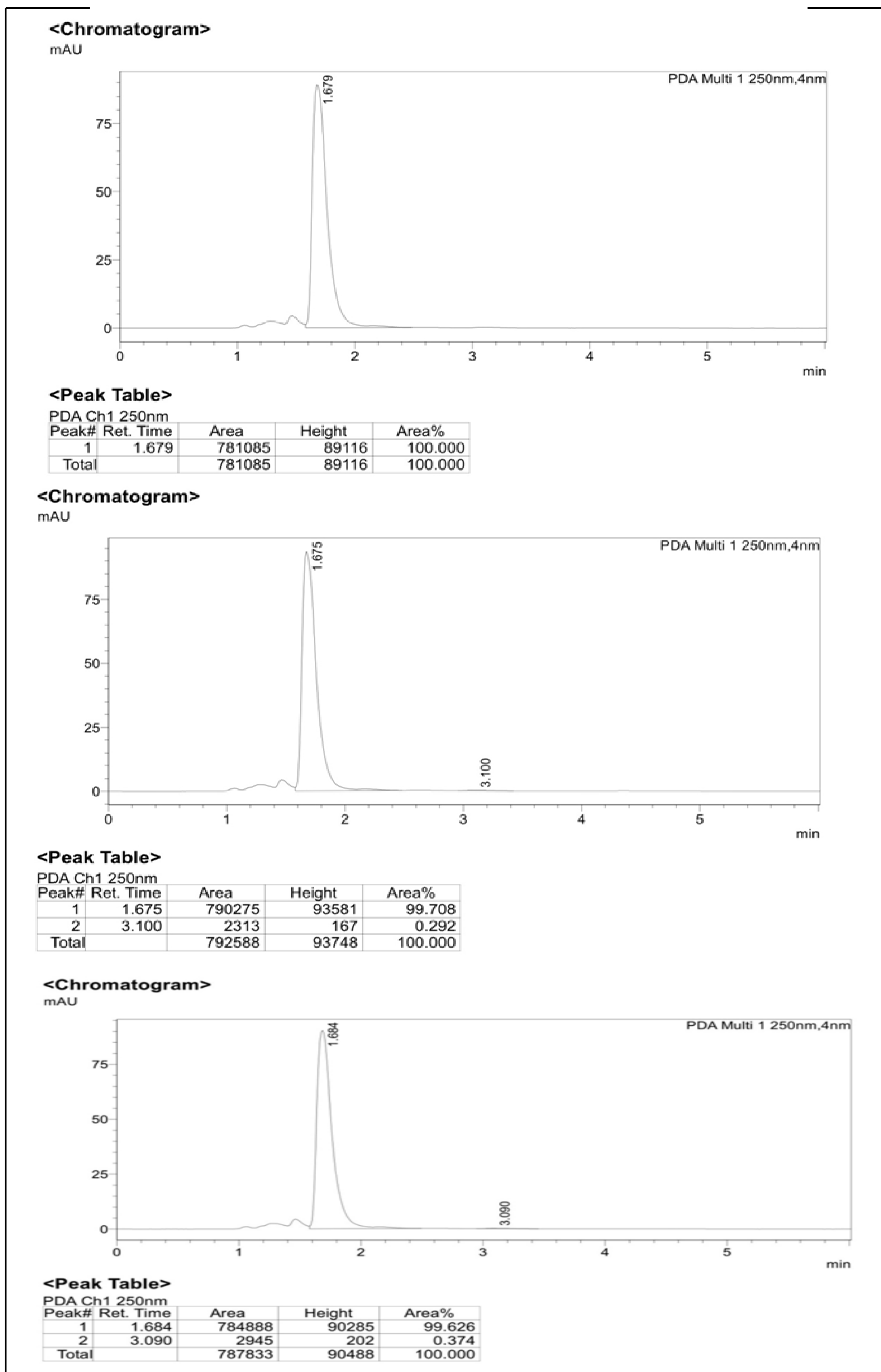
<Peak Table>
PDA Ch1 250nm

Peak#	Ret. Time	Area	Area%
1	1.679	804571	98.182
2	3.069	14898	1.818
Total		819469	100.000

<Peak Table>
PDA Ch1 250nm

Peak#	Ret. Time	Area	Area%
1	1.674	817812	98.101
2	3.068	15827	1.899
Total		833639	100.000

Figure (4.2.7): Quinine ProD 2 at 0.1N HCl at 1N HCl at zero time (A), after 24 (B) and 168 hours (C) respectively.



<Peak Table>

PDA Ch1 250nm

Peak#	Ret. Time	Area	Height	Area%
1	1.679	781085	89116	100.000
Total		781085	89116	100.000

<Peak Table>

PDA Ch1 250nm

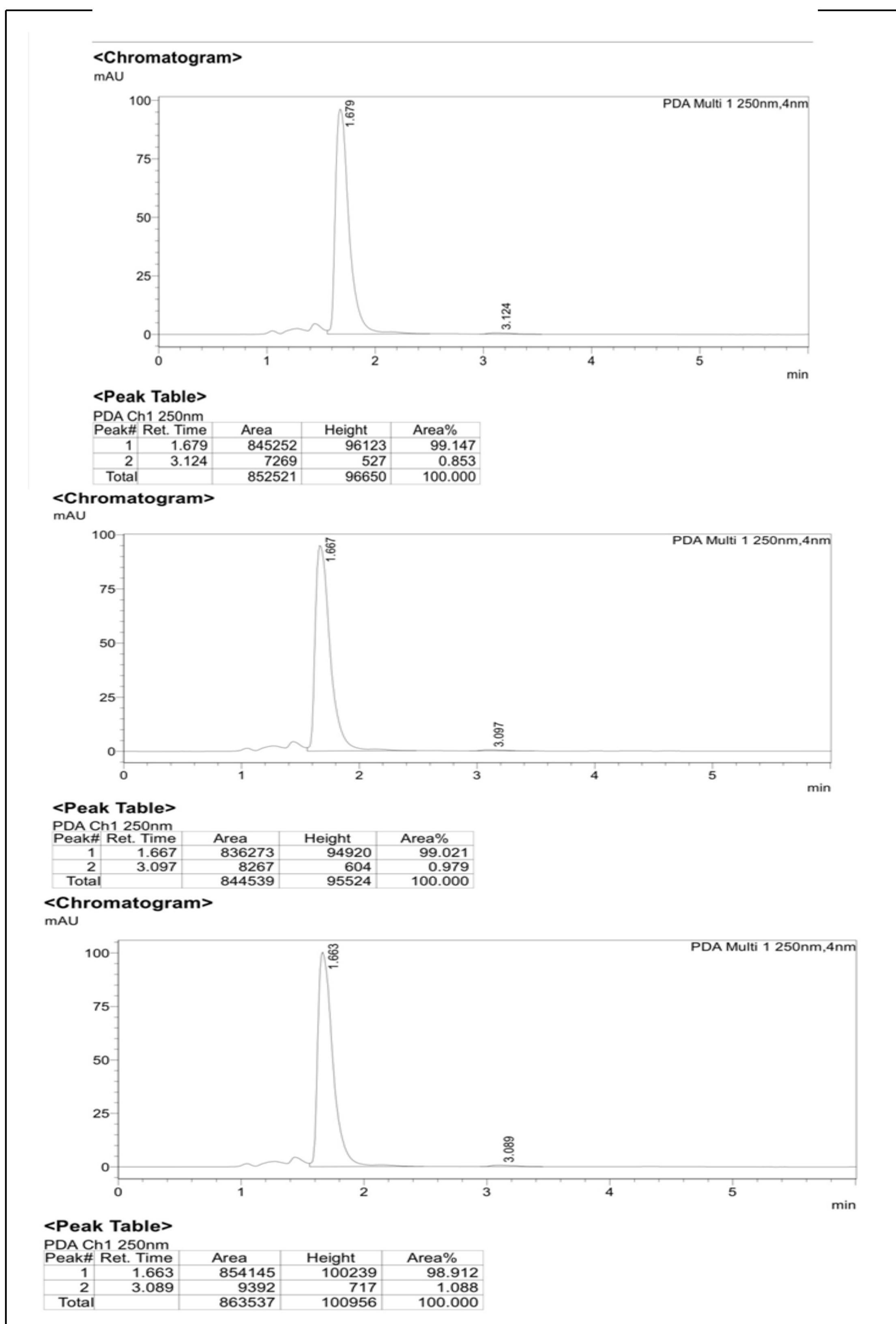
Peak#	Ret. Time	Area	Height	Area%
1	1.675	790275	93581	99.708
2	3.100	2313	167	0.292
Total		792588	93748	100.000

<Peak Table>

PDA Ch1 250nm

Peak#	Ret. Time	Area	Height	Area%
1	1.684	784888	90285	99.626
2	3.090	2945	202	0.374
Total		787833	90488	100.000

Figure (4.2.8): Quinine ProD 2 at pH 2.2 at t=0 (A), after 24 (B) and 168 hours (C) respectively.



<Peak Table>

PDA Ch1 250nm

Peak#	Ret. Time	Area	Height	Area%
1	1.679	845252	96123	99.147
2	3.124	7269	527	0.853
Total		852521	96650	100.000

<Peak Table>

PDA Ch1 250nm

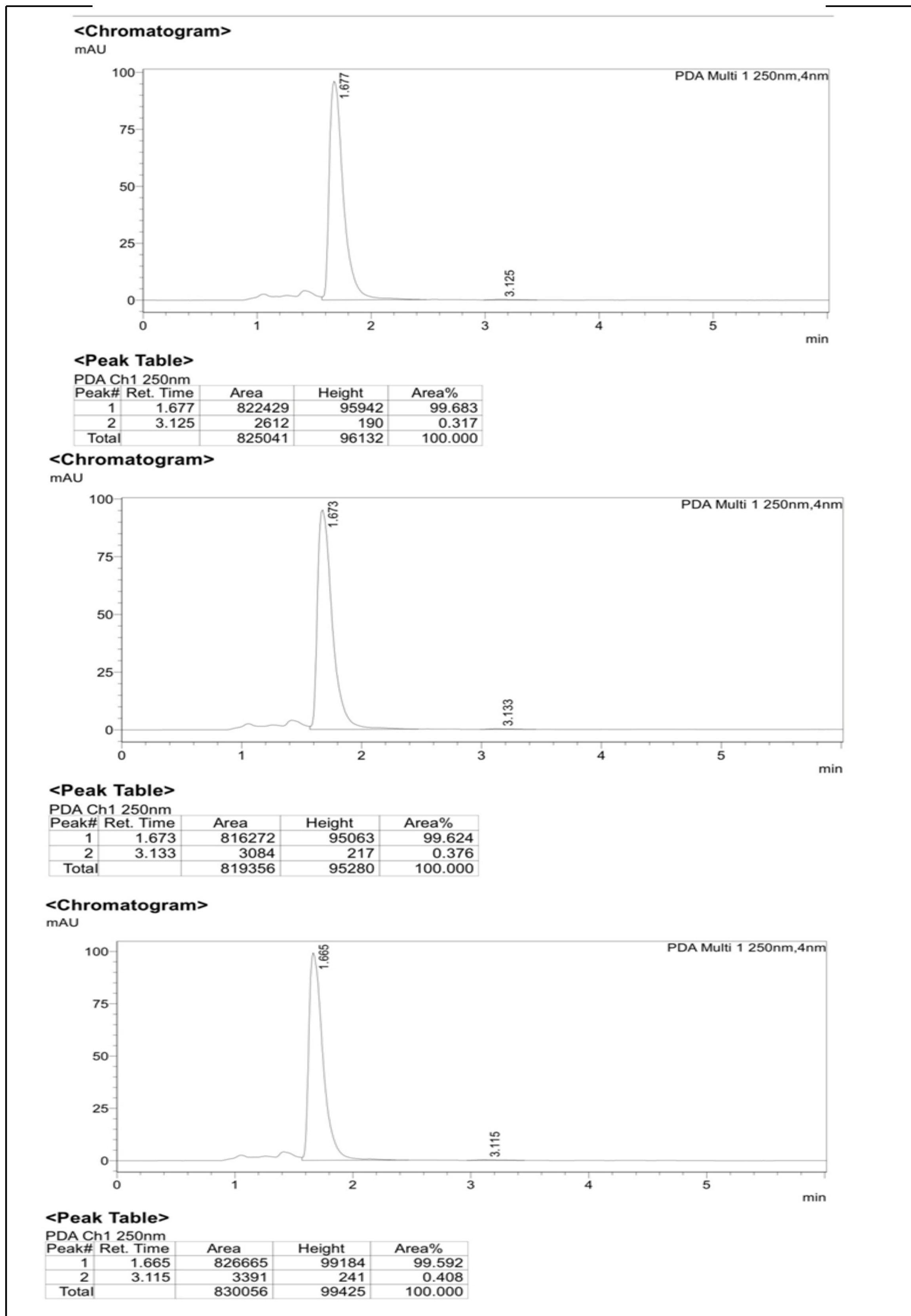
Peak#	Ret. Time	Area	Height	Area%
1	1.667	836273	94920	99.021
2	3.097	8267	604	0.979
Total		844539	95524	100.000

<Peak Table>

PDA Ch1 250nm

Peak#	Ret. Time	Area	Height	Area%
1	1.663	854145	100239	98.912
2	3.089	9392	717	1.088
Total		863537	100956	100.000

Figure (4.2.9): Quinine ProD 2 at pH 5.5 at t=0 (A), after 24 (B) and 168 hours (C) respectively.



<Peak Table>

PDA Ch1 250nm

Peak#	Ret. Time	Area	Height	Area%
1	1.677	822429	95942	99.683
2	3.125	2612	190	0.317
Total		825041	96132	100.000

<Peak Table>

PDA Ch1 250nm

Peak#	Ret. Time	Area	Height	Area%
1	1.673	816272	95063	99.624
2	3.133	3084	217	0.376
Total		819356	95280	100.000

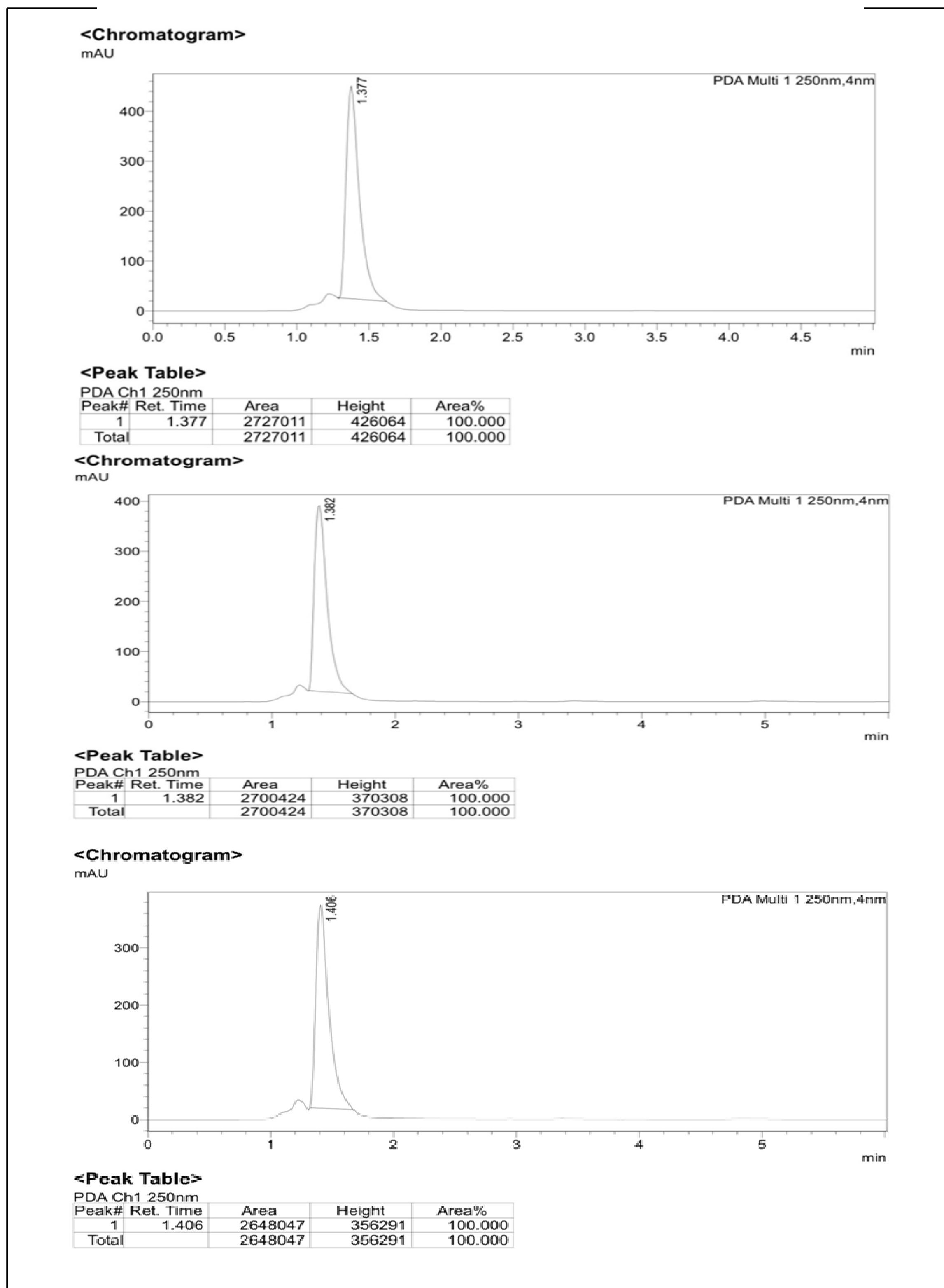
<Peak Table>

PDA Ch1 250nm

Peak#	Ret. Time	Area	Height	Area%
1	1.665	826665	99184	99.592
2	3.115	3391	241	0.408
Total		830056	99425	100.000

Figure (4.2.10): Quinine ProD 2 at pH 7.4 at t=0 (A), after 24 (B) and 168 hours (C) respectively.

Quinine ProD 3 kinetic study at 0.1N HCl, pH 2.2, pH 5.5, and pH 7.4.



<Peak Table>

PDA Ch1 250nm

Peak#	Ret. Time	Area	Height	Area%
1	1.377	2727011	426064	100.000
Total		2727011	426064	100.000

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PDA Ch1 250nm

Peak#	Ret. Time	Area	Height	Area%
1	1.382	2700424	370308	100.000
Total		2700424	370308	100.000

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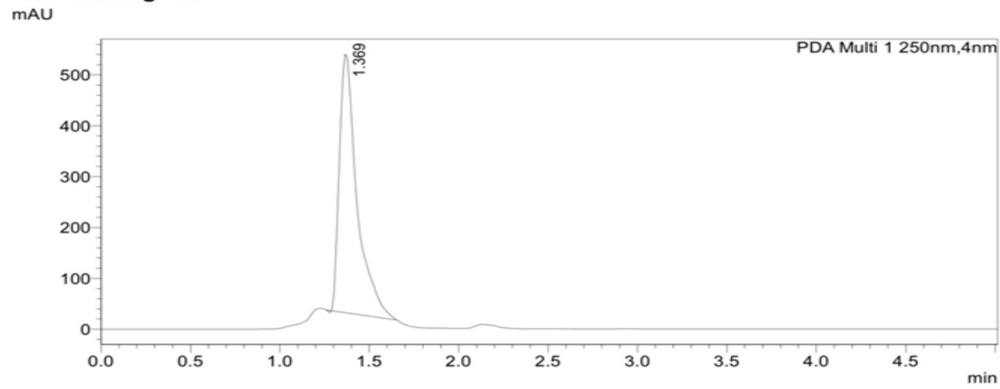
PDA Ch1 250nm

Peak#	Ret. Time	Area	Height	Area%
1	1.406	2648047	356291	100.000
Total		2648047	356291	100.000

Figure (4.2.11): Quinine ProD 3 at 0.1 N HCL at t=0 (A), after 24 (B) and 168 hours (C) respectively.

<Chromatogram>



<Peak Table>

PDA Ch1 250nm

Peak#	Ret. Time	Area	Height	Area%
1	1.369	3468126	508021	100.000
Total		3468126	508021	100.000

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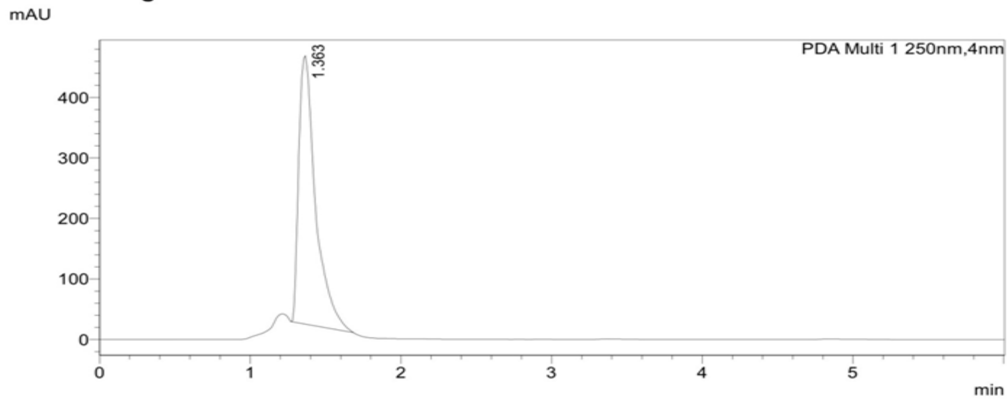

<Peak Table>

PDA Ch1 250nm

Peak#	Ret. Time	Area	Height	Area%
1	1.363	3430620	443158	100.000
Total		3430620	443158	100.000

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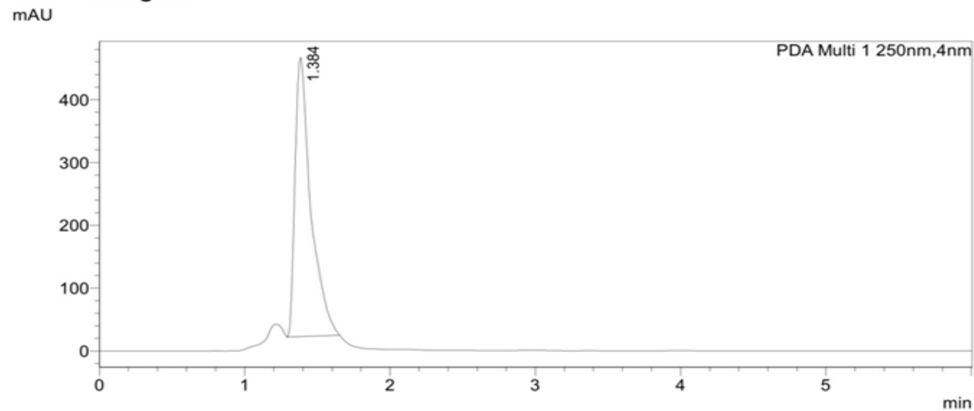
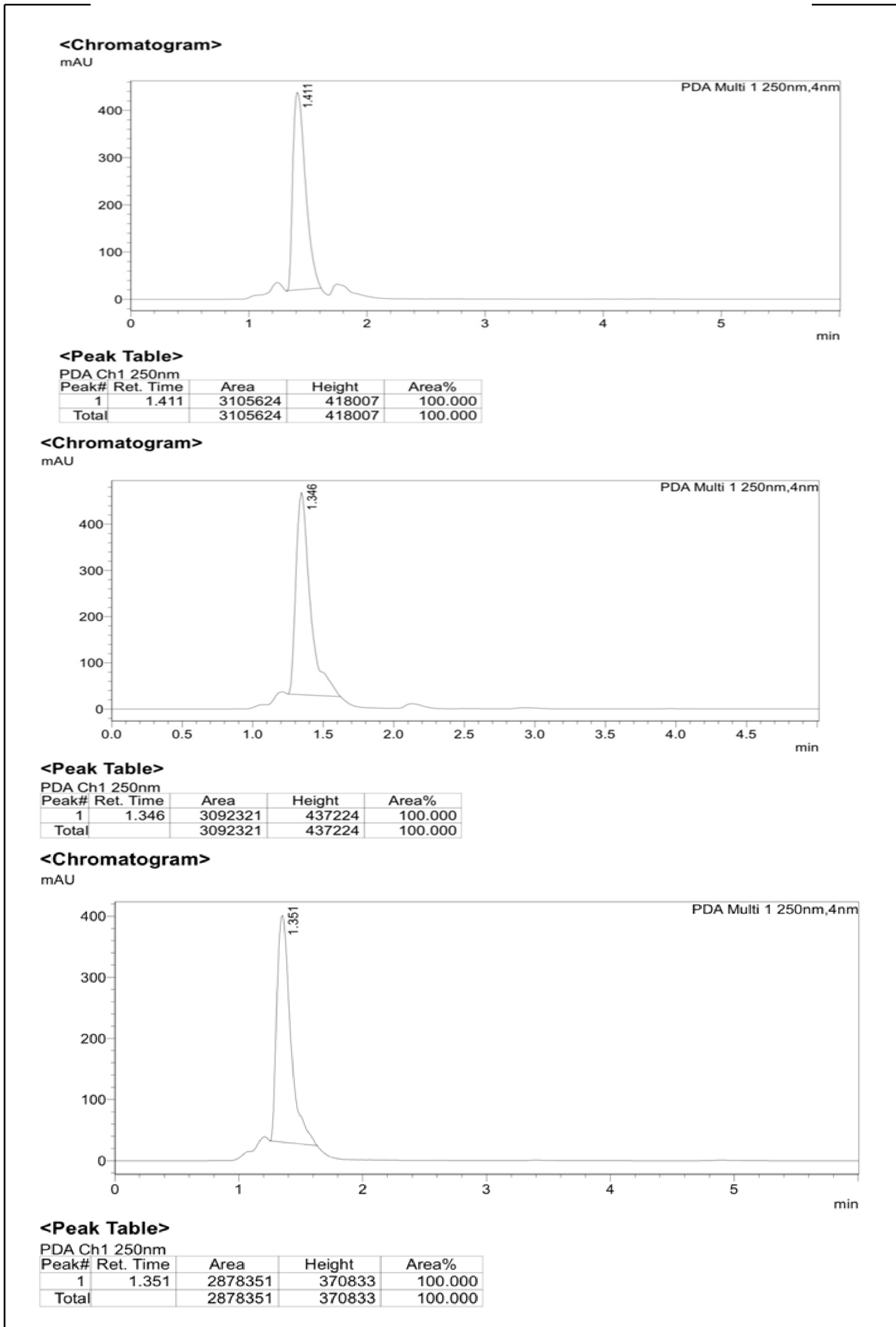


<Peak Table>

PDA Ch1 250nm

Peak#	Ret. Time	Area	Height	Area%
1	1.384	3357071	443873	100.000
Total		3357071	443873	100.000

Figure (4.2.12): Quinine ProD 3 at pH 2.2 at t=0 (A), after 24 (B) and 168 hours (C) respectively.



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PDA Ch1 250nm

Peak#	Ret. Time	Area	Height	Area%
1	1.411	3105624	418007	100.000
Total		3105624	418007	100.000

<Peak Table>

PDA Ch1 250nm

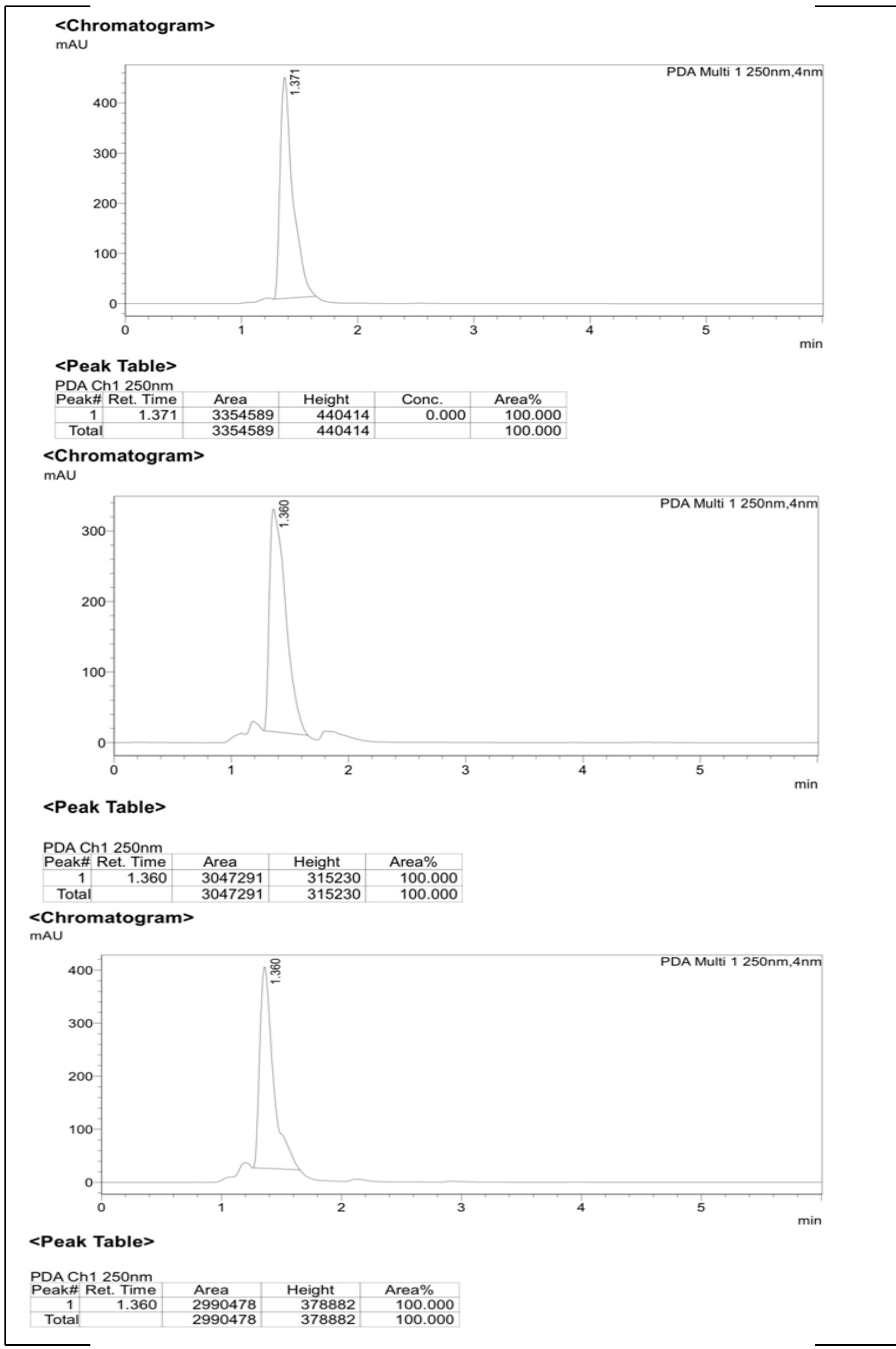
Peak#	Ret. Time	Area	Height	Area%
1	1.346	3092321	437224	100.000
Total		3092321	437224	100.000

<Peak Table>

PDA Ch1 250nm

Peak#	Ret. Time	Area	Height	Area%
1	1.351	2878351	370833	100.000
Total		2878351	370833	100.000

Figure (4.2.13): Quinine ProD 3 at pH 5.5 at t=0 (A), after 24 (B) and 168 hours (C) respectively.



Peak#	Ret. Time	Area	Height	Conc.	Area%
1	1.371	3354589	440414	0.000	100.000
Total		3354589	440414		100.000

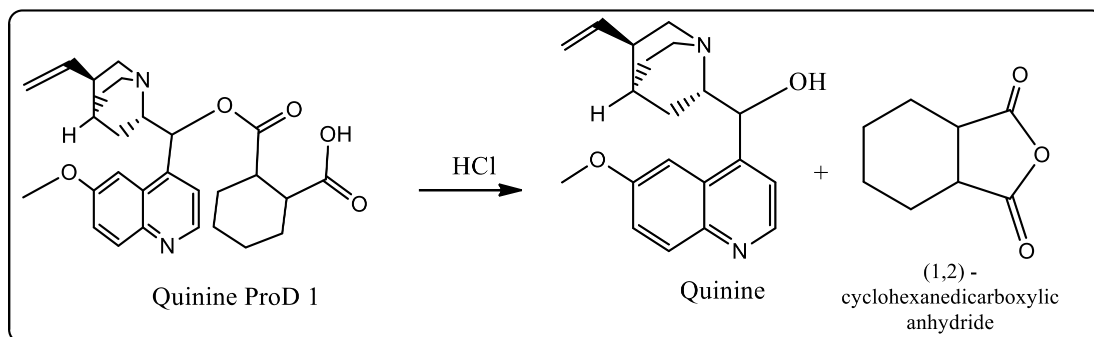
Peak#	Ret. Time	Area	Height	Area%
1	1.360	3047291	315230	100.000
Total		3047291	315230	100.000

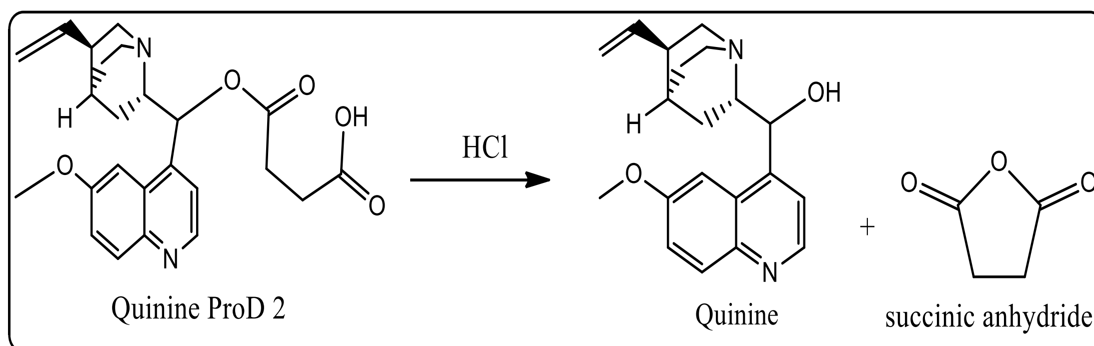
Peak#	Ret. Time	Area	Height	Area%
1	1.360	2990478	378882	100.000
Total		2990478	378882	100.000

Figure (4.2.14): Quinine ProD 3 at pH 7.4 at t=0 (A), after 24 (B) and 168 hours (C) respectively.

4.2.1 In *vitro* intraconversion of quinine ProD 1-3 to their parent drug:

In order to find out whether quinine prodrugs undergo hydrolysis in an aqueous medium and assess the rate of hydrolysis if it does, as well as to determine the destiny of the prodrugs in the system, kinetic investigations of hydrolysis of Quinine ProD 1-3 were carried out in an aqueous buffer. Four distinct aqueous media were used to study the kinetics of the acid-catalyzed hydrolysis of the produced quinine proD 1-3: 0.1 N HCl, buffer pH 2.2, buffer pH 5.5, and buffer pH 7.4. The stomach simulated pH (1-3) reflects the mean fasting stomach state of the adult, and the pH may increase by up to 5 upon ingesting meals, hence buffered solutions were chosen to test the intraconversion of quinine prodrugs. pH5, on the other hand, resembles the start of the small intestine route. The intraconversion of the studied quinine prodrugs in the blood circulation system was investigated at pH 7.4. **Figures (4.2.3) to (4.2.14)** provide an overview of the kinetic study's outcomes and chromatograms.





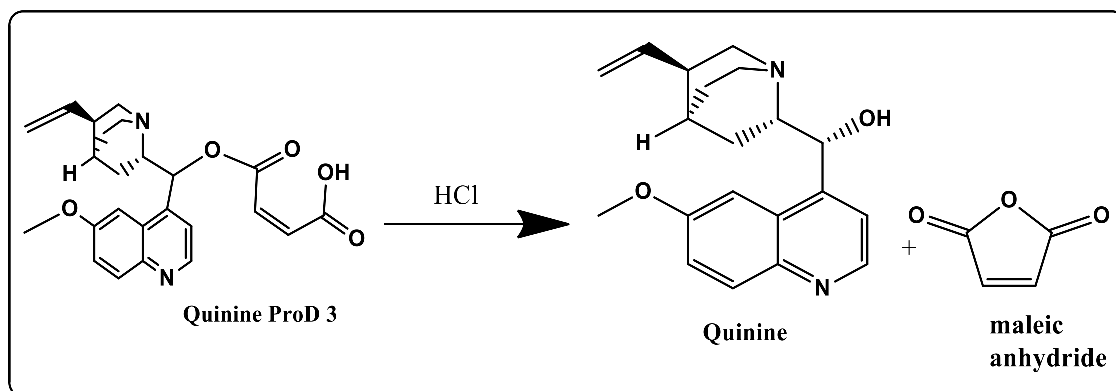


Figure (4.2.1a): Acid-catalyzed hydrolysis of Quinine prodrugs.

At constant temperature and pH, the prodrugs 1-3 were stable and no release of the parent drug was observed in all aqueous media, as evident by the HPLC analysis. As shown in **Tables 1, 2 and 3** time vs. concentration for prodrugs in all aqueous media.

ProD 1 at 0.1 N HCL

time	peak area	conc.
0	4176933	4.856255
1	4183828	4.86315
2	4178782	4.858104
24	4172898	4.85222
72	4173278	4.8526
96	4177054	4.856376
168	4183828	4.86315

ProD 1 at pH 2.2

Time (hr)	Peak area	Conc. ppm
0	4122260	4.801582
1	4124567	4.803889
2	4120796	4.800118
24	4132823	4.812145
72	4118687	4.798009
96	4088115	4.767437
168	4050915	4.730237

ProD 1 at pH 5.5

Time (hr)	Peak AREA	Conc. (ppm)
0	3916112	4.595434
1	3909432	4.588754
2	3909796	4.589118
24	3909322	4.588644
72	3901220	4.580542
96	3890805	4.570127
168	3844911	4.524233

ProD 1 at pH 7.4

Time (hr)	Peak area	Conc.(ppm)
0	3724006	4.403328
1	3710055	4.389377
2	3692682	4.372004
24	3678722	4.358044
72	3608795	4.288117
96	3677321	4.356643
168	3723491	4.402813

Table 1: Time, Peak area and Concentration for ProD 1 in all aqueous media.

ProD 2 at 0.1 N HCL

time	peak area	conc.
0	807962	1.642639
1	808301	1.642978
2	806221	1.640898
24	804571	1.639248
72	808681	1.643358
96	805301	1.639978
168	817812	1.652489

ProD 2 at pH 2.2

Time	Peak area	Conc.
0	781085	1.615762
1	783711	1.618388
2	775679	1.610356
24	790275	1.624952
72	784277	1.618954
96	784120	1.618797
168	784888	1.619565

ProD 2 at pH 5.5

time	peak area	conc.
0	845252	1.679929
1	842583	1.67726
2	834272	1.668949
24	836273	1.67095
72	849732	1.684409
96	841011	1.675688
168	854145	1.688822

ProD 2 at pH 7.4

time	peak area	conc.
0	822429	1.657106
1	821433	1.65611
2	815026	1.649703
24	816272	1.650949
72	817790	1.652467
96	818384	1.653061
168	826665	1.661342

Table 2: Time, Peak area and Concentration for ProD 2 in all aqueous media.

ProD 3 at 0.1 N HCL

time	peak area	conc.
0	2727011	3.561688
1	2761402	3.596079
2	2696350	3.531027
24	2700424	3.535101
72	2654169	3.488846
96	2651101	3.485778
168	2648047	3.482724

ProD 3 at pH 2.2

time	peak area	conc.
0	3468126	4.302803
1	3426083	4.26076
2	3426600	4.261277
24	3430620	4.265297
72	3337087	4.171764
96	3322322	4.156999
168	3357071	4.191748

ProD 3 at pH 5.5

time	peak area	conc.
0	3105624	3.940301
1	3104907	3.939584
2	3090809	3.925486
24	3092321	3.926998
72	3008281	3.842958
96	2914633	3.74931
168	2878351	3.713028

ProD 3 at pH 7.4

time	peak area	conc.
0	3354589	4.189266
1	3142270	3.976947
2	3127609	3.962286
24	3047291	3.881968
72	3139550	3.974227
96	2996704	3.831381
168	2990478	3.825155

Table 3: Time, Peak area and Concentration for ProD 3 in all aqueous media.

Chapter five

Summary and conclusions

Chapter Five

Summary and Conclusions

Quinine is a potent bitter chemical whose use has had a deleterious impact on pediatric and geriatric formulations. In this thesis, we used the linker technique to create a tasteless prodrug in order to disguise the better taste of this chemical. Three quinine prodrugs that we created have been completely characterized by ^{1}H-NMR, LC-MS, and FT-IR. The three quinine prodrugs had no intra-conversion to the parent medication, quinine, according to the kinetic analysis of the samples.

There was no intra-conversion of the prodrugs to the parent substance, and all three prodrugs were completely stable. This could be a result of the quinine anion's (R-O-) poor leaving group properties. The quinine prodrugs' in *vitro* binding to the bitter taste receptor demonstrated that these prodrugs lacked agonist action. In contrast, quinine, the drug's parent, has demonstrated high agonist action.

Since the three created novel prodrugs were completely stable in neutral pH and in the stomach (an acidic environment), the ester link could be broken by blood enzymes such esterase to furnish the parent drug, quinine. To test the viability of this strategy, in *vivo* research (on mice) will be carried out in the near future.

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تصنيع ودراسة مواصفات طلائع الأدوية لمركب الكواينين والتي يمكن أن تكون مفيدة.

إعداد: تسنيم محمد محمود أبودية

إشراف: أ.د.رفيق قرمان

الملخص:

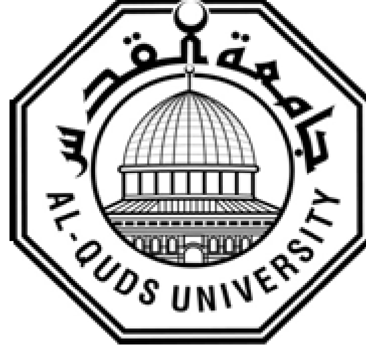
الكواينين: دواء ذو طعم مر قوي يؤثر تأثير سلبي في تقبل المرضى له سواء لعلاج الملاريا او التشنج العضلي وغيره .

هدفنا في هذه الدراسة هو اخفاء الطعم المر لدواء الكواينين وتحويله الى دواء طالع (prodrug) بلا طعم مر ومقبول لدى المرضى وذلك بإستخدام روابط أو مركبات مختلفة ترتبط عليه.

تم تصنيع طلائع الأدوية هذه او prodrug عن طريق ربط بعض الاحماض الانهيدرتية (1,2 - cyclohexanedicarboxylic anhydride, succinic anhydride, maleic anhydride) ,وذلك للحصول على طلائع الأدوية وتم دراسة مواصفات هذه الادوية المساعدة المقترحة عن طريق (FT-IR,H-NMR, LC-MS, HPLC, melting point) للتأكد من نقائها.

وتم كذلك اختبار وتحلل طلائع الأدوية هذه (prodrugs) داخل المختبر ودراسة تحويلها الى الدواء الاصلي دون اي انزيمات بإستخدام جهاز الHPLC على درجة حرارة ثابتة $37^{o}C$ ودرجات الحموضة المختلفة مثل0.1 N HCl ,pH 2.2, pH 5.5 pH 7.4 التي تطابق درجات الحموضة في جسم الانسان.

وكانت نتائج التحلل لطلائع الأدوية هذه للأسف في جميع الدراسات على درجات الحموضة ثابتة ومستقرة ولم تتحلل و تتحول الى الدواء الاصلي.



عمادة الدراسات العليا

جامعة القدس

تصنيع ودراسة مواصفات طلائع الأدوية لمركب الكواينين والتي يمكن أن تكون مفيدة.

اعداد
تسنيم محمد محمود أبودية

رسالة ماجستير

فلسطين – القدس

2022/1444

تصنيع ودراسة مواصفات طلائع الأدوية لمركب الكواينين والتي يمكن أن تكون مفيدة.

اعداد

تسنيم محمد محمود أبودية

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المشرف الرئيسي: بروفيسور رفيق قرمان

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

202/14442