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Synthesis and Characterization of Designed Guaifenesin Prodrugs

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M .Sc. Thesis

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Synthesis and Characterization of Designed Guaifenesin Prodrugs

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Synthesis and Characterization of Designed Guaifenesin Prodrugs

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Dedication

I dedicate this treatise to my dear country, Palestine. My dear land that I believe strongly will return to us some day.

I dedicate it to all our martyrs who sacrificed and gave their soul to our beloved country and to our prisoners who spent their life in the occupation's prisons.

To all the above mentioned, I dedicate this work and this success with pride.

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 bother university or institution.

Signed: .....

Ameen Mahmoud Thewabteh.

Date: .....

Abstract:

Guaifenesin is an extremely bitter taste substance which affects its usage in pediatric and geriatric formulations. In this thesis we aimed to mask the bitter taste of guaifenesin by converting it to a potential tasteless prodrugs using different linkers. The prodrugs were synthesized by esterification of carboxylic acid anhydrides and guaifenesin. Maleic anhydride, succinic anhydride, and glutaric anhydride, respectively, were used to synthesize guaifenesin ester prodrugs (guaifenesin maleate, guaifenesin succinate, guaifenesin glutarate), ^{1} H-NMR, LC-MS, and FT-IR have confirmed the identity and purity of the new prodrugs.

In vitro kinetic studies for the above mentioned prodrugs were done in four different aqueous media: 1 N HCl and buffers pH 3.3, pH 5.5 and pH 7.4. Under the experimental conditions the target prodrug was hydrolyzed to release the parent drug, guaifenesin, as was confirmed by HPLC determination. The k_{obs} and the corresponding $t_{1/2}$ values for guaifenesin prodrugs in 1N HCl were calculated from the linear regression equation correlating the log concentration of the prodrug versus time. The rate constant (k_{obs}) was found to be $7.2x10^{-4}$ for guaifenesin maleate prodrug, $2.54x10^{-4}$ guaifenesin succinate prodrug, and $2.36x10^{-4}$ guaifenesin glutarate prodrug. Half-lives values ($t_{1/2}$) were 2.01 hours for guaifenesin maleate prodrug, 7.03 hours for guaifenesin succinate prodrugs, and 7.17 hours for guaifenesin glutarate prodrug. On the other hand, at pH 3.3, 5.5 and 7.4, guaifenesin maleate, guaifenesin succinate, and guaifenesin glutarate prodrugs were entirely stable and no release of the parent drug, guaifenesin, was observed.

Acknowledgments:

At the beginning and before anything I would like to thank **God (Allah)** for this success. As without his support I would not have managed to accomplish this treatise.

Then, I would like to thank my dear university, Al-Quds University and its professional staff that provided me with every source and advice that needed to attain my BS degree in chemistry and M.Sc. degree in the Applied and Industrial Technology Program. For this great establishment and its staff, I am grateful.

In addition, I would like to thank my supervisor of this treatise Prof. Rafik Karaman, his help and support. I consider myself lucky to have such support and advice from them and to have them around me.

And a lot of thanks to Beit Jala Pharmaceutical Co. to help and support me, represented by general manager Dr. Angele Zaboura, quality control manager Mr. Abdel Fttah Hroub, and all stuff there.

In this special day I would like to thank all who supported me, encouraged me and had the tolerance to wait for this success to be made. My great parents, my brothers, Anas, and Mohammad, my beloved sisters Ala'a and Aya and every member of my dear family.

And great thanks to Dr. Mohmmad Thewabteh and Ms. Ala' Faroun to help me in grammar and spelling checking.

All respect, love and appreciation to all of you. Wishing all of you happiness and joy.

Jerusalem, Palestine, Feb. 2014. Ameen Thewabteh.

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Introduction

1.1 Background

Many curative drugs have adverse properties that may become pharmacological, pharmaceutical or pharmacokinetics barriers in the clinical drug application. Among the various approaches to minimize these adverse drug properties while preserving the beneficial therapeutic outcome, perhaps the chemical approach that uses drugs derivatization offers the highest flexibility and has been demonstrated as a very important means for improving drug efficacy.

The prodrug approach is useful in optimizing the clinical application of the drug, and has gained attention as a technique for improving drug therapy in the early 1950's.

The term "prodrug" or "pro-agent" was first introduced in 1958 by Albert to describe compounds that undergo biotransformation prior to their therapeutic activity.[1] Prodrugs are bioreversible derivatives of drug molecules that undergo an enzymatic or chemical transformation in vivo to release the active parent drug, which can then exert the desired pharmacological effect.[2] According to IUPAC (International Union of pure and applied chemistry); prodrug is defined as any compound that undergoes biotransformation before exerting its pharmacological effect.[3] Such drug-derivatives have also been called "latentiated drugs", "bioreversible derivatives", and "congeners", but "prodrug" is now the most commonly accepted term.[4-6] There are also so called co-drugs or mutual prodrugs where a prodrug consists of two pharmacologically active drugs, coupled together to act as a promoiety to each other.[7,8] In drug discovery and development, prodrugs have become an established tool for improving physicochemical, biopharmaceutical or pharmacokinetic properties of pharmacologically active agents.[9,10] By applying prodrug technology, the clinical usefulness of a drug molecule may be enhanced without modifying the

pharmacological activity of a parent drug. However, the design of an appropriate prodrug molecule should ideally be considered at the early stages of preclinical development, bearing in mind that prodrugs, while not common, may alter the tissue distribution, efficacy and the toxicity of the parent drug. Moreover, promoieties used should ideally be safe and rapidly excreted from the body. The schematic representation of the prodrug concept is shown in **Figure 1.1**.

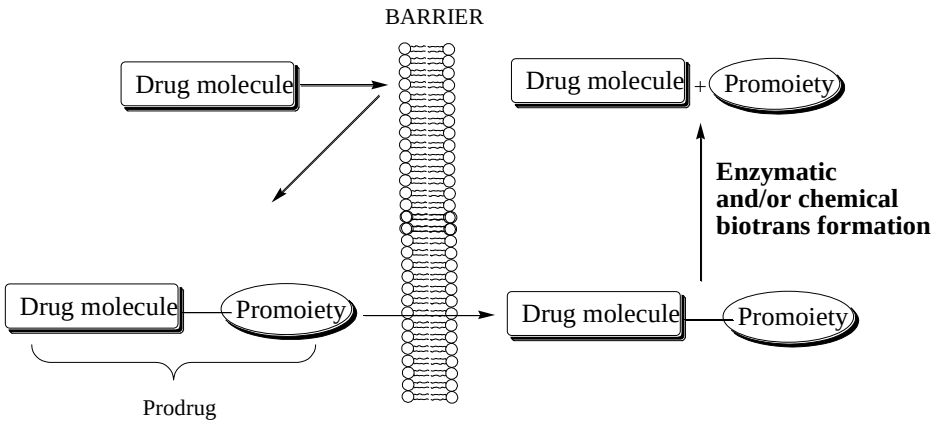


Figure (1.1): Schematic representation of the prodrug concept

The choice of promoiety should be considered with respect to the disease state, dose, and the duration of therapy. The prodrug approach can be exploited for almost all administration routes and dosage forms, and it can be applied to a wide variety of existing medicines on the market, as well as to novel drug molecules in the lead optimization step early in the drug discovery process.[11-13]

About 5–7% of drugs approved worldwide can be classified as prodrugs, and the implementation of a prodrug approach in the early stages of drug discovery is a growing trend.[14-16] The application of modern discovery technologies such as high-throughput screening and combinatorial chemistry can produce novel lead compounds with high pharmacological potency, but the physicochemical and biopharmaceutical aspects of the

initial leads have frequently been neglected. This can lead to drug candidates with poor drug-like properties that face significant problems later in the drug development process.[17] This is done to increase the usefulness of a drug by improving the physicochemical, biopharmaceutical or pharmacokinetic properties of the compound. By chemically modify an active agent various barriers can be overcome such as poor aqueous solubility, chemical instability, insufficient oral absorption, rapid pre-systemic metabolism, inadequate brain penetration, toxicity, local irritation and change the physical form of drug. Prodrugs can also prolong the duration of drug action i.e. highly lipophilic prodrugs of steroids and neuroleptics that are administrated intramuscular. [15]

In most cases a prodrug requires only one or two enzymatic or chemical transformation steps to become an active drug.

1.2 Pharmaceutical application of prodrug approach

The pharmaceutical application can be considered as the phase of expansion involving the identification of a new chemical entity with measured or proposed therapeutic potential, and its incorporation into a drug delivery system. The delivery system may be one of the conventional forms (injections, capsules, tablets, creams or ointments), or a new mode of drug delivery such as liposomes or implants.[18] Two barriers were identified in the development phase of commercially usable drug products include:

(i) Problems of drug formulation: enhancement of permeability and absorption; adverse physicochemical properties such as solubility and polarity.

(ii) Aesthetic properties: odor, taste (particularly when intended for pediatric usage or oral administration).

1.2.1 Enhancement of solubility and dissolution rate of drugs

Water solubility is required when dissolution is the rate limiting step in the absorption of poorly soluble aqueous agents, or when parental or ophthalmic formulation of such agents is desired. Many drugs are hydrophobic in nature and have poor bioavailability.

The prodrug approach can be applied as a solution for problems such as solubility. Hydrophilic drug forms can be produced by reacting half esters such as hemisuccinate, hemiglutarates or hemipthalates with the hydroxyl drug functional group. The other half of these acidic carriers can form sodium, potassium or amine salts and render the moiety more water soluble. For example, chloramphenicol succinate and chloramphenicol palmitate, ester prodrugs of chloramphenicol, have enhanced and reduced aqueous solubility respectively. On the basis of altered solubility, chloramphenicol sodium succinate prodrug is found suitable for parenteral administration.[19]

1.2.2 Enhancing permeability and absorption

The transport of a drug to its site of action usually requires passage through several lipid membranes; therefore, membrane permeability has a considerable influence on drug efficacy.[20]

Prodrug strategies are most commonly employed to increase permeability of compounds by masking the polar functional groups and hydrogen bonds with ester or amide linkers and therefore increasing lipophilicity. Both permeability by passive diffusion and the transporter-mediated process have been addressed with prodrug approaches. Oral delivery of ester/amide prodrugs to the curative target is confronted with many physiological, chemical, and biochemical barriers. In general, the highest oral bioavailability values that ester prodrugs can achieve clinically are 40% to 60%. This is due to incomplete membrane permeation, P-glycoprotein efflux, hydrolysis in the GI lumen and intestinal cells, esterase

metabolism in the liver, biliary excretion, and metabolism of the parent. Thus, a successful prodrug approach must consider a balance of all these issues.[21]

Prodrugs with lipophilic pro-moieties have also been used to promote topical absorption for transdermal and ocular drugs. The stratum corneum, the outermost layer of the epidermis, represents a high resistance barrier against topical drug delivery. Only the drugs with balance of both water and lipid solubility can efficiently penetrate through the layers of the skin.[22, 23]

1.2.3 Masking taste

An unfavorable taste emerges due to sufficient solubility and interaction of a drug with taste receptors, a problem which can be solved by lowering the solubility of drug or prodrug in the saliva. For example, Chloramphenicol is an extremely bitter substance inhibiting its usage in pediatric formulations. Chloramphenicol palmitate, a sparingly soluble ester of chloramphenicol, is practically tasteless because of its low aqueous solubility. Since the interaction of a drug or prodrug with taste receptors requires the drug to be sufficiently soluble in saliva, by lowering the aqueous solubility we can mask the unfavorable taste. Later, this prodrug is activated by in vivo hydrolysis to chloramphenicol by the action of pancreatic lipase.[24]

Other examples of the use of prodrugs to mask bitter taste are listed in **Table 1.1**.

Table (1.1): Prodrug for bitter taste masking[24, 25]

Drug	Prodrug	Reference	Year
Clindamycin	Alkyl ester	Sinkula et al.	(1973)
Chloramphenicol	Palmitate or phosphate ester	Glazko et al.	(1952)
Erythromycin	Ethyl succinate or ethyl carbonate	Murphy	(1953)
Lincomycin	Phosphate or alkyl ester	Morozowich et al.	(1969, 1973)

1.3 Taste

The tongue and the roof of the mouth are covered with thousands of tiny taste buds. While eating, the saliva in the mouth helps break down food. Taste buds contain receptors for taste. The perception of taste in humans occurs when molecules interact with these taste receptors on the surface of taste buds located in the mouth, mainly on the surface of the tongue. This process triggers signals in the mouth which are then sent to the brain where a specific taste sensation is recognized and translated as sweet, sour, bitter or salty.

The receptors for these four tastes are located on different areas of the tongue. Receptors responsible for sweet taste are located at the tip of the tongue, receptors for sour taste are located along the sides of the tongue, receptors for bitter taste are at the back of the tongue, and salty taste receptors are located at the sides and tip of the tongue (**Figure 1.2** and **Table 1.2**). These receptors bind molecules by saliva and transmit electrical impulses by cranial nerves to the brain where they are translated to the perception of taste.[26]

Table (1.2): Specific area of tongue.[27]

Taste	Area of tongue
Sweet	Tip
Salt	Tip and sides
Sour	Sides
Bitter	Back

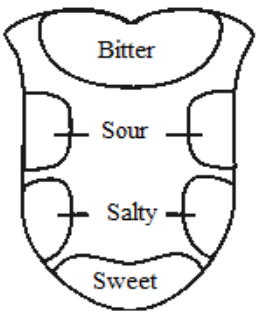


Figure (1.2): Locations of taste sensors on the tongue.[26]

1.4 Taste according to chemical structure

The solubility of a substance in the saliva determines the strength of taste; the greater the solubility of a drug, the greater the concentration of the drug in the saliva at the receptor site and thus, an increased sensation of taste.[27]

Sweet: the sense of this taste is given by many compounds having structural similarity. Sugar and glycerin (**Figure 1.3A**), the two chemical entities are the most common sweet substances, consist of polyhydric alcohols containing (CH_2OH) groups. Sodium or calcium salts of cyclohexyl sulfamic acid (**Figure 1.3A**) and the dipeptide ester aspartame, used as sugar substitutes in the preparation of mouth dissolved tablets, are sweet but require less quantity than sugar.[28]

Sour: the concentration of the hydrogen ion of the tastant is the most important factor that determines the strength of sour taste. Therefore, the higher the concentration of hydrogen ions in the compound, the stronger is the sense of sour taste. Chemical substances present in food such as acetic acid, citric acid and maleic acid (see **Figure 1.3B**) are responsible for stimulating sour taste as these acids are ionized in aqueous solution to produce hydrogen ions.[29]

Bitter: compounds that contain phenolic or alcoholic hydroxyl groups, nitro or amino groups, esters of aromatic acids, lactones, and sulfur containing aliphatic compounds exhibit bitterness. Most pharmaceutical active ingredients such as guaifenesin, acetaminophen, ibuprofen, (**Figure 1.3C**) naproxen, naproxen sodium, psyllium, chlorpheniramine, astemizole, loperamide, famotidine, ranitidine, cimetidine, and pseudoephedrine are bitter in taste.[30]

Salty: most of the halide salts such as sodium chloride, sodium bromide, potassium chloride and sodium iodide have a salty taste. The sensations of salts shift to bitterness

with an increase in molecular weight. For example, potassium bromide and ammonium iodide have a salty, bitter taste while potassium iodide is intensely bitter.[27, 31]

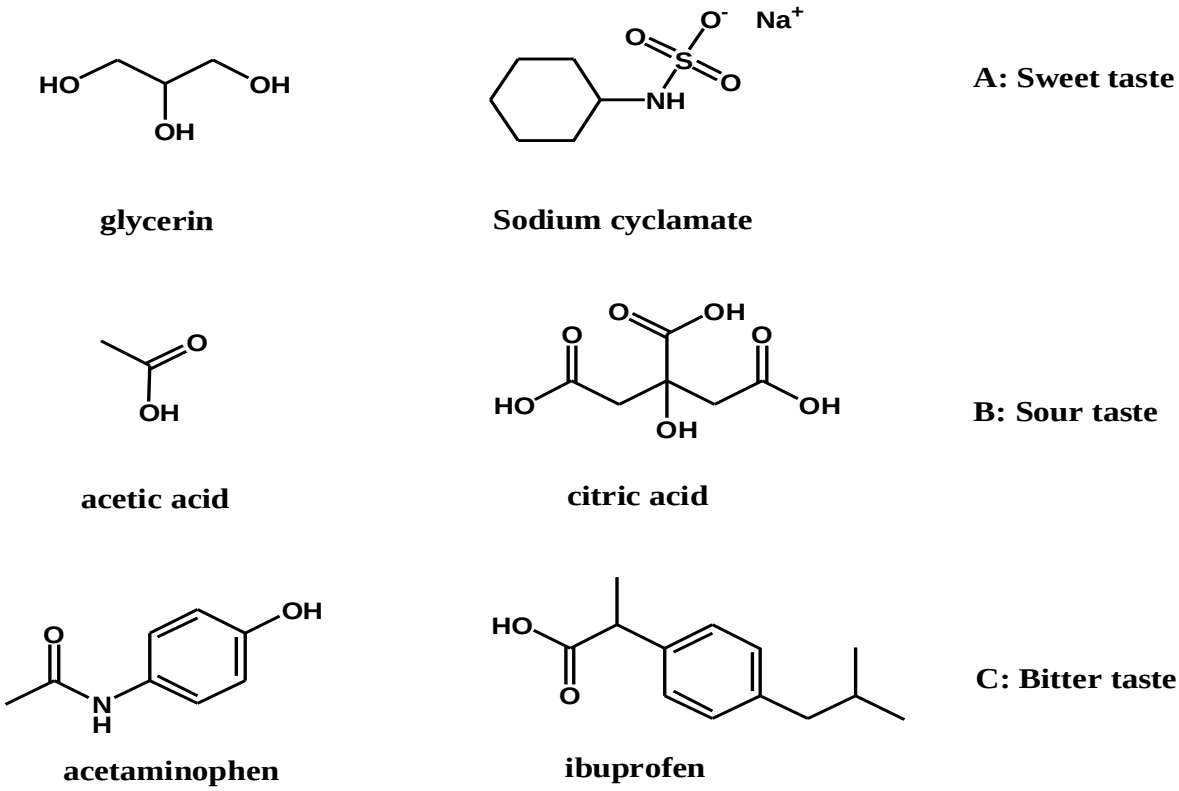


Figure (1.3): Sweet (A), sour (B), and bitter (C) taste compounds structures

1.5 Quantitative evaluation of taste

Human sensory evaluation, taking a sample and testing its taste, is the main process to measure the taste of a pharmaceutical active ingredient and its formulation. Nevertheless, this process is not favored for early stage drug development due to the cost of testing using humans, and the fact that the taste of a drug elected might not be important to the final product. Therefore, for a long time there has been a demand for taste-sensing detection and analytical devices.[32]

Electronic tongue or e-Tongue is an automated taste sensing device to detect the extent of bitterness of drug substance. This device has a transducer composed of various kinds of

lipid/polymer membranes with different characteristics that can be used to detect taste. Taste response is transformed into a pattern composed of the electronic signals of the lipid membrane potentials. Different response electric potential patterns are obtained for substances producing different taste qualities.[33]

The bitterness quantitative evaluation of medicines is measured by this technique. Basic drugs with amine groups in the molecule such as quinine, and anionic drugs as diclofenac sodium or salicylic acid, show a comparatively good correlation between the relative response electric potential (mV) of channels 1 or 2 for positive drug substance, and channel 5 or 6 for negative drug substance of the taste sensor.[34]

1.6 Masking bitter taste

There are numerous pharmaceutical preparations containing active ingredients that are bitter in taste. With respect to over the counter (OTC) preparations such as cough and cold syrups, the bitterness of the preparation is a major problem leading to lack of patient compliance, especially in pediatric and geriatric populations, and presents a challenge to the pharmacist. In order to ensure patient compliance bitterness masking becomes essential.

Taste masking is not an easy or simple procedure**.** Effort is required before bitter drugs are acceptable for market trials. The development of an appropriate formulation involves the investment of time, money and resources by pharmaceutical industries to develop palatable and pleasant tasting products using various taste masking techniques.

Various methods and technologies are used to mask the unpleasant taste of a drug. Flavor enhancers, adding natural flavors, such as anise oil, cardamom, lemon and orange, or artificial flavors are the simplest and oldest methods used. Excipient sweetening flavors

such as menthol and chloroform anaesthetize taste receptors, but this method fails to mask 70% of products.[35]

Polymer coating and micro-encapsulation are other techniques used to mask the unpleasant taste by forming a barrier around the drug particles. By using the right type of coating material, the interaction between the drug and taste buds is minimized. The micro-encapsulation technique is important in taste masking of bitter drugs through encapsulating the drug particles by a suitable polymer; the drug has no contact with the taste buds in the mouth when dosage forms are given orally to patients.[36,37]

The unpleasant taste of Diclofenac sodium (DS) was masked by the micro-encapsulation technique without any change in the rate of drug release. DS powder was mixed with cellulose and lactose and then converted into a spherical core to form a thin, uniform microcapsule wall. Diethylphthalate (DEP) and polyethyleneglycol (PEG) were used as plasticizers. Ten volunteers tasted the microcapsules. The results demonstrated that the extent of taste masking was influenced by the presence of additives (cellulose and lactose) within the core; the plasticizer, (DEP and PEG), and core size had an effect on the release rate.[38]

The bitter taste can be masked by inclusion complexes such as cyclodextrins (CDs), see **Figure 1.4**. The drug molecule is suitable to accommodate the core of a complexing agent, and the cyclodextrins wraps the bad tasting molecule to inhibit its interaction with the taste buds.[39]CD's are cyclic oligosaccharides containing six to twelve monosaccharides connected by 1 and 4 carbon atoms, were carbon confirmation which in the glucose units all secondary hydroxyl groups are located on the wider edge of the ring, and all primary hydroxyl groups are located on the narrower edge to form a conical cylinder with a hydrophilic outer surface and hydrophobic inner surface.

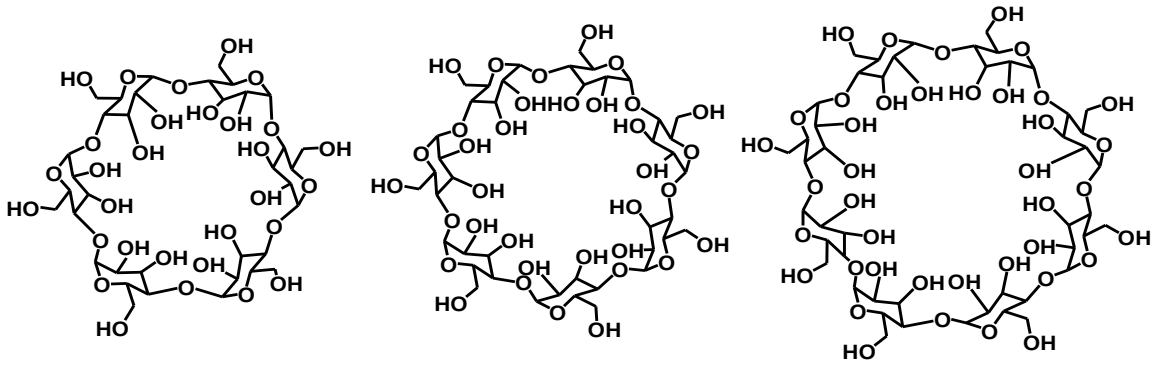


Figure (1.4): Cyclodextrins chemical structures

The prodrug approach can be used for masking the bitter taste of active pharmaceutical ingredients by changing the molecular configuration of the origin molecule and the contact of the molecule with taste receptors.

Most of the above mentioned approaches to mask bitter taste were found to be limited. Thus, different strategies should be developed in order to overcome this serious problem.

In the following study a bitter tastant, guaifenesin (see **Figure 1.5**), was chosen as a drug candidate to be masked by a linker to provide a prodrug without the bitterness of the parental drug.

1.7 Guaifenesin

Guaifenesin, the glyceryl ether of guaiacol, is a component of numerous cough and cold preparations available worldwide, termed as an expectorant. This medication is most commonly used to loosen mucus and phlegm and eventually clear the symptoms of congestion resulting from a cold or allergy.[40] It works by thinning mucus and phlegm in the body, and the thinning action makes it easier for the body to expel excess mucus and phlegm, generally through coughing or the blowing of the nose.[41]

Guaifenesin is a white to slightly gray, crystalline powder, derived from the resin of guaiacum trees, with IUPAC name (RS)-3-(2-methoxyphenoxy)propane-1,2-diol. It is soluble in water, freely soluble in alcohol, chloroform and propylene glycol, and sparingly soluble in glycerin. It is readily absorbed from the gastrointestinal tract and is rapidly metabolized and excreted in the urine (**Figure 1.5**).[42]

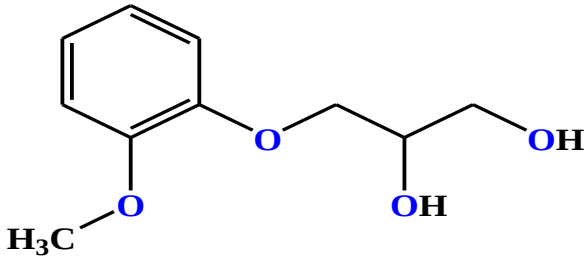


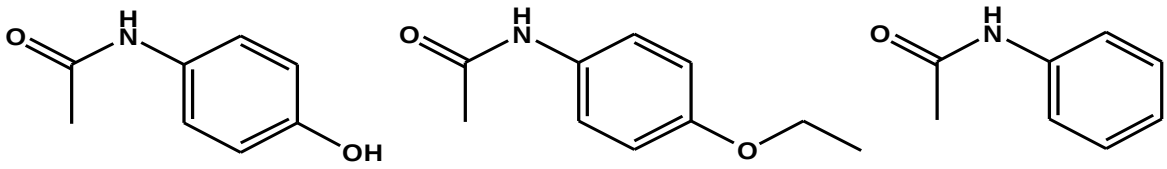
Figure (1.5): Guaifenesin chemical structure.

Guiafenesin was discovered in the 1500's, but its strong bitter taste made it unacceptable for pediatric and geriatric patients, as the bitterness of pharmaceutical medicines plays a critical role in patient compliance in prescribing orally administrated drugs.[43]

The bitter taste of guiafenesin is most likely due to the presence of the hydroxyl groups which may interact with the bitter taste receptors forming inter molecular interactions (hydrogen bonding). Blocking the hydroxyl groups of guaifenesin has the potential of masking its bitterness by decreasing the solubility of guaifenesin in saliva as was reported on paracetamol. Paracetamol is a pain killing drug with a strong bitter taste, but its derivatives, by which the phenolic group of paracetamol was blocked by an alkyl group, lacked the bitterness characteristic of paracetamol.[44]

Examination of the structures of paracetamol and phenacetin reveals that the only difference in the structural features in both is the nature of the group on the para position of

the benzene ring. While in the case of paracetamol the group is hydroxyl, in phenacetin it is ethoxy. Another related example is acetanilide that has a chemical structure similar to that of paracetamol and phenacetin but it lacks the group in the para position of the benzene ring. **Figures 1.6** illustrate these points. The combined facts described above suggest that the presence of hydroxyl group on the para position is the major contributor for the bitter taste of paracetamol.



Paracetamol ***Phenacetin*** ***acetanilide***

Figure (1.6): Paracetamol, phenacetin and acetanilide chemical structures.

"Hence, it is expected that blocking the hydroxyl group in paracetamol with a suitable linker could inhibit the interaction of paracetamol with its bitter taste receptor/s and hence masking its bitterness." [44]

It seems reasonable to assume that the aliphatic hydroxyl group in guaifenesin is crucial for obtaining the bitter taste characteristic; this might be due to the ability of guaifenesin to interact through hydrogen bonding with bitter taste receptors.

1.8 Guaifenesin dosing information[45-47]

It is available in the form of 200 mg or 400 mg tablets and liquid form (syrup). Each 5ml contains 100 mg guaifenesin.

Usual Adult Dose of guaifenesin for Cough:

Immediate release formulation: 200 to 400 mg tablet orally every 4 hours as needed, not to exceed 2.4 g/day. Sustained release formulation: 600 to 1200 mg tablet orally every 12 hours, not to exceed 2.4 g/day.

Usual Pediatric Dose of guaifenesin for Cough:

Immediate release formulation:

- less than 2 years: 12 mg/day syrup orally in 6 divided doses
- 2 to 5 years: 50 to 100 mg syrup orally every 4 hours as needed, not to exceed 600 mg/day
- 6 to 11 years: 100 to 200 mg syrup orally every 4 hours as needed, not to exceed 1.2 g/day
- 12 years or older: 200 to 400 mg tablet orally every 4 hours as needed, not to exceed 2.4 g/day

Sustained release formulation:

- 2 to 5 years: 300 mg syrup orally every 12 hours, not to exceed 600 mg/day
- 6 to 11 years: 600 mg syrup orally every 12 hours, not to exceed 1.2 g/day
- 12 years or older: 600 to 1200 mg tablet orally every 12 hours, not to exceed 2.4 g/day.

1.9 Objectives of the study:

The aims of this research were:

1. To mask the intensely bitter taste of guaifenesin by synthesizing prodrugs with suitable linkers that can release the parent drug (guaifenesin) when exposed to a physiological environment.

2. To conduct kinetic studies for the intraconversion of the synthesized novel prodrugs to their parent drug, guaifenesin using HPLC, and to measure the intraconversion rates and the half-lives (t ½) at different buffers 1N HCl, pH 3, pH 5, and pH 7.

Chapter two

Literature review

Chapter Two

Literature review

2.1 Chemical Modification of Clindamycin

Clindamycin is a lincosamide antibiotic. It is usually used to treat infections with anaerobic bacteria, but can also be used to treat some protozoa diseases, such as malaria. It is a common topical treatment for acne and can be useful against some methicillin-resistant infections.[48] It might be extremely bitter tasting, therefore to make it suitable for intake, the dosage is usually given in capsules or coated tablets, but unsuitable for a pediatric suspension or chewable tablet dosage form.

To improve taste properties, a series of 2 and 3 monoesters and some 2,3- dicarbonate ester of clindamycin were synthesized. The long chain clindamycin (palmitate and hexadecyl carbonate) is virtually devoid of the bitter taste characteristic of clindamycin. [48]

The synthesis of bitterness clindamycin prodrug was achieved by protection of 3,4 hydroxyl group of clindamycin with acidic anisaldehyde to produce 3,4- anisylidene clindamycin. The produced prodrug was then treated by esterfication of hydroxyl group number 2 with acid to afford pure clindamycin 2-monoesters ester as a scheme in **Figure2.1**.

Selective esterification of the 3 hydroxyl groups is achieved by adopting low temperature reaction with the use of pyridine as solvent under -25 °C and alkyl chloroformate to produce clindamycin 3-monoesters ester as per the scheme in **Figure 2.2**.

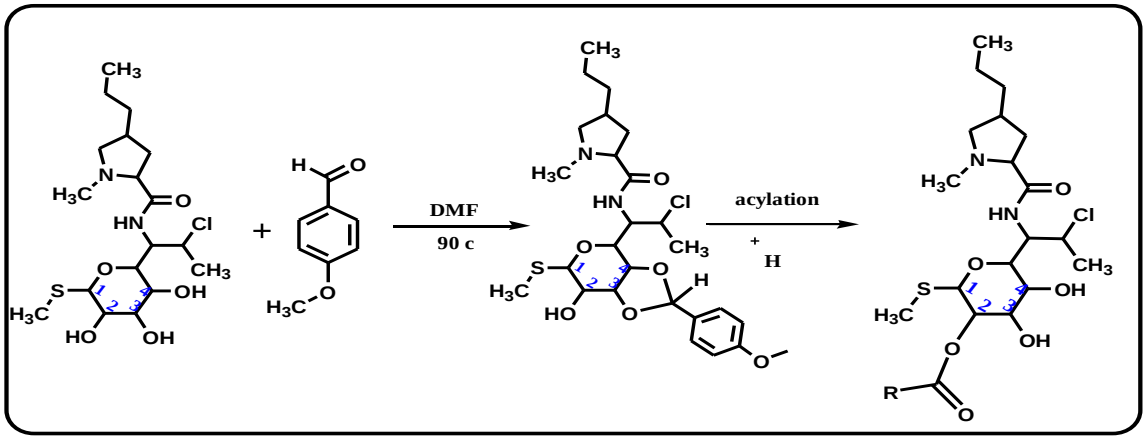


Figure (2.1): Clindamycin-2-monoesters

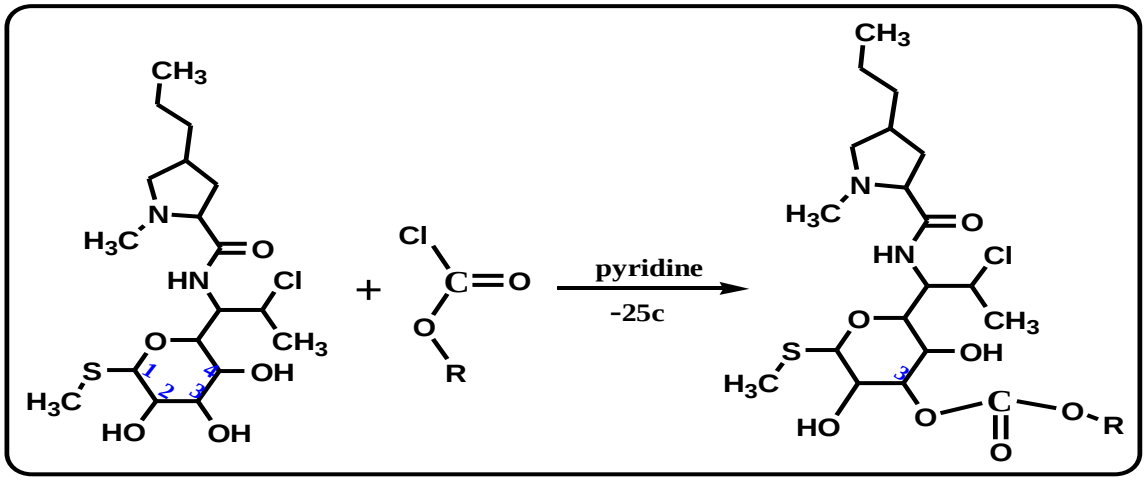


Figure (2.2): Clindamycin-3-monoesters

Taste studies:

Four derivatives of 2 monoesters, Clindamycin 2-hexylcarbonate HCl, Clindamycin 2-laurate HCl, Clindamycin 2-palmitate HCl, and Clindamycin 2-diphenylacetate were prepared, then dissolved in 30% sucrose solutions and 5-ml of samples of each derivatives were given to a taste panel of 26 people. The usual protocol was followed, with 1 hr. span between samples. The same protocol was employed for 3 monster derivatives, were the 2 and 3 monoester derivatives group each tested separately.

The average scores shown in **Figure 2.3** indicate a linear trend of taste improvement with increasing chain length. The palmitate ester is significantly better than the laurate at the 5 level of confidence. The laurate, in turn, is significantly better than both the hexanoate and acetate. The latter two were ranked equally low, being very bitter-tasting compounds.

Clindamycin 2 and 3 -palmitate hydrochloride are essentially tasteless, for these reasons, it is being extensively tested in humans as a tasteless pediatric formulation.

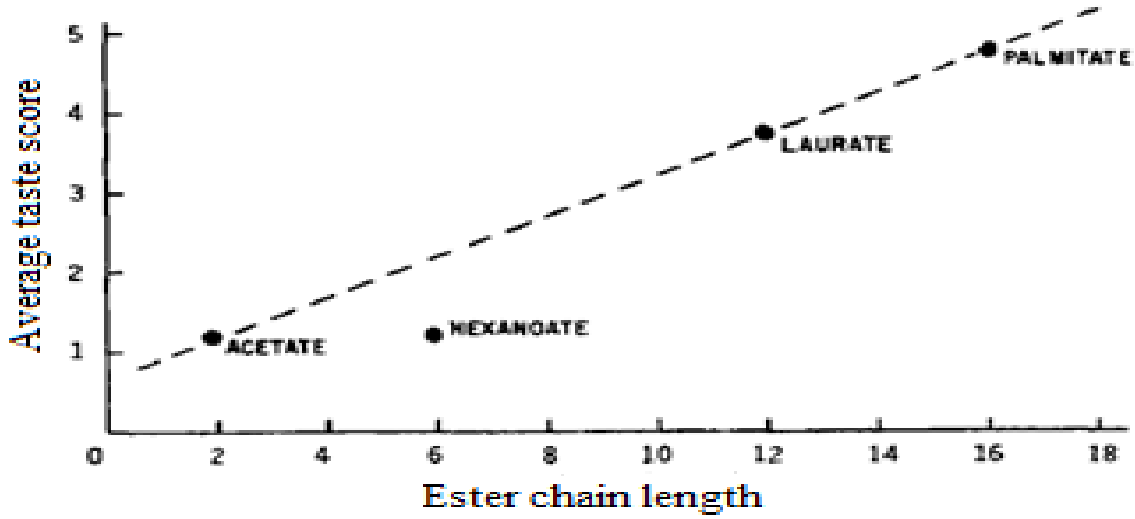


Figure (2.3): Effect of clindamycin ester chain length on taste of ester in syrup.

2.2 Masking the Bitter taste of atenolol.

Based on DFT, MP2, and the density functional from Truhlar group (hybrid GGA: MPW1k) calculations for an acid-catalyzed hydrolysis of nine Kirby's N-alkylmaleamic acids and two atenolol prodrugs were designed, **Figure 2.4**. The calculations demonstrated that the amide bond cleavage is due to intramolecular nucleophilic catalysis by the adjacent carboxylic acid group and the rate-limiting step is determined based on the nature of the amine leaving group. [50] In addition, a linear correlation of the calculated and experimental rate values has drawn credible basis for designing atenolol prodrugs that are bitterless, are stable in neutral aqueous solutions, and have the potential to release the parent drug in a

sustained release manner, **Figure 2.5**. For example, based on the calculated B3LYP/6-31 G (d,p) rates, the predicted (a time needed for 50% of the prodrug to be converted into drug) values for atenolol prodrugs ProD 1-ProD 2 at pH 2 were 65.3 hours (6.3 hours as calculated by GGA: MPW1K) and 11.8 minutes, respectively. *In vitro* kinetic study of atenolol prodrug ProD 1 demonstrated that the $t_{1/2}$ was largely affected by the pH of the medium. The determined $t_{1/2}$ values in 1N HCl, buffer pH 2, and buffer pH 5 were 2.53, 3.82, and 133 hours, respectively. [51]

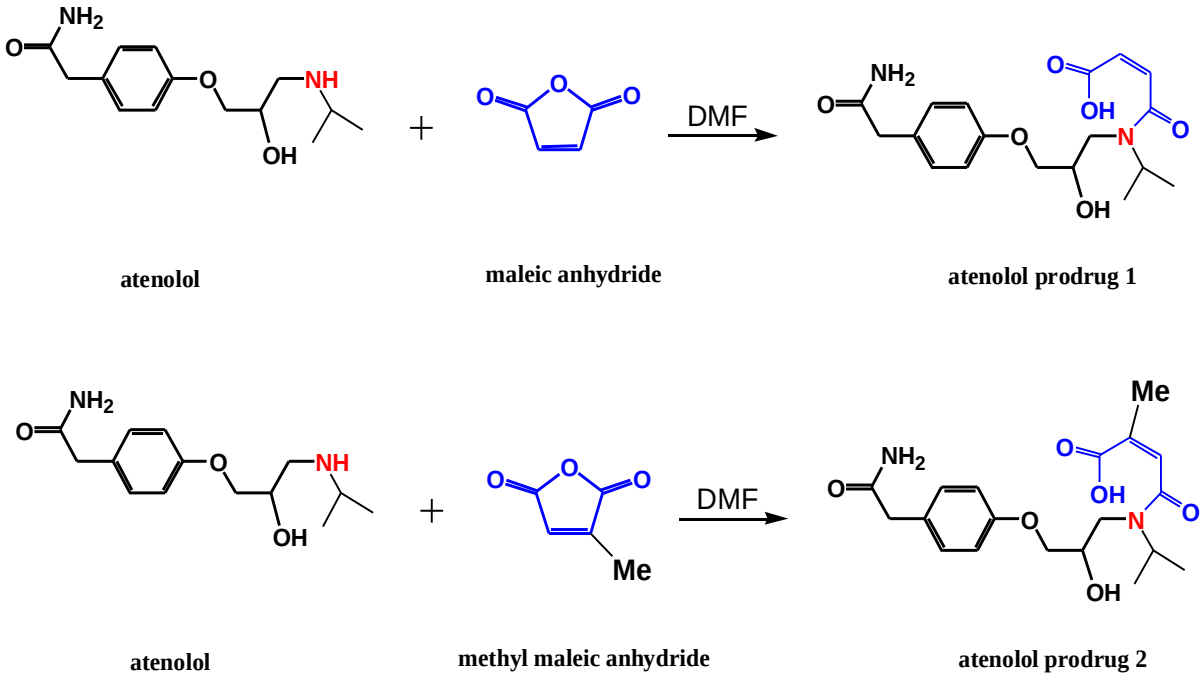


Figure 2.4: Atenolol prodrugs synthesis

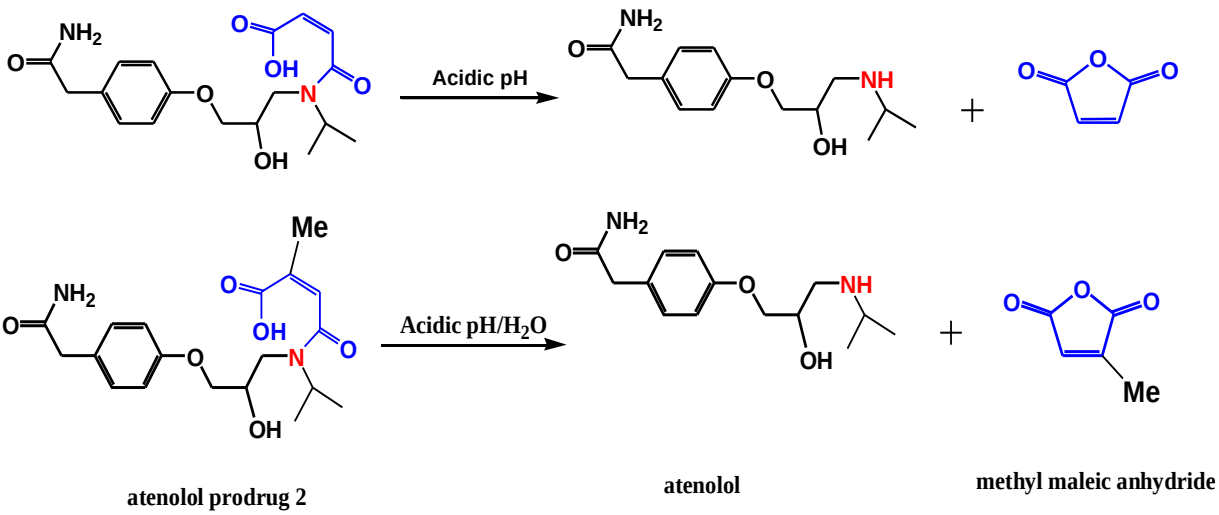


Figure (2.5): Atenolol prodrugs hydrolysis to parent drug.

2.3 Masking the bitter taste of amoxicillin and cephalexin.[52- 57]

Based on previously reported DFT calculations, amoxicillin ProD 1-2 and cephalexin ProD 1-2 were designed and synthesized, **Figure 2.6**. For the intraconversion of both antibacterial prodrugs the k_{obs} and $t_{½}$ values in different media were calculated from the linear regression equation obtained from the correlation of log concentration of the residual prodrug verses 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. Kinetics studies in 1N HCl, pH 2.5 and pH 5 were selected to examine the intraconversion of both prodrugs to their parent drugs, **Figure 2.7**. The acid-catalyzed hydrolysis of the prodrugs was found to be much higher in 1N HCl than in pH 2.5 and pH 5. Amoxicillin released from its prodrug after 3 h in 1 N HCl, 7 h in pH 2.5, and 81 h using pH 5, with ($k_{obs's}$), $2.47x10^{-4}$ in HCl, $9.60x10^{-5}$ in pH 2.5, and $7.55x10^{-6}$ in

pH 5. The released process for cephalexin from its prodrug took 2 h using 1N HCl and 14 h in pH 2.5, with ($k_{obs's}$), $2.57x10^{-4}$ in HCl and $4.17x10^{-5}$. The two prodrugs were not released to drugs at pH 7.4 and at pH 5 for cephalexin prodrug were quite stable and no release of the parent drugs was observed. At pH 5 the hydrolysis of the prodrugs was too slow. The two 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.

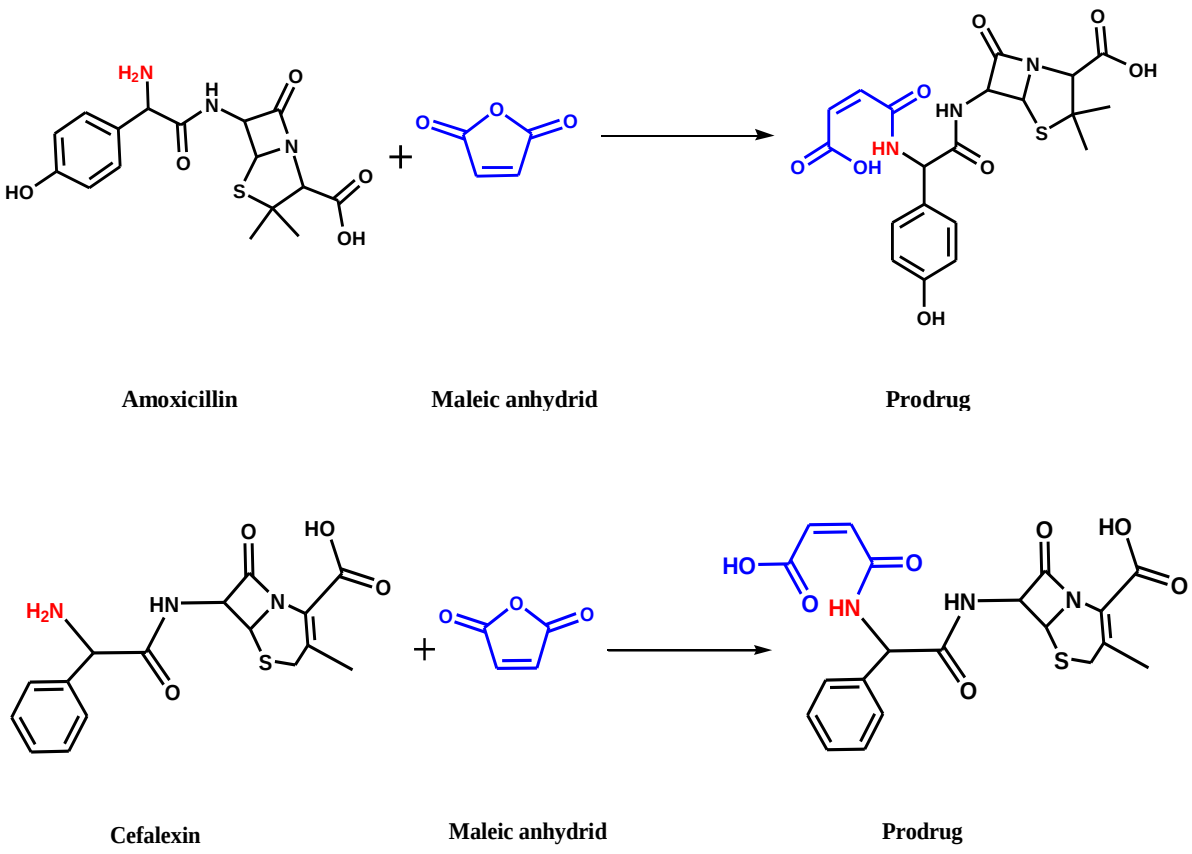


Figure (2.6): Amoxicillin and cephalexin prodrug synthesis

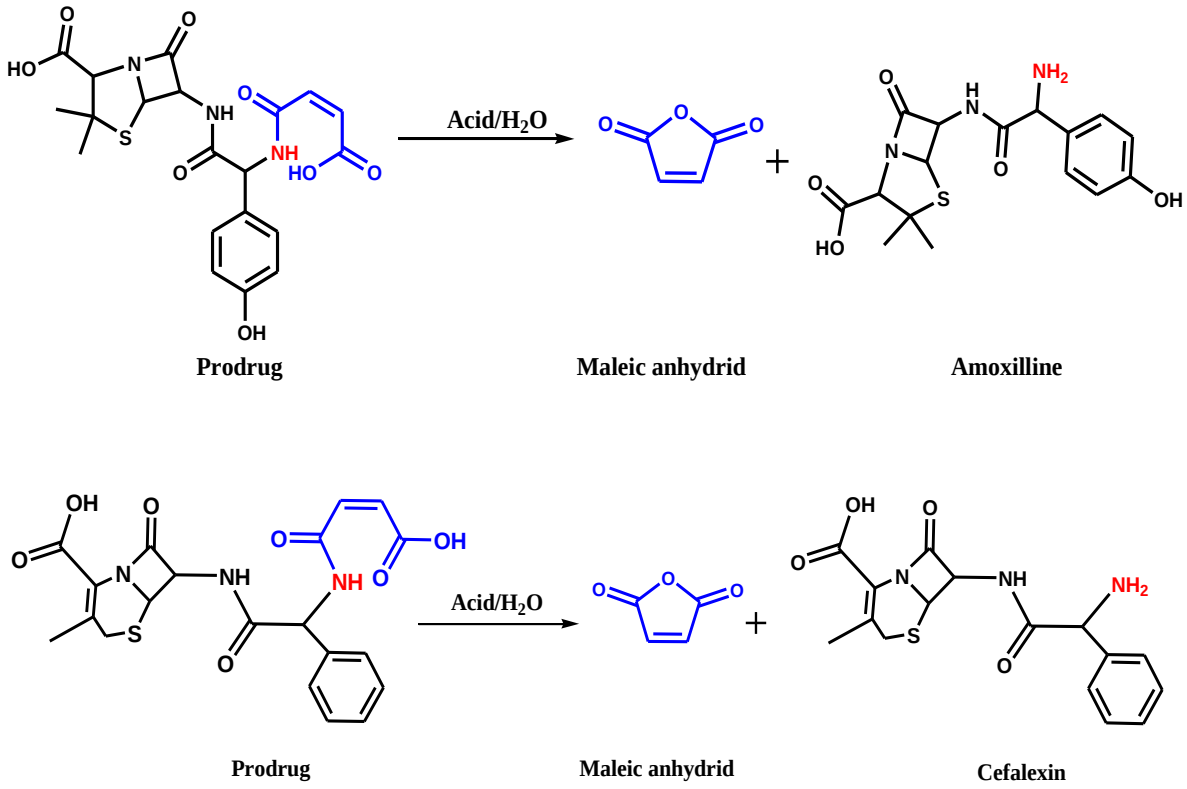


Figure (2.7): Amoxicillin and cephalexin prodrug hydrolysis to parent drug

Chapter three

Materials and Methods

Chapter three

Materials and Methods

3.1 General

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

The LC-MS system used was Agilent 1200 series liquid chromatography coupled with a 6520 accurate mass quadruple-time of flight mass spectrometer (Q-TOF LC/MS). The analysis was performed in the negative electrospray ionization mode, (Hebrew University). The high pressure liquid chromatography (Al-Quds University, HPLC) system consisted of an Alliance 2695 module equipped with 2996 Photodiode array detector from Waters (Germany). Data acquisition and control were carried out using Empower 2 ™ software (Waters, Germany). Analyses were separated with a 4.6 mm x250 mm XBridge® C18 column (5 μm particle size) used in conjunction with a 4.6x20 mm, XBridge® C18 guard column. Micro filters of 0.45μm porosity were normally used (Acrodisc® GHP, Waters).

^{1}H-NMR experiments were performed with a Bruker AvanceII 400 spectrometer equipped with a 5 mm BBO probe (Hebrew University). pH values were recorded on pH meter model HM-30G: TOA electronics ™ was used in this study to measure the pH value of each sample, thin-layer chromatography (TLC) was carried out on TLC plastic sheets silica gel, 20x20 cm, layer thickness 0.2 mm, the spots on the chromatograms were localized by UV light.

3.2 Chemicals and Reagents

Pure standards of guaifenesin were obtained from Merck Ltd., Maleic anhydride, succinic anhydride, glutaric anhydride, sodium dihydrogen phosphate anhydrous, sodium hydroxide, concentrated hydrochloric acid (36%), sodium hydride (60%) and magnesium sulfate anhydrous were commercially obtained from Sigma Aldrich. HPLC grade solvents of methanol, acetonitrile, and water were purchased from J.T. Paker. High purity chloroform, dimethylformamide (DMF), acetone and diethyl ether (> 99%) were purchased from Merck.

3.3 Preparation of guaifenesin prodrugs

Synthesis of the guaifenesin prodrrugs was accomplished using Bruice's procedures (**Figure 3.1 (a, b, and c)**). In a 250 mL round-bottom flask guaifenesin (10 mmol) was dissolved in 50 ml of dry dimethylformamide (DMF), 0.8 gm of sodium hydride was added, the resulting solution was stirred for 30 minutes then (20 mmol) of maleic anhydride, succinic anhydride or glutaric anhydride was slowly added to the mixture, then the mixture was stirred overnight. 1N HCl (50 mL) was added while the round-bottom flask is setting in an ice bath. The aqueous layer was extracted with ether (3x90 mL) and the combined ether layers was dried over $MgSO_4$ anhydrous, filtered and evaporated to dryness. The product was washed with hexane and dried. Reactions of guaifenesin with maleic anhydride, succinic anhydride or glutaric anhydride provided guaifenesin maleate, guaifenesin succinate, and guaifenesin glutarate respectively in yields of 61.5% (1.8 gm) for guaifenesin maleate, 58% (1.7 gm) for guaifenesin succinate and 58% (2.12 gm) for guaifenesin glutarate. The resulted guaifenesin prodrugs were tested by ^{1}H-NMR, FTIR, LC-MS and HPLC.

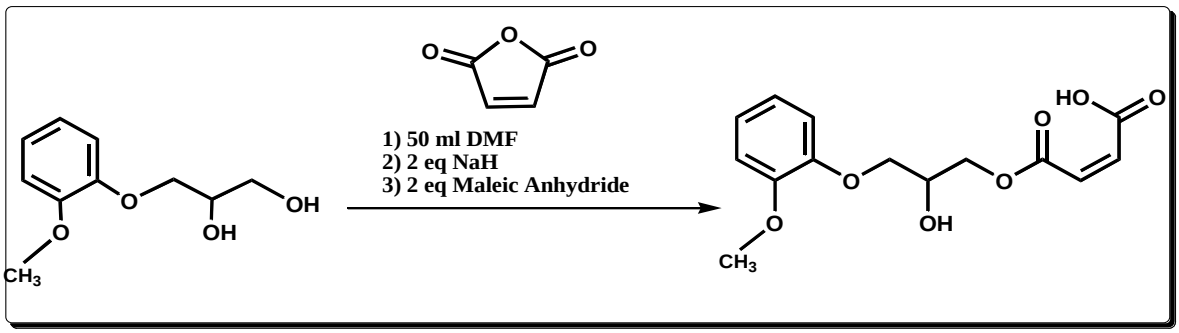


Figure (3.1a): Synthesis scheme for the preparation of guaifenesin maleate prodrug.

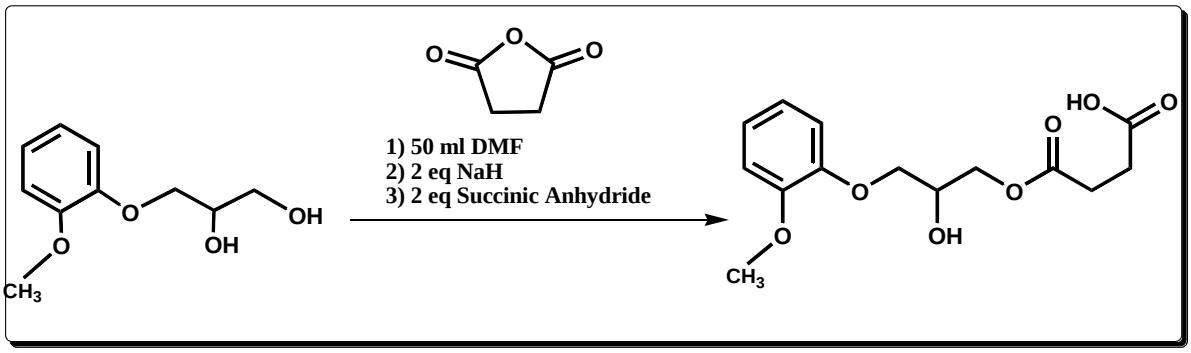


Figure (3.1b): Synthesis scheme for the preparation of guaifenesin succinate prodrug.

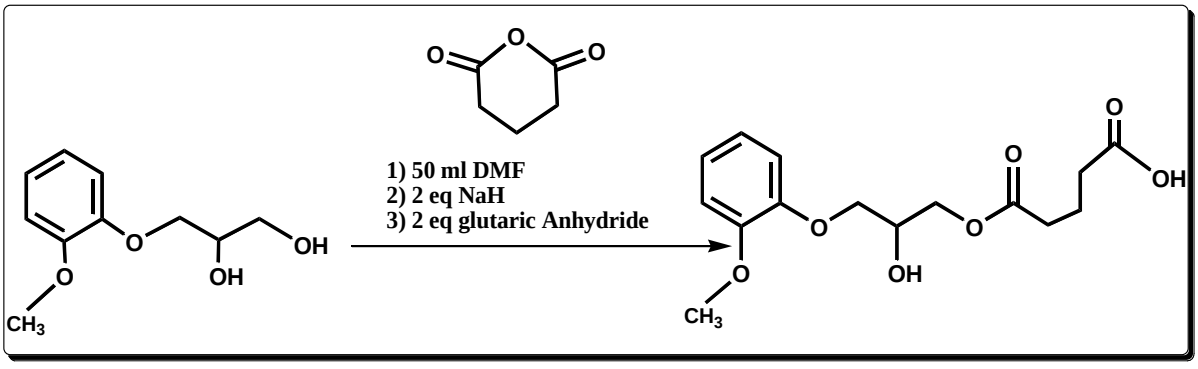


Figure (3.1c): Synthesis scheme for the preparation of guaifenesin glutarate prodrug.

Guaifenesin [$CH_3OC_6H_4OCH_2CHOHCH_2OH$]:

^{1}H-NMR ($CDCl_3$, δ ppm): $CH_3O\underline{C_6H_4}$: 6.88-7.10 (m, 4H), $OCH_2\underline{CH}OH$: 4.13 (m, 1H), $O\underline{CH_2}CHOH$: 4.06 (m, 2H), $\underline{CH_3}OC_6H_4$: 3.85 (s, 3H), $HOCH\underline{CH_2}OH$: 3.79 (m, 2H).

Guaifenesin maleate [$CH_3OC_6H_4OCH_2CHOHCH_2O.CHCHOH$ 2(CO)]:

Yield: 1.80 gm (61.5%)

^{1}H NMR ($CDCl_3$, δ ppm): $CH_3O\underline{C_6H_4}$: 6.88-7.15 (m, 4H), $\underline{CHCH}OH$ 2(CO): 6.31-6.14 (m, 2H), $OCH_2\underline{CH}OH$: 4.27 (m, 1H), $HOCH\underline{CH_2}O$: 4.03-4.23 (m, 2H), $O\underline{CH_2}CHOH$: 3.98-4.10 (m, 2H), $\underline{CH_3}OC_6H_4$: 3.86 (s, 3H).

FT-IR: group frequency (cm^{-1}): $OCH_2CH\underline{OH}$: 3063 cm^{-1}, C=O stretch of carboxylic acid: 1738 cm^{-1}, CH=CH: 1634 cm^{-1}), M/z 295 gm/mol were analyzed using LC-MS.

Guaifenesin succinate [$CH_3OC_6H_4OCH_2CHOHCH_2OCH_2CH_2OH$ 2(CO):

Yield: 1.70 gm (58%)

^{1}H-NMR ($CDCl_3$, δ ppm): $CH_3O\underline{C_6H_4}$: 6.88-7.12 (m, 4H), $OCH_2\underline{CH}OH$: 4.48 (m, 1H), $HOCH\underline{CH_2}O$: 4.09-4.31 (m, 2H), $O\underline{CH_2}CHOH$: 4.03-4.24 (m, 2H), $\underline{CH_3}OC_6H_4$: 3.84 (s, 3H), $\underline{CH_2CH_2}OH$: 2.97 (m, 4H).

FT-IR: group, frequency (cm^{-1}): $OCH_2CH\underline{OH}$: 3017 cm^{-1}, C=O stretch of carboxylic acid: 1738 cm^{-1}, and M/z 297 gm/mol were analyzed using LC-MS.

Guaifenesin glutarate [$CH_3OC_6H_4OCH_2CHOHCH_2OCH_2CH_2CH_2OH$ 2(CO)]:

Yield: 2.12 gm (58%)

^{1}H-NMR ($CDCl_3$, δ ppm): $CH_3O\underline{C_6H_4}$: 6.87-7.09 (m, 4H), $OCH_2\underline{CH}OH$: 4.50 (m,1H), $HOCH\underline{CH_2}O$: 4.16-4.33 (m, 2H), $O\underline{CH_2}CHOH$: 4.05-4.24 (m, 2H), $\underline{CH_3}OC_6H_4$: 3.70 (s, 3H), $\underline{CH_2}CH_2\underline{CH_2}OH$: 2.39-2.45 (m, 4H), $CH_2\underline{CH_2}CH_2OH$: 1.91-1.97 (m, 2H).

FT-IR: group, frequency(cm^{-1}): OCH_2CHOH: 3066 cm^{-1},C=O stretch of carboxylic acid: 1736 cm^{-1}], and M/z 311 gm/mol were analyzed using LC-MS.

3.4 Kinetic Methods

3.4.1 Buffer preparation

Buffer pH 3.3: 6.8 g potassium dihydrogen phosphate were dissolve in 900 mL water for HPLC and the pH was adjusted by diluted phosphoric acid, water was added to reach a final volume of 1000 mL. The same procedure was done for the preparation of buffers pH 5.5 and 7.4, however in these two cases the pH was adjusted using 1 N NaOH.

3.4.2 Calibration curve for guaifenesin and guaifenesin prodrugs

To construct a calibration curve for guaifenesin prodrugs and the parent drug, guaifenesin, several concentrations (600, 500, 400, 300, 200 and 100 ppm) were prepared. All samples were injected into HPLC-PDA. The optimal HPLC conditions used for the analysis of guaifenesin: 4.6 mmx250mm, 5 μm, XBridge ® C18 column, a mixture of water: acetonitrile (water pH adjusted to 5.5 using diluted phosphoric acid) (75:25 v/v) as a mobile phase, a flow rate of 1 mL/minute and a UV detection at a wavelength of 275 nm. Peak area vs. concentration of the pharmaceutical (ppm) was then plotted, and R^2 value of the plot was recorded.

3.4.3 Preparation of standard and sample solution

500 ppm of standard guaifenesin was prepared by dissolving 50 mg of drug in 100 mL 1N HCl, buffer pH 3.3, buffer pH 5.5 or buffer pH 7.4. The sample was injected into HPLC to

detect the retention time of guaifenesin. The same procedure was followed for the preparation of 500 ppm of each guaifenesin prodrugs.

3.4.4 Hydrolysis of guaifenesin prodrugs

Guaifenesin prodrugs hydrolysis rate were studied at 25^0C in buffer solutions at different pHs (1N HCl, pH 3.3, pH 5.5 and pH 7.4); samples of the reaction mixtures were analyzed directly by HPLC, the decreased area percentage of the prodrug peaks and the increased area of guaifenesin peaks was monitored each 30 minutes, then area under the peaks of guaifenesin and its prodrugs vs. time were plotted. Furthermore the decreased area of the prodrugs peaks and the increased area of guaifenesin peaks were monitored in other buffers each hour then the area under the peaks of guaifenesin and its prodrugs vs. time were plotted.

Chapter four

Results and discussion

Chapter Four

Result and discussion

4.1 Results and discussion

We have successfully obtained three guaifenesin prodrugs with three different linkers (guaifenesin maleate, guaifenesin succinate, and guaifenesin glutarate). They were characterized by FT-IR, ^{1}H-NMR and LC-MS techniques, to guarantee pure guaifenesin prodrugs that were bitterless taste and were capable of releasing their parental drugs in a sustained-release manner as proposed.

4.2 Prodrugs characterization using different analytical techniques

4.2.1. Fourier transforms infrared spectroscopy (*FTIR*)

The FTIR spectra for the synthesized prodrugs (guaifenesin maleate, guaifenesin succinate, and guaifenesin glutarate), **Figure 4.2**, **Figure 4.3**, and **Figure 4.4**, respectively, showed prominent peaks that were analyzed and compared to guaifenesin spectra, **Figure 4.1**.

The peaks observed in guaifenesin maleate at 3063 cm^{-1}, 3017 cm^{-1} in guaifenesin succinate, and at 3066 cm^{-1}in guaifenesin glutarate are characteristic of the O-H stretching seen in carboxylic acids, compared to the peak produced in guaifenesin standard at 3244 cm^{-1} which in fact comes from the O-H stretch seen in alcohol.

The prodrugs peak produced at 1455-1454 $cm^{-1}$is characteristic of the CH_2 bend of the alkyl group. The same peak pattern for the band produced by the guaifenesin standard at 1455 cm^{-1}, and the same for the peaks produced at 1376-1378 cm^{-1} in prodrugs and parent drug, guaifenesin, that is characteristic of the CH_3 bend. The peaks produced at 1588 cm^{-1} in guaifenesin maleate, 1595 cm^{-1}in guaifenesin succinate, and at 1593 cm^{-1}in guaifenesin

glutarate, are characteristic of the C=C stretching of the benzene ring in the prodrugs compare with the peak produced in the guaifenesin standard at 1594 cm^{-1}.

On the other hand, the peak characteristic of the carbonyl group was seen at 1738 to 1726cm^{-1} in the three prodrugs only, while the peak at 1634 cm^{-1} is characteristic of the C=C stretch (conjugation), found in the guaifenesin maleate IR spectrum only.

In a conclusion; the gathered information shows that the FT-IR spectrums of the prodrugs matches the guaifenesin spectrum in most peaks observed except the peaks of carbonyl groups that ranged between 1738-1726 cm^{-1}, and C=C stretch (conjugation) at 1634 cm^{-1}in guaifenesin maleate, in addition to a variation in the range of hydroxyl functional groups stretching frequencies between carboxylic acid prodrug functional group and an alcoholic OH group of guaifenesin.

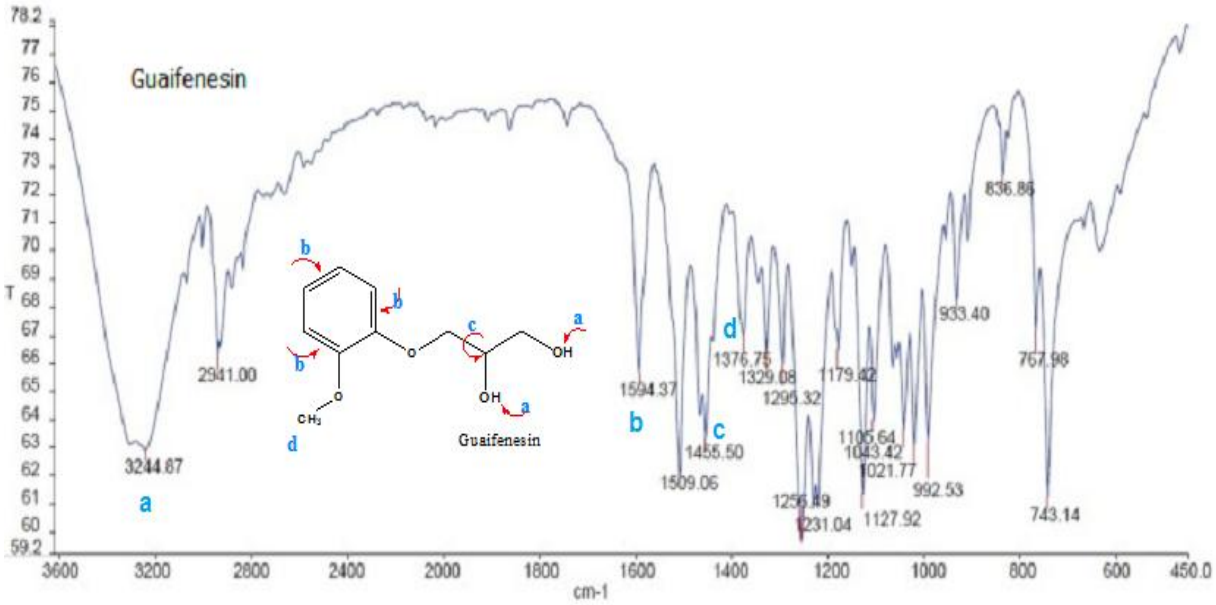


Figure (4.1): FT-IR spectrum of guaifenesin standard.

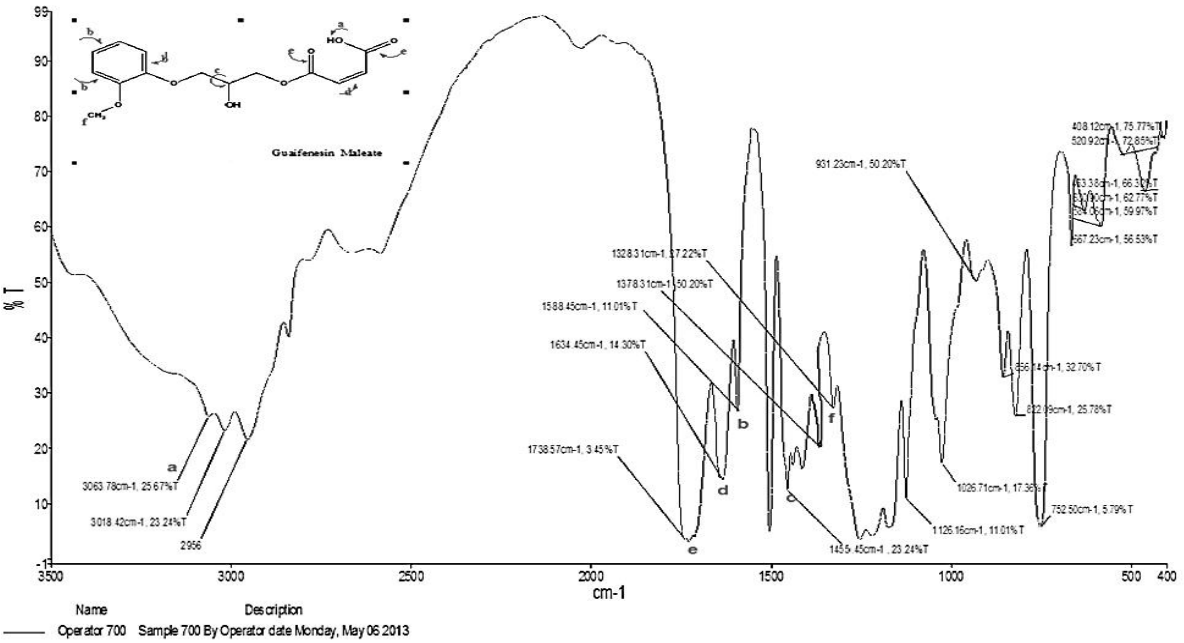


Figure (4.2): FT-IR spectrum of guaifenesin maleate.

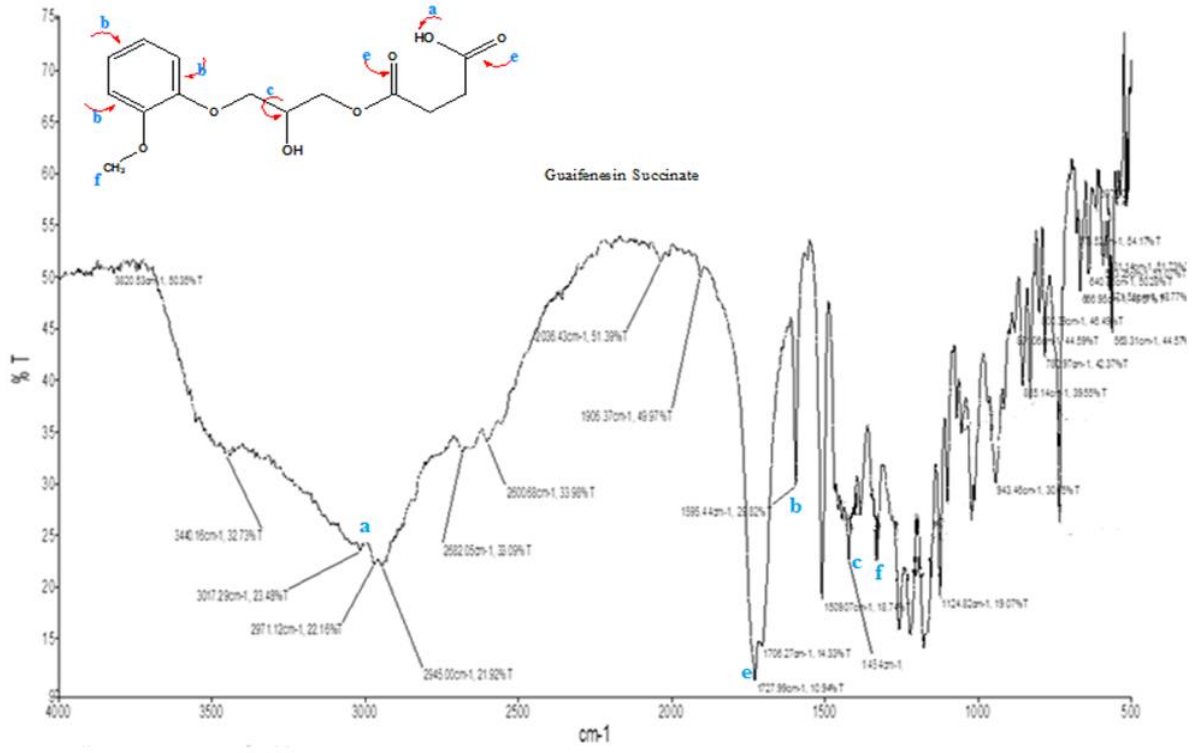


Figure (4.3): FT-IR spectrum of guaifenesin succinate.

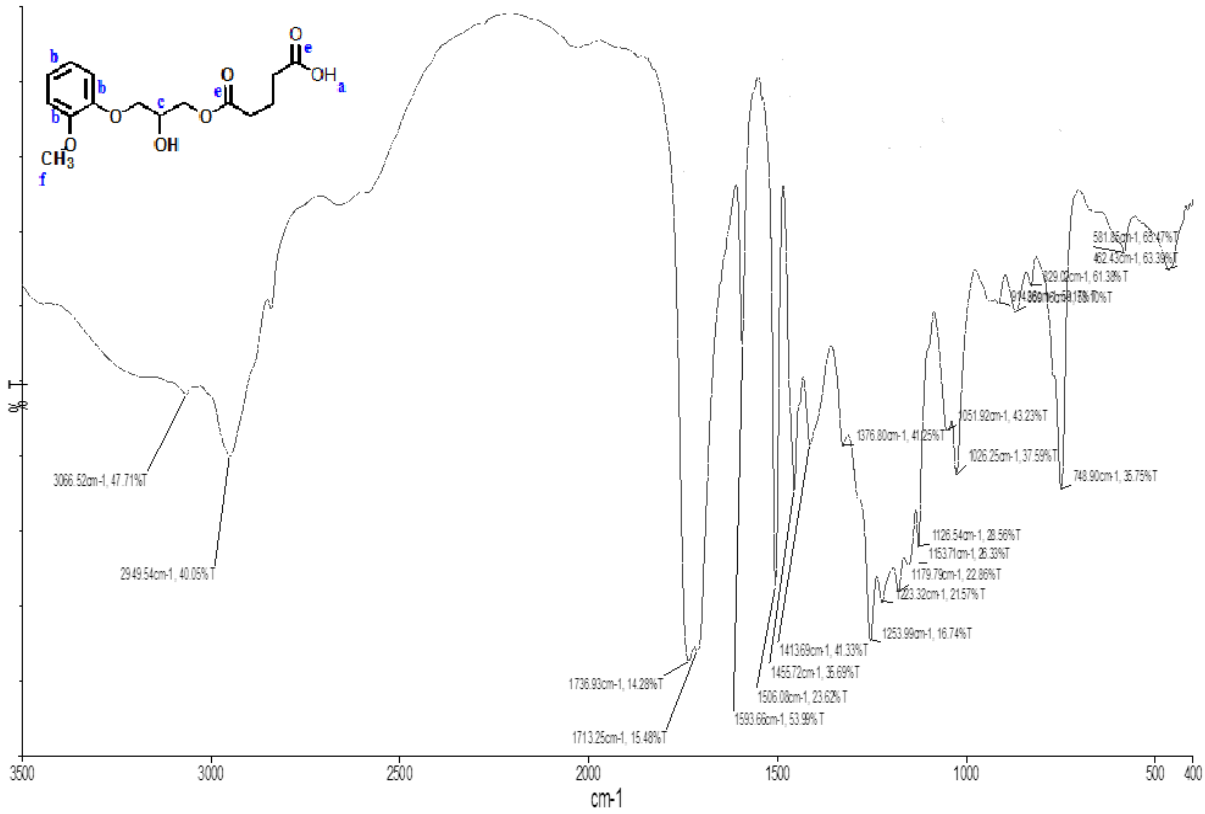


Figure (4.4): FT-IR spectrum of guaifenesin glutarate.

4.2.2: <u>Nuclear magnetic resonance (NMR)</u>

The H-NMR spectrum of guaifenesin prodrugs, (guaifenesin maleate, guaifenesin succinate, and guaifenesin glutarate), **Figure 4.6**, **Figure 4.7**, and **Figure 4.8**, respectively, showed characterstic proton chemical shifts when compared with that of guaifenesin spectrum, **Figure 4.5.**

The methylene proton of (—CH_2OH) group appearing at 3.79 ppm in guaifenesin, shifted to 4.03-4.23 ppm in guaifenesin maleate prodrug, to 4.09-4.31 ppm in guaifenesin succinate, and to 4.16-4.33 ppm in guaifenesin glutarate, this deshielding was mainly due to the carbon carbonyl of the acid anhydride linker.

The singlet at 3.85 ppm is typical of (—OCH_3) protons. These protons appeared in the same chemical shift range for guaifenesin, and its three prodrugs.

The multiplet characteristic of the four aromatic benzene ring protons appeared between 6.85-7.15 ppm in the guaifenesin prodrugs almost in the same chemical shift range as guaifenesin.

On the other hand, the multiplet signals at 2.67 ppm characteristic of the four methylene protons of the (—CH_2CH_2—) were only seen in guaifenesin succinate. The signals produced in guaifenesin glutarate at 1.94 ppm and 2.42 ppm referred to the protons of the methylene protons (—$CH_2CH_2CH_2$—). While the multiplet signals at 6.31–6.41 ppm are characteristic of the two ethylene protons (—CH=CH —), found in the guaifenesin maleate only. The protons of the methylene group bonded to the alcoholic hydroxyl group (—CH_2OH) were observed at 4.13 ppm in guaifenesin, at 4.27 ppm in guaifenesin maleate, at 4.48 ppm in guaifenesin succinate, and at 4.50 ppm in guaifenesin glutarate.

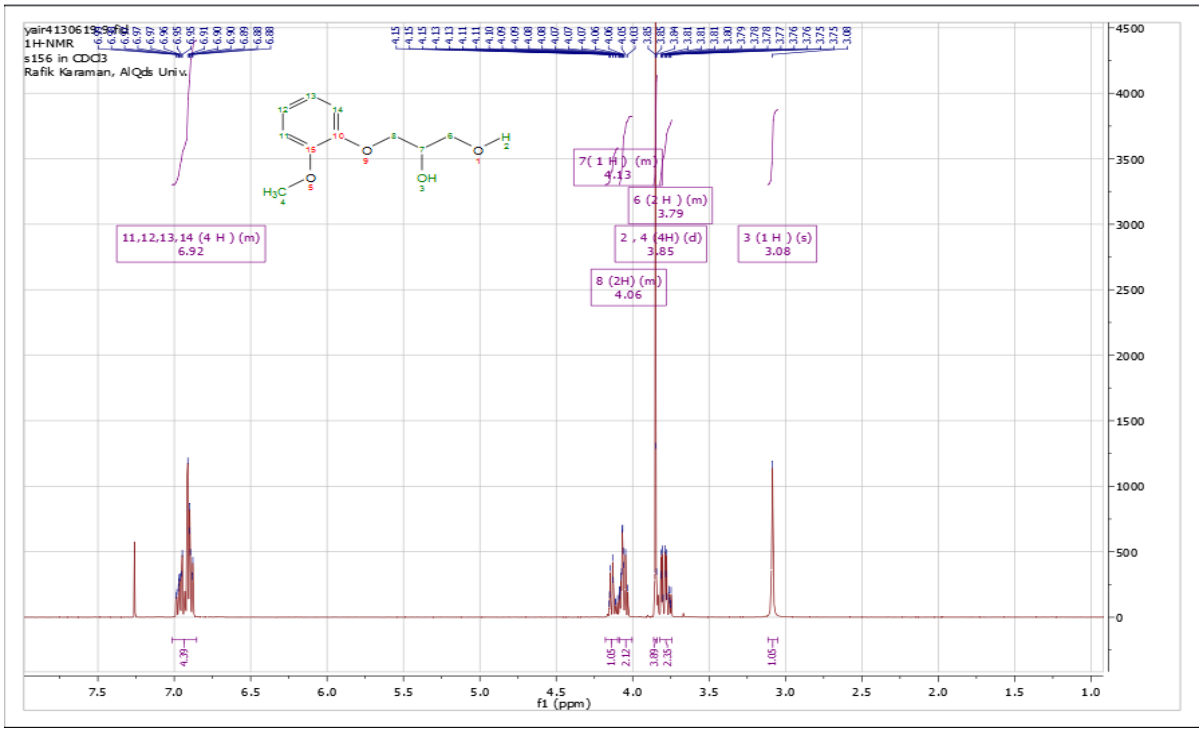


Figure (4.5): H-NMR spectrum of guaifenesin.

Figure (4.6): H-NMR spectrum of guaifenesin maleate.

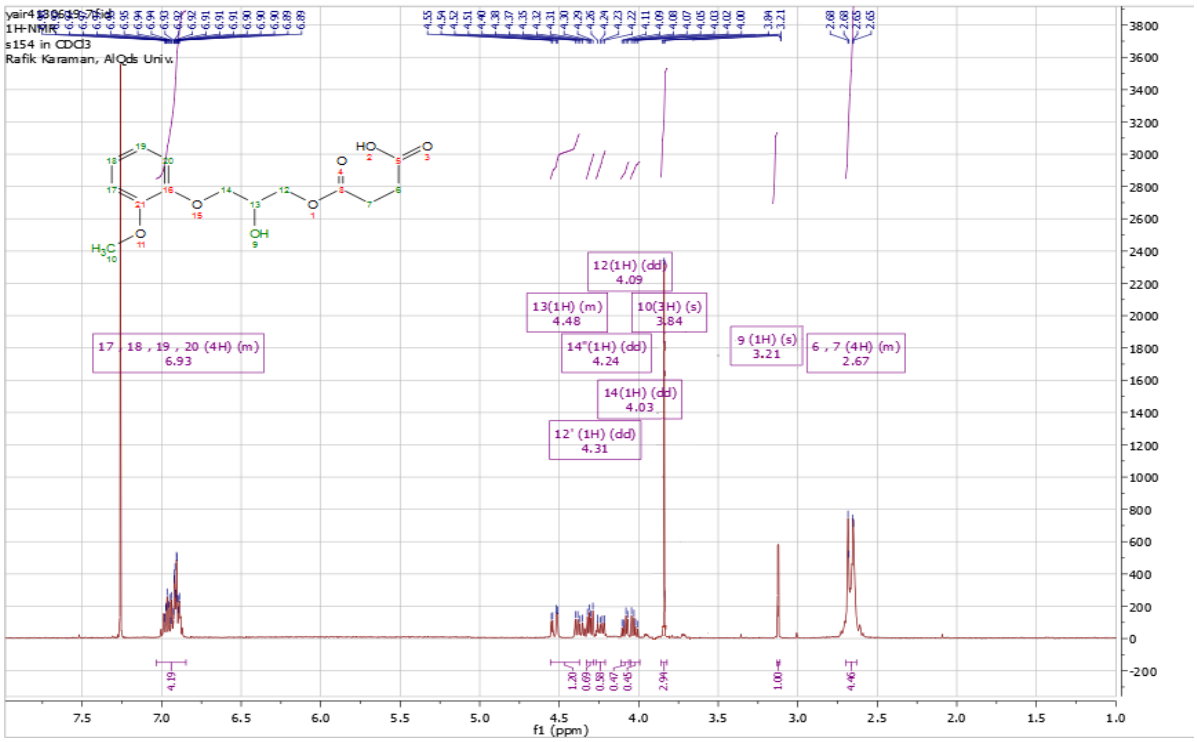


Figure (4.7): H-NMR spectrum of guaifenesin succinate

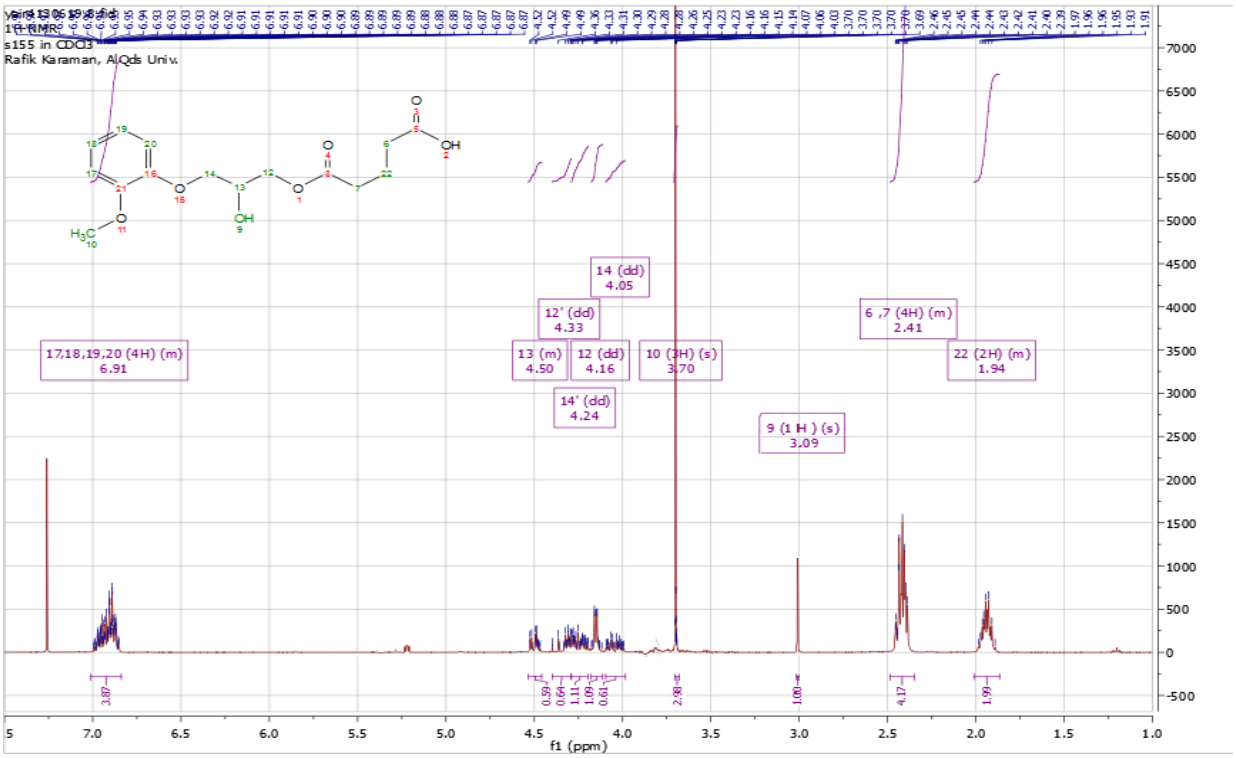


Figure (4.8): H-NMR spectrum of guaifenesin glutarate.

4.2.3: Liquid chromatography–mass spectrometry (LC-MS)

To further characterize the synthesized prodrugs (guaifenesin maleate, guaifenesin succinate, and guaifenesin glutarate) samples were analyzed by liquid chromatography coupled with mass quadruple-time of flight mass spectrometer. The analysis was performed in the negative electrospray ionization mode. The corresponding molecular masses were obtained (**Figure 4.9, 4.10, 4.11**).

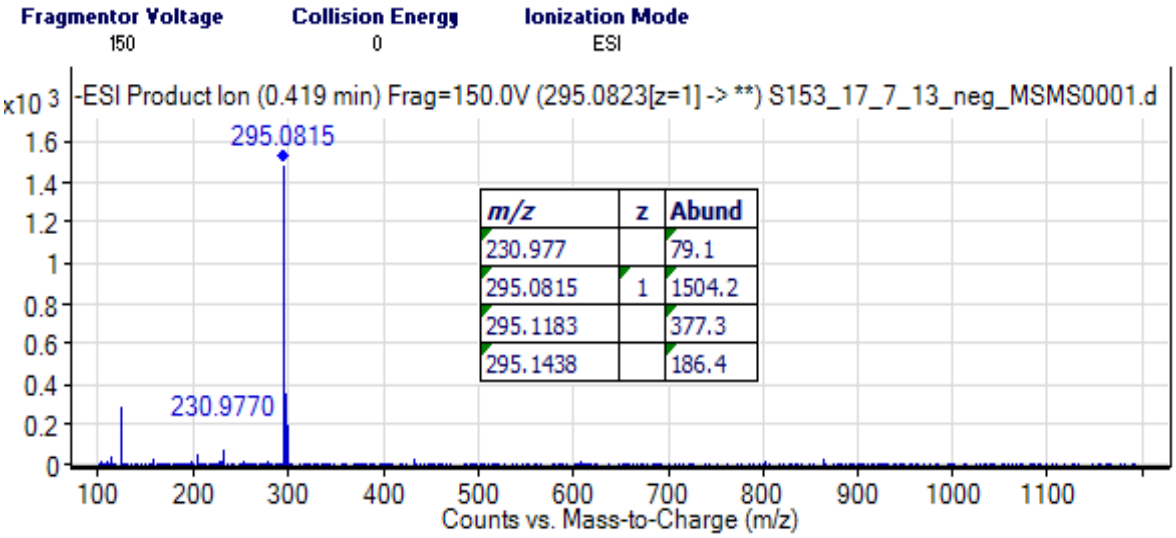


Figure (4.9): LC-MS spectrum of guaifenesin maleate.

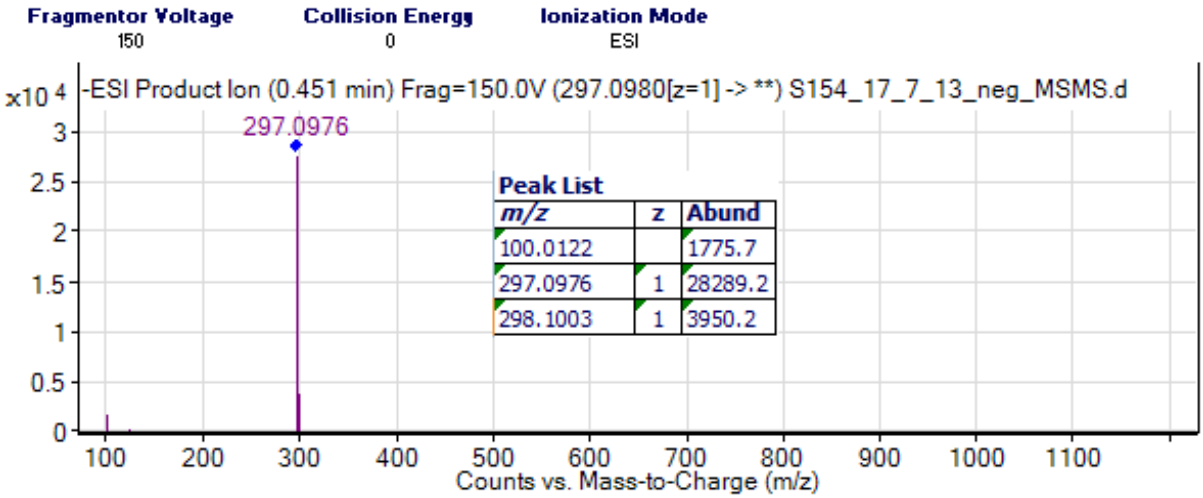


Figure (4.10): LC-MS spectrum of guaifenesin succinate.

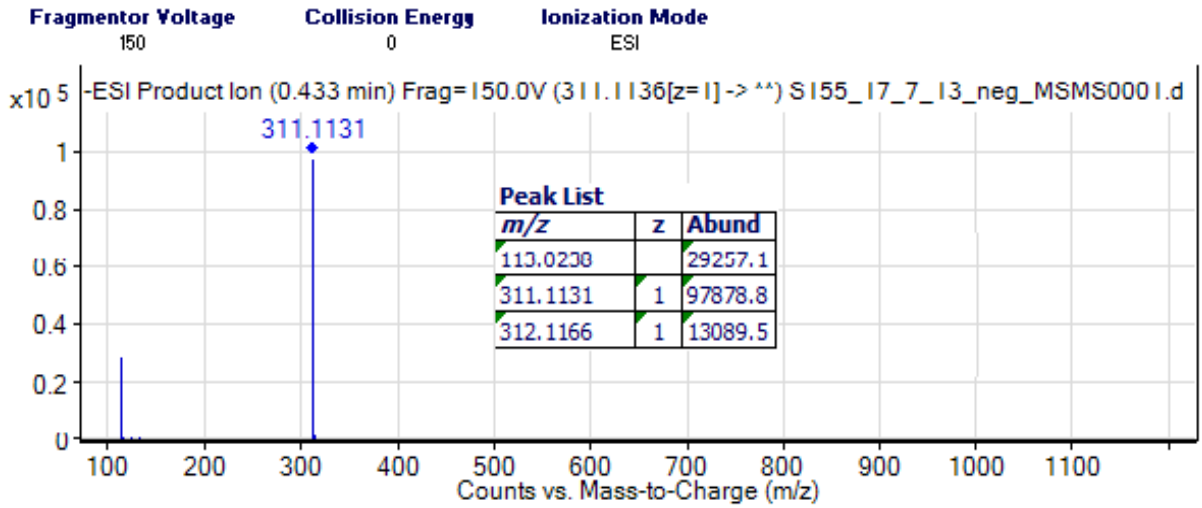


Figure (4.11): LC-MS spectrum of guaifenesin glutarate.

4.3 Hydrolysis studies:

In this part of the study, the stability of the guaifenesin prodrugs (guaifenesin maleate, guaifenesin succinate, and guaifenesin glutarate) was investigated using high-performance liquid chromatography (HPLC). Appearance of the parent drug (guaifenesin) peak (after 5.944 min) and disappearance of the prodrug peak (14.999, 10.961, 14.142 min) were monitored to determine the rate of conversion for each of the three prodrugs. Kinetic studies were performed at constant temperature (37 ^{o}C) and at the ambient pressure (2421-2443 psi) in different buffers particularly 1N HCl, pH 3.3 (stomach), pH 5.5 (intestine), and pH 7.4 (blood) which corresponds to the physiological environments in the human body.

Calibration curves were made for the three prodrugs. The results show that R^2 values were above 0.95 for all the prodrugs as indicated in **Figure 4.12**.The hydrolysis monitoring for the three prodrugs in 1N HCl, pH 3.3, pH 5.5, and pH 7.4 was conducted and the results of the study are summarized in **Figures 4.13-4.30**.

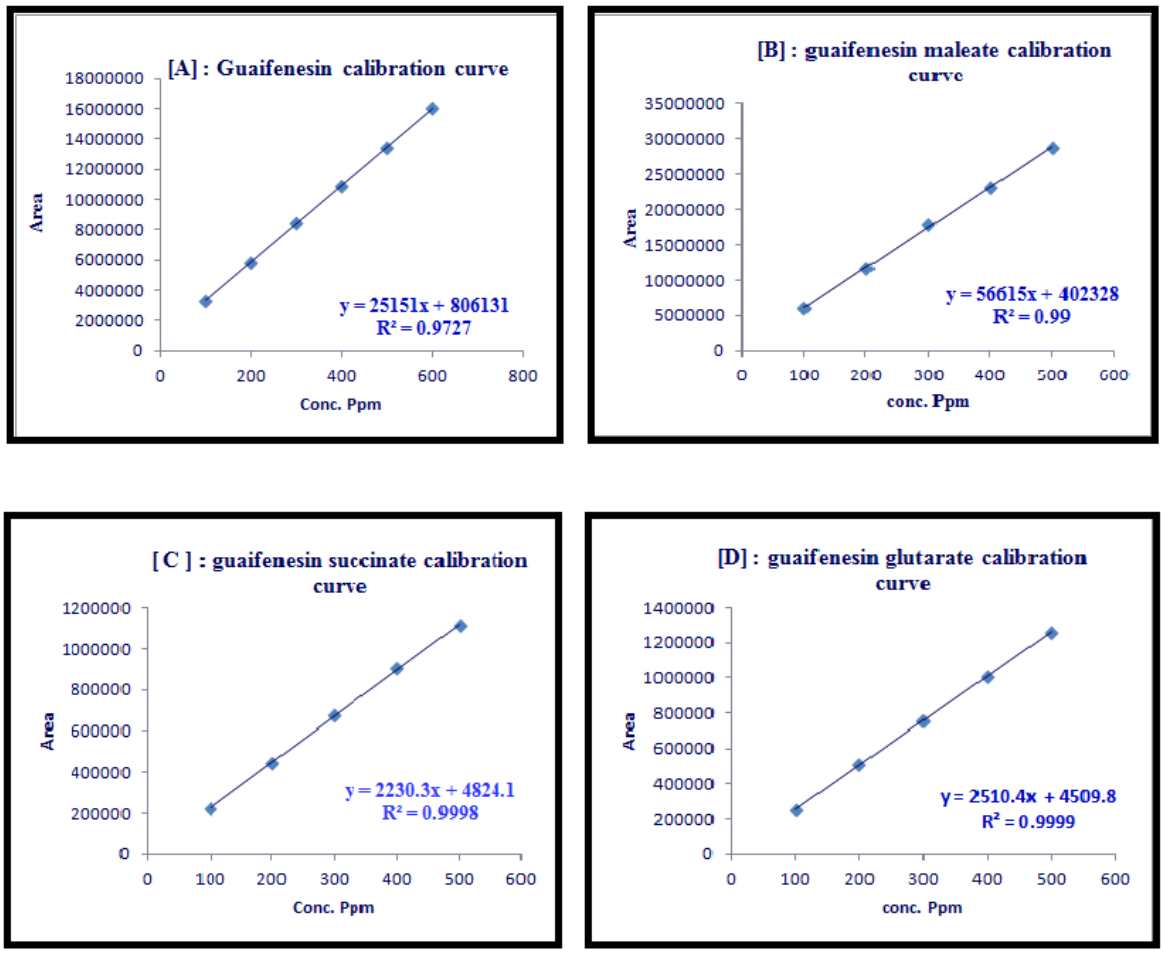


Figure (4.12): Calibration curves of a: guaifenesin, b: guaifenesin maleate, c: guaifenesin succinate and d: guaifenesin glutarate.

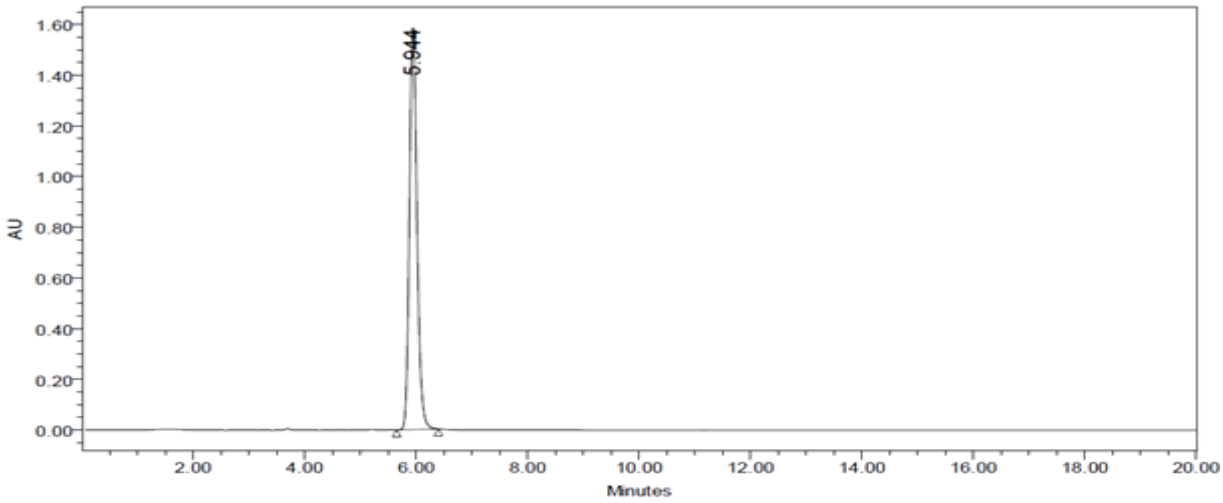


Figure (4.13): Guaifenesin standard.

1) Guaifenesin maleate kinetic study at 1N HCl, pH 3.3, pH 5.5 and pH 7.4**.**

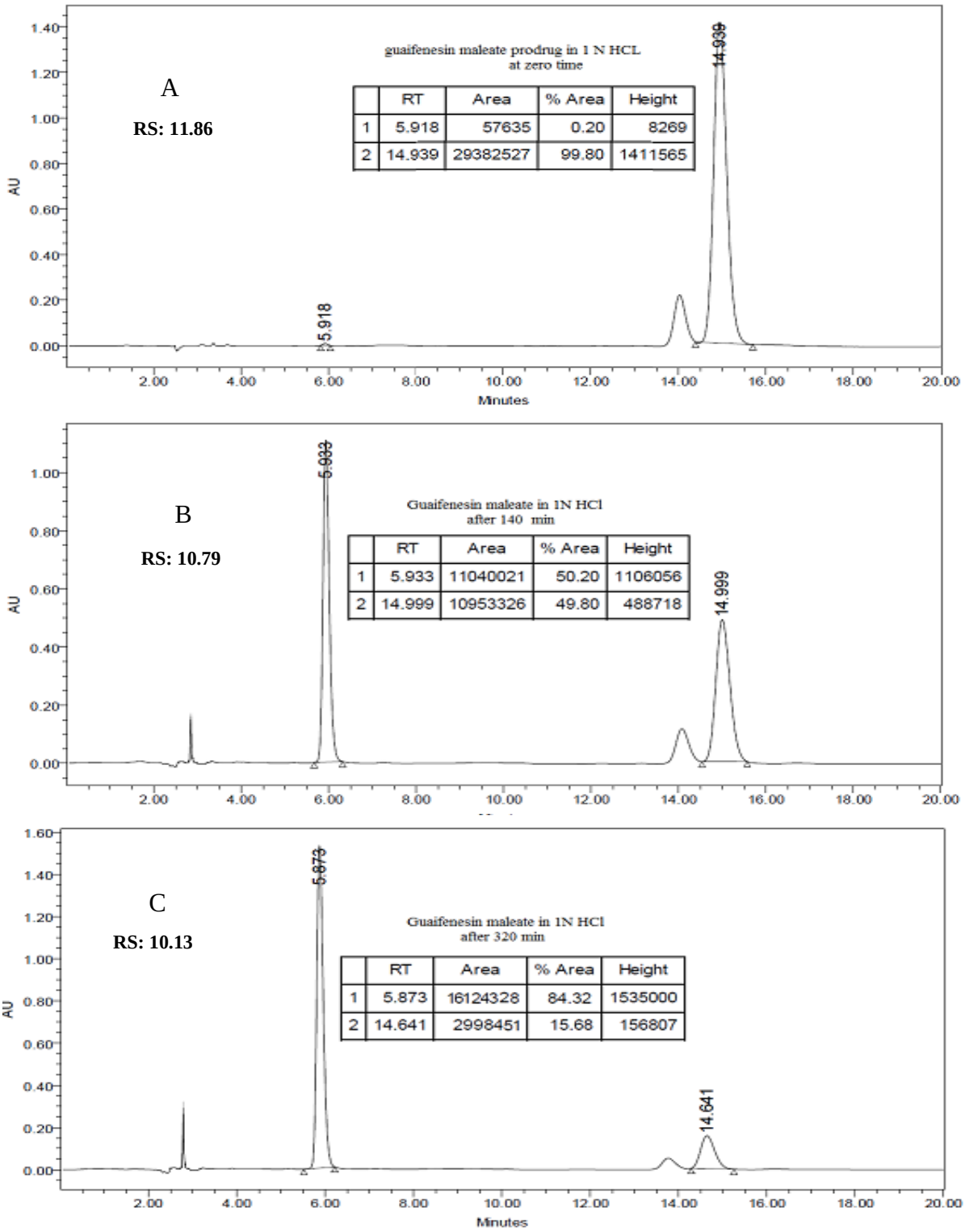


Figure (4.14): Guaifenesin maleate prodrug at 1N HCl at zero time (A), after 2.3 (B) and 5.3 hours (C), **[RS: Resolution value].**

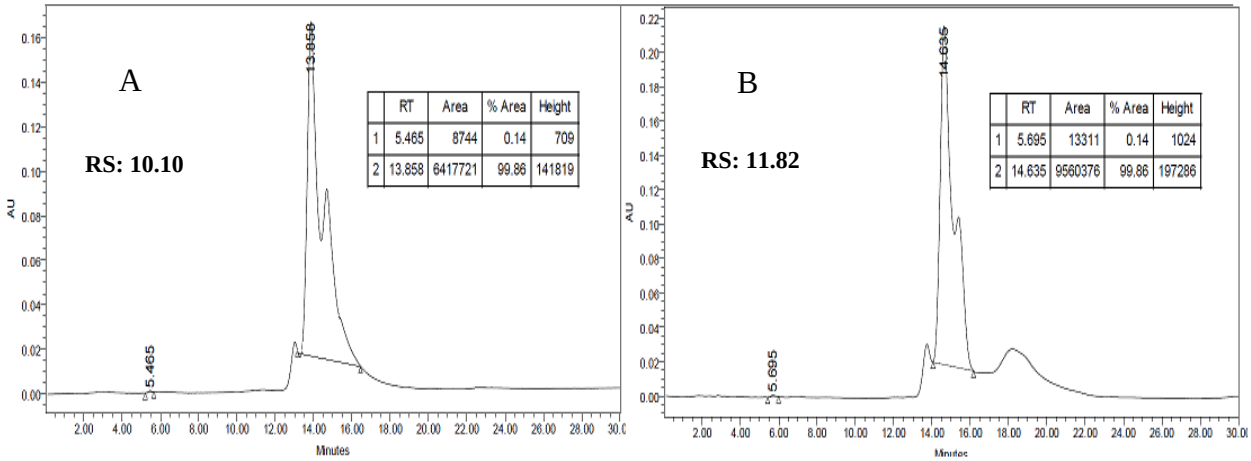


Figure (4.15): Guaifenesin maleate prodrug at pH 3.3 at t = 0 (A), and after 39 hours (B),

[RS: Resolution value].

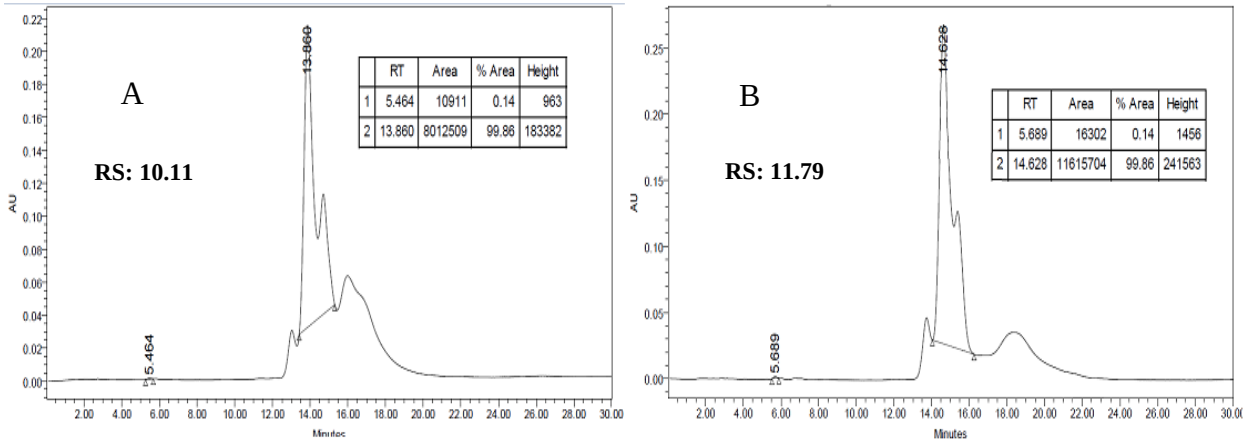


Figure (4.16): Guaifenesin maleate prodrug at pH 5.5 at t = 0 (A), and after 39 hours (B),

[RS: Resolution value].

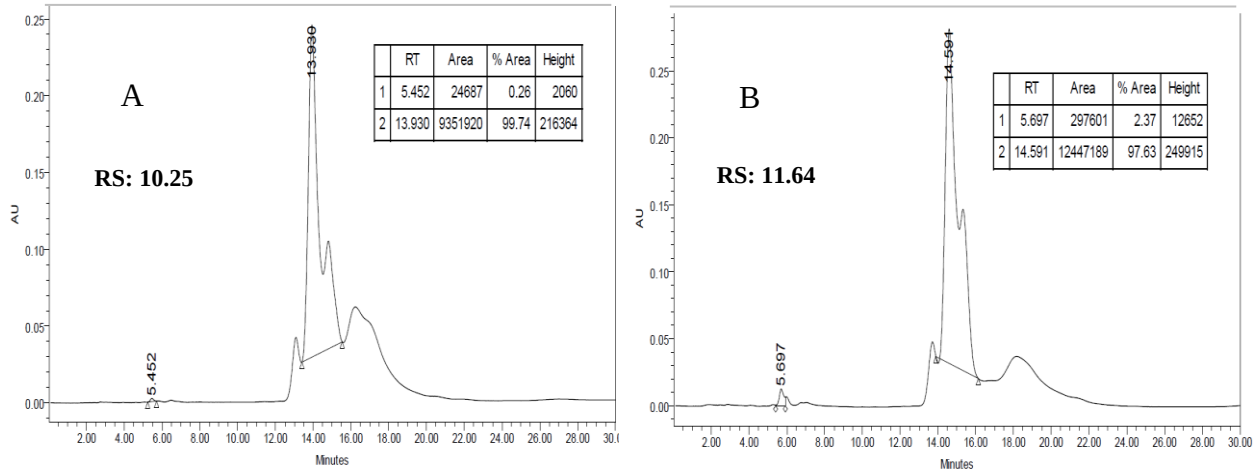


Figure (4.17): Guaifenesin maleate prodrug at pH 7.4 at t = 0 (A), and after 39 hours (B),

[RS: Resolution value].

2) Guaifenesin succinate kinetic study at 1N HCl, pH 3.3, pH 5.5 and pH 7.4.

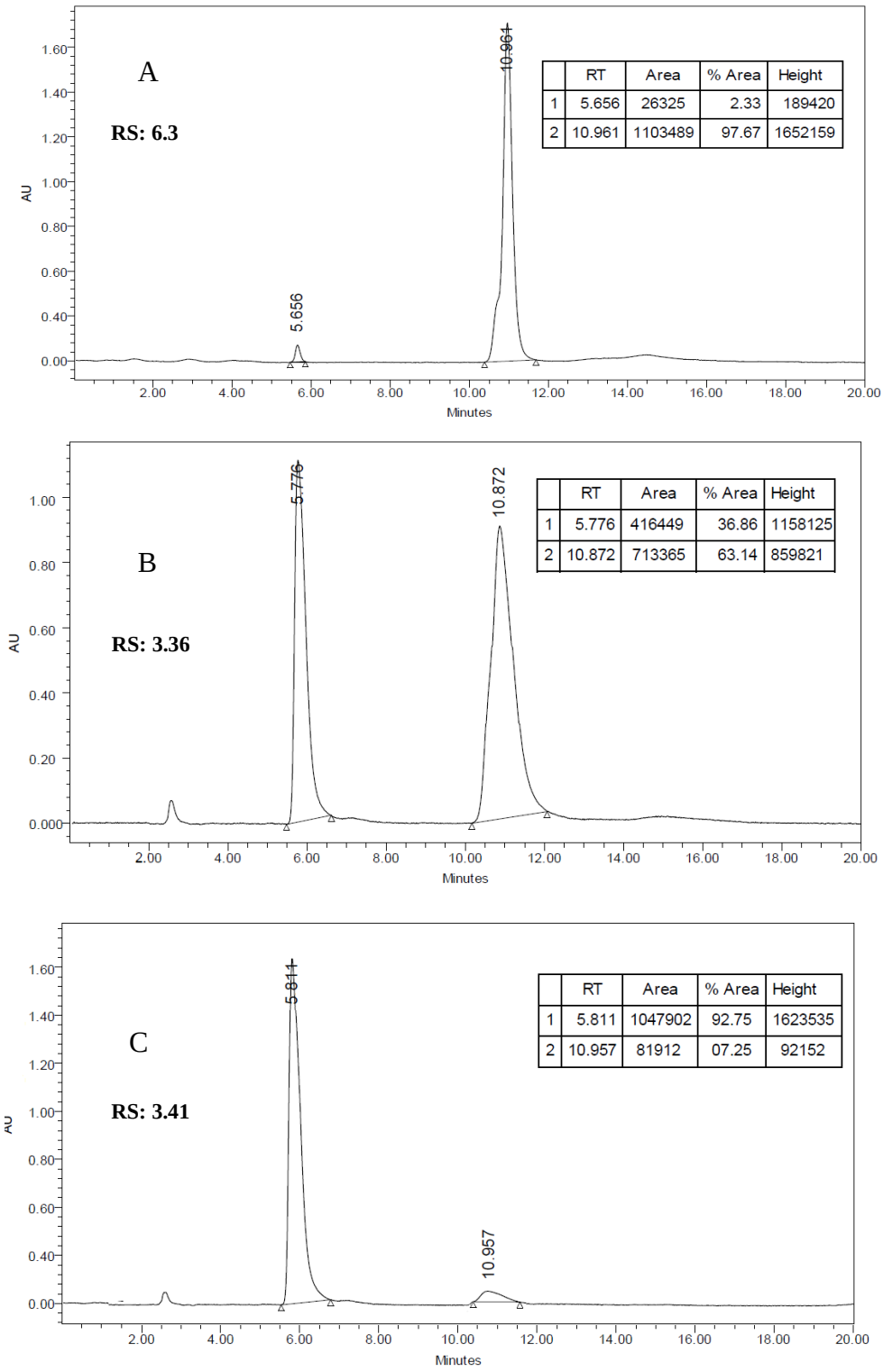


Figure (4.18): Chromatograms showing the conversion of Guaifenesin succinate to parent drug in 1N HCl at zero time (A), after 6 (B), and 12.8 hours (C), **[RS: Resolution value].**

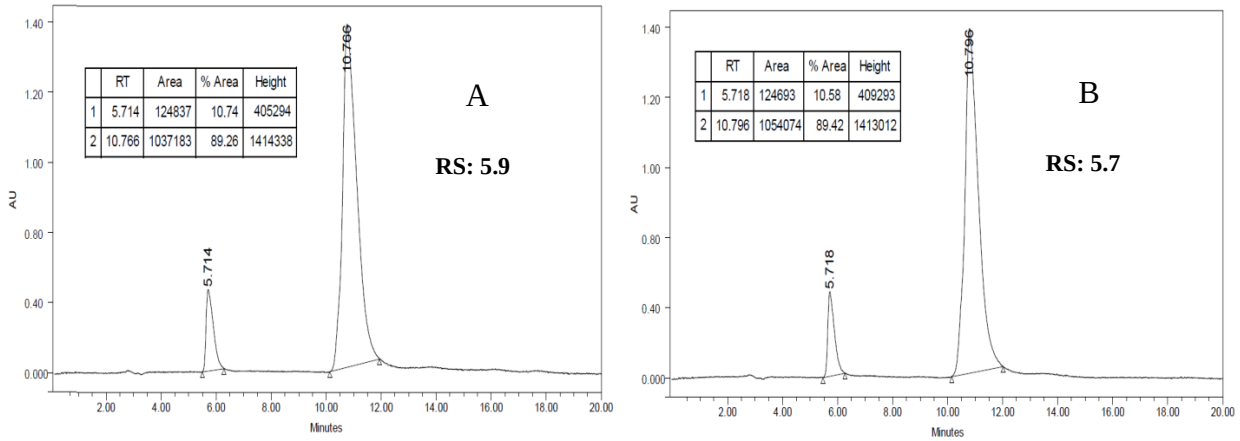


Figure (4.19): Guaifenesin succinate prodrug at pH 3.3 at t = 0 (A), after 40 hours (B), **[RS: Resolution value].**

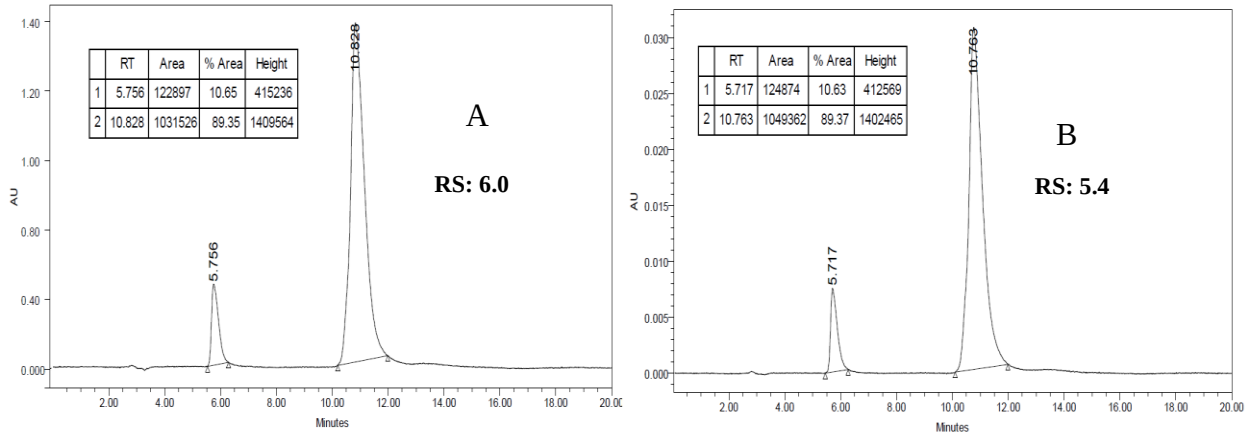


Figure (4.20): Guaifenesin succinate prodrug at pH 5.5 at t = 0 (A), after 40 hours (B), **[RS: Resolution value].**

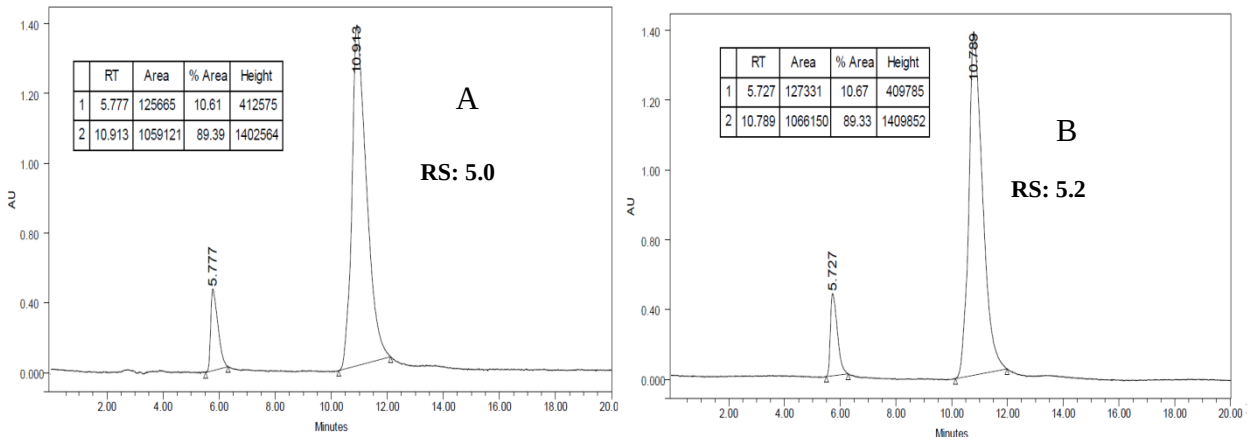


Figure (4.21): Guaifenesin succinate prodrug at pH 7.4 at t = 0 (A), after 40 hours (B), **[RS: Resolution value].**

3) Guaifenesin glutarate kinetic studies at 1N HCl, pH 3.3, pH 5.5 and pH 7.4**.**

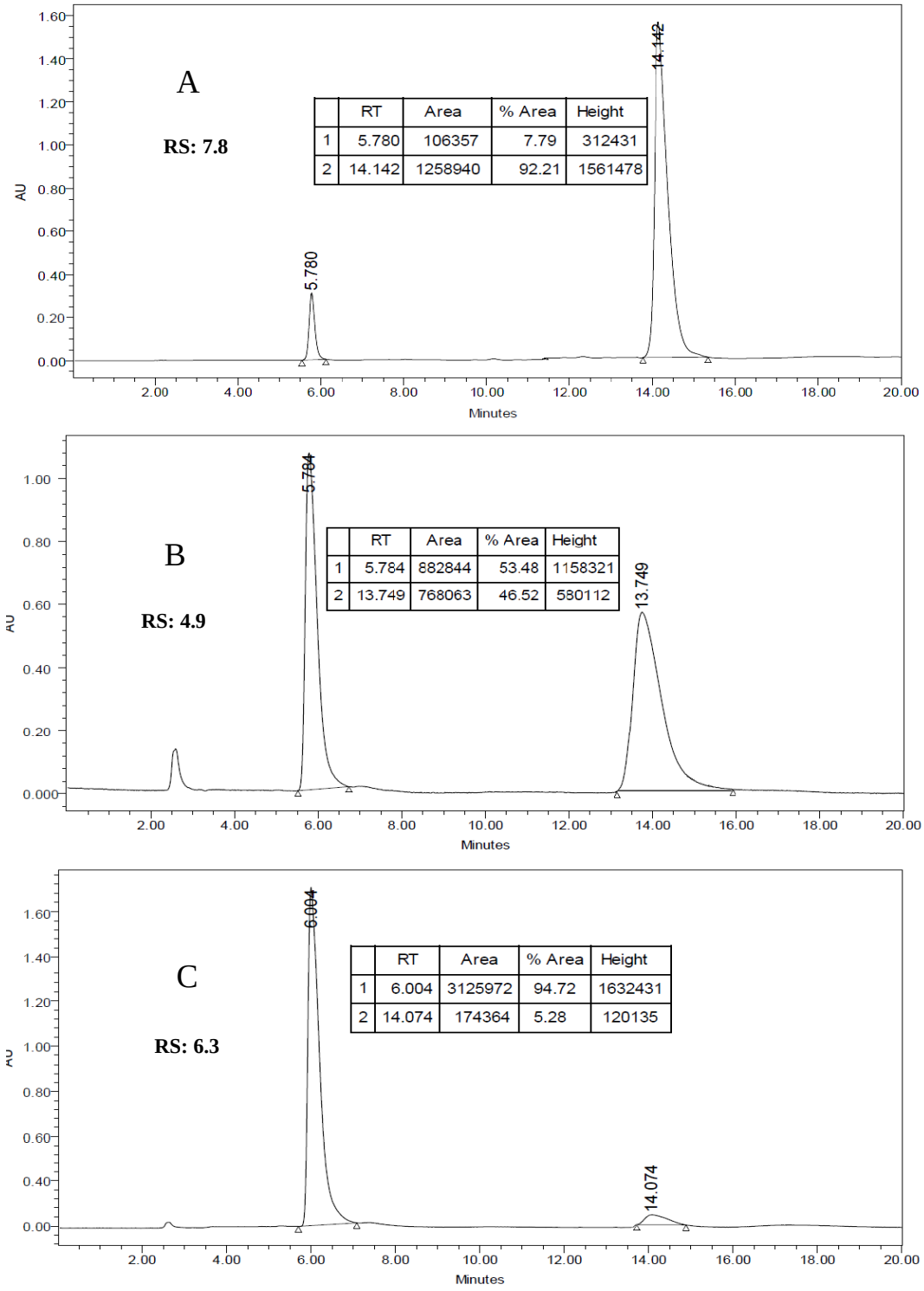


Figure (4.22): Chromatograms showing the conversion of Guaifenesin glutarate to parent drug in 1N HCl at zero time (A), after 6 (B) and 13.75 hours (C), **[RS: Resolution value].**

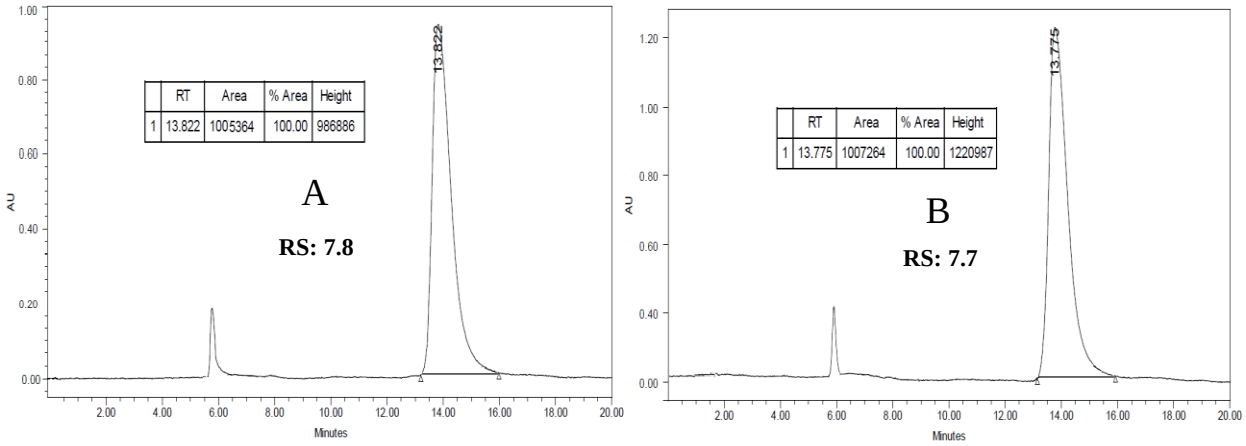


Figure (4.23): Guaifenesin glutarate prodrug at pH 3.3 at t = 0 (A), after 40 hours (B), **[RS: Resolution value].**

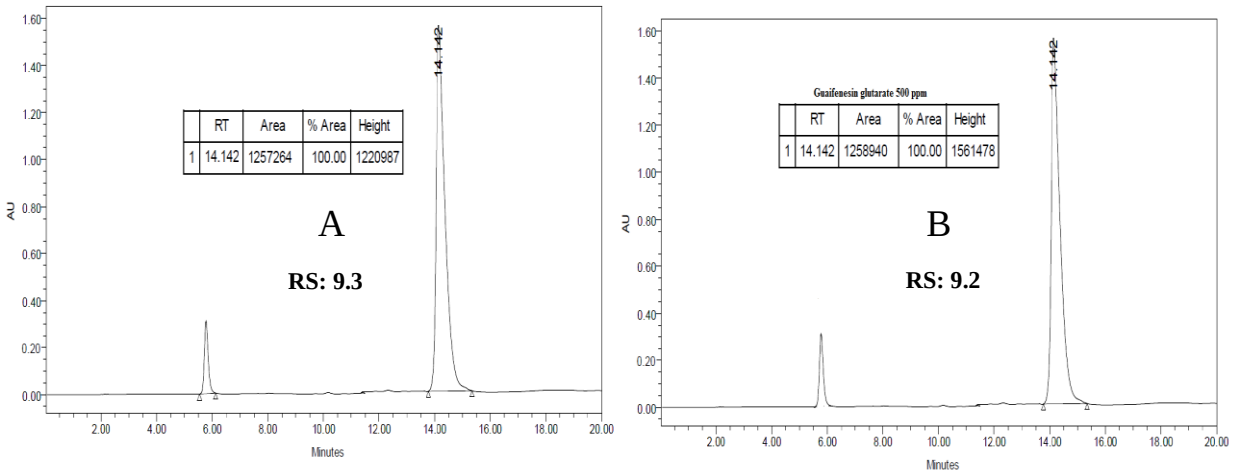


Figure (4.24): Guaifenesin glutarate prodrug at pH 5.5 at t = 0 (A), after 40 hours (B), **[RS: Resolution value].**

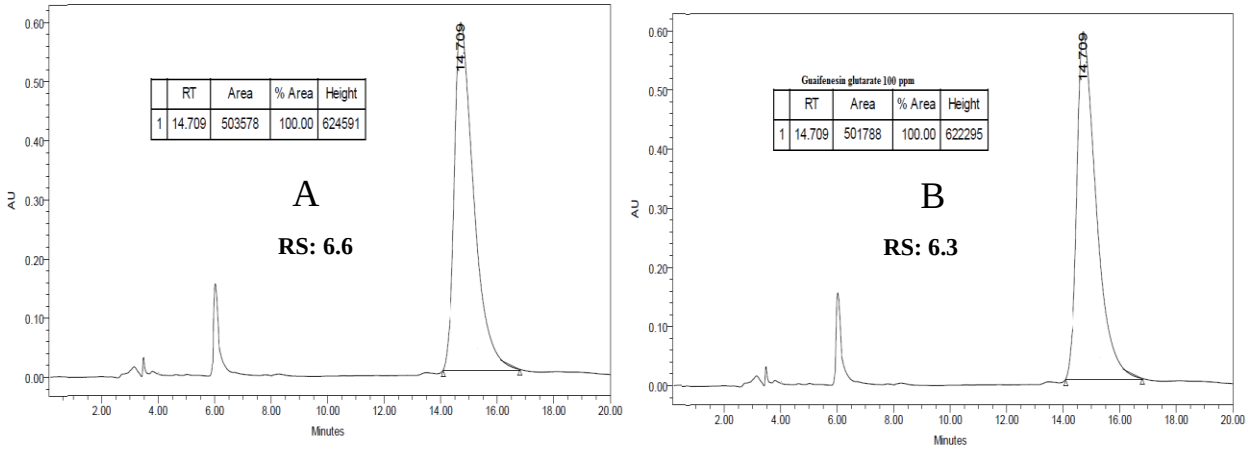


Figure (4.25): Guaifenesin glutarate prodrug at pH 7.4 at t = 0 (A), after 40 hours (B), **[RS: Resolution value].**

4.4 In vitro intraconversion of guaifenesin prodrugs to their parent drug.

The kinetics of the acid-catalyzed hydrolysis for guaifenesin prodrugs were carried out in aqueous buffers in a manner similar to that done by Bruice on Methyl Phenyl Acetals of Formaldehyde [58] This study investigates whether guaifenesin prodrugs are hydrolyzed to release the parent drug, guaifenesin in aqueous medium and to what extent. Acid-catalyzed hydrolysis of the synthesized guaifenesin prodrugs were studied in four different aqueous media: 1N HCl and buffers pH 3, pH 5 and pH 7.4 and the reaction was monitored by HPLC instrument, **Figure 4.26**.

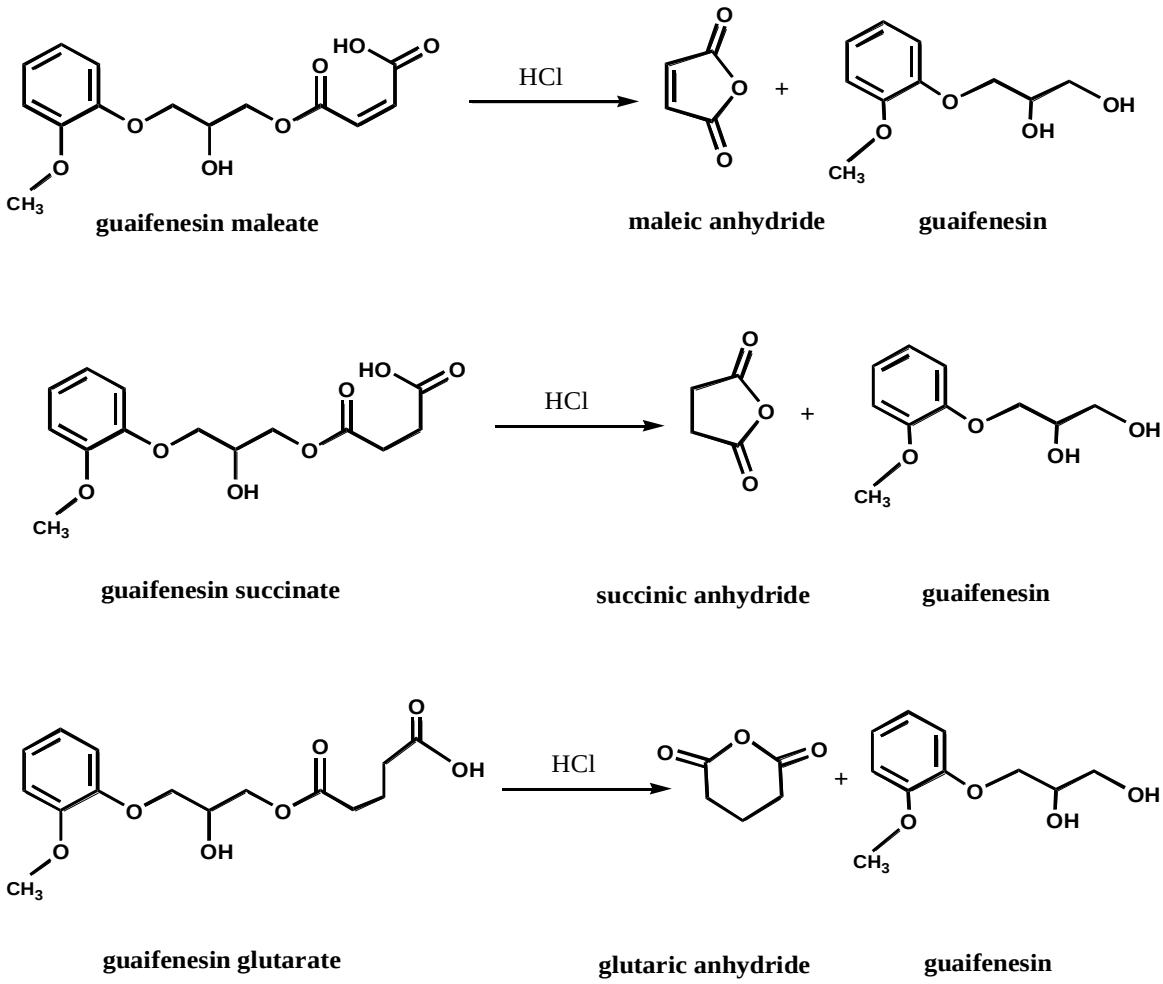


Figure (4.26): Acid-catalyzed hydrolysis of guaifenesin prodrugs.

The buffered solutions, 1N HCl, pH 3.3 and pH 5.5 were selected to examine the interconversion of guaifenesin prodrugs as a stomach simulated pH because as we know

pH 1-3 reflects the mean fasting stomach state of the adult, and the pH may increase up to 5 following ingestion of food. In addition, the second buffer system with pH 5 mimics the beginning of the small intestine pathway. The medium, pH 7.4 was selected to examine the interconversion of the tested guaifenesin prodrugs in the blood circulation system.

For guaifenesin prodrugs, at constant temperature and pH, the reaction displayed strict first-order kinetics as the k_{obs} was fairly constant and a straight line was obtained by plotting log concentration of the guaifenesin prodrugs versus time. The rate constant (k_{obs}) for guaifenesin prodrugs in 1N HCl was calculated from the linear regression equation correlating the log concentration of the prodrug versus time. The rate constant (k_{obs}) was found to be 7.2 x 10^{-4} for maleate prodrug, 2.36 x $10^{-4}$for succinate prodrug, and 2.54 x 10^{-4} for glutarate prodrug (see **Figure 4.27**). Complete hydrolysis of the guaifenesin prodrugs to their parent drug, guaifenesinin in 1N HCl requred 6.5 hours for maleate prodrug, 12.8 hours for succinate prodrug, and 13.75 hours for the glutarate prodrug (see **Figure 4.28)**.

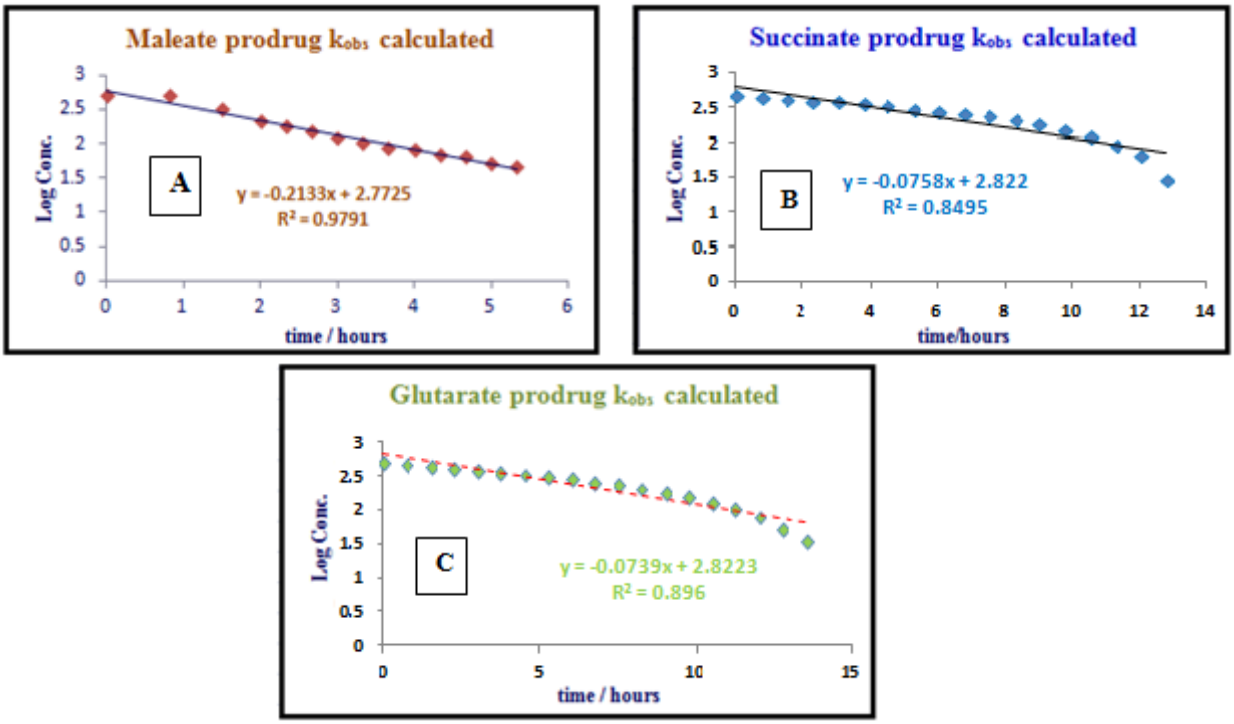


Figure (4.27): log concentration of the prodrug versus time to calculate the rate constant (k_{obs}).

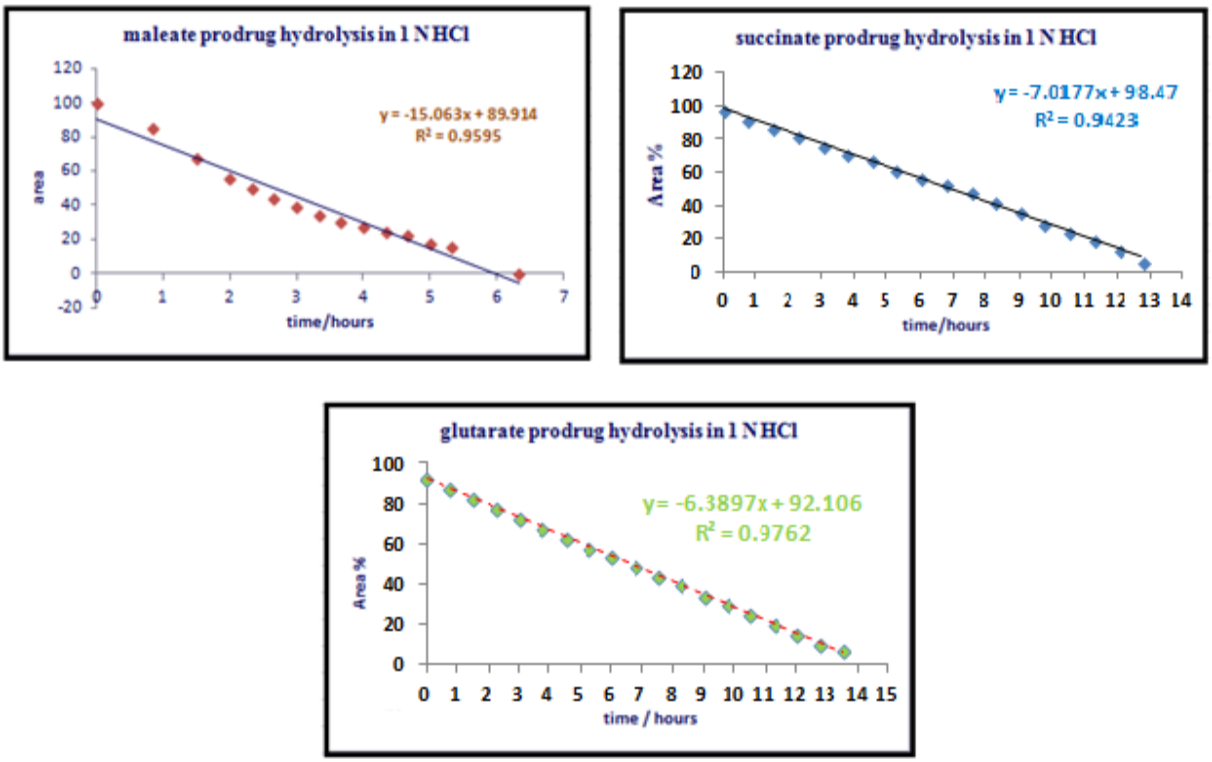
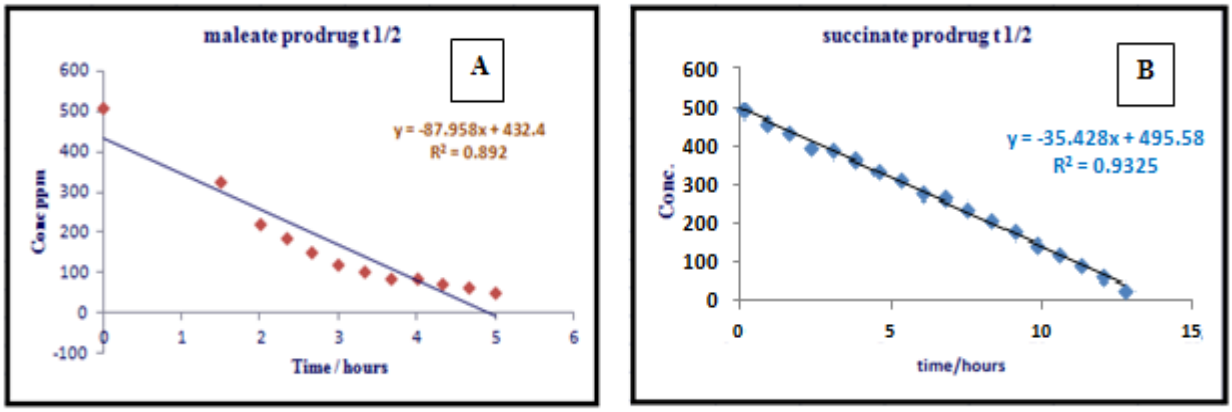


Figure (4.28): Acid-catalyzed hydrolysis of the guaifenesin prodrugs in 1N HCl.

Half-lives ($t_{1/2}$) for guaifenesin prodrugs in 1N HCl were calculated from the linear regression equation correlating the concentration (ppm) of the prodrugs with time. The half-lives determined were 2.01 hours for maleate prodrug, 7.03 hours for succinate prodrug, and 7.17 hours for the glutarate prodrug (see **Figure 4.29).**



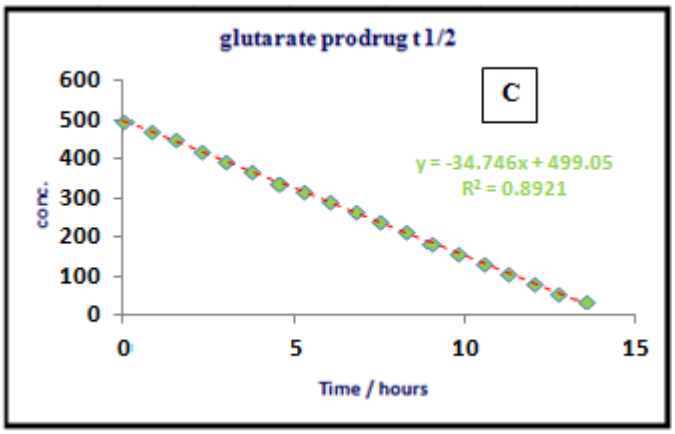


Figure (4.29): concentration of the prodrug versus time to calculate half-lives ($t_{1/2}$).

On the other hand, at pH 3.3, 5.5 and 7.4, the prodrugs were entirely stable and no release of the parent drug was observed as shown in **Figure 4.30 (A, B, C)**.

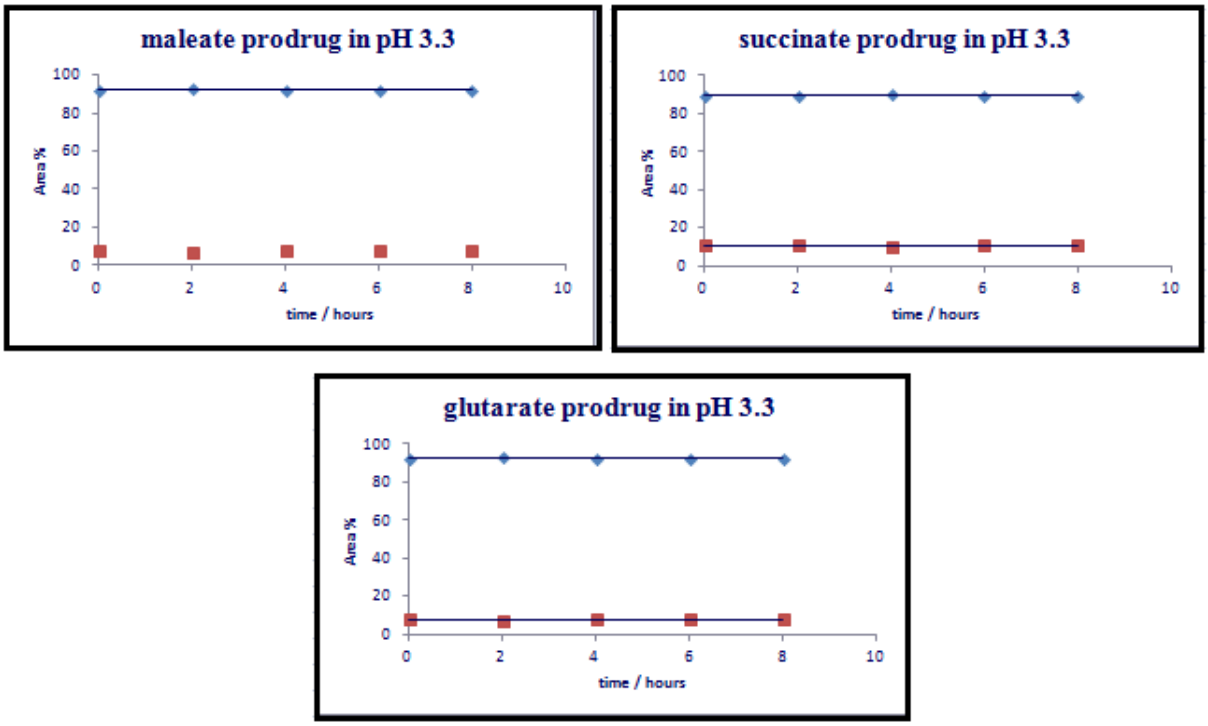


Figure (4.30A): Kinetic study of guaifenesin prodrugs in pH 3.3,(♦: prodrug, ■: drug).

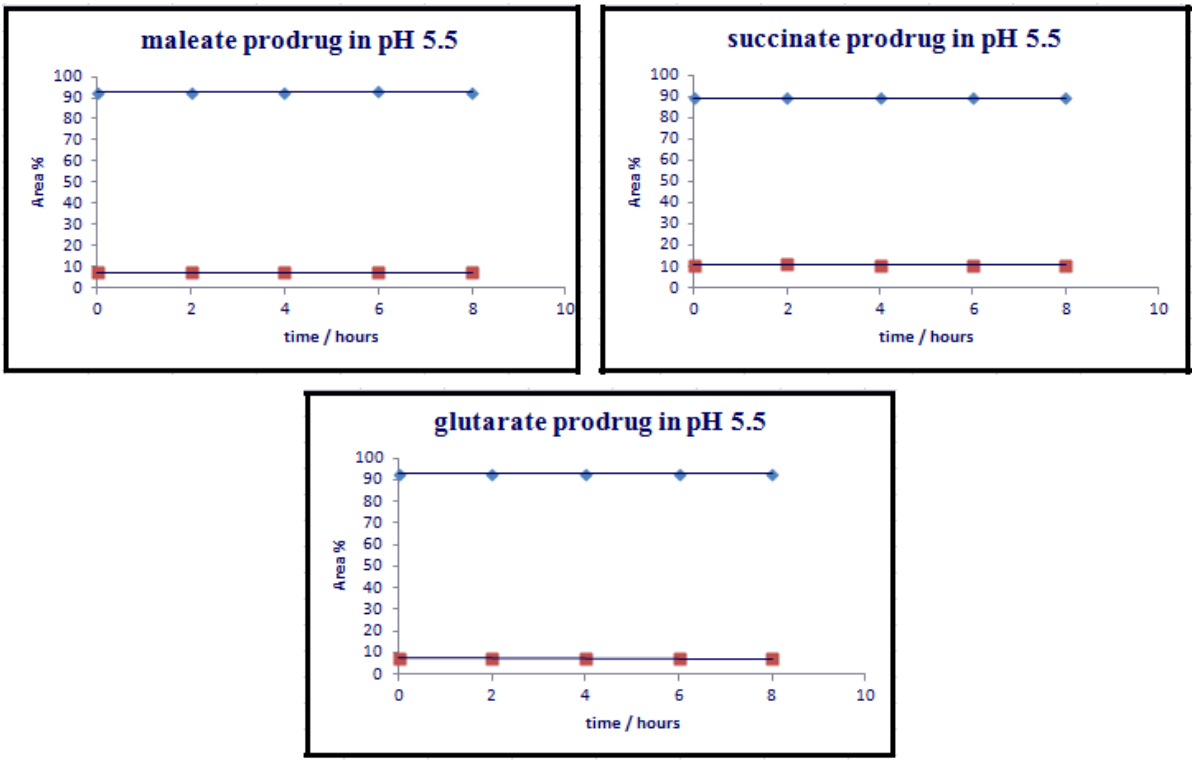


Figure (4.30B): Kinetic study of guaifenesin prodrugs in pH 5.5, (♦: prodrug, ■: drug).

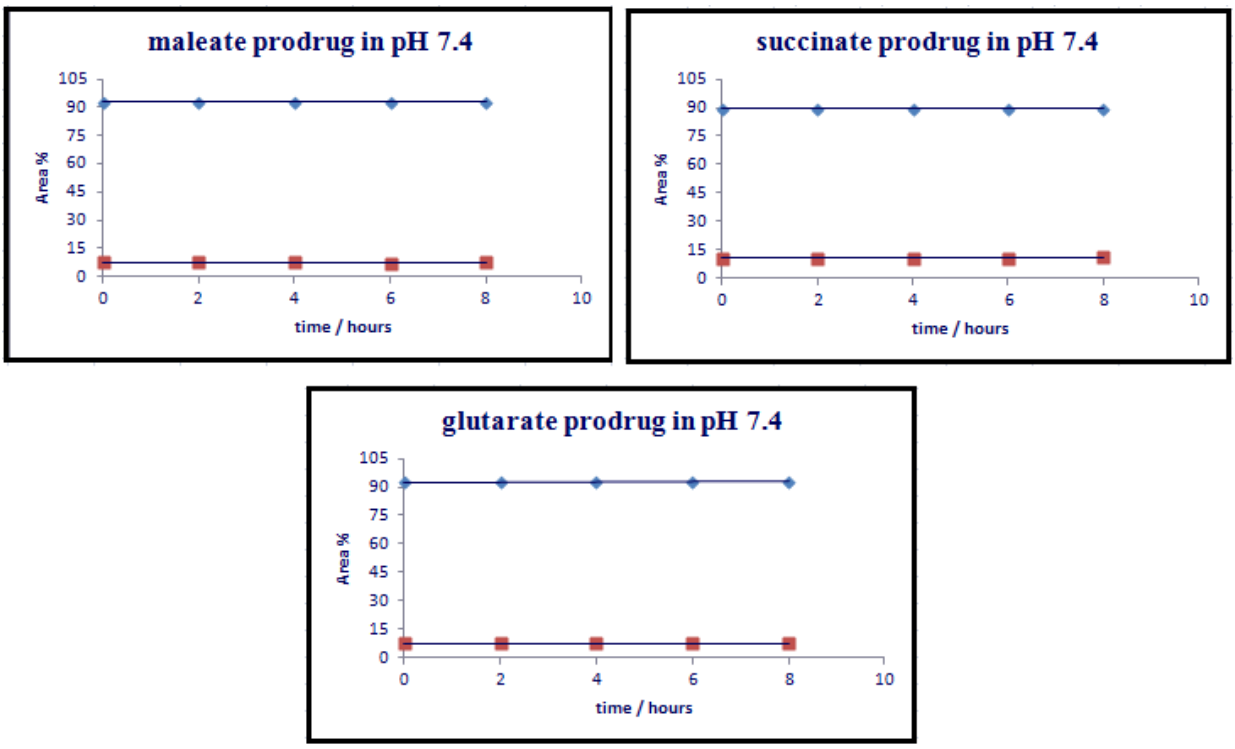


Figure (4.30C): Kinetic study of guaifenesin prodrugs in pH 7.4, (♦: prodrug, ■: drug).

Chapter five

Summary and conclusions

Chapter Five

Summary and Conclusions

Guaifenesin is an extremely bitter taste substance which negatively affects its usage in pediatric and geriatric formulations. In this thesis we aimed to mask the better taste of this substance by producing a tasteless prodrug using the linker approached. We have synthesized three guaifenesin prodrugs (guaifenesin maleate, guaifenesin succinate, guaifenesin glutarate) which were fully characteristic by ^{1} H-NMR, LC-MS, and FT-IR.

Kinetic study of the three guaifenesin prodrugs demonstrated that all of them underwent intraconversion to the parent drug, guaifenesin. At 1N HCL the half-life values for guaifenesin maleate, guaifenesin succinate, and guaifenesin glutarate 2.01 hours, 7.03 hours and 7.17 hours, respectively. The hydrolysis constant rate for the intraconversion of guaifenesin maleate was the highest rate, $7.2X10^{-4}$, among all others and that for glutarate prodrug was the lowest rate, $2.36X10^{-4}$. This is because in the maleic prodrug there is a double bond that creates more strain which results in a shorter distance between the neucleophile and electrophile than in the succinate and glutarate prodrugs. It is worth noting that at pH 3.3, 5.5 and 7.4, all three prodrugs were entirely stable and no intraconversion of the prodrug to the parent drug was observed. This might be due to the fact that the guiafenesen anion ($R\text{-}O^{-}$) leaving group is a bad leaving group

In vitro binding to bitter taste receptor 14 (BTR 14) of prodrugs guaifenesin maleate and succinate revealed that both prodrugs did not show any agonist activity. This is in contrast to the parent drug, guaifenesin, which has shown a strong agonist activity.

Since the three synthesized novel prodrugs have shown to be cleaved in the stomach (acidic medium), where in neutral pH they were entirely stable, formulations of the products in natural solutions or syrups are afforded and a high potential to have effective

clinical profile. Administration of the prodrugs in syrup form enables the pediatric and geriatric to administer the drug without any bitter sensation. Once the prodrug reaches the stomach it undergoes hydrolysis to give the parent drug in high yield.

We conclude that the prodrug approach utilized in this study (thesis) is an efficient strategy to mask the better taste sensation of guaifenesin and yet provides potential prodrug candidates with predictable and acceptable pharmacokinetic profiles.

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Appendix

Appendix

Appendix A: Synthesis Calculations

- **Guaifenesin:**

$$\text{No. of moles} = \frac{\text{Mass (g)}}{\text{Molecular Mass } (\frac{\text{g}}{\text{mol}})} \quad \ldots\ldots\ldots\ldots\ldots\ldots (\text{A})$$

$$\text{No. of moles} = \frac{1.98\ \text{g}}{198\ \text{g/mole}} = 0.010\ \text{moles}$$

- **Sodium hydride (NaH):**

For 1:1 equivalent

No. of moles NaH = No. of moles guaifenesin

No. of moles NaH = 0.010 mole

Then for 1:2 equivalent

No. of moles NaH = No. of moles guaifenesin X 2.0

No. of moles NaH = 0.020 mole

According to equation (A)

Mass of 100 % NaH = No. of moles x Molecular Mass

Mass of 100 % NaH = 0.020 mole x 24 g/mole

Mass of 100 % NaH = 0.480 grams

$$\text{Mass of 60 \% NaH} = \frac{0.480\ \text{grams X } 100}{60} = 0.80\ \text{grams}$$

- **Maleic Anhydride:**

  For 1:1 equivalent

  No. of moles Maleic Anhydride = No. of moles guaifenesin

  No. of moles Maleic Anhydride = 0.010 mole

  Then for 1:2 equivalent

  No. of moles Maleic Anhydride = No. of moles guaifenesin X 2.0

  No. of moles Maleic Anhydride = 0.020 mole

  According to equation (A)

  Mass of Maleic Anhydride = No. of moles X Molecular Mass

  Mass of Maleic Anhydride = 0.020 mole X 98.06 g/mole

  Mass of Maleic Anhydride = 1.96 grams

- **Succinic Anhydride:**

  For 1:1 equivalent

  No. of moles Succinic Anhydride = No. of moles guaifenesin

  No. of moles Succinic Anhydride = 0.010 mole

  Then for 1:2 equivalent

  No. of moles Succinic Anhydride = No. of moles guaifenesin X 2.0

  No. of moles Succinic Anhydride = 0.020 moles

  According to equation (A)

Mass of Succinic Anhydride = No. of moles x Molecular Mass

Mass of Succinic Anhydride = 0.020 mole x 100.07 g/mole

Mass of Succinic Anhydride = 2.0 grams

- **Glutaric anhydride:**

For 1:1 equivalent

No. of moles glutaric anhydride = No. of moles guaifenesin

No. of moles glutaric anhydride = 0.010 mole

Then for 1:2 equivalent

No. of moles glutaric anhydride = No. of moles guaifenesin X 2.0

No. of moles glutaric anhydride = 0.020 mole

According to equation (A)

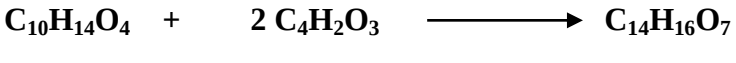
Mass of glutaric anhydride = No. of moles X Molecular Mass

Mass of glutaric anhydride = 0.020 mole X 114 g/mole

Mass of glutaric anhydride = 2.28 grams

Appendix B: Percent yield (PY) calculation

- **Guaifenesin Maleate prodrug:**



1.98 gm (0.01 mol , 198 gm/mol) of $C_{10}H_{14}O_4$ was mixed with excess $C_4H_2O_3$ (0.02 mol, 98 gm/mol) and 1.80 gm of $C_{14}H_{16}O_7$ (0.01 mol, 296.3 gm/mol) was produced .

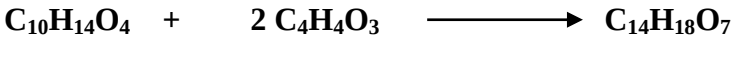
$$TY = 1.98gm\ C_{10}H_{14}O_4 * \frac{0.01\ mol\ C_{10}H_{14}O_4}{198\ gm\ C_{10}H_{14}O_4} * \frac{0.01\ mol\ C_{14}H_{16}O_7}{0.01\ mol\ C_{10}H_{14}O_4} * \frac{296.3\ gm\ C_{14}H_{16}O_7}{0.01\ mol\ C_{14}H_{16}O_7}$$

TY = 2.96 gm of $C_{14}H_{16}O_7$ was expected.

Percent yield = 61.5%

Were TY = theoretical yield

- **Guaifenesin succinate prodrug:**



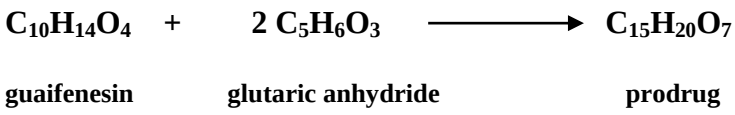
1.98 gm (0.01 mol , 198 gm/mol) of $C_{10}H_{14}O_4$ was mixed with excess $C_4H_4O_3$ (0.02 mol, 98 gm/mol) and 1.70 gm of $C_{14}H_{18}O_7$ (0.01 mol, 298.3 gm/mol) was produced .

$$TY = 1.98gm\ C_{10}H_{14}O_4 * \frac{0.01\ mol\ C_{10}H_{14}O_4}{198\ gm\ C_{10}H_{14}O_4} * \frac{0.01\ mol\ C_{14}H_{18}O_7}{0.01\ mol\ C_{10}H_{14}O_4} * \frac{298.3\ gm\ C_{14}H_{18}O_7}{0.01\ mol\ C_{14}H_{18}O_7}$$

TY = 2.98 gm of $C_{14}H_{18}O_7$ was expected.

Percent yield = 58 %

- **Guaifenesin glutarate prodrug:**



1.98 gm (0.01 mol , 198 gm/mol) of $C_{10}H_{14}O_4$ was mixed with excess $C_5H_6O_3$ (0.02 mol, 98 gm/mol) and 2.12 gm of $C_{14}H_{18}O_7$ (0.01 mol, 312.3 gm/mol) was produced .

$$TY = 1.98gm\ C_{10}H_{14}O_4 * \frac{0.01\ mol\ C_{10}H_{14}O_4}{198\ gm\ C_{10}H_{14}O_4} * \frac{0.01\ mol\ C_{15}H_{20}O_7}{0.01\ mol\ C_{10}H_{14}O_4} * \frac{312.3\ gm\ C_{15}H_{20}O_7}{0.01\ mol\ C_{15}H_{20}O_7}$$

TY = 3.12 gm of $C_{15}H_{20}O_7$ was expected.

Percent yield = 58 %

Appendix C: Calibration curves:

Table (S1): HPLC data for guaifenesin and prodrugs calibration curves.

Concentration (ppm)	Guaifenesin AUC	guaifenesin maleate AUC	guaifenesin succinate AUC	guaifenesin glutarate AUC
500	13381831	28703612	1113489	1260140
400	10859151	23106157	902987	1007264
300	8502337	17796481	678094	755364
200	5836530	11773719	450196	513578
100	3321331	5993449	224752	251788
Equations Y=	25151X+806131	56615X+402328	2230.3X+4824.1	2510.4X+4509.8

Appendix D: Hydrolysis data and calculations

- **Guaifenesin maleate prodrug:**

Table (S2): HPLC data of guaifenesin maleate hydrolysis.

Time/h	drug %	prodrug %	Area	Conc. Ppm	Log Conc.
0	0.2	99.8	29382527	511	2.7
0.83	15.25	84.75	23726339	321	2.71
1.5	33	67	18757355	324	2.51
2	44.33	55.67	12927791	221	2.34
2.33	50.2	49.67	10953326	186	2.27
2.66	56.24	43.76	9013694	152	2.18
3	61.3	38.7	7365445	122	2.08
3.33	65.63	34.37	6338279	104	2.02
3.66	70.34	29.66	5424482	88	1.94
4	72.63	27.37	5250100	85	1.93
4.33	75.46	24.54	4494666	72	1.85
4.66	77.42	22.58	4148793	66	1.82
5	82.33	17.67	3285501	50	1.71
5.33	84.32	15.68	2998451	45	1.66
6.33	99.9	0.01	1489		

The rate constant (k_{obs}) for guaifenesin maleate in 1N HCl was calculated from the linear regression equation correlating the log concentration of the prodrug versus time, this equation is Y= -0.2133X+2.7725. (**Figure 4.27 A**).

Then K_{obs} = 0.2133/296 , were 296gm mol^{-1} is the molar mass of maleate prodrug

K_{obs} = **$7.2x10^{-4}$**

Half-life ($t_{1/2}$) for guaifenesin maleate in 1N HCl was calculated from the linear regression equation correlating the concentration (ppm) of the prodrugs versus time, this equation is

Y= -87.958X + 432.4**. (Figure 4.29 A**).

Y= concentration / 2, and X = $t_{½}$.

Then $t_{½}$ = 255.5 – 432.4 / 87.958, were 255.5 is the initial concentration divided by 2 .

$t_{½}$ of guaifenesin maleate is 2.01 hours.

- **Guaifenesin succinate prodrug:**

Table (S3): HPLC data of guaifenesin succinate hydrolysis.

Time/h	drug %	prodrug %	Area	Conc. Ppm	Log Conc.
0	2.33	97.97	1103489	492.6	2.69
0.75	9.07	90.93	1027339	458.4	2.66
1.5	11.05	88.95	972882	434	2.63
2.25	13.89	86.11	948669	423.1	2.62
3	15.76	84.24	914358	407.8	2.61
3.75	17.39	82.61	913563	407.4	2.61
4.5	19.07	80.93	861371	384	2.58
5.25	23.76	76.24	850404	379.1	2.57
6	28.44	71.56	808495	360.3	2.55
6.75	31.57	68.43	773132	344.4	2.53
7.5	36.86	63.14	713365	317.6	2.5
8.25	41.55	58.45	660376	293.9	2.46
9	51.15	48.85	551914	245.2	2.38
9.75	55.04	44.96	507964	225.5	2.35
10.5	61.64	38.36	433397	192.1	2.28
11.3	65.56	34.32	387752	171.6	2.23
12	70.85	29.14	329228	145.4	2.16
12.8	80.07	19.93	217376	95.3	1.97
13.5	92.79	7.25	81912	34.5	1.53

The resulted linear equation, Y= -0.0758x + 2.8134, shown in **Figure 4.27 B**, It represents the rate constant (k_{obs}), which equal $2.54x10^{-4}$.

The equation Y= -34.428x + 495.85, that indicated in **Figure 4.29 B**, used to calculated the succinate prodrug half-life ($t_{1/2}$), which equal 7.03 hours.

- **Guaifenesin glutarate prodrug:**

Table (S4): HPLC data of guaifenesin glutarate hydrolysis.

Time/h	drug %	prodrug %	Area	Conc. Ppm	Log Conc.
0	7.79	92.21	1258940	499.6	2.69
0.75	19.42	80.58	1100141	436.4	2.63
1.5	29.26	70.74	1011439	401.1	2.6
2.25	37.99	62.01	943212	373.9	2.57
3	53.48	46.52	768063	304.1	2.48
3.75	57.35	42.65	757792	300	2.47
4.5	65.46	34.54	691502	273.6	2.43
5.25	70.73	29.27	614757	243	2.38
6	73.62	26.38	579581	229	2.35
6.75	77.4	22.6	535724	211.6	2.32
7.5	78.81	21.19	521765	206	2.31
8.25	80.12	19.86	513579	202.7	2.3
9	81.5	18.5	487640	192.4	2.28
9.75	83.71	16.29	442497	174.4	2.24
10.5	85.87	14.13	404041	159.1	2.2
11.3	89.03	10.97	327971	128.8	2.1
12	92.4	7.6	241192	94.2	1.97
12.8	94.72	5.28	174364	67.6	1.82

(k_{obs}) =$2.36x10^{-4}$, was calculated from the eqution Y= -0.0739x + 2.8223. (See **Figure 4.27 C**) And ($t_{1/2}$) was calculated used the equation Y= -34.746x + 499.0**5** that shown in **Figure 4.29 C.**

➢ **Resolution values data and calculation:**

Table (S5): HPLC data of prodrugs chromatograms.

Figure	Chromatogram	RT_1	RT_2	W_1	W_2.	Rs
4.14 A	Guaifenesin maleate prodrug at 1N HCl at 0.0 h	5.91	14.93	0.21	1.31	11.86
4.14 B	Guaifenesin maleate prodrug at 1N HCl after 2.3 h	5.93	14.99	0.65	1.03	10.79
4.14 C	Guaifenesin maleate prodrug at 1N HCl after 5.3 h	5.87	14.64	0.83	0.90	10.13
4.15 A	Guaifenesin maleate prodrug at pH 3.3 at 0.0 h	5.46	13.85	0.11	1.55	10.10
4.15 B	Guaifenesin maleate prodrug at pH 3.3 after 39 h	5.69	14.63	0.13	1.38	11.82
4.16 A	Guaifenesin maleate prodrug at pH 5.5 at 0.0 h	5.46	13.86	0.09	1.57	10.11
4.16 B	Guaifenesin maleate prodrug at pH 5.5 after 39 h	5.68	14.62	0.08	1.43	11.79
4.17 A	Guaifenesin maleate prodrug at pH 7.4 at 0.0 h	5.45	13.93	0.12	1.53	10.25
4.17 B	Guaifenesin maleate prodrug at pH 7.4 after 39 h	5.69	14.59	0.13	1.39	11.64
4.18 A	Guaifenesin succinate prodrug at 1N HCl at 0.0 h	5.65	10.96	0.38	1.30	6.30
4.18 B	Guaifenesin succinate prodrug at 1N HCl after 6.0 h	5.77	10.87	1.13	1.90	3.36
4.18 C	Guaifenesin succinate prodrug at 1N HCl after 12.8 h	5.81	10.95	1.25	1.76	3.41
4.19 A	Guaifenesin succinate prodrug at pH 3.3 at 0.0 h	5.71	10.76	0.53	1.18	5.90
4.19 B	Guaifenesin succinate prodrug at pH 3.3 after 40 h	5.71	10.79	0.56	1.20	5.70
4.20 A	Guaifenesin succinate prodrug at pH 5.5 at 0.0 h	5.75	10.82	0.48	1.21	6.00
4.20 B	Guaifenesin succinate prodrug at pH 5.5 after 40 h	5.71	10.76	0.53	1.31	5.40
4.21 A	Guaifenesin succinate prodrug at pH 7.4 at 0.0 h	5.77	10.91	0.69	1.35	5.00

4.21 B	Guaifenesin succinate prodrug at pH 7.4 after 40 h	5.72	10.78	0.63	1.31	5.20
4.22 A	Guaifenesin glutarate prodrug at 1N HCl at 0.0 h	5.78	14.14	0.56	1.58	7.80
4.22 B	Guaifenesin glutarate prodrug at 1N HCl after 6.0 h	5.78	13.74	1.2	2.03	4.90
4.22 C	Guaifenesin glutarate prodrug at 1N HCl after 13.75 h	6.00	14.07	1.38	1.16	6.30
4.23 A	Guaifenesin glutarate prodrug at pH 3.3 at 0.0 h	5.98	13.82	0.16	1.83	7.80
4.23 B	Guaifenesin glutarate prodrug at pH 3.3 after 40 h	5.99	13.77	0.12	1.81	7.70
4.24 A	Guaifenesin glutarate prodrug at pH 5.5 at 0.0 h	5.98	14.14	0.18	1.56	9.30
4.24 B	Guaifenesin glutarate prodrug at pH 5.5 after 40 h	5.99	14.14	0.23	1.53	9.20
4.25 A	Guaifenesin glutarate prodrug at pH 7.4 at 0.0 h	6.10	14.70	0.26	2.35	6.60
4.25 B	Guaifenesin glutarate prodrug at pH 7.4 after 40 h	6.20	14.70	0.30	2.40	6.30

The resolution between two peaks in all chromatograph is given by:

$Rs = 2 (RT_2 – RT_1) / (W_1 + W_2)$

where RTs are the retention times (min) and Ws are the widths at the baseline of the peaks (min).

تصنيع ودراسة لمواصفات الأدوية المساعدة المصممة لمادة الغايفينيسين

إعداد: أمين محمود عبد المنعم ثوابتة

إشراف : أ.د. رفيق قرمان

الملخص:

الغايفينيسين هي مادة ذات طعم مر جدا مما يؤثر ذلك في قبولية استخدامه لدى الاطفال والكبار في السن. هدفنا في هذه الدراسة هو اخفاء الطعم المر لمادة الغايفينيسين وتحويله الى دواء مساعد (Prodrug) بلا طعم مر ومستساغ لدى الاطفال والكبار في السن باستخدام روابط و مركبات مختلفة.

تم تصنيع الدواء المساعد عن طريق استرة بعض الحوامض الكربوكسيلية الانهيدراتية (انهيدريد المالئيك، انهيدريد السكسينيك، انهيدريد الغلوتاريك) والغايفينيسين وذلك للحصول على الدواء المؤستر؛ الغايفينيسين المالئيت والغايفينيسين السكسنيت وأيضاً الغايفينيسين الغلوتاريت. كما وأكدت نتائج فحوصات H-NMR و FT-IR و LC-MS نقاء وهوية الادوية المساعدة المؤسترة التي تم تصنيعها.

خضعت الادوية المساعدة المذكورة اعلاه لدراسات حركية مخبرية في اربع اوساط مائية حمضية وأخرى معتدلة (1N HCl ، pH 3.3 ، pH 5.5 ، و ايضا pH 7.4) باستخدام جهاز التحليل الطيفي (HPLC) و تحت ظروف معيارية معينة تحللت هذه الادوية المساعدة الى الدواء الاصلي الغايفينيسين.

عند تحليل القيم التي تم الحصول عليها من هذه الدراسات الحركية المخبرية في حمض الهيدروكلوريك (1N HCl) و تم حساب معدل الثبوتية (K_{obs}) و انصاف الحياة ($t_{1/2}$) باستخدام العلاقة الخطية بين لوغاريتم التراكيز (Log concentrations) والوقت المستغرق لتحليل الدواء المساعد كليا الى الدواء الاصلي الغايفينيسين.

وكانت نتائج حسابات معدل الثبوتية على النحو التالي: $10X7.2^{-4}$ لغايفينيسين المالئيت، و$10X2.54^{-4}$ لغايفينيسين السكسنيت، و$10X2.36^{-4}$ لغايفينيسين الغلوتاريت. اما قيم انصاف الحياة فهو 2.01 ساعة لغايفينيسين المالئيت، و 7.03 ساعة لغايفينيسين السكسنيت، وأيضاً 7.17 ساعة لغايفينيسين الغلوتاريت .

كما و أوضحت الدراسات ان الادوية المساعدة لغايفينيسين التي تم تصنيعها مستقرة وثابتة في الاوساط الحمضية (pH3.3 و pH5.5) والوسط المعتدل pH 7.4 .

عمادة الدراســات العليــا

جامــعة القــدس

تصنيع ودراسة لمواصفات الأدوية المساعدة المصممة لمادة الغايفينيسين

إعداد:

أمـــين محمـــود ثــوابتة

رسالـــة ماجـــستير

القدس – فلســطين

1435/2014

تصنيع ودراسة لمواصفات الأدوية المساعدة المصممة لمادة الغايفينيسين

إعداد:

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بكالوريوس:كيمياء-جامعة القدس-فلسطين

إشراف:

أ. البروفيسور رفيق قرمان

جامعة القدس-فلسطين

قدمت هذه الاطروحة استكمالا لمتطلبات درجة الماجستير في التكنولوجية التطبيقية و الصناعية من كلية الدراسات العليا في جامعة القدس- فلسطين

1435/2014