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Investigation of Enalapril Maleate Hydrolysis Kinetics in Extemporaneous Solution Using Infrared Spectroscopy (FTIR)

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Dedication

To my beloved family—my father, mother, brothers, and sisters—whose love, prayers, and unwavering support have been the foundation of my journey.

To my cherished friends, for their understanding, patience, and kind words during both the challenging and joyful moments along this path.

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This achievement reflects your support, and I share it with all of you.

Aya Ameen Ali Halabiya.

Declaration

I confirm that this thesis, submitted for the Master's degree in Applied Industrial Technology, is my original work, except where specific references are made. I also declare that it has not been submitted, in whole or in part, for any academic degree at any other university or institution.

Name: Aya Ameen Ali Halabiya

Signed: 

Date: 14/1/2026

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Aya Ameen Ali Halabiya

Abstract:

This study investigates the **hydrolytic stability and degradation kinetics of enalapril maleate** in extemporaneous oral liquid formulations, focusing on the stabilizing effect of **propylene glycol** and the application of Fourier Transform Infrared Spectroscopy **(FTIR)** as a rapid analytical tool. Enalapril maleate, a commonly used ACE inhibitor, demonstrates chemical instability in aqueous media, resulting in rapid hydrolysis to enalaprilat and, under specific conditions, cyclization to diketopiperazine (DKP).

Calibration curves indicated excellent linearity for both FTIR ($R^2 > 0.99$) and HPLC ($R^2 > 0.999$). Under **alkaline conditions** (0.1 M NaOH), water based solutions degraded within 60 minutes. In contrast, the presence of 10% propylene glycol allowed for the detection of the enalapril maleate peak signal, with approximately 103% recovery by HPLC at the 60-minute mark.

Homogeneity testing revealed significant stratification in water based suspensions, where the upper layer lacked detectable drug (0%), while the lower layer contained approximately 12.5% (FTIR), with HPLC confirming variability (upper 84.64%, central 109.03%, lower 129.5%). In contrast, PG-based suspensions, and PG + syrup formulations demonstrated uniform concentrations, even when samples were obtained exclusively from the **upper** layer, the practical site of dose withdrawal for patients. FTIR indicated approximately 100% drug content for PG (upper: 100%, lower: 101.7%), while HPLC confirmed nearly complete uniformity (upper PG: ~98.9%; upper PG + syrup: ~95.5%). This indicates that propylene glycol ensures dose consistency at the clinically relevant point of administration, unlike water based systems that risk sub-therapeutic dosing.

FTIR effectively monitored the disappearance of the **ester carbonyl band at 1741 cm^{-1},** yielding degradation profiles consistent with HPLC results. This validates FTIR as a rapid, cost-effective screening method, while HPLC remains the confirmatory reference method.

In **conclusion**, this research demonstrates that enalapril maleate is physiochemically unstable in aqueous extemporaneous formulations. Propylene glycol significantly enhances stability and ensures homogeneity, establishing it as a practical stabilizer. The validated application of FTIR in conjunction with HPLC provides a robust analytical framework for improving the safety, efficacy, and reliability of extemporaneous enalapril suspensions.

Keywords: Enalapril maleate, Extemporaneous formulations, FTIR-ATR, HPLC, Hydrolytic degradation, Propylene glycol, Stability kinetics.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

Abbreviation	Meaning
ACE	Angiotensin-Converting Enzyme
API	Active Pharmaceutical Ingredient
ATR	Attenuated Total Reflectance
DKP	Diketopiperazine
EM	Enalapril maleate
FDA	Food and Drug Administration
FTIR	Fourier Transform Infrared (Spectroscopy)
HPLC	High-Performance Liquid Chromatography
ICH	International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use
IR	Infrared
NaOH	Sodium Hydroxide
HCL	Hydrochloric Acid
PG	Propylene Glycol
RAAS	Renin–Angiotensin–Aldosterone System
R^2	Correlation Coefficient (Coefficient of Determination)
UV	Ultraviolet
USP	United States Pharmacopeia
WHO	World Health Organization
BUD	Beyond-Use Date
$t_{½}$	Half time
k_{obs}	Observed Rate Constant
t_R	Retention Time
HPMC	Hydroxypropyl Methylcellulose

COOH	Carboxylic acid group
CONH	Amide group (carbonyl attached to nitrogen)
NH	Amine linkage
OH^-	Hydroxide ion (base, nucleophile in alkaline hydrolysis)
C/C_0	Concentration ratio (remaining concentration at time *t* relative to initial concentration)

Chapter One:

Introduction

1.1 Background

1.1.1 Hypertension: Epidemiology and Clinical Significance

Hypertension is a major global health concern, affecting over 1.39 billion adults worldwide. Its prevalence is rising due to factors such as aging populations, sedentary lifestyles, and poor dietary habits (Mills et al., 2020). As a leading modifiable risk factor for cardiovascular disease, hypertension significantly contributes to the development of ischemic heart disease, stroke, heart failure, chronic kidney disease, and premature death (Levine et al., 2018). According to the World Health Organization (WHO), fewer than half of those with hypertension are aware of their condition, and less than 20% achieve adequate blood pressure control, highlighting a critical clinical need (World Health Organization, 2021).

Epidemiological data indicate that hypertension is responsible for approximately 10 million deaths annually, accounting for nearly 20% of global mortality (Forouzanfar et al., 2017). The impact of this disease is particularly severe in low- and middle-income countries, where healthcare resources and access to long-term medications are often limited (Geldsetzer, 2019). Beyond cardiovascular issues, uncontrolled hypertension causes microvascular damage, leading to complications such as retinopathy, nephropathy, and cognitive impairment. This underscores hypertension as a systemic disorder affecting multiple organs rather than just one (Iadecola & Davisson, 2008).

The clinical implications of hypertension extend beyond morbidity and mortality; it significantly increases healthcare expenditures and imposes a considerable burden on both individuals and healthcare systems. (Kearney, 2005).

1.1.2 The Renin–Angiotensin–Aldosterone System (RAAS)

The Renin–Angiotensin–Aldosterone System (RAAS), as shown in Figure 1.1, plays a vital role in regulating cardiovascular and renal physiology. It is essential for maintaining vascular tone, electrolyte balance, and fluid homeostasis. This system is activated when the juxtaglomerular apparatus in the kidney releases renin in response to reduced renal perfusion pressure, sympathetic nervous system activation, or decreased sodium delivery to the distal tubule (Hall, 2020).

Renin cleaves angiotensinogen, a plasma protein synthesized by the liver, to form angiotensin I. This inactive decapeptide is then converted into the potent vasoconstrictor angiotensin II by angiotensin-converting enzyme (ACE), which is primarily located in the vascular endothelium, especially in the lungs (Ferrario, 2006). Angiotensin II binds to AT1 receptors, resulting in direct vasoconstriction, stimulation of aldosterone secretion from the adrenal cortex, increased sympathetic outflow, and enhanced sodium and water retention. These combined effects elevate systemic vascular resistance thereby contributing to the restoration blood pressure (Atlas, 2007).

Beyond its hemodynamic effects, the renin-angiotensin-aldosterone system (RAAS) significantly contributes to the pathological remodeling of the cardiovascular system. Angiotensin II induces smooth muscle hypertrophy, myocardial fibrosis, oxidative stress, and endothelial dysfunction, all of which contribute to conditions like hypertension, heart failure, and chronic kidney disease (Mehta et al., 2007). In addition, aldosterone promotes sodium retention and exerts profibrotic and proinflammatory effects on both the myocardium and vasculature, further heightening cardiovascular risk (Brown, 2008).

Therapeutic inhibition of the renin-angiotensin-aldosterone system (RAAS) through the use of ACE inhibitors, angiotensin receptor blockers (ARBs), and mineralocorticoid receptor antagonists has been shown to improve survival and reduce morbidity in patients with hypertension, heart failure, and diabetic nephropathy. This underscores the significance of RAAS as both a physiological regulator and a pharmacological target (Brenner, 2001; Pitt, 2003; Yusuf, 2000) .

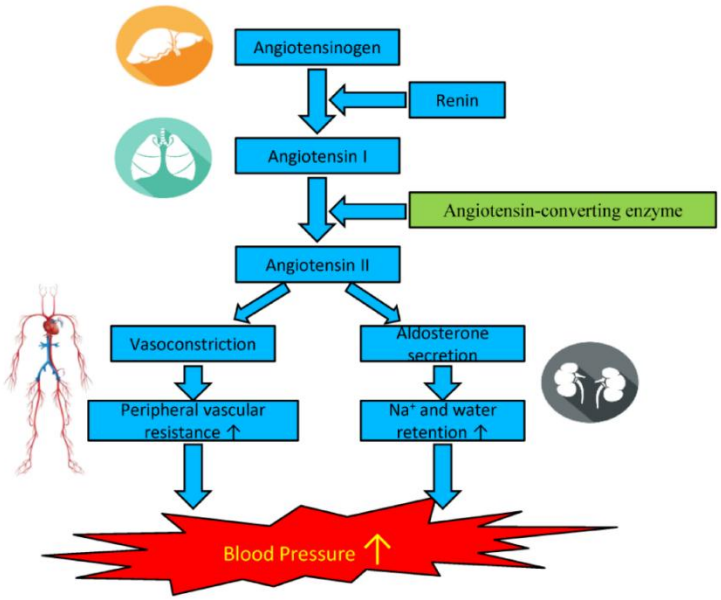

Figure 1.1: The renin-angiotensin-aldosterone system (RAAS) in the regulation of blood pressure and the function of angiotensin-converting enzyme (ACE) (Jiang et al., 2021)

1.1.3 Angiotensin-Converting Enzyme (ACE) Inhibitors

Angiotensin-converting enzyme (ACE) inhibitors are essential in the treatment of hypertension, heart failure, chronic kidney disease, and post-myocardial infarction therapy. They function by inhibiting the angiotensin-converting enzyme, thereby prevention the conversion of angiotensin I to angiotensin II. This inhibition leads to decreased vasoconstriction, reduced aldosterone secretion, and ultimately lower blood pressure (Dzau, 2001; Whelton et al., 2018).

ACE inhibitors exert hemodynamic effects and prevent structural changes in the cardiovascular system induced by angiotensin II, including myocardial hypertrophy, vascular smooth muscle proliferation, and fibrosis (Mehta et al., 2007). Additionally, ACE inhibitors inhibit the degradation of bradykinin, a potent vasodilatory peptide that stimulates the endothelial release of nitric oxide (NO) and prostacyclin. This enhancement of bradykinin-mediated signaling contributes to improved vasodilation and endothelial function (Brown & Vaughan, 1998). However, the accumulation of bradykinin may also lead to common side effects, such as cough and angioedema (Israili & Hall, 1992).

ACE inhibitors have been clinically demonstrated to slow the progression of diabetic nephropathy and delay the onset of end-stage renal disease in high-risk patients (Lewis et al., 1993). Their positive effects are particularly notable in high-risk cardiovascular patients, underscoring the importance of ACE inhibition as a key therapeutic and preventive strategy (Yusuf, 2000).

These medications are widely used in clinical practice, with several options available, including captopril, enalapril, Lisinopril, ramipril, and perindopril. Although they share a common

mechanism of action, their pharmacokinetics—such as absorption, metabolism, and duration of action—vary, affecting dosing regimens and clinical applications (Hilal-Dandan et al., 2022).

1.1.4 Drug Profile of Enalapril Maleate

1.1.4.1 Chemical and structural characteristics

Enalapril maleate, as illustrated in the Figure 1.2, is a widely prescribed angiotensin-converting enzyme (ACE) inhibitor classified as a **prodrug** to enhance oral bioavailability. It is the ethyl ester of enalaprilat, its pharmacologically active metabolite. After oral administration, enalapril undergoes hydrolysis by hepatic esterases, converting it into enalaprilat, which exerts its therapeutic effect by inhibiting the renin–angiotensin–aldosterone system (Arafat et al., 2005; Faruqi et al., 2024).

The maleate salt form of enalapril significantly enhances **its water solubility and chemical** stability, making it ideal for oral administration. Its molecular structure includes key functional groups, such as an **ester** group, **an amide** linkage, and a **secondary** amine, as illustrated in Figure 1.3, all of which influence its physicochemical and pharmacokinetic properties.

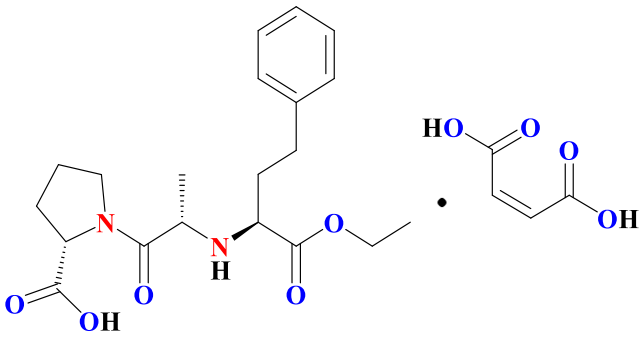

Figure 1.2: Chemical structure of Enalapril maleate (National Center for Biotechnology Information, 2025)

These features provide sufficient stability during storage while facilitating effective hydrolysis in vivo to release the active metabolite. The primary functional groups of enalapril maleate influencing its stability and pharmacokinetic properties include the ester and amide groups, which are susceptible to hydrolytic degradation.(Bhardwaj & Singh, 2008).

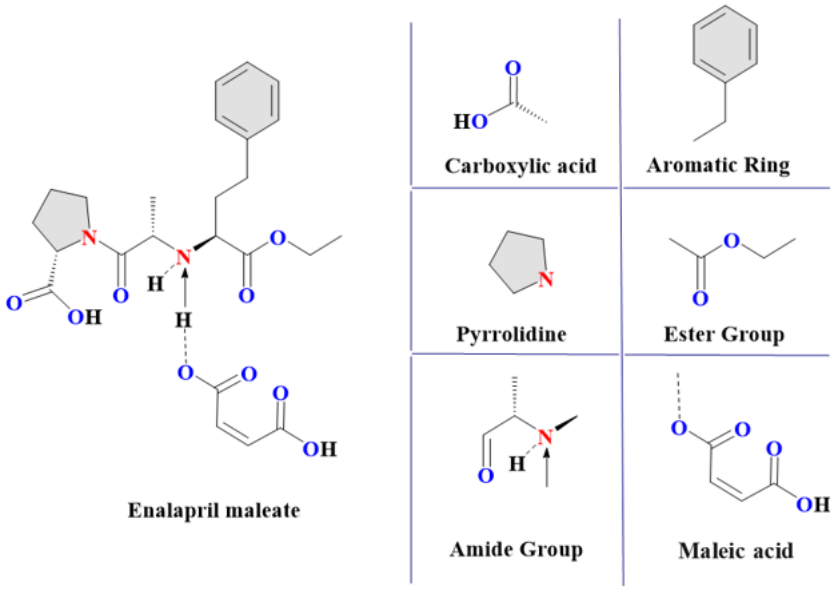

Figure1.3: Chemical structure of Enalapril maleate with highlighted functional groups. (adapted from PubChem, CID 5388961) (National Center for Biotechnology Information, 2025)

Enalaprilat functions as a competitive inhibitor of angiotensin-converting enzyme (ACE), effectively obstructing the conversion of angiotensin I to angiotensin II, a powerful vasoconstrictor. This inhibition leads to vasodilation, reduced blood pressure, decreased aldosterone secretion, and improved protection of both the renal and cardiovascular system. The formulation of enalapril maleate strikes an optimal balance between hydrophilicity, which enhances solubility, and lipophilicity, which promotes gastrointestinal absorption. This combination ensures efficient systemic bioavailability and favorable therapeutic outcomes in conditions such as hypertension, heart failure, and diabetic nephropathy (Hilal-Dandan et al., 2022).

1.1.4.2 FDA Approval Specifications for Enalapril Maleate

In 1985, the Food and Drug Administration (FDA) approved Enalapril Maleate for the treatment of hypertension and heart failure. This approval was accompanied by stringent specifications concerning safety, efficacy, and quality. The approval process involved evaluating clinical trial data, ensuring proper formulation and dosage, and meeting established standards for impurities, dissolution, and stability. These regulatory quality-related characteristics are summarized in Table 1.1 (U.S. Food and Drug Administration, 1985).

Table 1.1: Physicochemical properties of Enalapril maleate Based on (U.S. Food and Drug Administration, 2015; DrugBank Online, 2024)

Properties	Description / value
Appearance	White to off-white crystalline powder
Molecular Weight	492.53 g/mol
Molecular Formula	$C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$
Melting Point	Approximately 145–150°C (with decomposition)
Odor	Odorless
Solubility	Sparingly soluble in water; slightly soluble in Ethanol
pH	Acidic (approximately pH 2.5 in aqueous solution)
Stability	Sensitive to hydrolysis, Heat, Light, and moisture

1.1.4.3 Pharmacodynamics

Enalaprilat functions as a competitive inhibitor of angiotensin-converting enzyme (ACE), effectively preventing the conversion of angiotensin I to angiotensin II, a powerful vasoconstrictor. This inhibition leads to a reduction in systemic vascular resistance, resulting in decreased blood pressure and suppression of aldosterone secretion, which subsequently diminishes sodium and water retention. Additionally, ACE inhibition raises bradykinin levels, promoting vasodilation but also contributing to adverse effects, including a persistent dry cough (Hall, 2020; Israili & Hall, 1992).

1.1.4.3.1 Pharmacological Mechanism of Enalapril Maleate

Enalapril maleate is administered as a prodrug and is hydrolyzed by hepatic esterases, leading to the formation of its active metabolite, enalaprilat, as depicted in Figure 1.4. At the active site, the carboxylate group ($–COO^-$) of enalaprilat is essential for binding to angiotensin-converting enzyme (ACE), thereby facilitating its inhibitory effect (Davies et al., 1984).

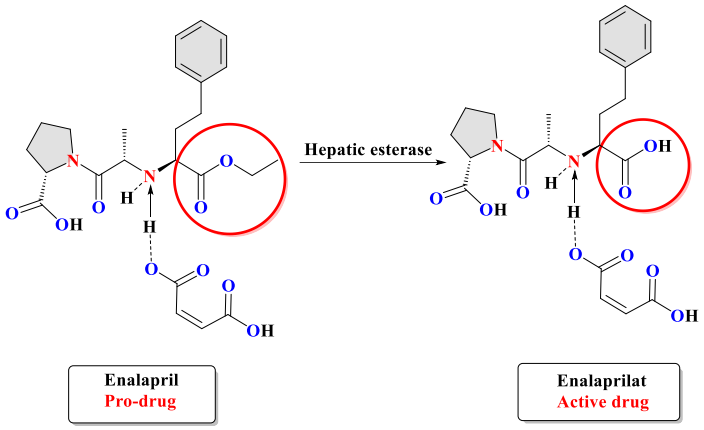

Figure 1.4: Schematic representation of the biotransformation of enalapril (a prodrug) into its pharmacologically active metabolite, enalaprilat, via hepatic esterase-mediated ester hydrolysis (Hilal-Dandan et al., 2022)

Angiotensin I is converted into **angiotensin II** by angiotensin-converting enzyme (ACE), a zinc-dependent metalloproteinase that necessitates a zinc ion for its catalytic activity. The active metabolite, enalaprilat, interacts with the zinc ion at the active site of ACE via its carboxylate group, establishing strong coordination interactions that lead to competitive inhibition of the enzyme. Inhibition of ACE prevents the formation of **angiotensin II**, thereby interfering with the physiological processes responsible for vasoconstriction and aldosterone release, as demonstrated in Figure 1.5 (Davies et al., 1984).

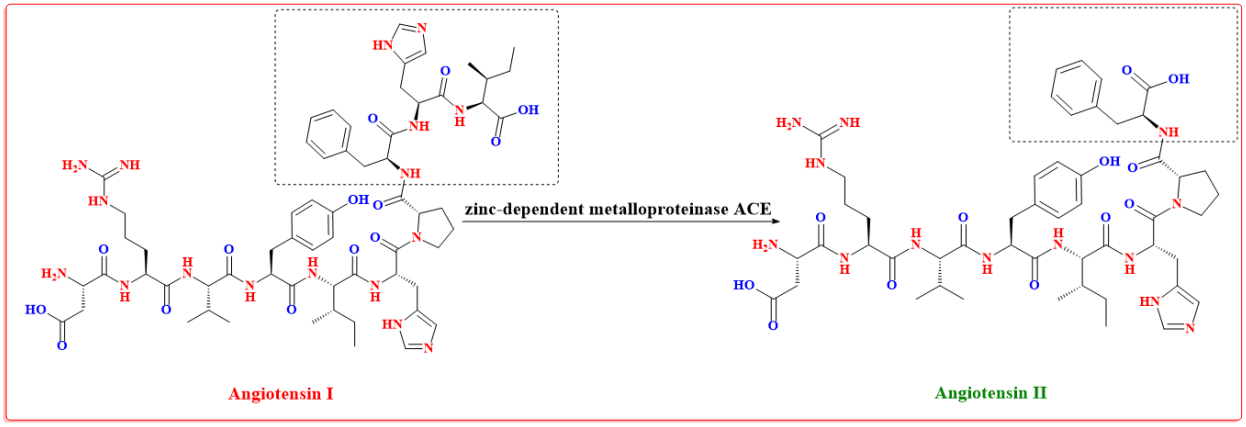

Figure 1.5: Schematic representation of the enzymatic conversion of angiotensin I into the potent vasoconstrictor angiotensin II by angiotensin-converting enzyme (ACE), a zinc-dependent metalloproteinase (Hilal-Dandan et al., 2022)

1.1.4.4 Pharmacokinetics

Enalapril is rapidly absorbed following oral administration, demonstrating an average bioavailability of approximately 60%. Peak plasma concentrations are reached within approximately 1 hour for enalapril and 3 to 4 hours for its active metabolite, enalaprilat. The drug

exhibits a biphasic elimination profile, with enalaprilat having an effective half-life of approximately 11 hours, facilitating once- or twice-daily dosing. Approximately 60% of the administered dose is eliminated through the kidneys, primarily as enalaprilat (MacFadyen et al., 1993).

1.1.4.5 Clinical Indications

Enalapril is indicated for the management of essential and renovascular hypertension, symptomatic heart failure, and asymptomatic left ventricular dysfunction. Additionally, it is commonly employed to slow the progression of heart failure and diabetic nephropathy. Its established clinical efficacy, extensive clinical experience, and favorable safety profile have contributed to its widespread use as a well-characterized angiotensin-converting enzyme inhibitor (Remuzzi et al., 2002; Yusuf, 2000).

1.1.4.6 Metabolism and Elimination

Enalapril is metabolized in the liver to form enalaprilat, with renal clearance being the primary elimination pathway. In patients with renal impairment, drug accumulation may occur, necessitating dosage adjustments. Food does not significantly affect absorption, allowing for convenient administration (U.S. Food and Drug Administration, 2015).

1.1.4.7 Adverse Effects

Common adverse reactions include dry cough, dizziness, and hypotension. Severe but rare side effects can include angioedema and hyperkalemia. Enalapril is contraindicated during pregnancy due to potential risks of fetal toxicity, such as renal dysgenesis and fetal death (Faruqi et al., 2024).

1.1.5 Mechanism of Hydrolysis and Degradation Pathways of Enalapril

Enalapril maleate, like other ester-containing prodrugs, is chemically unstable and susceptible to hydrolytic degradation. The drug undergoes hydrolysis of its ethyl ester group, resulting in enalaprilat, the pharmacologically active diacid metabolite. While this transformation is beneficial in vivo, it poses a significant challenge for extemporaneous preparations and aqueous formulations, as uncontrolled hydrolysis can lead to substantial loss of potency (Bhardwaj & Singh, 2008).

1.1.5.1 Primary Hydrolytic Pathway

In aqueous environments, the hydrolysis of enalapril is influenced by pH and temperature. Under acidic or neutral conditions, the ester group is cleaved, yielding enalaprilat (Bakshi & Singh, 2002). However, this process accelerates in alkaline conditions due to base-catalyzed hydrolysis, a

phenomenon consistently observed in forced degradation studies (Bhardwaj & Singh, 2008; Su et al., 2021).

1.1.5.2 Secondary Degradation: Diketopiperazine (DKP) Formation

In addition to hydrolysis to enalaprilat, enalapril is prone to intramolecular cyclization, producing diketopiperazine (DKP) as illustrated in Figure 1.6. This degradation product results from the nucleophilic attack of the secondary amine on the ester linkage, forming a cyclic dipeptide derivative. The formation of DKP is significantly influenced by temperature, moisture, and the presence of excipients. Since DKP is pharmacologically inactive, its formation contributes to potency loss and stability concerns (Lin et al., 2004).

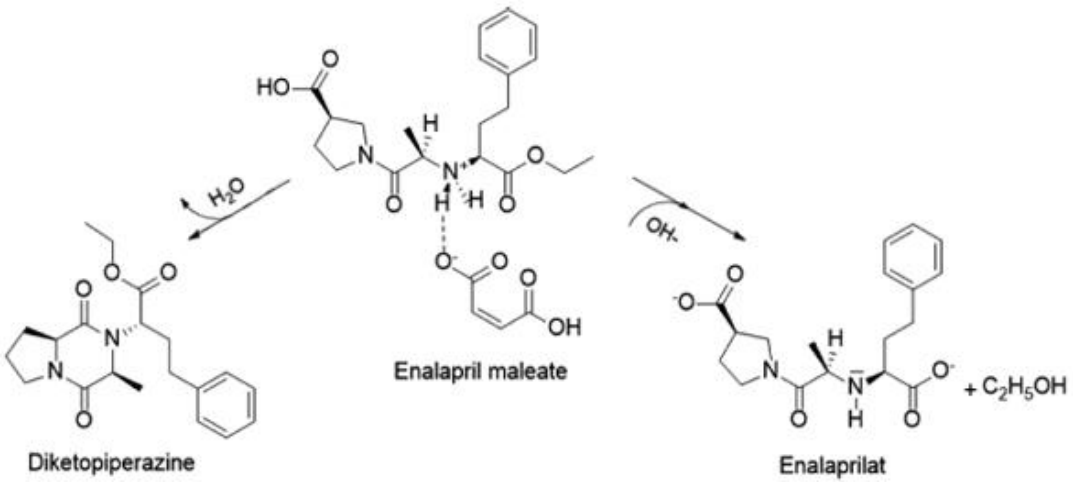

Figure 1.6: Molecular structure of enalapril maleate and the degradation products diketopiperazine and enalaprilat. Enalapril is bound to maleate through hydrogen bonding (Bout & Vromans, 2022)

1.1.5.3 Environmental and Formulation Factors

- **pH:** Hydrolysis is minimized in mildly acidic conditions (pH 3–4), while alkaline pH greatly accelerates ester cleavage (Bhardwaj & Singh, 2008).
- **Temperature:** Elevated temperatures increase both hydrolysis and DKP formation, in accordance with Arrhenius kinetics (Bout & Vromans, 2022).
- **Moisture & Excipients:** The presence of condensed water and hygroscopic excipients can enhance instability in solid formulations. Interactions with excipients such as lactose have been reported to increase degradation rates (Bout & Vromans, 2022).
- **Light & Oxidative Stress:** Although less significant than hydrolysis, photo degradation and oxidative reactions may contribute to minor instability under inadequate storage conditions (Blessy et al., 2014).

1.1.5.4 Clinical and Pharmaceutical Implications

These degradation pathways pose challenges in the development of liquid formulations and extemporaneous preparations. To ensure chemical stability, it is essential to implement strategies such as pH adjustment, the use of stabilizing agents, controlled storage conditions, and protective packaging. Stability-indicating HPLC methods are widely employed to detect and quantify enalapril and its degradation products, thereby ensuring reliable quality control (Blessy et al., 2014).

1.1.6 Stability of Enalapril in Solid and Liquid Dosage Forms

The stability of enalapril maleate is a significant concern for both industrial formulations and extemporaneous preparations due to its chemically labile ester functionality. Instability primarily arises from hydrolysis and cyclization to diketopiperazine (DKP), which are influenced by the type of formulation, environmental conditions, and interactions with excipients (Bout & Vromans, 2022)**.**

1.1.6.1 Solid-State Stability

Enalapril maleate in tablet or capsule form is prone to degradation due to moisture, heat, and certain excipients. The presence of condensed water within the solid matrix accelerates hydrolysis, while hygroscopic excipients like lactose and microcrystalline cellulose further compromise stability. Research indicates that the formation of diketopiperazine (DKP) is the primary degradation pathway in solid dosage forms, particularly under high humidity and elevated temperature conditions (Bout & Vromans, 2022; ICH, 2003). Forced degradation studies have shown that enalapril is significantly unstable in solid forms, with degradation rates influenced by the compatibility of excipients (Baertschi et al., 2016).

1.1.6.2 Liquid-State Stability

Extemporaneous liquid formulations of enalapril exhibit inherent instability due to the aqueous medium, which promotes ester hydrolysis. Solutions stored at room temperature are susceptible to rapid degradation, resulting in the formation of enalaprilat and diketopiperazine (DKP). The rates of degradation are significantly influenced by pH and storage temperature. Enhanced stability has been documented for extemporaneously prepared oral liquid formulations when pH is meticulously controlled and stabilizing agents are utilized. Nonetheless, the shelf life of these formulations remains limited, typically ranging from 7 to 14 days when stored under refrigerated conditions (Nahata & Allen, 2008).

1.1.6.3 Influence of pH and Temperature

The stability of enalapril in aqueous solution is significantly influenced by pH levels. Enhanced stability has been observed under mildly acidic conditions (approximately pH 2.5–4), where the hydrolysis of esters is minimized. Conversely, degradation rates increase substantially at alkaline pH values due to base-catalyzed ester cleavage (Blessy et al., 2014). Additionally, temperature is a crucial factor affecting stability, as storage under refrigerated conditions (2–8 °C) markedly extends shelf life compared to room temperature, in accordance with established principles of chemical degradation kinetics (Grimm, 1998).

1.1.6.4 Implications for Extemporaneous Preparations

The inherent instability of enalapril maleate presents significant challenges for extemporaneous compounding. Factors such as moisture, temperature, pH, and interactions with excipients can accelerate hydrolysis and intramolecular cyclization, resulting in decreased potency and shortened beyond-use dates (Bout & Vromans, 2022). Therefore, extemporaneous oral liquid preparations should be compounded under controlled conditions, dispensed in protective packaging, and stored under refrigeration whenever possible. Furthermore, the implementation of clear labeling, conservative beyond-use dating, and stability-indicating analytical methods, such as high-performance liquid chromatography (HPLC), is crucial to ensure product quality, patient safety, and therapeutic efficacy in clinical practice (Blessy et al., 2014).

1.1.7 Extemporaneous Preparations

1.1.7.1 Importance of Extemporaneous Preparations

Extemporaneous compounding is an essential practice in both hospital and community pharmacies, particularly when commercially manufactured dosage forms are unavailable or unsuitable for specific patient populations, such as pediatric and geriatric patients. Customized formulations, including oral liquid preparations of medications like enalapril maleate, are often necessary when children are unable to swallow solid dosage forms (Sosnowska, Winnicka, & Czajkowska-Kośnik. A, 2009). This approach allows for flexible dosing, optimization of therapeutic outcomes, and the accommodation of individual clinical needs. Additionally, extemporaneous preparations are crucial in resource-limited settings, where access to appropriate commercially available dosage forms may be limited (Standing & Tuleu, 2005).

1.1.7.2 Challenges and Objectives in Extemporaneously Prepared Drug Products

Extemporaneous preparations, while providing significant clinical advantages, are accompanied by several practical challenges. The lack of standardized formulations raises concerns regarding product quality, reproducibility, and patient safety. Stability is a critical limitation, as many active

pharmaceutical ingredients (APIs), such as enalapril maleate, exhibit chemical instability in aqueous environments, which can lead to hydrolysis and subsequent loss of potency (Glass & Haywood, 2006). Moreover, liquid preparations that do not incorporate appropriate preservative systems are vulnerable to microbial contamination, further jeopardizing patient safety (Nahata & Allen, 2008). Variability in compounding practices across different pharmacies may also result in inconsistencies in drug concentration, bioavailability, and therapeutic efficacy (Nunn & Williams, 2005). Therefore, the primary objectives of extemporaneous compounding are to ensure chemical stability, microbiological safety, and accurate dosing, while also maintaining acceptable palatability and promoting patient adherence (Nahata & Allen, 2008).

1.1.7.3 Oral Liquid Categories

Oral liquid preparations are primarily categorized into two main types: solutions and suspensions.

- Solutions are homogeneous systems in which all solid components, including the active pharmaceutical ingredient, are fully dissolved in one or more solvents, which may be aqueous, organic, or a combination of both. Common examples of oral solutions include syrups, elixirs, linctuses, and aromatic waters, as detailed in Table 1.2(Allen, 2011). Solutions provide immediate drug availability and ensure uniform dosing; however, their chemical stability may be compromised, as dissolved drug molecules are more prone to degradation processes such as hydrolysis and oxidation (Attebäck et al., 2022).

Table 1.2: Classification and definitions of common oral solution dosage forms (Allen, 2011)

Type of Solution	Definition
Syrup	Aqueous preparation containing a high proportion of sucrose or another sweetener
Elixir	A clear, sweetened Hydroalcoholic solution containing flavoring agents
Linctus	Viscous liquid containing sucrose, intended for small volume administration
Aromatic waters	Saturated aqueous solutions of volatile aromatic compounds

- **Solutions** provide numerous advantages as oral liquid dosage forms, including rapid drug absorption, uniform dosing, and ease of administration. However, their utilization is frequently constrained by poor chemical stability, particularly due to susceptibility to hydrolytic degradation, difficulties in masking unpleasant tastes, and limited solubility for certain drugs (Allen, 2011).
- **Suspensions** in contrast, are biphasic systems consisting of insoluble solid particles (the dispersed phase) uniformly distributed within a liquid vehicle (the dispersion medium).

They are particularly beneficial for medications with limited aqueous solubility, such as enalapril maleate, and offer flexible dosing options, rendering them suitable for pediatric and geriatric populations (Doye et al., 2017).

Characteristics of an Ideal Suspension

An optimal pharmaceutical suspension should demonstrate rapid and complete redispersion of particles upon gentle agitation, without the formation of a hard cake during sedimentation. The formulation must be free from grittiness to ensure patient acceptability, particularly for oral and topical applications. Furthermore, both chemical and physical stability must be preserved throughout storage to maintain product quality, safety, and therapeutic efficacy (Florence & Attwood, 2016).

1.1.8 Suspending Agents

Suspending agents are critical excipients in pharmaceutical suspensions, designed to enhance physical stability by promoting the uniform distribution of insoluble drug particles throughout the dispersion medium. Their primary functions include reducing the sedimentation rate, preventing caking, and facilitating the easy and complete re-dispersion of particles upon shaking. These properties contribute to consistent dosing, improved patient compliance, and an acceptable shelf life for liquid dosage forms (Martin & Bustamante, 1993).

1.1.8.1 Mechanism of Action

Suspending agents primarily function by increasing the viscosity of the continuous phase, thereby reducing the settling velocity of dispersed particles, as outlined by Stokes' law. Furthermore, they offer steric and electrostatic stabilization, which minimizes particle aggregation and flocculation, ultimately enhancing the physical stability of pharmaceutical suspensions (Martin & Bustamante, 1993).

1.1.8.2 Types of Suspending and Stabilizing Agents

Suspending agents utilized in pharmaceutical suspensions are typically categorized into three primary groups: natural, semi-synthetic, and synthetic polymers. These excipients are primarily incorporated to enhance physical stability by increasing the viscosity of the continuous phase and ensuring uniform particle dispersion.

Natural suspending agents, including acacia, tragacanth, guar gum, and xanthan gum, are frequently employed due to their biocompatibility and availability. However, their use may be constrained by batch-to-batch variability and vulnerability to microbial contamination (Rowe, 2009).

Synthetic suspending agents, such as carbomers, polyvinyl alcohol, and poloxamers, offer consistent rheological properties and enhanced chemical stability. Nonetheless, certain synthetic

polymers necessitate neutralization or pH adjustment to attain optimal thickening and suspension efficacy (Sharma et al., 2022) .

Semi-synthetic suspending agents, including sodium carboxymethyl cellulose (NaCMC), hydroxypropyl methylcellulose (HPMC), and methylcellulose, provide a balance between stability, cost-effectiveness, and patient acceptability. These polymers are extensively utilized in both industrial and extemporaneous pharmaceutical formulations (Rowe, 2009).

In addition to polymeric suspending agents, certain excipients serve as stabilizing **co-solvents** in suspension formulations. Propylene glycol (PG) is a multifunctional excipient that acts as a wetting agent by reducing interfacial tension between hydrophobic drug particles and the aqueous vehicle, thereby enhancing dispersion. At elevated concentrations, PG may also increase viscosity and decrease water activity, which can slow the hydrolytic degradation of labile drugs such as enalapril maleate (Rowe, 2009). In pediatric and extemporaneous preparations, PG is often incorporated to improve physical stability and extend refrigerated shelf life when utilized within recommended concentration limits (Nahata & Allen, 2008).

1.1.8.3 Factors Affecting the Choice of Suspending Agent

Selecting an appropriate suspending agent depends on several factors, including:

- Drug properties: such as solubility, particle size, and pH sensitivity.
- Route of administration: oral versus parenteral.
- Patient considerations: differences in pediatric and geriatric populations, including taste masking, viscosity, and ease of redispersion.
- Stability requirements: compatibility with other excipients, resistance to microbial growth, and effects on chemical stability (Hatwar et al., 2023).

1.1.8.4 Clinical and Pharmaceutical Relevance

In pediatric suspensions, such as extemporaneously prepared enalapril maleate formulations, the selection of suspending agents is crucial for ensuring accurate dosing and extended shelf life under refrigeration. Suboptimal suspension performance can lead to dose variability, diminished therapeutic efficacy, and an increased risk of adverse effects, particularly in pediatric populations. Consequently, it is essential to optimize both the type and concentration of suspending agents to guarantee safety and therapeutic effectiveness in extemporaneous preparation (Belayneh & Tessema, 2021).

1.1.8.5 Propylene Glycol as a Co-solvent and Excipient

Propylene glycol (PG) (Figure 1.7) is a colorless, odorless, hygroscopic liquid that is completely miscible with water and many organic solvents. It is widely recognized as a safe and versatile

pharmaceutical excipient, officially listed in the United States Pharmacopeia (USP), the European Pharmacopoeia (Ph. Eur.), and the Japanese Pharmacopoeia (JP). Furthermore, PG has been designated as generally recognized as Safe (GRAS) by the U.S. Food and Drug Administration (FDA) for use in pharmaceutical products (U.S. Food and Drug Administration, 2023).

PG is extensively utilized as a co-solvent, humectant, and stabilizing excipient in oral, parenteral, and topical dosage forms. Regulatory guidance indicates that PG is commonly employed within defined concentration limits, which vary based on the route of administration and formulation requirements (Medicines Agency, 2017). In extemporaneous liquid preparations, PG enhances dispersion characteristics and improves the physical stability of suspensions. From a physicochemical perspective, co-solvents such as PG can modify the aqueous environment of the formulation, potentially influencing drug stability and reducing the rate of hydrolytic degradation of labile compounds (Rowe, 2009). These attributes reinforce its role as a functional excipient in the development of enalapril maleate oral liquid formulations.

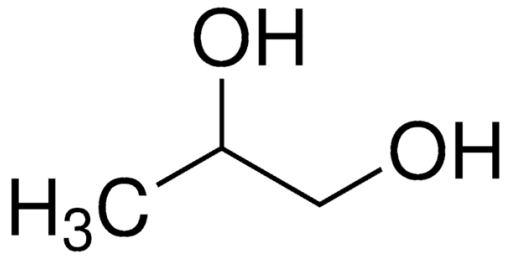

Figure 1.7: Structure of Propylene Glycol (Sigma-Aldrich, 2025)

1.1.9 Challenges and Limitations

The extemporaneous compounding of enalapril and other unstable drugs presents several challenges that hinder their widespread use and affect clinical outcomes. The primary concern is **chemical instability**, as enalapril rapidly hydrolyzes in aqueous environments and can cyclize into diketopiperazine, reducing its potency and therapeutic efficacy. This instability necessitates frequent re-dispensing, imposes short beyond-use dates, and requires strict storage conditions, which may be impractical in typical clinical settings (Nahata & Allen, 2008).

Another significant limitation is the **lack of standardized formulations**. Unlike commercially manufactured medications, compounded preparations can vary considerably in excipients, preparation techniques, and storage protocols, leading to substantial batch-to-batch differences in stability and bioavailability. These variations increase the risk of sub-therapeutic dosing, particularly in vulnerable populations such as pediatrics and geriatrics (Rood et al., 2014).

Analytical challenges also emerge, as stability-indicating methods like HPLC or FTIR are often unavailable in community or hospital pharmacies. Without adequate monitoring, degradation products such as enalaprilat or diketopiperazine may accumulate unnoticed, potentially

jeopardizing patient safety (Bhardwaj & Singh, 2008). Furthermore, excipient incompatibilities—including lactose, basic buffers, or reducing sugars—can exacerbate degradation and complicate formulation development (Bout & Vromans, 2022).

From a regulatory perspective, compounded formulations encounter challenges due to the absence of robust clinical trials demonstrating their efficacy and safety (Batchelor & Marriott, 2015; Kairuz et al., 2007). Regulatory guidelines often establish beyond-use dates (BUDs) based on general stability data rather than drug-specific studies, resulting in conservative expiration timelines (American Society of Health-System Pharmacists [ASHP], 2017). Additionally, requirements for clear labeling, appropriate storage conditions, and, in some cases, refrigeration create logistical challenges for pharmacists and patients, particularly in resource-limited settings (Institute for Safe Medication Practices (ISMP) Canada, 2016).

Lastly, palatability and patient adherence present additional challenges, particularly in pediatric patients. Extemporaneous oral formulations may acquire an unpleasant taste due to drug degradation or excipient-related effects during the dosing interval. This can diminish patient acceptance and adherence, ultimately compromising therapeutic effectiveness (Naser Zaid et al., 2022).

1.1.10 Disadvantages of Using Commercially Manufactured Products as a Source

Pharmacists often utilize commercially manufactured tablets or capsules as a source of the active pharmaceutical ingredient (API) when preparing extemporaneous formulations. While this practice is prevalent due to the limited availability of licensed liquid dosage forms, it presents several disadvantages that may adversely affect product quality, safety, and therapeutic efficacy.

Formulation Variability: Commercial solid dosage forms contain a range of excipients, such as binders, fillers, disintegrates, and lubricants, which are optimized for tablet manufacturing rather than liquid formulations. When dispersed in aqueous vehicles, these excipients may contribute to sedimentation, poor resuspendability, or an increased risk of microbial contamination, ultimately leading to inconsistent dosing (Glass & Haywood, 2006)**.**

Stability Concerns: The active drug obtained from commercially manufactured tablets is generally less stable in aqueous vehicles than pure API powders. Enalapril maleate, in particular, is highly susceptible to hydrolytic degradation when formulated into solutions or suspensions prepared from tablets, with degradation potentially accelerated by the presence of tablet excipients (Richey et al., 2012). Studies have indicated that extemporaneous liquid formulations prepared from tablets often exhibit shorter shelf lives compared to those prepared directly from raw API materials (Sosnowska et al., 2009).

Dosing Accuracy: Crushing and dispersing tablets may result in variability in drug content uniformity. Losses of the API can occur due to adhesion to mortars, pestles, or container surfaces, leading to under-dosing. For drugs with narrow therapeutic indices, such as angiotensin-converting enzyme inhibitors, even minor deviations in dose can result in clinically significant outcomes (Nahata & Allen, 2008).

Palatability and patient adherence: Tablet excipients, including lactose or starch, may impart an unpleasant taste or gritty texture when suspended in water, negatively affecting palatability. Pediatric patients are particularly sensitive to such organoleptic properties, which can reduce acceptance and adherence to therapy (Batchelor & Marriott, 2015).

1.1.11 Analytical Instruments for Stability Studies

1.1.11.1 Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) is a modern analytical technique widely employed in pharmaceutical sciences for both qualitative and quantitative analysis of drugs. Unlike traditional dispersive infrared spectroscopy, FTIR utilizes a **Michelson interferometer**, which is the fundamental component of the system (Griffiths & De Haseth, 2007).

The interferometer consists of a **fixed mirror, movable mirror, and beam splitter**. The incoming infrared radiation is divided into two beams by the beam splitter. One beam is reflected by the fixed mirror, while the other is reflected by the movable mirror. Upon recombination, the difference in optical path generates an **interference pattern** (interferogram) that is detected and subsequently transformed into an infrared spectrum through the Fourier transform. The figure illustrates light intensity as a function of mirror displacement. By applying the Fourier transform, the interferogram is converted into a spectrum, which provides structural information regarding the molecular functional groups (Chai et al., 2020).

For enalapril maleate, (FTIR) enables the monitoring of functional groups that are prone to degradation, including the **ester, amide, and carboxyl** groups. This technique offers several advantages:

- **Rapid** analysis with minimal sample preparation.
- **Non-destructive** nature, allowing for repeated assessments.
- **Quantitative capabilities**, facilitating the construction of calibration curves to track hydrolysis kinetics (Stuart, 2004).

In this study, FTIR-Attenuated Total Reflectance (ATR) was employed as a **novel, efficient, and complementary tool** alongside High-Performance Liquid Chromatography (HPLC) to monitor the hydrolysis kinetics and distribution of enalapril in suspensions.

1.1.11.2 High Performance Liquid Chromatography (HPLC)

High Performance Liquid Chromatography (HPLC) is regarded as the **standard** reference method for stability-indicating analysis of pharmaceutical compounds, including enalapril maleate. HPLC separates analytes based on their interactions with the **stationary phase** and their solubility in the **mobile phase**, facilitating precise quantification of the intact drug as well as its degradation products.

The instrument comprises four primary components:

1. **Pump system** – delivers the mobile phase at high pressure.
2. **Injector** – introduces a precise volume of sample into the system.
3. **Column** – packed with stationary phase (e.g., C8 silica) where the separation of analytes occurs.
4. **Detector** – typically a UV or photodiode array, which measures the absorbance of analytes at specific wavelengths (Dong, 2019).

In stability studies, HPLC has been extensively employed to quantify enalapril and its major degradation products, namely **enalaprilat** and **diketopiperazine (DKP)** (De Diego et al., 2011). Its advantages include:

- **High sensitivity and specificity**.
- The ability to differentiate between the **parent compound and degradation products** (Dong, 2019).
- Validation in accordance with ICH guidelines, establishing it as a reliable regulatory standard (International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use, 2005).

1.1.11.3 Comparison of FTIR and HPLC

FTIR and HPLC are compared in this section (Mehrotra, 2000; Selamat et al., 2021), and some aspects of this comparison are illustrated in Table 1.3

Table 1.3: Comparison of FTIR and HPLC

Aspect	FTIR	HPLC
Technique	Non-destructive technique	Destructive technique
Sample preparation	Minimal-no sample preparation required	Sample preparation required
Analysis time	Short analysis time	Depending upon the method use
Sample type handling	Liquids and solids	Liquids

1.2 Problem Statement

Enalapril maleate is a crucial ACE inhibitor commonly employed in the management of hypertension and heart failure. However, its chemical instability in aqueous environments poses significant challenges for the safe preparation of extemporaneous formulations. In practice, community pharmacies frequently prepare oral liquids by dispersing crushed tablets in water, often without conducting stability evaluations. This method may lead to rapid hydrolysis into enalaprilat and cyclization into diketopiperazine (DKP), thereby compromising both potency and safety, and potentially resulting in sub-therapeutic dosing or exposure to inactive degradation products. Although stability studies have been documented in the literature, evidence regarding formulation-based stabilizing strategies directly applicable to extemporaneous liquid preparations remains limited, particularly concerning excipients suitable for pediatric and geriatric populations. Propylene glycol, a widely accepted pharmaceutical co-solvent, may enhance solubility, improve homogeneity, and modify the aqueous microenvironment, potentially reducing hydrolytic degradation; however, its stabilizing role in enalapril formulations has not been systematically investigated.

Therefore, this study addresses three critical gaps:

- The inherent instability of enalapril maleate in water-based extemporaneous oral solutions.
- The stabilizing, solubilizing, and homogenizing effects of propylene glycol in liquid formulations.
- The validation of FTIR/ATR spectroscopy as a quantitative, stability-indicating analytical method, benchmarked against HPLC as the reference technique.

1.3 Aim of the Study

The aim of this study is to investigate the hydrolytic stability of enalapril maleate in extemporaneous oral solutions, evaluate the stabilizing effect of propylene glycol, and develop FTIR-based analytical method benchmarked against the established HPLC assay.

1.4 Objectives of the Study

1. To establish calibration curves for enalapril maleate using both FTIR/ATR and HPLC methods, validating their linearity and accuracy.
2. To evaluate the degradation kinetics of enalapril maleate in aqueous solutions under alkaline stress (NaOH model system).
3. To assess the impact of varying concentrations of propylene glycol (10–15%) on the hydrolytic stability of enalapril maleate.
4. To test the homogeneity of drug distribution within extemporaneous formulations by sampling the upper, middle, and lower layers.

5. To validate FTIR/ATR as a quantitative and stability-indicating method, using HPLC as the reference standard.
6. To propose practical, evidence-based recommendations for safer and more stable extemporaneous compounding practices in community pharmacies.

1.5 Research Questions and Hypotheses

Research Questions (RQs):

- **RQ1**: What are the primary stability challenges associated with aqueous extemporaneous formulations of enalapril?
- **RQ2:** Does the incorporation of propylene glycol improve both chemical stability and homogeneity compared to water-based formulations?
- **RQ3:** Can FTIR/ATR spectroscopy be validated as a reliable and quantitative stability-indicating method for monitoring the degradation of enalapril, with HPLC serving as the reference technique?

Hypotheses (H):

- **H1:** Water-based formulations of enalapril undergo rapid hydrolytic degradation with poor homogeneity.
- **H2:** Propylene glycol significantly delays degradation and enhances uniformity of drug distribution in suspensions.
- **H3:** FTIR/ATR offers a precise, reproducible, and rapid quantitative assessment of enalapril degradation, exhibiting strong agreement with HPLC-based measurements.

1.6 Significance of the Study

This study is significant as it integrates formulation science with advanced analytical methodology. By validating FTIR-ATR as a quantitative, stability-indicating analytical technique, it demonstrates its applicability for rapid and cost-effective stability assessment. Importantly, FTIR-ATR is positioned as a complementary quantitative approach that enhances analytical flexibility, while HPLC remains the reference method for high-resolution separation and definitive quantification.

Clinically, this research addresses a **critical gap in pediatric and geriatric care**, where liquid dosage forms are often essential but typically lack robust stability data. By systematically examining the **effect of propylene glycol as a stabilizer and homogenizer**, this study provides practical insights that can enhance patient safety and therapeutic outcomes.

1.7 Scopes

- The study focuses on the hydrolytic degradation of enalapril maleate in water-based and propylene glycol–containing formulations.
- Both **HPLC (reference method)** and **FTIR/ATR (novel method)** were employed for quantitative analysis.
- The study evaluates the homogeneity of formulations by assessing the distribution of enalapril maleate within the prepared formulations, with a particular emphasis on the role of propylene glycol as a co-solvent.

Chapter Two:

Literature Review

Characterization of Liquid Dosage Forms of Atenolol and Enalapril Maleate for Oral and Enteral Feeding Administration

Mota et al. (2024) conducted a comprehensive study on the formulation and stability of liquid dosage forms of cardiovascular medications, specifically atenolol and enalapril maleate, to address the absence of commercially available options for pediatric, geriatric, and enteral feeding patients. The research focused on the physicochemical, microbiological, and chemical stability of extemporaneously prepared liquid formulations, underscoring the significance of vehicle selection in preserving drug stability, palatability, and suitability for long-term administration.

In this study, oral liquid formulations of enalapril maleate (0.5 mg/mL) were developed using a proprietary aqueous suspending vehicle (SuspendIt®), designed to provide thixotropic properties and compatibility with enteral feeding tubes. The formulations were stored under refrigerated (5 °C) and ambient (25 °C) conditions for a duration of 180 days. Chemical stability was evaluated using validated stability-indicating HPLC methods capable of separating enalapril maleate from its degradation products, while pH, rheological behavior, organoleptic properties, and microbiological stability were evaluated concurrently.

The results indicated that enalapril maleate formulations maintained satisfactory physical and microbiological stability throughout the 180-day storage period at both temperatures, with minimal changes observed in pH and rheological parameters. However, chemical stability was found to be highly temperature-dependent. Under refrigerated conditions, enalapril maleate concentrations remained within the acceptable pharmacopeial range (90–110%) for the entire 180 days. Conversely, formulations stored at 25 °C exhibited progressive degradation, with drug

content falling below 90% after approximately 90 days, confirming enalapril maleate's susceptibility to hydrolytic degradation in aqueous systems at elevated temperatures.

The study further highlighted the importance of formulation design in mitigating instability. The controlled acidic pH of the vehicle, the structured polymeric network, and the effective preservative system collectively contributed to minimizing both chemical and microbiological degradation. These findings reinforce prior observations that the stability of enalapril maleate in liquid formulations is influenced by storage temperature, vehicle composition, and microenvironmental pH, rather than concentration alone.

From the perspective of the current research, this study provides contemporary evidence that extended stability of extemporaneously prepared enalapril maleate liquids is achievable when appropriate formulation strategies. Nonetheless, the reliance on HPLC-based analytical techniques suggests potential for alternative spectroscopic methods. The documented temperature-driven degradation behavior highlights the need for further investigation into solvent systems and analytical methodologies, such as FTIR-based monitoring, to elucidate degradation pathways and assess stability in extemporaneous formulations prepared with alternative co-solvents (Mota et al., 2024).

Stability of Extemporaneous Enalapril Maleate Suspensions for Pediatric Use Prepared from Commercially Available Tablets:

Sosnowska, Winnicka, and Czajkowska-Kośnik (2009) conducted a significant and frequently cited study on the stability of extemporaneously prepared enalapril maleate oral suspensions. This issue is particularly pertinent in pediatric therapeutics, where no commercial liquid formulation is available. The authors aimed to determine whether suspensions prepared from crushed tablets could serve as a chemically and physically stable alternative for pediatric administration.

In this investigation, the researchers prepared suspensions at concentrations of 0.1 mg/mL and 1.0 mg/mL using two different vehicles: a sugar-free hydroxyethylcellulose system and a sugar-containing raspberry syrup-based vehicle. Acknowledging that enalapril maleate exhibits maximum stability at approximately pH 3, as indicated by its pKa values (3.0 and 5.4), all formulations were meticulously adjusted to pH 3 with citric acid. The suspensions were stored under controlled conditions at 4°C and 25°C for 30 days in amber glass bottles to minimize light-induced degradation.

Chemical stability was assessed on days 7, 14, 21, and 30 using a validated HPLC method, which demonstrated confirmed linearity ($r^2 > 0.999$). Additional physicochemical evaluations, including pH and viscosity measurements, were conducted for each sample. At all-time points, both sugar-free and sugar-containing formulations maintained at least 98% of the initial enalapril concentration at both temperatures, with no detectable degradation products such as enalaprilat or diketopiperazine. The pH remained largely unchanged throughout the storage period, and viscosity exhibited only minimal variations, confirming adequate physical stability. Notably, the

raspberry syrup effectively masked the drug's bitterness without adversely affecting stability or suspension performance.

These findings indicate that enalapril maleate demonstrates excellent short-term stability in acidic oral suspensions, provided that an appropriate vehicle and pH adjustment are utilized. The study reinforces fundamental formulation principles: maintaining an acidic microenvironment, selecting an appropriate suspending medium, and ensuring preservative compatibility to mitigate microbial growth. Additionally, it underscores the practicality of producing reliable pediatric suspensions directly from commercially available tablets, a method commonly employed in clinical practice when ready-made liquid formulations are unavailable.

For the present research, this study serves as a foundational reference, elucidating how formulation variables—specifically vehicle composition and microenvironmental pH—impact the chemical stability of enalapril in liquid systems. It also advocates for the exploration of alternative vehicles, such as propylene glycol, and supports the need for analytical tools, including FTIR, to assess degradation behavior in extemporaneous preparations (Sosnowska, 2009).

Enhancement of Water Solubility for Lopinavir by Co-solvency Approach:

Manikandan et al. (2022) examined the effects of various pharmaceutical co-solvents—including propylene glycol (PG), polyethylene glycol 400 (PEG 400), and glycerin—on the aqueous solubility of the poorly water-soluble antiretroviral drug Lopinavir. Their research provides a foundational understanding of how co-solvency systems modify solvent polarity and hydrogen-bonding interactions to enhance solubility, thereby supporting the rationale for using PG as a vehicle in extemporaneous liquid preparations.

The study quantified solubility across graded mixtures of water with each co-solvent. The results demonstrated a significant, concentration-dependent increase in solubility when PG or PEG 400 was incorporated, with the greatest enhancement observed in PEG 400-rich systems. Notably, PG also exhibited a pronounced solubilizing effect, outperforming glycerin and underscoring its capacity to disrupt the hydrogen-bond network of water while reducing the interfacial tension around hydrophobic drug molecules. This mechanism enables PG to create a more favorable microenvironment for the dissolution of drugs that are otherwise poorly soluble in aqueous media.

The authors further highlight that co-solvents possess both hydrophilic groups (facilitating miscibility with water) and hydrophobic regions that disrupt structured water clusters. This dual property allows co-solvents such as PG to mitigate water’s exclusion of nonpolar solutes, thereby enhancing molecular dispersion and solubility. This concept is crucial for understanding the widespread use of PG in pharmaceutical liquid formulations, particularly in preparations that require improved solubility without chemically modifying the active pharmaceutical ingredient (API).

These findings are directly relevant to the current study on Enalapril Maleate. Similar to Lopinavir, Enalapril is poorly soluble in water and susceptible to degradation via hydrolysis. The co-solvency mechanisms described by Manikandan et al. provide a scientific basis for the protective role of PG observed in the present experimental work. By participating in hydrogen-bonding and modifying the water microenvironment, PG not only enhances the solution's homogeneity but may also reduce the extent of water-mediated hydrolysis, resulting in improved stability profiles detectable via FTIR. Thus, the co-solvency framework supports the hypothesis that PG can function as more than an inert solvent; it may actively modulate the physicochemical environment of Enalapril, influencing both solubility and degradation kinetics (Manikandan et al., 2022).

Study on the stability and compatibility mechanism of enalapril maleate based on pKa and pH microenvironment:

Su et al. (2021) conducted a comprehensive mechanistic study to elucidate how microenvironmental pH and excipient interactions govern the chemical stability of enalapril maleate (EM) in solid dosage forms. The study employed an integrated panel of thermoanalytical techniques, including thermogravimetric analysis (TGA), differential scanning calorimetry (DSC), and thermomechanical analysis (TMA). Their findings provide critical insights into how formulation components modify the degradation pathways of EM, particularly its conversion to diketopiperazine (DKP) and enalaprilat (ET), the two major hydrolytic and cyclization-related impurities.

Through DSC-based compatibility studies, the authors demonstrated that enalapril maleate reacts with sodium bicarbonate (a common excipient in reference formulations), producing notable thermal transitions that indicate incomplete reactions in some commercial reference tablets. These findings suggest that excipient–active pharmaceutical ingredient (API) interactions may persist beyond manufacturing, influencing the drug’s stability during storage. TGA data further confirmed that EM is thermally stable by itself up to approximately 140 °C; however, when incorporated into a tablet matrix containing excipients, decomposition patterns shift, reinforcing the importance of excipient selection. TMA results revealed structural expansion and contraction of the tablet matrix during heating—consistent with CO_2 release from $NaHCO_3$ decomposition—highlighting how microenvironmental pH and moisture sensitivity contribute to mechanical instability during storage.

A significant contribution of this study lies in its systematic evaluation of degradation as a function of microenvironmental pH. By exposing EM and tablet powders to buffer systems of varying pH under accelerated stress conditions (60 °C for 24 hours), the authors demonstrated that EM exhibits optimal stability at pH ≈ 3 and pH ≈ 7, whereas both acidic and alkaline extremes accelerate the formation of DKP and ET. This pH dependency aligns with EM’s two pKa values (3.0 and 5.4), which modulate the protonation state of the secondary amine and carbonyl groups, thereby influencing intramolecular cyclization. The authors propose mechanistic degradation

pathways in which protonation or deprotonation alters nucleophilicity and electrophilicity, ultimately dictating whether DKP or ET predominates as the degradation product.

Importantly, the study highlights that stabilizers such as maleic or tartaric acid, which maintain a mildly acidic microenvironment, significantly reduce DKP formation, while alkaline excipients like sodium bicarbonate promote hydrolytic degradation. This reinforces the principle that formulation design must control microenvironmental pH rather than rely solely on bulk pH measurements. In this context, maleic and tartaric acids serve as acidifying excipients in solid-state formulations, specifically to regulate the microenvironmental pH, rather than functioning as formulation additives in liquid systems.

Relevance to the Present Study:

Although Su et al. focused exclusively on solid-state formulations, their mechanistic findings are directly applicable to liquid compounding practices. The demonstrated sensitivity of EM to microenvironmental pH—and its rapid degradation in unbuffered or aqueous conditions—supports the rationale for exploring alternative vehicles such as propylene glycol (PG). While their work does not investigate co-solvents, it clearly establishes that water-rich environments accelerate hydrolysis, whereas modified microenvironments can suppress degradation pathways. Accordingly, the present research extends this mechanistic framework by evaluating whether PG can alter the solution-phase microenvironment of EM, reduce water-mediated hydrolysis, and enhance stability as detected by FTIR (Su et al., 2021).

Kinetics of Degradation of Enalapril Maleate in Dosage Forms:

Stanisz (2004) conducted a comprehensive kinetic and thermodynamic investigation into the degradation behavior of Enalapril Maleate (ENA) in solid-state dosage forms, focusing on the impacts of humidity, temperature, and packaging integrity. This study represents one of the earliest and most thorough assessments of how storage conditions accelerate ENA degradation, providing mechanistic insights pertinent to both industrial formulation and extemporaneous compounding practices.

In this research, commercially manufactured ENA tablets (10 mg) were stored either within blister packs or unprotected under high relative humidity (76.4% RH). Samples were subjected to controlled temperatures ranging from 313 K to 333 K, with degradation monitored using a stability-indicating HPLC method optimized for the selective detection of ENA and its principal degradation products. The author quantified first-order degradation rate constants, calculated activation energies, and derived thermodynamic parameters utilizing the Arrhenius model.

The findings demonstrated a significant effect of environmental exposure on ENA stability. Tablets stored without blister protection exhibited markedly faster degradation, characterized by higher rate constants and shorter $t_{0.1}$ and $t_{0.5}$ values compared to blister-packed tablets. The study

confirmed that both humidity and elevated temperature accelerate the hydrolysis of the ester group, facilitating the formation of enalaprilat and the diketopiperazine derivative (DKP). Notably, the activation energy for degradation was substantially lower in unprotected tablets, indicating increased susceptibility to environmental stress. Across all conditions, ENA degradation adhered to first-order kinetics, and the derived thermodynamic parameters (ΔH‡, ΔS‡) supported a degradation pathway significantly influenced by moisture-induced molecular mobility within the solid matrix.

This research underscores the critical importance of packaging integrity and environmental control in preserving ENA stability. While conducted on solid-state tablets, the mechanistic conclusions are directly applicable to extemporaneous compounding: any prior degradation in tablets utilized for suspension preparation may compromise the final product, and interactions with excipients or moisture exposure can considerably accelerate hydrolysis. Consequently, these kinetic insights warrant the implementation of protective formulation strategies in liquid preparations and emphasize the necessity of selecting vehicles and conditions that minimize water-driven degradation pathways (Stanisz, 2004).

Advances in Simultaneous DSC–FTIR Micro spectroscopy for Rapid Solid-State Chemical Stability Studies: Some Dipeptide Drugs as Examples:

Lin and Wang (2012) conducted a comprehensive evaluation of a hybrid analytical approach—simultaneous differential scanning calorimetry and Fourier-transform infrared micro spectroscopy (DSC–FTIR)—aimed at enhancing and expediting solid-state stability testing of peptide-based pharmaceuticals. Their research sought to overcome the limitations of traditional stability methods by facilitating real-time monitoring of thermal events and corresponding chemical transformations within a single experimental run.

In their study, the authors investigated three representative dipeptide drugs—Aspartame hemihydrate, Lisinopril dihydrate, and Enalapril maleate—to characterize dehydration processes, phase transitions, and the formation of significant degradation products such as diketopiperazine (DKP). The DSC component captured endothermic and exothermic events associated with structural changes, while FTIR concurrently recorded variations in functional group vibrations, allowing for direct correlation between thermal behavior and molecular degradation. For all three drugs, the technique effectively identified stepwise dehydration followed by intramolecular cyclization to DKP, with Enalapril maleate exhibiting a distinct degradation profile and an activation energy of approximately 195 kJ/mol. Furthermore, compatibility studies with polymeric excipients—such as Eudragit E—revealed that excipient interactions could lower the onset temperature of degradation and introduce new spectral features indicative of chemical reactions within the formulation matrix.

The primary contribution of this research lies in demonstrating that DSC–FTIR offers a rapid, sensitive, and mechanistically informative platform for assessing solid-state stability and drug–

excipient interactions. Unlike separate thermal and spectroscopic analyses, this simultaneous method enables precise temporal alignment between thermal events and structural modifications, facilitating early detection of degradation pathways such as DKP formation.

Relevance to the Present Study

While Lin and Wang's study primarily focuses on solid-state systems, its mechanistic relevance extends to liquid dosage forms, particularly extemporaneous preparations of Enalapril maleate. Their findings underscore the inherent instability of Enalapril and its susceptibility to cyclization and hydrolysis, thereby supporting the need for stabilizing environments and alternative analytical techniques. The demonstrated sensitivity of FTIR in detecting early structural changes validates its application as a quantitative, stability-indicating tool in solution systems. Consequently, this research provides a scientific basis for employing FTIR-based methods in the current study to monitor Enalapril degradation and to evaluate whether propylene glycol can modulate microenvironmental conditions and reduce hydrolytic breakdown (S. Y. Lin & Wang, 2012).

The reviewed literature clearly indicates that Enalapril Maleate is a chemically sensitive drug whose stability is significantly influenced by its pH microenvironment, solvent composition, and exposure to moisture. While prior studies have thoroughly characterized its degradation pathways—particularly hydrolysis to enalaprilat and cyclization to DKP—most have concentrated on solid-state systems or short-term aqueous suspensions, primarily analyzed using HPLC and thermoanalytical techniques. However, a notable gap exists regarding the stabilization of Enalapril in extemporaneous liquid preparations, particularly concerning functional excipients such as propylene glycol. Furthermore, despite the established sensitivity of FTIR to structural changes, no previous research has investigated its quantitative application for monitoring solution-phase degradation of Enalapril.

Therefore, the present study aims to expand upon these mechanistic insights by examining whether propylene glycol can modify the aqueous microenvironment to mitigate hydrolytic degradation, while concurrently validating FTIR as a rapid, practical, and stability-indicating analytical tool. This integrated approach addresses a critical unmet pharmaceutical need: the development of more stable, accessible, and analytically manageable liquid formulations of Enalapril Maleate for routine clinical compounding.

Chapter Three:

Methodology:

3.1 Study Approach

This chapter presents an overview of the methodology employed in this study. It delineates the experimental design, preparation procedures, and analytical techniques used to investigate the stability and hydrolysis of enalapril maleate. Additionally, it describes the method validation process undertaken to ensure accuracy and reliability, along with the protocols followed for data collection and analysis. Subsequent sections will provide a detailed elaboration of these methodologies.

3.2 Materials

The following raw materials, reagents, and solvents were utilized in the study:

- **Enalapril maleate (raw powder)**, sourced from Jerusalem Pharmaceuticals, Palestine.
- **Enalapril maleate tablets** (20 mg each), obtained as Enaladex®, Dexxel Pharma, Palestine.
- **Distilled water**, produced using an MRC distilled water purification system.
- **Hydrochloric acid (HCl, 37%)**, supplied by Sigma-Aldrich.
- **Sodium hydroxide (NaOH, pellets)**, also from Sigma-Aldrich.
- **Phosphoric acid**, provided by Sigma-Aldrich.
- **Acetonitrile (HPLC grade)**, sourced from Sigma-Aldrich.
- **Methanol (HPLC grade)**, obtained from Sigma-Aldrich.
- **Ethanol (absolute)**, supplied by Sigma-Aldrich.
- **Monobasic sodium phosphate**, acquired from Sigma-Aldrich (for phosphate buffer, pH 2.2).

- **Propylene glycol (99% pure)**, obtained from Sigma-Aldrich.
- **Simple syrup (85%)**, prepared extemporaneously.

3.3 Laboratory Tools and Accessories

The following laboratory glassware and equipment were utilized for preparing and handling solutions:

- **Glassware**: Beakers, flasks, graduated cylinders, and volumetric pipettes for solution preparation.
- **Hotplate with magnetic stirrer**: Used for dissolving and mixing solutions.
- **Spatulas and weighing dishes**: For handling powdered samples.
- **Analytical balance**: With ±0.1 mg precision for weighing materials and excipients.
- **pH meter**: Calibrated for adjusting the pH of solutions.
- **Sonicator**: Ensures complete dissolution of drugs and excipients.
- **Vortex mixer**: For homogenizing solutions.
- **Silicon ATR window**: For FTIR measurements.
- **Amber glass bottles**: For sample storage.
- **Micropipettes**: Ranging from 5–1000 µL with sterile tips for accurate sample transfer.
- **Filtration system**: Includes a vacuum pump, filter apparatus, filter syringes, and filters with a 0.45 µm pore size for buffer and sample preparation.
- **Sample vials**: Available in amber and clear, HPLC grade, for storage and injection.

3.4 Analytical Instruments

The primary analytical instruments used in this study included:

- **FTIR spectrophotometer**: With ATR accessory (Bruker, Germany; equipped with a silicon windows).
- **HPLC System**: (Shimadzu), featuring a UV detector for quantitative analysis and method validation.

3.5 Methods/Experimental Procedure

3.5.1 Calibration Curve and Validation

3.5.1.1 FTIR/ATR Calibration Curve

A stock solution of enalapril maleate was prepared by dissolving 120 mg in 10 mL of ethanol, resulting in a concentration of 12 mg/mL. Dilutions were then made to achieve concentrations of 10, 8, 6, 4, and 2 mg/mL. Each dilution (with a final volume of 1.5 mL) was placed into

Eppendorf tubes and dried on the ATR crystal. Calibration curves were generated by plotting absorbance against concentration, confirming linearity within the tested range.

3.5.1.2 HPLC Calibration Curve

A stock solution was prepared by dissolving 150 mg of enalapril maleate in 100 mL of phosphate buffer at pH 2.2, yielding a concentration of 1.5 mg/mL. Dilutions of 0.1, 0.2, 0.4, 0.7, and 1.0 mg/mL were prepared in 25 mL volumetric flasks using micropipettes. Each solution was injected in triplicate into the HPLC system, and calibration curves were constructed by plotting concentration against peak area**.**

3.5.2 Reference Spectra and Assay

3.5.2.1 FTIR Functional Group Characterization

The powdered enalapril maleate was analyzed directly in the solid state using ATR-FTIR. The resulting spectrum served as a reference fingerprint for identifying functional groups.

3.5.2.2 HPLC Assay of Enalapril (Standard vs. Tablet)

A standard solution was prepared with a concentration of 0.2 mg/mL enalapril maleate in buffer.

The tablet solution was created by powdering ten Enaladex® tablets (20 mg each), suspending the powder in buffer, and processing according to USP guidelines. This involved sonication for 15 minutes, shaking for 30 minutes, an additional 30 minutes of sonication, and finally, filtration using a 0.45 µm filter. The final solution was adjusted to a concentration of 0.2 mg/mL.
The retention times for both the tablet and the standard were approximately 3.9 minutes, and the assay confirmed that the content was within the USP acceptance range of 98–102%.

3.5.3 Solvent Effect and Structural Changes

Two model solutions, each at a concentration of 4 mg/mL, were prepared:

1. Enalapril in water.
2. Enalapril in a 10% propylene glycol solution.

The spectra obtained were compared with that of the raw powder to assess solubility and peak definition.

3.5.4 Kinetic Degradation Studies

3.5.4.1 FTIR Alkaline Hydrolysis (Aqueous) 0.1 M NaOH

Samples of 1 mg/mL enalapril maleate in water were treated with 0.1 M NaOH. Aliquots (200 µL) were collected at 0, 10, and 60 minutes, neutralized with HCl, dried on an ATR crystal, and subsequently analyzed.

3.5.4.2 FTIR Alkaline Hydrolysis (10% PG) 0.1 M NaOH

Samples of 1 mg/mL enalapril maleate in a 10% propylene glycol (PG) aqueous solution were treated with 0.1 M NaOH. Aliquots were collected at 0, 10, 60, and 90 minutes, neutralized with HCl, dried overnight on silicon windows, and analyzed.

3.5.4.3 HPLC: Effect of Propylene Glycol Concentration on Alkaline Degradation (0.2 M NaOH)

Enalapril maleate solutions were prepared at a concentration of 1 mg/mL and exposed to 0.2 M NaOH. To evaluate the stabilizing effect of propylene glycol (PG), various concentrations of PG were tested, resulting in the following adjusted drug concentrations:

- 10% PG: 5.3 mg/mL.
- 15% PG: 4.0 mg/mL.

Aliquots were withdrawn at scheduled time points, neutralized, diluted with buffer, and analyzed using HPLC.

3.5.4.4 HPLC: Kinetic Degradation in 10% PG

Two independent experiments were conducted using 1 mg/mL enalapril maleate in a 10% PG solution:

- Experiment A: Samples were exposed to 0.1 M NaOH, with aliquots taken at predetermined times, neutralized, diluted, and analyzed.
- Experiment B: Samples were exposed to 0.001 M NaOH and treated in an identical manner.

3.5.5 Homogeneity Studies (Extemporaneous Formulations)

3.5.5.1 FTIR Homogeneity

Two Formulations (10 tablets each, 20 mg/tablet):

- **F1:** 50 mL distilled water.
- **F2:** 5 mL propylene glycol (PG) + water to 50 mL.
  Aliquots were taken from the upper and lower layers, dried overnight on silicon windows, and subsequently analyzed.

3.5.5.2 HPLC Homogeneity

Three Formulations (10 tablets each, 20 mg/tablet):

- **F1 (Aqueous):** 100 mL distilled water.
- **F2 (PG):** 90 mL water + 10 mL PG.
- **F3 (PG + Syrup):** 67 mL water + 10 mL PG + 23 mL syrup (85%).
  Aliquots were collected from different layers (upper/middle/lower for F1; upper only for F2 and F3). Each 1 mL sample was diluted to 10 mL with buffer and injected, resulting in a final concentration of 1 mg/mL.

Formulation Preparation Procedure:

For each formulation, ten enalapril maleate tablets (20 mg/tablet) were accurately weighed and finely triturated using a mortar and pestle. The resulting powder was transferred to an amber glass bottle. For the aqueous formulation (F1), 100 mL (50 mL in the FTIR case) of distilled water was added while continuously shaking until a uniform mixture was achieved. In the propylene glycol-containing formulation (F2), propylene glycol was initially mixed with the triturated powder to form a smooth paste, followed by the gradual addition of distilled water to achieve a final volume of 100 mL. For the formulation containing both propylene glycol and syrup (F3), the powder was initially combined with propylene glycol to ensure wetting and partial solubilization, after which syrup was added, followed by distilled water to reach the final volume. All formulations were thoroughly shaken to ensure uniform dispersion prior to aliquot collection.

Rationale for Formulation Selection:

The three formulations were chosen to represent clinically relevant extemporaneous preparations:

- **Aqueous solution** served as the baseline vehicle (Simulation of regular pharmacy practice).
- **Propylene glycol (PG)** was included due to its common application as a co-solvent, potentially providing stabilizing effects against hydrolysis.
- **Propylene glycol + syrup** represented a pediatric-friendly vehicle, where syrup enhances palatability while PG improves solubility and stability.

These formulations enabled a systematic evaluation of enalapril maleate stability under various vehicle conditions, closely simulating real-world pharmaceutical compounding scenarios.

3.5.6 Analytical Procedures

FTIR/ATR Analysis:

Spectroscopic analysis was performed using a Bruker FTIR system equipped with an Attenuated Total Reflectance (ATR) accessory. Spectra were recorded over the range of 4000 to 400 cm^{-1} with a resolution of 4 cm^{-1}, averaging 60 scans per sample. For calibration curve samples prepared in ethanol, 1.5 µL aliquots were directly applied to the ATR crystal and allowed to dry for approximately 2 minutes before measurement whereas kinetic sample were allowed to dry for approximately 20 minutes prior to analysis (Chrisikou et al., 2020). Stability and forced degradation samples prepared in aqueous or propylene glycol media had 20 µL aliquots placed on silicon windows, which were permitted to dry overnight prior to measurement. Silicon windows were specifically employed for samples containing propylene glycol to ensure uniform film formation.

HPLC Analysis:

The quantitative analysis of enalapril maleate was conducted based on the official USP method. A Shimadzu HPLC system equipped with a UV detector set to 215 nm was utilized for this analysis. Separation was achieved using an L7 column (C8, 4.6 mm × 25 cm, 5 µm particle size) maintained at 50 °C. The mobile phase consisted of a 75:25 (v/v) mixture of phosphate buffer (pH 2.2) and acetonitrile, delivered at a flow rate of 2.0 mL/min. The injection volume was 50 µL, and all solutions were filtered through 0.45 µm filters prior to injection.

Data Processing and Plotting:

All graphical representations, including calibration curves, degradation profiles, and comparative stability charts, were generated using OriginPro software (OriginPro, 2024). This software facilitated curve fitting, regression analysis, and data visualization, ensuring accurate presentation of results.

Chapter Four:

Results and Discussion

4.1 Introduction

This chapter presents the experimental findings and their interpretation, situating the results within the broader context of enalapril maleate stability in chemical and pharmaceutical applications. The analysis begins with the calibration and validation of analytical methods, progresses through spectroscopic and chromatographic characterization, and concludes with an evaluation of degradation kinetics and formulation homogeneity.

Enalapril maleate is recognized for its chemical lability in aqueous environments, where it undergoes hydrolysis of its ethyl ester moiety to produce enalaprilat. In certain instances, this process can also yield diketopiperazine (DKP) via intramolecular cyclization. These degradation pathways can undermine therapeutic efficacy and pharmaceutical quality, particularly in extemporaneous formulations derived from crushed tablets. Additionally, practical challenges such as stratification, uneven dosing, and chemical instability are prevalent in water-based vehicles used for pediatric suspensions.

In this context, the chapter addresses three interconnected questions:

- Can FTIR/ATR spectroscopy, when properly calibrated, provide quantitative stability monitoring comparable to HPLC, the reference method?
- How does propylene glycol (PG), a widely used pharmaceutical co-solvent, impact the hydrolytic stability and physical uniformity of enalapril suspensions?
- What are the mechanistic links between molecular functional groups, solvent environment, and the observed kinetic profiles?

By integrating FTIR and HPLC data with mechanistic chemical reasoning, this chapter not only validates analytical approaches but also offers valuable insights into practical compounding strategies.

4.2 Calibration Curve by FTIR and Validation by HPLC

4.2.1 FTIR Calibration Curve of Enalapril Maleate (2–12 mg/mL in Ethanol)

The ATR-FTIR spectra of enalapril maleate solutions prepared in ethanol, covering a concentration range of 2–12 mg/mL, are presented in Figure 4.1. A distinct ester carbonyl absorption was observed at 1741 cm^{-1} (Wang et al., 2004), exhibiting a sharp and well-defined profile in ethanol. This band was selected as the analytical marker for this study and was subsequently used for calibration and stability monitoring.

In the solid state, this absorption typically manifests as two bands at 1751 cm^{-1} (ester C=O) and 1726 cm^{-1} (carboxylic acid C=O). However, upon dissolution in ethanol, these bands merged into a single sharp peak at 1741 cm^{-1}.

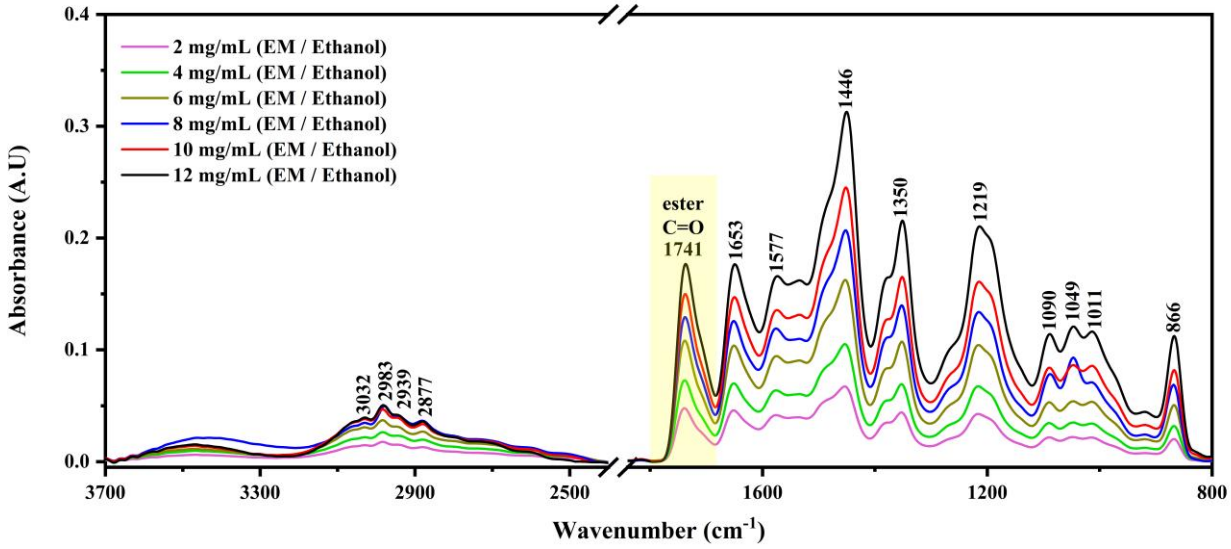

Figure 4.1: ATR-FTIR spectra of enalapril maleate solutions in ethanol (2–12 mg/mL)

The absorbance at **1741 cm^{-1}** increased proportionally with concentration, confirming adherence to the Beer–Lambert law. The calibration curve (Figure 4.2) demonstrated excellent linearity ($R^2 >$ 0.99), indicating reliable quantitative performance across the tested range. Importantly, no saturation effects were observed on the ATR crystal, which supports the robustness of the method.

Ethanol was chosen as the solvent due to its rapid evaporation on the ATR crystal, which enabled efficient sample drying prior to spectral acquisition. This characteristic made ethanol more appropriate for ATR-FTIR measurements compared to water. Furthermore, the use of ethanol reduced spectral interferences, facilitating the acquisition of clear and well-defined carbonyl

bands, whereas aqueous solutions displayed intense O–H stretching absorptions that overlapped with and obscured the carbonyl region. The suitability of ethanol as a solvent for the FTIR characterization of enalapril has been documented in the literature (Chrisikou et al., 2020).

The calibration confirmed that FTIR, when combined with appropriate solvent selection and careful peak assignment, can be extended from qualitative fingerprinting to **quantitative stability monitoring**. This approach offers a cost-effective and rapid preliminary tool for degradation studies, complementing confirmatory HPLC analysis.

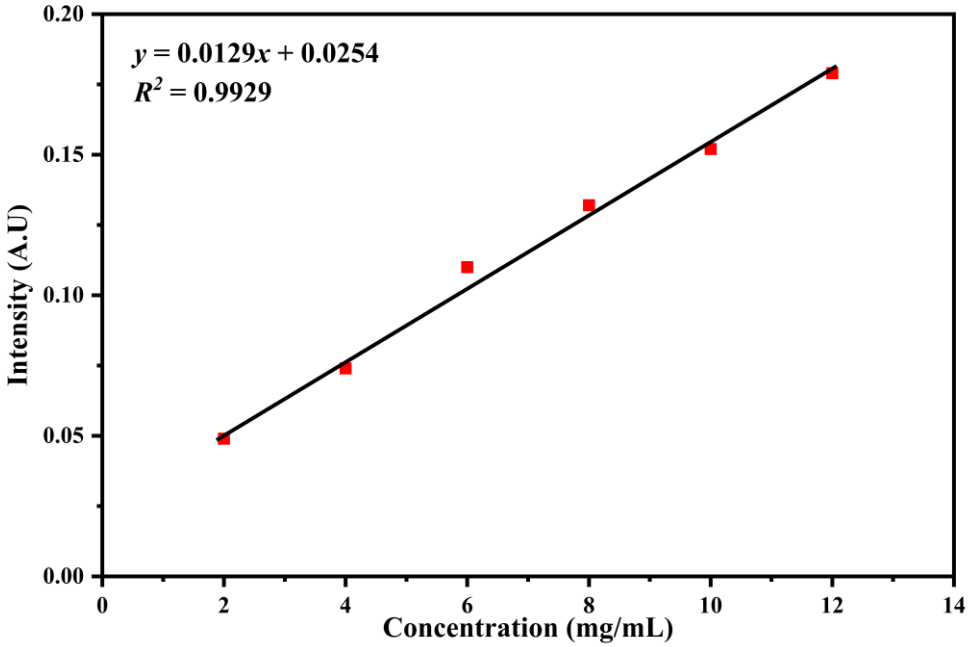

Figure 4.2: FTIR/ATR Calibration curve displaying the absorbance at 1741 cm^{-1} in relation to the concentration of enalapril maleate (2–12 mg/mL in ethanol)

4.2.2 HPLC Calibration Curve of Enalapril Maleate (0.1–1.5 mg/mL)

The HPLC calibration curve for enalapril maleate was established over a concentration range of 0.1–1.5 mg/mL (Figure 4.3). The plot demonstrated near-perfect linearity, with an R^2 value greater than 0.999, confirming the method's precision and reliability. This strong linearity reflects both the detector's sensitivity and the optimization of chromatographic conditions.

HPLC was selected as the reference analytical method because it effectively separates the parent drug, enalapril, from its hydrolytic products, including enalaprilat and diketopiperazine (DKP). In contrast to FTIR, which measures the collective absorption of functional groups, HPLC distinguishes individual molecules based on their polarity and retention time. This capability

establishes HPLC as the reference method for stability-indicating assays, particularly in complex degradation studies.

The chemical form of the drug also enhanced the robustness of the calibration. The maleate salt of enalapril improved aqueous solubility, ensuring complete dissolution in the mobile phase and preventing variability that could compromise linearity.

When comparing the HPLC calibration to FTIR, both techniques exhibited excellent linearity, though their mechanistic implications differ. FTIR quantifies functional group vibrations, providing rapid, indirect monitoring of drug concentration, while HPLC directly measures the intact parent compound, serving as a confirmatory and regulatory-grade method. Together, they provide complementary insights: FTIR acts as a fast screening tool, while HPLC offers validated, stability-indicating quantification.

In summary, the results establish the concentration range of 0.1–1.5 mg/mL as both scientifically justified and pharmaceutically practical. This range encompasses the expected concentrations encountered during degradation studies and meets regulatory expectations for assay linearity, reinforcing HPLC as the analytical cornerstone of this study.

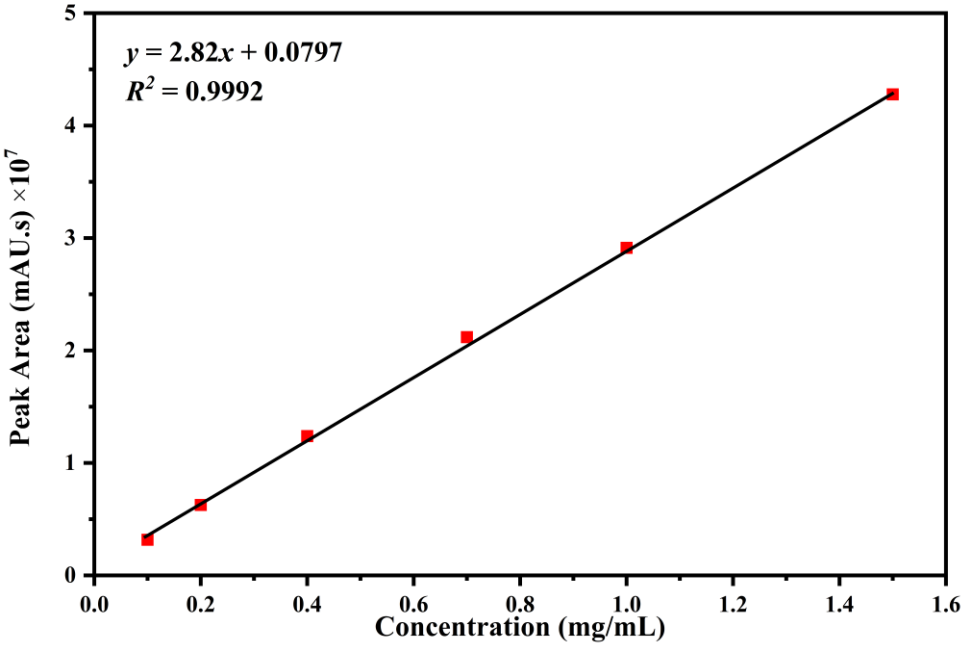

Figure 4.3: HPLC Calibration Curve for Enalapril Maleate (Concentration Range: 0.1 – 1.5 mg/mL)

4.3 Reference Spectra and Assay

4.3.1 FTIR Characterization of Functional Groups in Pure Enalapril Maleate

Figure 4.4 illustrates the ATR-FTIR spectrum of pure enalapril maleate, utilized to establish the baseline fingerprint of the drug. In the solid state, the carbonyl region typically displays multiple absorptions resulting from the presence of ester, carboxylic acid, and amide carbonyl groups, each contributing unique vibrational bands.

The characteristic wavenumbers of pure enalapril maleate are summarized in Table 4.1, with each absorption assigned to its corresponding functional group. This provides a comprehensive vibrational fingerprint of the compound, while the 1741 cm^{-1} band was prioritized for quantitative evaluation due to its sharpness and reproducibility.

It is important to note that while Figure 4.4 presents the spectrum in its standard absorbance format, the precise peak positions reported in Table 4.1, the position (wavenumber) of these bands were assigned using the method of second derivative, which improved the resolution of overlapping signals and ensured accurate assignments (S.-Y. Lin et al., 2004; Refat et al., 2014; Srinivasarao & Gopinath, 2021; Widjaja et al., 2007).

Table 4.1: Characteristic FTIR absorption bands of pure enalapril maleate with their corresponding functional group assignments, determined by second derivative peak picking (S.-Y. Lin et al., 2004; Refat et al., 2014; Srinivasarao & Gopinath, 2021; Widjaja et al., 2007)

Peak position (cm^{-1})	Functional Groups
3211	Stretching vibration of N–H band of aromatic ring
3024	Stretching vibration of C-H band of aromatic ring
2980	Asymmetric CH_3 stretching vibration
2928	Asymmetric CH_2 stretching vibration
1751	Carbonyl stretching of ester
1726	Carbonyl stretching of carboxylic acid
1648	Carbonyl stretching of tertiary amide
1598	Asymmetric stretching of carboxylate
1572	Carboxylate of maleate and/or ring mode of benzene
1496	Carboxylate of maleate and/or ring mode of benzene
1448	CH_2 scissoring
1379	C-H bending/symmetric carboxylate
1360	Symmetric stretching of carboxylate
1299	C-N stretching (L-proline) vibration
1270	C-N stretching (L-proline) vibration
1226	C-C-O stretching band of acetate
1190	C -C-O stretching band of ester
874	C-H out-of-plane bending

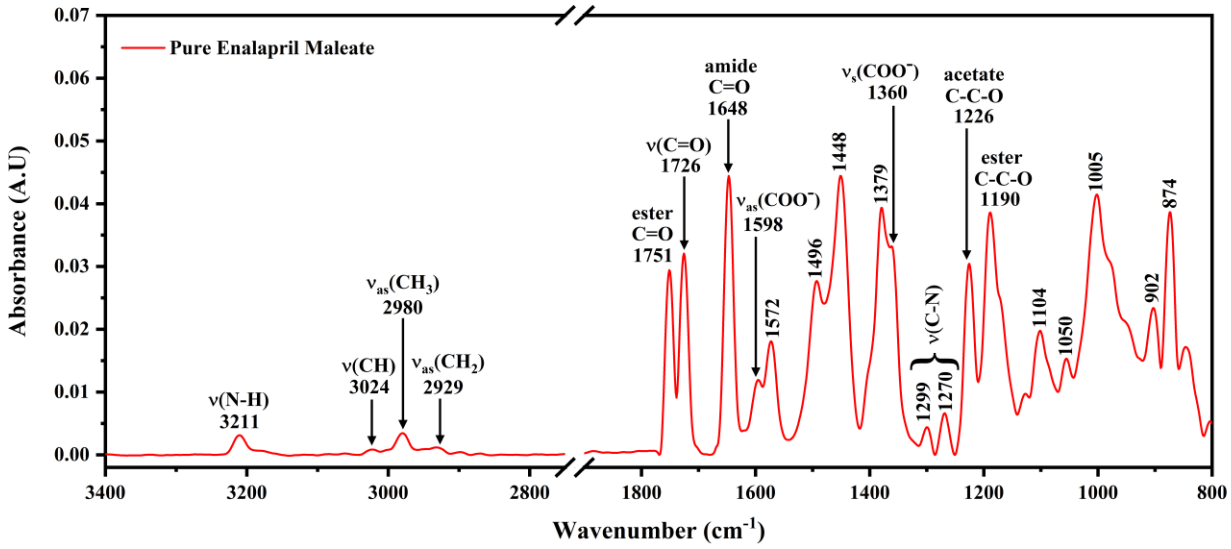

Figure 4.4: ATR-FTIR Spectrum of Pure Enalapril Maleate

4.3.2 HPLC Assay of Enalapril (Standard vs. Tablet)

The HPLC chromatogram for the enalapril maleate standard and Tablet solution is illustrated in Figure 4.5. The average peak area for the standard, calculated from three replicate injections (n = 3), was determined to be 6,262,361. In comparison, the peak area for the tablet solution analyzed under the same conditions was recorded at 6,264,756. This comparison yielded a recovery of 100.04%, which falls within the USP acceptance criteria of 90–110%, thereby confirming compliance with compendial standards.

Both the standard and tablet samples exhibited consistent retention times of approximately 3.8–3.9 minutes. This consistency signifies method specificity, indicating that the tablet excipients did not interfere with the enalapril signal. The similarity in peak areas further corroborates that the assay procedure effectively liberated enalapril from the tablet matrix without any significant degradation during preparation.

These results affirm that the assay method is accurate, specific, and robust. From a pharmaceutical perspective, this outcome is critical; ensuring that extemporaneous formulations derived from crushed tablets contain the correct labelled dose is essential for safe and effective therapy.

The observed peak broadening may be associated with the ionizable nature of enalapril maleate in aqueous media. Several mobile phase types and compositions were evaluated; however, the selected HPLC method provided the best overall chromatographic performance. Ionizable compounds may exist in multiple ionization states during drug separation, which can result in broadened peaks despite appropriate method optimization (Snyder et al., 2010; Dong, 2019).

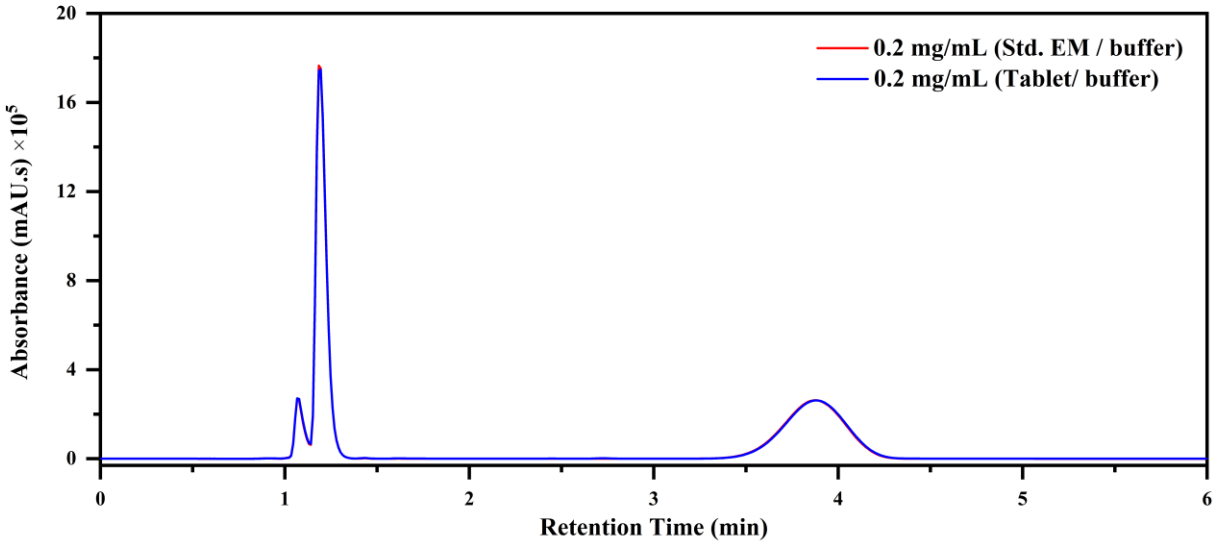

Figure 4.5: HPLC Chromatograms of Standard Solution (0.2 mg/mL)

4.4 Solvent Effect and Structural Changes

4.4.1 Structural Changes of Carbonyl Bands

Enalapril maleate is a prodrug distinguished by the presence of an ester functional group. Upon hydrolysis, the ester bond is cleaved, yielding the active diacid form, enalaprilat, along with the corresponding enalapril alcohol derivative, as illustrated in Equation 4.1. The overall reaction can be represented as follows:

Ester (RCOOR') + H_2O → Carboxylic acid (RCOOH) + Alcohol (R'OH) (Equation 4.1)

Hydrolysis specifically targets the ester carbonyl group; thus, monitoring the C=O stretching region in FTIR spectra serves as a sensitive indicator of structural degradation. The progressive decrease and eventual disappearance of the ester carbonyl band signify the consumption of the ester functionality during hydrolysis.

Upon dissolution in ethanol and propylene glycol, the two distinct carbonyl bands observed in the solid state at 1751 and 1726 cm^{-1} merged into a single, sharper band near 1743 cm^{-1}. This merging is attributed to solvent–solute hydrogen bonding, as both ethanol and propylene glycol contain hydroxyl groups that serve as hydrogen bond donors to the carbonyl oxygens of enalapril maleate. These interactions homogenize the local carbonyl environments associated with the ester and maleate moieties, leading to a reduction in vibrational differentiation and resulting in a dominant absorption peak in solution.

This reproducible spectral behavior offers a robust analytical marker for quantifying hydrolysis, with the 1741 cm^{-1} band designated as the primary indicator of ester bond integrity throughout the kinetic study.

The carbonyl stretching region of enalapril maleate was analyzed in three states: solid (powder), aqueous solution, and aqueous solution with 10% (PG) (Figure 4.6).

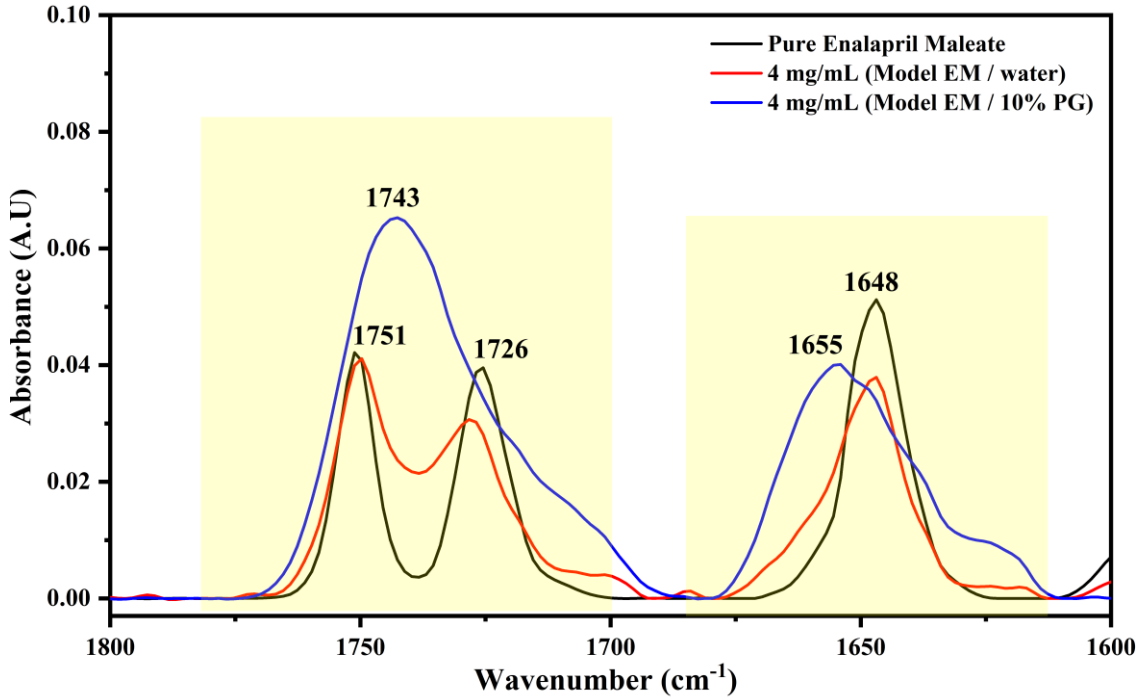

Figure 4.6: Comparative FTIR spectra of enalapril maleate in powder, aqueous solution, and aqueous solution with 10% propylene glycol (PG)

The powder spectrum reveals two distinct peaks at 1751 cm^{-1}, corresponding to the ester C=O stretching, and 1726 cm^{-1}, associated with the carboxylic acid C=O stretching. This band splitting is frequently observed in crystalline systems and may be attributed to lattice packing interactions that create slightly different microenvironments for the carbonyl functionalities, resulting in multiple, yet well-defined, vibrational states (Widjaja et al., 2007).

Upon dissolution in water, both carbonyl peaks remained detectable and retained their original positions, with only a slight merging of intensities. Importantly, no significant wavenumber shift was observed, indicating that aqueous solvation does not markedly perturb the local vibrational environment of the ester or acid groups. This observation indicates that aqueous solvation does not facilitate strong, specific interactions with the carbonyl moieties.

By contrast, in the **PG-containing system**, the two carbonyl peaks observed in the powder and aqueous states at 1751 cm^{-1} and 1726 cm^{-1} **merged into a single sharp band at 1743 cm^{-1}**,

which is primarily assigned to ester C=O stretching. This merging indicates a reorganization of the carbonyl vibrational environment due to specific interactions with PG. The hydroxyl groups of PG function as hydrogen bond donors, selectively associating with the ester and carboxyl carbonyl oxygens. Such interactions result in a homogenization of the vibrational response, producing a sharper and more reproducible spectral marker compared to the broader features observed in the aqueous medium.

Furthermore, the tertiary amide C=O band, consistently observed at 1648 cm^{-1} in both solid-state and aqueous spectra, exhibited a positive shift to 1655 cm^{-1} (+7 cm^{-1}) in the propylene glycol-containing system. Within the context of this study, this spectral shift may be attributed to the involvement of the amide carbonyl group in solvent–solute interactions. The polar and dipolar characteristics of propylene glycol likely modify the local vibrational environment of the amide functionality, resulting in the observed frequency shift. These findings align with previous reports that describe the high solubilizing capacity of propylene glycol and its ability to engage in intermolecular interactions comparable to those observed with ethanol (S. Y. Lin & Wang, 2012; Wang et al., 2004).

The comparison between water and propylene glycol (PG) reveals that while water retained a powder-like spectral pattern with minimal perturbations, PG induced significant alterations, specifically peak merging and measurable shifts that underscore its direct interaction with the functional groups of enalapril maleate. Consequently, PG systems yield a well-defined, reproducible signal at 1743 cm^{-1}, which is particularly advantageous for kinetic monitoring.

In addition to assessing peak positions, a quantitative evaluation was performed by comparing the integrated band areas of the ester and amide C=O vibrations in water versus PG systems. The calculated Δ values (Table 4.2) represent the area and differences in area ($\Delta A = A_{EM/water} - A_{EM/PG}$), thereby reflecting the extent of solvent-induced spectral changes. For the ester C=O band, the area significantly increased substantially in PG ($\Delta A = +1.053$), indicating enhanced stabilization and improved band definition due to selective hydrogen bonding. Similarly, the amide C=O band showed a moderate increase ($\Delta A = +0.519$), confirming that PG not only shifted the band position but also intensified its vibrational response. These ΔA values provide quantitative support for the qualitative interpretation of peak merging and sharpening, demonstrating PG's role in establishing a more defined solvation environment.

Table 4.2: Peak positions and integrated band areas of ester and amide C=O stretching vibrations of enalapril maleate in different systems (water, and PG) by FTIR, with calculated Δ values indicating solvent effects

Band	$A_{EM/Water}$	$A_{EM/PG}$	$\Delta A = A_{EM/Water} - A_{EM/PG}$
Ester C=O	1.197	2.250	1.053
Amide C=O	0.761	1.280	0.519

4.5 Kinetic Degradation Studies

4.5.1 FTIR Alkaline Hydrolysis of Enalapril Maleate in Aqueous Media

The FTIR spectra of enalapril maleate (10 mg/mL) in 0.1 M NaOH, recorded at intervals of 0, 10, and 60 minutes, are presented in Figure 4.7. At the initial time point, the spectrum displayed a prominent ester carbonyl peak at 1741 cm^{-1}, indicating the presence of the intact prodrug. Over the duration of the experiment, the intensity of this peak gradually diminished, nearly disappearing by the 60-minute mark. This transformation can be explained by the classical mechanism of base-catalyzed hydrolysis. In an alkaline environment, hydroxide ions function as nucleophiles, attacking the carbonyl carbon of the ester group. This interaction results in the formation of a tetrahedral intermediate, which subsequently collapses, cleaving the ester bond and releasing ethoxide. The resulting product, enalaprilat, is the diacid metabolite and is fully hydrophilic in comparison to the parent ester.

The FTIR results reflect this chemical transformation directly. The disappearance of the ester C=O peak at 1741 cm^{-1} indicates the loss of prodrug functionality.

From a pharmaceutical perspective, the rapid degradation observed in this experiment underscores enalapril's instability in aqueous solutions. Hydrolysis occurs swiftly, effectively eliminating the ester group within one hour, and rendering water-based formulations unsuitable for extemporaneous compounding.

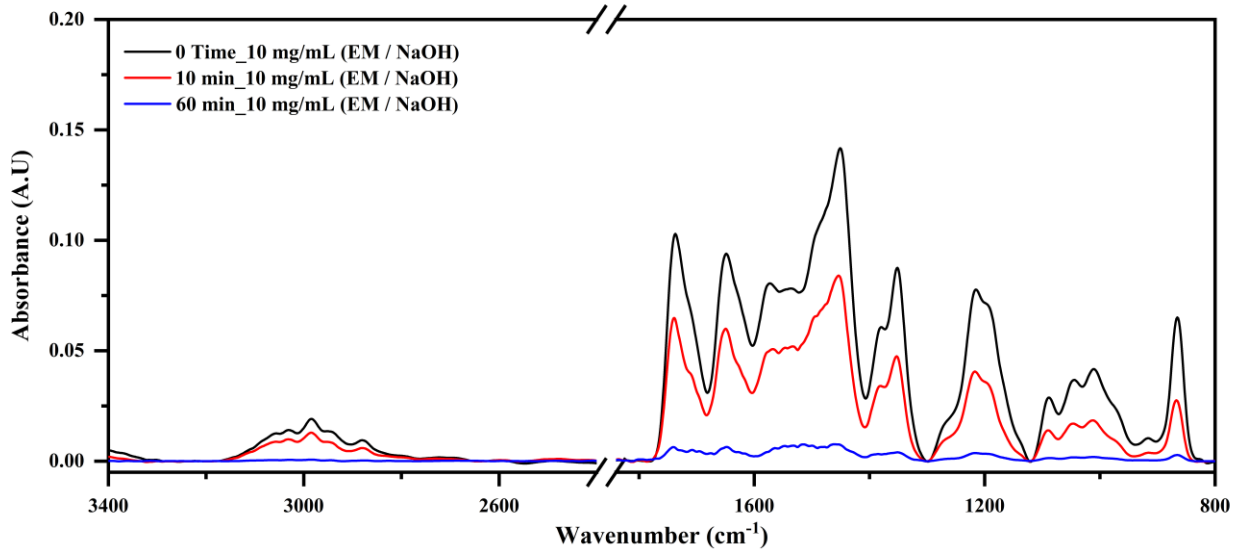

Figure 4.7: FTIR spectra of enalapril maleate (10 mg/mL) in 0.1 M NaOH at 0, 10, and 60 minutes

4.5.2 FTIR Alkaline Hydrolysis of Enalapril Maleate in 10% Propylene Glycol system

The alkaline hydrolysis profile of enalapril maleate (10 mg/mL in 0.1 M NaOH) dissolved in a 10% propylene glycol (PG) vehicle is presented in Figure 4.8 and Table 4.3. The absorption of the ester carbonyl, previously identified as the kinetic marker, was monitored at 0, 10, 60, and 90 minutes. In contrast to the purely aqueous medium, the ester peak remained stable in the PG system throughout the duration of the experiment. Even after 90 minutes, the signal showed no meaningful decline up to 90 min. Values slightly exceeding 100% (Table 4.3) are attributed to normal analytical variability (e.g., ATR deposition and baseline effects) rather than a genuine increase in drug content.

Several solvent effects contribute to the stability of enalapril maleate observed in the PG system. First, PG may reduce the effective water activity, thereby limiting the availability of "free" water molecules that could attack the ester group. Second, the increased viscosity of the medium slows the mobility of hydroxide ions, which reduces their interaction with the ester carbonyl. Third, PG provides specific solvation; its hydroxyl groups form stabilizing hydrogen bonds with the ester and amide moieties of enalapril, effectively shielding them from nucleophilic attack. Finally, the micro-heterogeneous environment created by PG modifies the effective polarity of the solution, supporting the persistence of the ester peak.

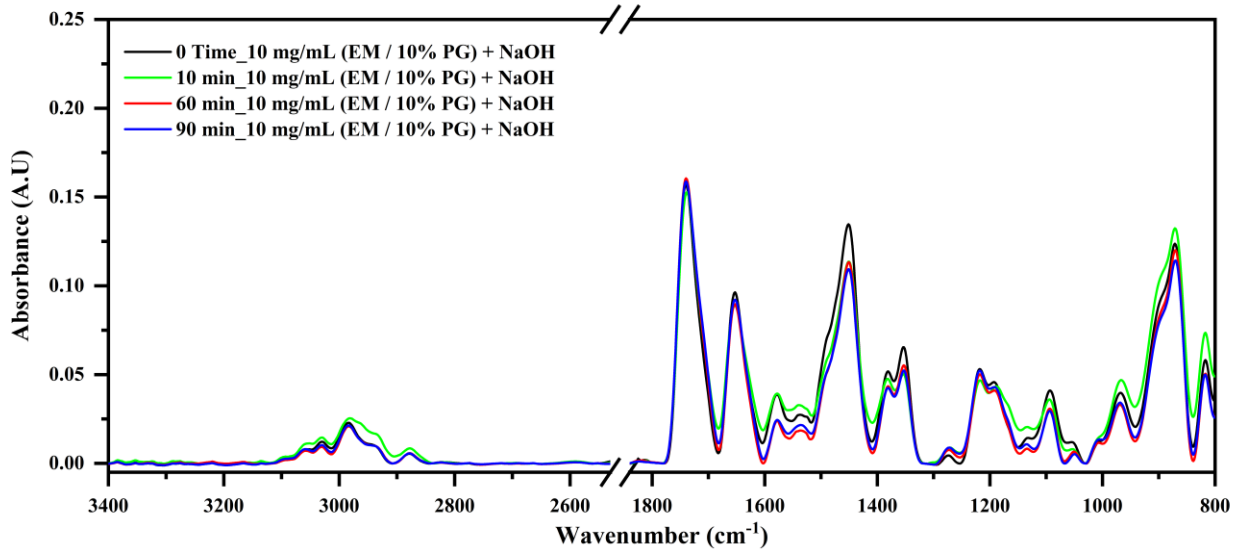

Figure 4.8: FTIR spectra of enalapril maleate (10 mg/mL) in 0.1 M NaOH with 10% propylene glycol at time intervals of 0, 10, 60, and 90 minutes

In the present study, the incorporation of propylene glycol led to a reduced extent of alkaline hydrolysis of enalapril, indicating improved formulation stability under basic conditions. From a pharmaceutical compounding perspective, this finding holds significant implications for extemporaneous preparations, as formulations containing propylene glycol supporting its

assessment as a stabilizing vehicle component. Literature reports (Nahata & Allen, 2008), have similarly indicated improvements in the stability of propylene glycol–containing extemporaneous liquid formulations compared to water-based vehicles.

Table 4.3: Percentage of enalapril maleate remaining over time in 0.1 M NaOH aqueous solution compared to a 10% PG and 0.1 M NaOH solution by FTIR

Time (min)	EM / NaOH	EM / NaOH+10% PG
0	60% ± 0.4%	102% ± 0.8%
10	34% ± 0.5%	95% ± 0.8%
60	0% ± 0.0%	103% ± 0.5%
90	0% ± 0.0%	102% ± 0.8%

4.5.3 Effect of Propylene Glycol Concentration on Alkaline Degradation in 0.2 M NaOH by HPLC

The impact of propylene glycol (PG) concentration on the alkaline hydrolysis of enalapril maleate in a 0.2 M NaOH solution is summarized in Table 4.4. Samples were analyzed at intervals of 10, 30, and 60 minutes with PG concentrations of 10% and 15%. The results demonstrate that an increase in PG concentration did not result in significant differences between the 10% and 15% formulations, indicating that 10% PG alone is adequate for stabilization.

The stabilization observed at higher PG levels can be attributed to several physicochemical factors. First, PG reduces the effective water activity, limiting the availability of free water molecules for nucleophilic attack on the ester carbonyl. Second, the increased viscosity at elevated PG concentrations impedes the diffusion of hydroxide ions, thereby decreasing the frequency of ester–OH^- collisions. Third, PG forms extensive hydrogen-bonding networks with the ester and amide groups, further protecting the ester moiety from hydrolytic attack. Collectively, these factors diminish the rate of base-catalyzed hydrolysis in a concentration-dependent manner.

Table 4.4: Effect of propylene glycol concentration (10+15%) on the percentage of enalapril maleate remaining over time (10, 30, and 60 min) in 0.2 M NaOH, determined by HPLC

Time (min)	10% PG	15% PG
10	111.16% ± 1.20%	102.34 % ± 0.80%
30	109.48% ± 1. 0%	101.22% ± 0.69%
60	103.86% ± 0.80%	102.35 % ± 0.70%

Mechanistically, these findings support the role of PG as a "hydrolysis damper." By combining reduced water activity, increased viscosity, and selective hydrogen bonding, PG provides a concentration-dependent protective effect against alkaline degradation. This underscores the practical utility of PG not only as a co-solvent but also as a stabilizing excipient capable of extending the effective stability window of enalapril formulations.

4.5.4 HPLC Chromatogram of Fully Hydrolyzed Sample

The HPLC chromatogram of enalapril maleate following complete alkaline hydrolysis is presented in Figure 4.9. The peak associated with enalapril, typically observed at a retention time (t_R) of 3.88 minute, is entirely absent in the hydrolyzed sample. Instead, a distinct peak corresponding to enalaprilat is clearly discernible at a retention time (t_R) of 1.6 minute. Under specific experimental conditions, an additional peak attributed to diketopiperazine (DKP), the intramolecular cyclization product of enalapril, may also be detected at a different retention time approximately 8 minute.

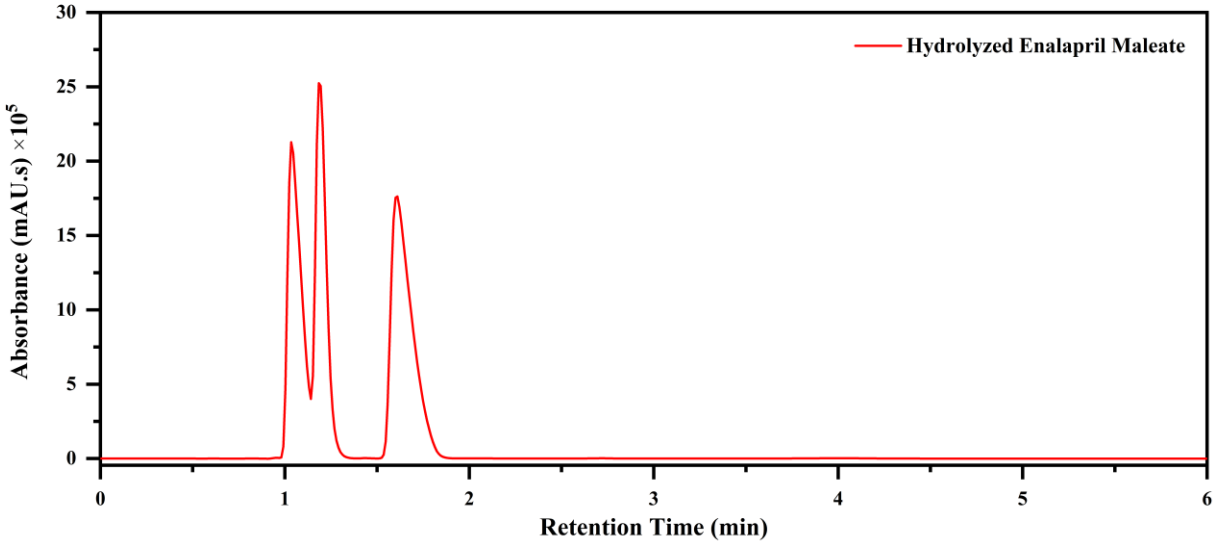

Figure 4.9: HPLC chromatogram of fully hydrolyzed enalapril sample demonstrating the disappearance of the parent peak (t_R1.6 min) and the appearance of enalaprilat

This chromatographic behavior highlights the chemical transformation of enalapril. The compound undergoes base-catalyzed ester hydrolysis, resulting in enalaprilat, which is more polar than the parent compound. Consequently, on a reversed-phase C8 column, the increased polarity of enalaprilat facilitates its earlier elution compared to enalapril, due to diminished hydrophobic interactions with the stationary phase. The absence of the enalapril peak, coupled with the emergence of enalaprilat, provides clear evidence of complete degradation and demonstrates the method's efficacy in distinguishing the parent drug from its degradation products.

From a pharmaceutical perspective, these findings affirm that the developed HPLC method is genuinely stability-indicating. The chromatogram not only quantifies intact enalapril but also separates and identifies its hydrolysis products. This selectivity ensures that the method can be reliably employed to monitor drug stability in compounded formulations and for regulatory-quality assays.

4.5.5 HPLC Kinetics ±10% PG at 0.2 M NaOH

Figures 4.10 and 4.11, along with Table 4.5, illustrate the comparative kinetic degradation of enalapril maleate in a strongly alkaline medium (0.2 M NaOH) both with and without the addition of 10% propylene glycol (PG). In the aqueous system devoid of PG, the parent enalapril peak (t_R 3.88 min) diminished rapidly, becoming nearly undetectable by 60 minutes, which indicates significant hydrolysis to enalaprilat. Conversely, the addition of 10% PG under the same alkaline conditions allowed the parent drug peak to retain nearly its initial intensity, correlating to approximately 100% recovery even after 60 minutes.

The protective effect of PG is corroborated by FTIR observations. PG reduces the effective water activity, thereby limiting the availability of free water molecules for nucleophilic attack. Furthermore, the increased viscosity of the PG-containing medium impedes the diffusion of hydroxide ions toward the ester carbonyl. PG also forms stabilizing hydrogen bonds with the ester and amide functionalities, which partially shields them from hydrolytic cleavage. These combined effects yield a shallower slope in the kinetic degradation curve, indicating a slower hydrolysis rate.

From a clinical and pharmaceutical perspective, the differences between the two systems are significant. In the absence of PG, enalapril is highly unstable under alkaline stress, decomposing nearly completely within one hour. However, with the incorporation of 10% PG, the drug remains virtually stable throughout the same time frame. This underscores PG's potential not only as a co-solvent but also as a stabilizing excipient that can significantly extend the shelf life of extemporaneous formulations.

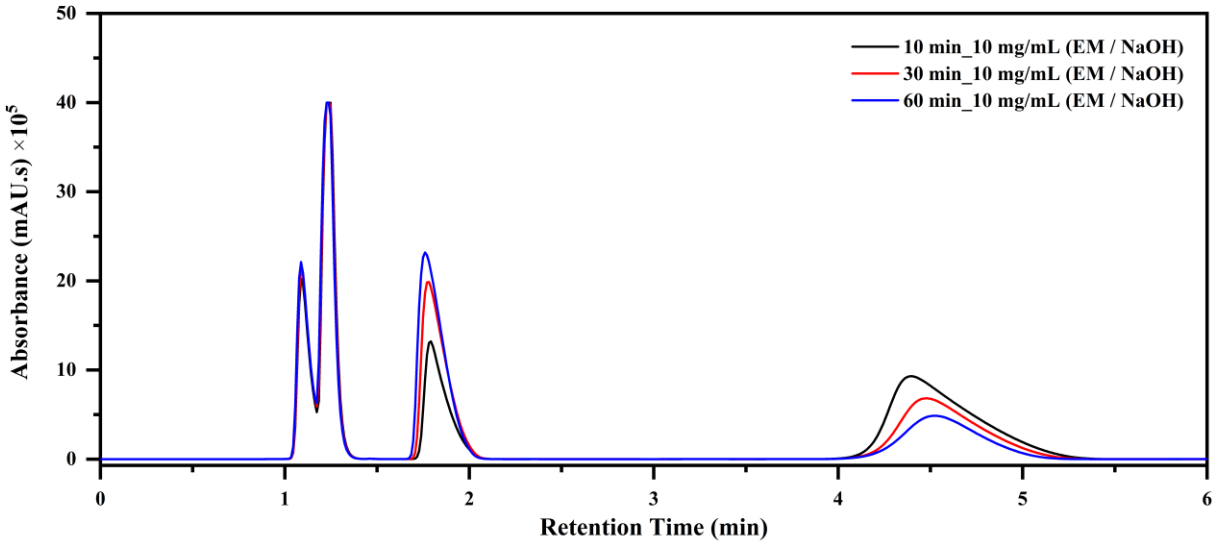

Figure 4.10: HPLC chromatogram of Degradation Profile of Enalapril (1 mg/mL) in 0.2 M NaOH as a function of Time

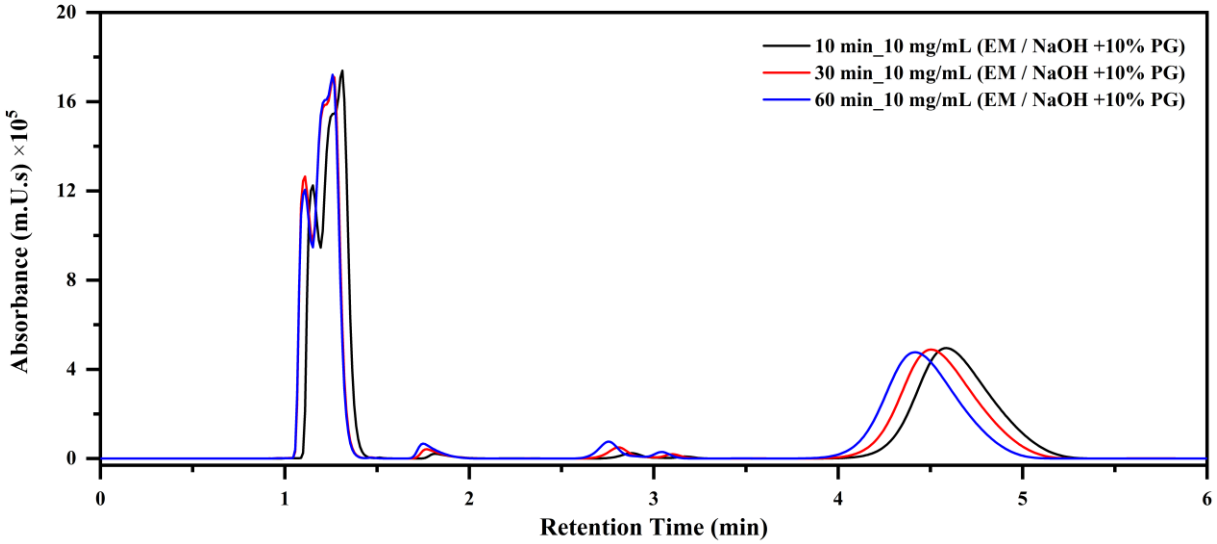

Figure 4.11: HPLC chromatogram of Degradation Profile of Enalapril (1 mg/mL) in 0.2 M NaOH Containing 10% Propylene Glycol as a Function of Time

Table 4.5: Percentage of enalapril maleate remaining over time in 0.2 M NaOH, with and without 10% PG, determined by HPLC

Time (min)	EM / NaOH	EM / NaOH +10% PG
0	116.26% ± 1.20%	111.16% ± 1.00%
10	80.20% ± 0.80%	109.47% ± 0.90%
60	-0.56% ± 0.10%	103.86% ± 0.80%

4.5.6 HPLC Kinetics and Degradation Behavior of Enalapril Maleate (1 mg/mL) under Alkaline Conditions: A Comparative Analysis of 0.1 M and 0.001 M NaOH, with 10% Propylene Glycol in the 0.001 M NaOH System

The degradation profiles of enalapril maleate (1 mg/mL) under two alkaline stress conditions (0.1 M and 0.001 M NaOH) are detailed in Table 4.6. Propylene glycol (10%) was included exclusively in the 0.001 M NaOH system to assess its potential stabilizing effect on the hydrolysis rate.

At a concentration of 0.1 M NaOH, hydrolysis was significant throughout the 60-minute observation period. In the aqueous NaOH system, the concentration of the parent drug decreased markedly, with **90.18% remaining at 10 minutes**, **23.77% at 30 minutes,** and only **15.13% at 60 minutes.** These results indicate rapid and extensive base-catalyzed cleavage of the ester linkage in enalapril maleate, **consistent with pseudo-first-order kinetics at elevated hydroxide** concentrations. The observed trend confirms that exposure to strong alkaline conditions leads to nearly complete degradation within one hour.

At **0.001 M NaOH**, degradation was minimal over the 90-minute period. Without PG, the drug exhibited a gradual decline, with **108.24% at 10 minutes**, **104.26% at 30 minutes**, **94.55% at 60 minutes**, and **67.10% at 90 minutes**. In the presence of PG, the parent drug remained remarkably stable, exhibiting **119.14% at 10 minutes**, **109.85% at 30 minutes**, **109.33% at 60 minutes**, and still above 100% (**102.45%**) at 90 minutes. The data support a sustained protective role of PG under mild alkaline conditions.

These results underscore the concentration-dependent nature of base-catalyzed ester hydrolysis. At a higher hydroxide concentration (0.1 M), the reaction proceeds rapidly, consistent with pseudo–first-order kinetics, whereas a reduction in hydroxide concentration (0.001 M) significantly decreases the hydrolysis rate. In both scenarios, PG mitigates the degradation process by lowering water activity, increasing viscosity, and forming stabilizing hydrogen bonds with functional groups in enalapril.

Table 4.6: Percentage of Enalapril Maleate Remaining Over Time in 0.1 M and 0.001 M NaOH Solutions, with 10% Propylene Glycol Included Only in the 0.001 M System, as Determined by HPLC

Time(min)	EM / (0.1 M) NaOH	EM / (0.001 M) NaOH	EM /(0.001 M) NaOH + 10% PG
10	90.18% ± 0.9 %	108.24% ± 0.9 %	119.14% ± 1.2 %
30	23.77% ± 0.7 %	104.26% ± 0.8 %	109.85% ± 1.00 %
60	15.13% ± 0.6 %	94.55% ± 0.9 %	109.33% ± 1.00 %
90	-	67.10% ± 1.00 %	102.45% ± 1.00 %

From a pharmaceutical perspective, these findings are highly pertinent. In practical extemporaneous preparations, products are typically stored at near-neutral to slightly acidic pH. Where the hydrolysis rate is naturally slower than under the alkaline stress conditions examined here. The observation that PG provides measurable stabilization even under mild alkaline stress strongly supports its inclusion in formulation strategies aimed at extending beyond-use dating (BUD) and ensuring consistent therapeutic dosing.

4.6 Results of Extemporaneous Formulations

4.6.1 FTIR Homogeneity Testing for Formulations

The homogeneity of extemporaneously prepared enalapril suspensions, formulated in either pure water or a 10% propylene glycol (PG) system, is demonstrated in Figures 4.12 and 4.13, as well as in Table 4.7.

In the aqueous suspension (10 tablets dispersed in 50 mL of water), the FTIR spectra from the upper and lower layers revealed significant differences. The upper layer exhibited an almost flat baseline with no significant ester C=O or amide absorptions, indicating a near absence of the drug. Conversely, the lower layer spectrum displayed detectable peaks, corresponding to approximately 12.5% of the expected drug content. This disparity indicates severe stratification, with the majority of the drug settling at the bottom of the container.

In the suspension containing PG (10% PG, same drug load), the FTIR spectra of the upper and lower layers were nearly identical. Both layers exhibited overlapping ester C=O and amide bands, suggesting that the drug was evenly distributed throughout the suspension. Quantitative data in Table 4.7 corroborated this finding: the upper layer retained approximately 100% of the drug, while the lower layer measured about 101.7%, virtually identical within the limits of analytical variability.

The underlying mechanism involves PG acting as a physical stabilizer. In water-based systems, enalapril tablets disperse poorly due to hydrophobic excipients that resist wetting. The low viscosity of the medium facilitates rapid sedimentation of undissolved particles, leading to depletion in the upper portion of the suspension and accumulation at the bottom. PG modifies this behavior through three key mechanisms: **(i)** it acts as a wetting agent, coating hydrophobic particles and reducing interfacial tension; **(ii)** it increases viscosity, which slows sedimentation according to Stokes' law; and **(iii)** it forms hydrogen-bonding interactions with polar functional groups of enalapril, enhancing apparent solubility and dispersion. Collectively, these effects promote uniform drug distribution across the suspension.

Clinically, these findings are critical. In water-based suspensions, taking a spoonful from the top could result in an almost drug-free dose, while sampling from the bottom could provide an excessive dose. This variability poses significant risks in pediatric and geriatric dosing, where

precise administration is vital. The inclusion of PG addresses this issue by ensuring consistent therapeutic dosing per milliliter and enhancing patient safety.

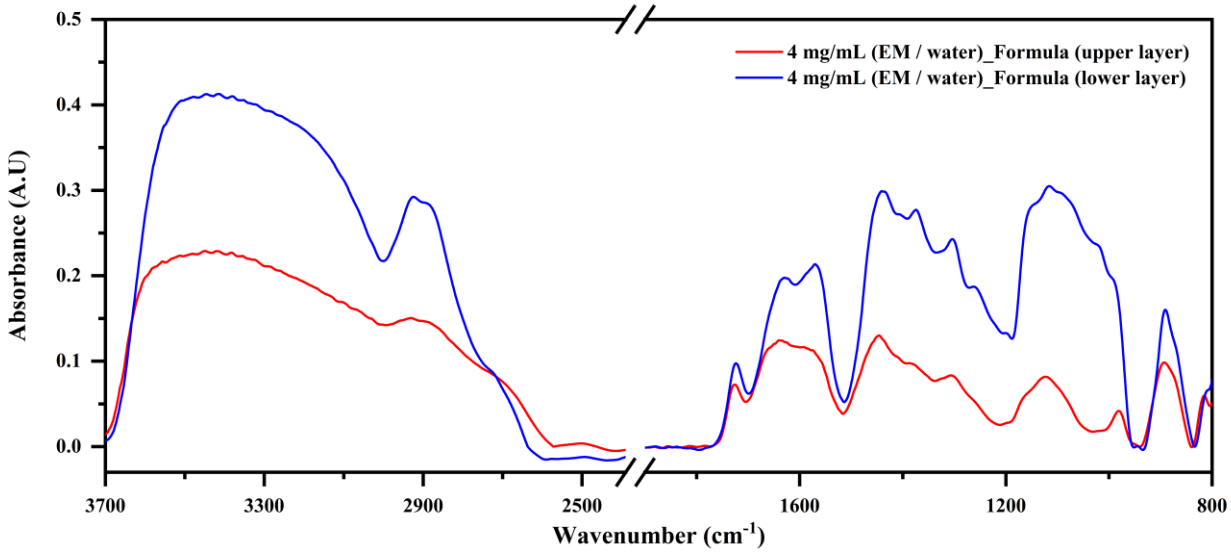

Figure 4.12: FTIR spectra of upper versus lower layers of aqueous enalapril suspension (10 tablets/50 mL water)

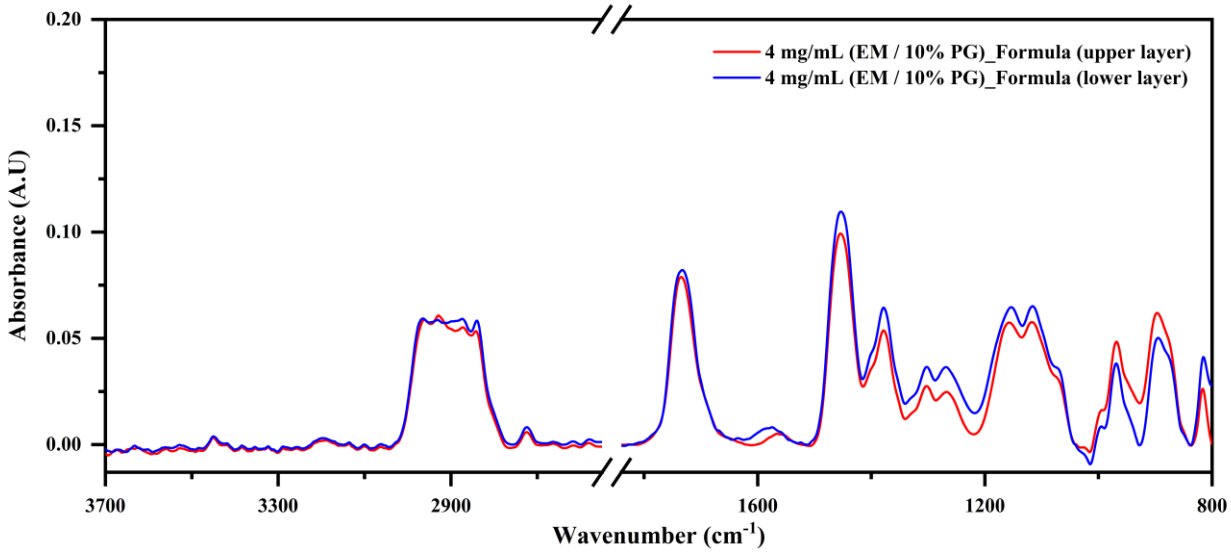

Figure 4.13: FTIR spectra of upper versus lower layers of enalapril suspension in 10% PG

Table 4.7: Percentage of enalapril maleate detected in upper and lower layers of aqueous versus PG-containing suspensions, as determined by FTIR

Sampling Point	Formula 1 (10 tablets/ 100% Water)	Formula 2 (10 tablets/ 10% PG)
Upper layer	0%	100%
Lower layer	12.5%	101.75 %

4.6.2 HPLC Homogeneity Testing For Formulations

The evaluation of homogeneity in various suspension vehicles using HPLC is presented in Table 4.8**.** The results reveal significant differences in dose uniformity based on the formulation medium.

In **Formula 1 (100% water)**, the drug content varied considerably across sampling positions: the upper layer contained only 84.64% of the expected concentration, the center measured 109.03%, and the lower layer exhibited 129.50%. This pronounced vertical gradient suggests sedimentation and poor physical stability. The elevated value in the center likely reflects the partial re-dispersion of drug-rich sediment during sampling, further highlighting the instability of aqueous suspensions.

In **Formula 2 (10% PG)**, the upper layer contained 98.93% of the theoretical concentration, closely approaching the desired 100%. This finding confirms that PG effectively prevents stratification and maintains dose uniformity throughout the suspension.

In **Formula 3 (10% PG + 20% syrup)**, the upper layer displayed 95.50% of the expected concentration. The addition of syrup did not compromise homogeneity; rather, it increased viscosity, providing a stabilizing effect analogous to that of PG while also enhancing palatability—an essential factor for pediatric formulations.

Table 4.8: Percentage of enalapril maleate remaining in various suspension vehicles (water only, 10% PG, and 10% PG + 20% syrup), demonstrating dose distribution at upper, central, and lower sampling points as analyzed by HPLC

Sampling Point	Formula 1 (10 tablets100% Water)	Formula 2 (10 tablets/ 10% PG)	Formula 3 (10 tablets/ 10% PG, 20% Syrup)
Upper layer	84.64%	98.93%	95.50%
Central layer	109.03%	-	-
Lower layer	129.50%	-	-

Mechanistically, these findings illustrate the relationship between solubility, interfacial energy, and viscosity. Enalapril maleate, being amphiphilic but not freely soluble, tends to undergo phase separation in pure water, resulting in the sedimentation of poorly wetted excipients. PG reduces interfacial energy, enhances the wetting of hydrophobic particles, and increases viscosity, which slows sedimentation and promotes homogeneous suspensions. The inclusion of syrup offers similar viscosity-driven stabilization while effectively masking the drug's bitterness.

The consistency between FTIR and HPLC results reinforces these conclusions. FTIR spectra demonstrated overlapping diagnostic peaks across upper and lower layers in PG-containing suspensions, while HPLC provided quantitative confirmation with values close to 100% at all sampling positions. Clinically, this is crucial: non-uniform suspensions can deliver sub-therapeutic or supratherapeutic doses, particularly in pediatric and geriatric populations. The use of PG ensures reproducible dosing and safer administration.

Key Takeaways for Sections 4.5 & 4.6:

- **Without PG:** suspensions experience both chemical instability (rapid hydrolysis) and physical instability (sedimentation, dose gradient).
- **With PG:** both issues are addressed-hydrolysis is slowed, and suspensions achieve homogeneity.
- **Adding syrup:** enhances taste and acceptability for pediatric use without compromising uniformity.
- **FTIR vs. HPLC:** FTIR facilitated rapid quantitative confirmation of concentration changes and homogeneity, whereas HPLC validated dose uniformity across layers and confirmed the separation of degradation products.
- Collectively, these findings demonstrate that PG is not merely a stabilizer but a **formulation enabler**, ensuring both chemical stability and physical homogeneity.

4.7 Cross-Technique Synthesis and Practical Implications

The integrated dataset from FTIR and HPLC analyses provides an overview of enalapril maleate's stability in extemporaneous solutions. A consistent conclusion drawn from various experiments is that enalapril is inherently unstable in aqueous environments due to the hydrolytic lability of its ester bond. However, the inclusion of propylene glycol (PG) significantly modifies both the chemical and physical properties of the formulation.

Analytical Complementarity and Concordance

FTIR and HPLC yielded concordant results, highlighting the rapid hydrolysis of enalapril in aqueous media and its systematic stabilization in the presence of propylene glycol. FTIR demonstrated its efficacy as a matrix-sensitive spectroscopic technique for monitoring molecular-level changes associated with degradation, notably the progressive reduction of the ester C=O band, thereby providing kinetic insights into the hydrolysis process. Conversely, HPLC facilitated high-resolution quantitative separation and accurate identification of enalapril and its degradation products (e.g., enalaprilat and, in some cases, DKP), with differences in retention times confirming product identity. Consequently, FTIR and HPLC fulfilled complementary analytical functions, with FTIR supporting mechanistic and trend-based assessments, while HPLC provided definitive quantification and specificity.

Mechanistic Linkage between Kinetics and Homogeneity. PG mitigates base-catalyzed hydrolysis by **(i)** reducing water activity (fewer "free" water molecules/hydroxide at the ester site), **(ii)** increasing viscosity (which impedes OH^- diffusion and the approach of reactants), and **(iii)** providing hydrogen bonding and solvation shielding around carbonyl and amide functionalities. These physicochemical effects also enhance suspension homogeneity by improving tablet powder wetting, reducing interfacial tension, and minimizing sedimentation and stratification. Consequently, both chemical stabilization (slower hydrolysis) and physical stabilization (uniform suspensions) arise from a shared PG-driven mechanism.

Clinical and Pharmaceutical Relevance. Water-based vehicles exhibit chemical instability, resulting in rapid ester cleavage into enalaprilat/DKP under stress, and physical non-uniformity, leading to dose gradients across layers. This can result in under- or over-dosing and safety concerns, particularly in pediatric and geriatric populations. The incorporation of approximately 10% PG offers a straightforward, pharmacy-ready solution that mitigates these risks by reducing degradation and ensuring uniform dose distribution.

Novelty and Role of FTIR as a Quantitative Tool. While HPLC is regarded as the standard reference method, this study demonstrates that FTIR can perform quantitatively ($R^2 > 0.99$) with matrix-matched calibration and appropriate solvent selection, rivaling HPLC in linearity for trend monitoring and rapid screening in community and hospital settings.

Integrated Practice Perspective:

- FTIR-ATR can serve as an efficient screening method to track stability-related spectral changes in enalapril formulations.
- HPLC will continue to serve as the confirmatory quantitative reference.
- The incorporation of approximately 10% PG should be adopted as a practical stabilizer, balancing chemical resistance to hydrolysis with improved suspension homogeneity.

Executive Takeaway. Enalapril maleate is inherently unstable in aqueous extemporaneous formulations. However, the simple addition of PG provides dual protection—chemical (slowed ester hydrolysis) and physical (enhanced homogeneity). The complementary use of FTIR and HPLC supports this approach, leading to safer, more consistent, and stable liquid preparations for users.

4.8 Future Directions and Refinements

This study successfully demonstrated the mechanistic degradation pathways of enalapril maleate and the stabilizing role of propylene glycol (PG). To further strengthen and expand these findings, future research could explore:

Broader Storage Conditions: Extending investigations beyond short-term alkaline stress to include near-neutral to slightly acidic pH conditions, refrigeration, and room temperature to better simulate real-world storage scenarios.

Kinetic Refinement: Calculating quantitative kinetic constants (k_{obs}, $t_{½}$) through C/C_0 normalization and model fitting to achieve greater precision in rate comparisons.

Practical Considerations: Evaluating microbiological stability with appropriate preservatives and assessing taste-masking or palatability for pediatric formulations to facilitate clinical translation.

4.9 Conclusion

The results of this study clearly demonstrate that enalapril maleate is inherently unstable in aqueous alkaline environments. The observed degradation profiles align with classical ester hydrolysis mechanisms, wherein hydroxide ions attack the ester carbonyl, yielding enalaprilat as the primary product and DKP as a secondary product under cyclization conditions. Both degradation pathways ultimately compromise therapeutic stability.

Both FTIR and HPLC calibration curves exhibited excellent linearity, confirming their effectiveness as quantitative methods. FTIR monitored spectral changes, such as the disappearance of ester carbonyl bands at 1741 cm^{-1}, while HPLC confirmed the quantitative loss of the drug alongside the appearance of enalaprilat. Together, these complementary techniques provided a comprehensive understanding of enalapril degradation and supported the use of FTIR as a rapid, cost-effective approach for stability assessment within a validated experimental framework.

The addition of propylene glycol consistently slowed hydrolysis and enhanced homogeneity across formulations. Mechanistically, PG protects enalapril through several pathways: (i) reducing effective water activity and decreasing the availability of free water molecules, (ii) increasing viscosity and limiting hydroxide diffusion toward ester sites, and (iii) forming hydrogen bonds with ester and amide groups, thereby partially shielding them from nucleophilic attack. In addition to its chemical stabilization, PG improved physical homogeneity, resulting in nearly uniform concentrations across suspension layers, in contrast to the steep gradients observed in water-based systems. This dual role—as both a stabilizer and homogenizer—positions PG as a practical excipient for safer and more reliable community compounding.

From a clinical perspective, these insights are particularly significant. In water-based systems, enalapril rapidly decomposes, posing a substantial risk of under-dosing or uneven dosing, especially for pediatric and geriatric patients. Conversely, formulations containing PG maintain stability over extended periods, ensuring consistent dosing per milliliter.

In conclusion, this chapter establishes three key insights:

- ✓ Hydrolysis pathway: Ester cleavage produces enalaprilat, with DKP as a side pathway.
- ✓ PG's protective role: Slows hydrolysis kinetics and improves dose uniformity through chemical (lower a_w, H-bonding) and physical (viscosity, solvation) stabilization.
- ✓ Analytical complementarity: FTIR provides rapid spectral monitoring, while HPLC offers quantitative, stability-indicating validation.

Collectively, these findings strongly indicate that propylene glycol is essential for the stability and reliability of extemporaneous enalapril formulations. By linking fundamental degradation chemistry with applied pharmaceutics, this chapter highlights the dual value of FTIR and HPLC in enhancing mechanistic understanding and clinical application.

Chapter Five:

General Conclusion and Recommendations

5.1 General Conclusion:

This thesis provides an investigation into the stability and degradation kinetics of enalapril maleate in extemporaneous liquid formulations, utilizing complementary analytical techniques—FTIR and HPLC. The key conclusions are as follows:

5.1.1 Intrinsic Instability in Aqueous Media

Enalapril maleate, as an ethyl ester prodrug, demonstrates inherent instability in aqueous alkaline environments. The ester carbonyl band at 1741 cm^{-1} was identified as the most chemically labile site, undergoing base-catalyzed hydrolysis to yield enalaprilat as the primary degradation product, along with diketopiperazine (DKP) as a secondary cyclization product. Both degradation pathways compromise the therapeutic stability of the drug.

5.1.2 Complementary Analytical Insights from FTIR and HPLC

FTIR spectroscopy proved to be a rapid and cost-effective method for monitoring degradation trends. The disappearance of the ester carbonyl band served as clear mechanistic indicator of hydrolysis.

HPLC provided quantitative confirmation through the loss of the parent peak and the appearance of enalaprilat (and occasionally DKP).

The high calibration linearity of both methods ($R^2 > 0.99$ for FTIR and $R^2 > 0.999$ for HPLC) supports their combined use, with FTIR serving as a practical screening tool and HPLC as the standard reference method.

5.1.3 Stabilizing and Homogenizing Role of Propylene Glycol (PG)

The inclusion of propylene glycol consistently reduced the rate of hydrolysis and significantly improved suspension homogeneity. PG exerted its protective effects through:

- ✓ **Chemical stabilization:** Reducing water activity, lowering hydroxide ion diffusion, and shielding ester and amide groups through hydrogen bonding.
- ✓ **Physical stabilization:** Enhancing the wetting of hydrophobic excipients, increasing viscosity to minimize sedimentation, and ensuring uniform dose distribution throughout the suspension.

5.1.4 Clinical and Pharmaceutical Implications

These findings are clinically significant. Water-based suspensions of enalapril maleate degrade rapidly and stratify, posing risks of sub-therapeutic or supra-therapeutic dosing, particularly in pediatric and geriatric patients. In contrast, PG-containing formulations maintained chemical integrity and ensured uniformity, providing safer and more reliable extemporaneous preparations.

In summary, this study establishes propylene glycol as both a **stabilizer** and a **homogenizer** for extemporaneous enalapril formulations while confirming the dual use of FTIR and HPLC as a robust analytical framework. These insights connect fundamental degradation chemistry with practical pharmaceutical applications.

5.2 Recommendations

Based on the experimental findings, the following recommendations are proposed:

5.2.1 for Pharmaceutical Practice

Avoid water-based suspensions of enalapril maleate due to their instability and non-uniformity.

Incorporate at least 10% PG into extemporaneous formulations to enhance both chemical stability and physical homogeneity.

Consider adding syrup to improve palatability, particularly for pediatric patients, without compromising uniformity.

5.2.2 for Analytical Applications

Following proper calibration and validation, FTIR can be utilized for the quantitative analysis of enalapril maleate within a specified concentration range, particularly for monitoring degradation-related changes during formulation development and stability studies.

HPLC remains the definitive reference method for high-precision quantification, ensuring regulatory compliance and determining beyond-use dates (BUD).

5.2.3 for Future Research

Conduct long-term stability studies under real-world storage conditions (neutral and acidic pH, refrigeration, and room temperature) to establish practical BUD guidelines.

Investigate additional degradation pathways, including oxidative, photolytic, and thermal stress, in line with ICH stability requirements.

Explore microbiological stability and the role of preservatives in PG-based formulations.

Evaluate palatability and patient acceptability through formal sensory studies, especially for pediatric populations.

Investigate alternative excipients (e.g., glycerin, sorbitol, HPMC) for potential synergistic stabilization effects.

Perform pharmacokinetic studies to confirm that laboratory stability improvements translate into consistent therapeutic outcomes in clinical practice.

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Appendix

Appendix A: USP Enalapril Maleate Monograph

The following pages provide the USP Monograph for Enalapril Maleate, which serves as the official reference for the preparation of solutions, assay methodology, system suitability criteria, and analytical procedures utilized in this study.

Enalapril Maleate Tablets

DEFINITION

Enalapril Maleate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of enalapril maleate ($C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$).

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Buffer: Dissolve 1.38 g of monobasic sodium phosphate in 800 mL of water, adjust with phosphoric acid to a pH of 2.2, and dilute with water to 1000 mL.

Mobile phase: Acetonitrile and *Buffer* (250:750)

Enalapril diketopiperazine solution: Place 20 mg of USP Enalapril Maleate RS in a 100-mL beaker to form a mound on the bottom of the beaker. Place the beaker on a hot plate at one-half the maximum hot plate temperature setting to melt the solid. When melting is observed (after 5–10 min of heating), immediately remove the beaker from the hot plate, and allow it to cool. Avoid overheating beyond the melting initially observed to prevent heat-induced degradation, which would give rise to a brown color. To the cooled residue in the beaker add 50 mL of acetonitrile, and sonicate for a few min to dissolve the residue. The solution typically contains between 0.2 and 0.4 mg/mL of enalapril diketopiperazine.

Enalaprilat stock solution: 0.4 mg/mL of USP Enalaprilat RS in water

Standard solution: 0.2 mg/mL of USP Enalapril Maleate RS and 0.002 mg/mL of USP Enalaprilat RS in *Buffer* prepared as follows. To a suitable amount of USP Enalapril Maleate RS in a suitable volumetric flask add an appropriate amount of *Enalaprilat stock solution* to the flask, and add 50% of the total volume of *Buffer* to dissolve. Sonicate if necessary, then dilute with *Buffer* to volume.

System suitability solution: Dilute 0.5 mL of *Enalapril diketopiperazine solution* with *Standard solution* to a final volume of 25 mL.

Sample solution: Nominally 0.2 mg/mL of enalapril maleate in *Buffer* prepared as follows. Transfer NLT 10 Tablets to a volumetric flask of capacity such that when filled to volume it will produce a 0.2-mg/mL solution. Add a volume of *Buffer* that is about one-half the nominal volume of the flask, sonicate for 15 min, and shake by mechanical means for 30 min. Dilute with *Buffer* to volume, shake well, and sonicate for another 15 min. Pass the solution through a suitable filter of 0.45-µm pore size, and discard the first portion of the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 25-cm; 5-µm packing L7

Column temperature: 50°

Flow rate: 2 mL/min

Injection volume: 50 µL

System suitability

Samples: *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for maleic acid, enalaprilat, enalapril, and enalapril diketopiperazine are about 0.3, 0.5, 1.0, and 1.5, respectively, *System suitability solution*. A peak response for a heat-induced degradation product of enalapril diketopiperazine (if present with a relative retention time of about 1.2) is NMT 15% of the response for enalapril diketopiperazine.]

Suitability requirements

Resolution: NLT 2.0 between maleic acid and enalaprilat; NLT 2.0 between enalaprilat and enalapril; NLT 2.0 between enalapril and enalapril diketopiperazine, *System suitability solution*

Column efficiency: NLT 1000 theoretical plates for enalaprilat; NLT 300 theoretical plates for enalapril; NLT 2500 theoretical plates for enalapril diketopiperazine, *System suitability solution*

Tailing factor: NMT 2.0 for enalapril, *System suitability solution*

Relative standard deviation

Enalapril peak: NMT 2.0%, *Standard solution*

Enalaprilat peak: Responses agree within 5%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of enalapril maleate ($C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Enalapril Maleate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of enalapril maleate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **DISSOLUTION** ⟨711⟩

Medium: pH 6.8 phosphate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*); 900 mL

Apparatus 2: 50 rpm

Time: 30 min

Standard solution: 0.1 mg/mL of USP Enalapril Maleate RS in *Medium*. Sonicate if necessary. Dilute in *Medium* per *Table 1*.

Table 1

Tablet Strength (mg)	Volume of Standard solution (mL)	Volumetric Flask Size (mL)
2.5	5	200
5	10	200
10	10	100
20	10	50
40	10	25

Sample solution: Pass a portion of solution under test through a suitable filter. Dilute as needed with *Medium* to a concentration that is similar to that of the *Standard solution*.

Analysis: Determine the amount of enalapril maleate ($C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$) dissolved as directed in *Uniformity of Dosage Units* ⟨905⟩.

Tolerances: NLT 80% (*Q*) of the labeled amount of enalapril maleate ($C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$) is dissolved.

Change to read:

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩: ▲Meet the requirements▲ (CN 1-Aug-2023)

Procedure for content uniformity

Buffer and **Mobile phase:** Prepare as directed in the *Assay*.

Standard solution: 0.1 mg/mL of USP Enalapril Maleate RS in *Buffer*

Sample solution: 0.1 mg/mL of enalapril maleate from 1 Tablet in *Buffer*. Add a volume of *Buffer* that is one-half the nominal volume of the flask, sonicate for 15 min, and shake by mechanical means for 30 min. Dilute with *Buffer* to volume, shake well, and sonicate for an additional 15 min. Pass through a suitable filter of 0.45-µm pore size, and discard the first portion of the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 25-cm; 5-µm packing L7

Column temperature: 50°

Flow rate: 2 mL/min

Injection volume: 50 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 300 theoretical plates

Tailing factor: NMT 2.0

Capacity factor, *k'*: NLT 1.5

Relative standard deviation: NMT 2.0%

[NOTE—The enalapril peak tailing factor may be minimized by controlling the column temperature between 45° and 50° and by raising the pH of the aqueous component of the *Mobile phase* from 2.2 to 2.6; the capacity factor may be increased by decreasing the amount of acetonitrile in the *Mobile phase*.]

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of enalapril maleate ($C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$) in the Tablet taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Enalapril Maleate RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of enalapril maleate in the *Sample solution* (mg/mL)

▲▲ (CN 1-Aug-2023)

IMPURITIES

• **ORGANIC IMPURITIES**

Buffer, Mobile phase, Enalapril diketopiperazine solution, Standard solution, System suitability solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Diluted standard solution: Dilute 1.0 mL of *Standard solution* with *Buffer* to 100 mL.

Analysis

Samples: *Buffer, Standard solution, Sample solution,* and *Diluted standard solution*

Measure the responses for all of the peaks in the *Sample solution* greater than 0.1% of the response of the enalapril peak that are not observed in the *Buffer*.

Calculate the percentage of anhydrous enalaprilat (as enalapril maleate) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Enalaprilat RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of enalapril maleate in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of enalapril maleate, 492.52
M_{r2} = molecular weight of anhydrous enalaprilat, 348.39

Calculate the percentage of enalapril diketopiperazine (as enalapril maleate) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of enalapril diketopiperazine from the *Sample solution*
r_S = peak response of enalapril from the *Diluted standard solution*
C_S = concentration of USP Enalapril Maleate RS in the *Diluted standard solution* (mg/mL)
C_U = nominal concentration of enalapril maleate in the *Sample solution* (mg/mL)
F = relative response factor of enalapril diketopiperazine, 1.25
M_{r1} = molecular weight of enalapril maleate, 492.52
M_{r2} = molecular weight of enalapril diketopiperazine, 358.44

Calculate the percentage of the sum of all other individual impurities in the portion of Tablets taken:

$$\text{Result} = (r_T/r_S) \times (C_S/C_U) \times 100$$

r_T = sum of the responses of all other individual impurities other than maleic acid, enalapril, enalaprilat, and enalapril diketopiperazine from the *Sample solution*
r_S = peak response of enalapril maleate from the *Diluted standard solution*
C_S = concentration of USP Enalapril Maleate RS in the *Diluted standard solution* (mg/mL)
C_U = nominal concentration of enalapril maleate in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 5.0% for the sum of all impurities including those from enalaprilat and enalapril diketopiperazine

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Enalaprilat RS
USP Enalapril Maleate RS

العنوان: دراسة حركية التحلل المائي لإنالابريل ماليات في محاليل محضرة آنياً باستخدام التحليل الطيفي بالأشعة تحت الحمراء (FTIR).

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الملخص

تبحث هذه الدراسة الاستقرار التحللي والحركية الانحلالية لمركب إنالابريل ماليات في المستحضرات الفموية السائلة المحضرة آنياً، مع التركيز على الدور المثبت للبروبيلين جلايكول، وتطبيق تقنية التحليل الطيفي بالأشعة تحت الحمراء بتحويل فوريه (FTIR) كأداة تحليلية سريعة. يُعتبر إنالابريل ماليات، وهو أحد مثبطات الإنزيم المحول للأنجيوتنسين (ACE) واسعة الاستخدام، مركباً غير مستقر كيميائياً في الأوساط المائية، حيث يتعرض لتحلل مائي سريع إلى إنالابريلات، وقد يخضع في ظروف معينة لعملية تَحلُّق داخلية تؤدي إلى تكوين ثنائي كيتوبيبيرازين (DKP).

أظهرت منحنيات المعايرة خطية ممتازة لكل من FTIR ($R^2 > 0.99$) و HPLC ($R^2 > 0.999$). وتحت ظروف الإجهاد القلوي (0.1 M NaOH) ، أظهرت المحاليل المائية فقداناً كاملاً للمركب خلال 60 دقيقة. وعلى النقيض، أدى وجود 10% من البروبيلين جلايكول إلى الحفاظ على الإشارة الطيفية لإنالابريل ماليات، مع استرداد يقارب 103% بواسطة HPLC عند الدقيقة 60.

كشفت اختبارات التجانس عن حدوث تدرج كبير في المستحضرات المائية فقط، حيث لم تظهر الطبقة العلوية محتوى دوائيا قابلا للكشف (0%)، بينما احتوت الطبقة السفلية على نحو (12.5 %) FTIR ، وأكدت نتائج HPLC التفاوت الكبير (الطبقة العلوية 84.64%، الوسطى 109.03%، والسفلية 129.5%). في المقابل، أظهرت المستحضرات المحتوية على البروبيلين جلايكول (PG) أو البروبيلين جلايكول مع الشراب تجانساً واضحاً، حتى عند أخذ العينات من الطبقة العلوية، وهي الموقع العملي لسحب الجرعات من قبل المرضى. حيث بيّن FTIR محتوى دوائي يقارب 100% (الطبقة العلوية 100%، السفلية 101.7%)، بينما أكّد HPLC درجة عالية من التجانس (الطبقة العلوية مع~98.9% :PG ، ومع PG + شراب: ~95.5 .%)

وتشير هذه النتائج إلى أن البروبيلين جلايكول يضمن ثبات الجرعة خلال الاستخدام السريري، على عكس المستحضرات المائية التي قد تؤدي إلى جرعات غير علاجية.

أثبت FTIR كفاءته في تتبع اختفاء حزمة الكربونيل للإستر عند 1741 سم$^{-1}$، مقدماً ملفات انحلال متوافقة مع نتائجHPLC ، مما يعزز اعتماده كأداة فحص سريعة وفعّالة من حيث التكلفة، بينما يبقى HPLC المرجع الذهبي التأكيدي.

خلصت الدراسة إلى أن إنالابريل ماليات غير مستقر كيميائياً وفيزيائياً في المستحضرات المائية المحضرة آنياً، وأن إضافة البروبيلين جلايكول تؤدي إلى تحسين ملحوظ في الاستقرار والتجانس، مما يرسّخ مكانته كمثبت عملي. كما أن التحقق من جدوى استخدام FTIR بالتوازي مع HPLC يوفر إطاراً تحليلياً متيناً يعزز سلامة وفعالية وموثوقية المستحضرات الدوائية المحضرة آنياً المحتوية على إنالابريل.

الكلمات المفتاحية :إنالابريل ماليات، مستحضرات محضرة آنياً، FTIR-ATR، HPLC، التحلل المائي، بروبيلين جلايكول، حركية التحلل.