Angiotensin – converting enzyme (ACE) inhibitors are widely prescribed as first – line therapies in the management of essential hypertension, particularly in patients with concomitant diabetes, renal disease, and congestive heart failure. Their efficacy in the treatment of hypertension has been well documented.

Enalapril is a powerful drug used to treat congestive heart failure in patients who are unresponsive to treatment with digitalis and diuretics. The polypeptide angiotensin, when influenced by a converting enzyme, forms angiotensin II, a very strong vasoconstrictive agent. As an angiotensin – converting – enzyme inhibitor, enalapril relaxes the vasoconstriction caused by angiotensin II, easing resistance to heart function.

It is very important to determine the concentration of ACE inhibitors in plasma with respect to pharmacokinetics and pharmacodynamics. To study the pharmacokinetics and disposition of this drug and its active metabolite, we needed to develop selective analytical methods for their measurements in plasma and various active medicinal compounds. In this report we discuss the analytical methodology developed for quantifying enalapril in plasma.¹

The significant of this study is to develop a reliable and simple method for the routine analysis of pharmaceutical dosage forms by high – performance liquid chromatography and gas chromatography – mass spectrometer. Standardized extraction procedures for drugs in various dosage forms need to be developed and applied to a wide range of current pharmaceutical formulations. The approach should increase the efficiency and
reduce the operating cost of the laboratories engaged in the quality control of pharmaceuticals by effective utilization of automated equipment.

1.1 The Role of the Angiotensin Converting Enzyme Inhibitor:

ACE inhibitor or angiotensin converting enzyme inhibitors (i.e., Enalapril, Captopril) reduce peripheral vascular resistance via blockage of the angiotensin converting enzyme. This action reduces the myocardial oxygen consumption, thereby improving cardiac output and moderating left ventricular and vascular hypertrophy. ²

The experience with ACE inhibitors in the treatment of high blood pressure in the elderly indicates that they are as effective as diuretics despite the fact that the elderly are characterized by low levels of plasma renin. There are a number of advantages of the treatment with ACE inhibitors. First, they are free of the undesirable biochemical side effects, such as hypokalaemia, hyperglycaemia, hypercholesterolaemia, and hyperuricaemia that are common consequences of diuretic therapy. It has been speculated that these adverse effects might be associated with increased risk of sudden death from low serum potassium or an increased rate of arthenogenesis from a rise in serum cholesterol ³. Second, ACE inhibitors are associated with an improvement in quality of life compared to treatment with propranolol or methyldopa ⁴.
1.2 ENALAPRIL MALEATE

Enalapril maleate (ENM) is chemically described as (S)-1-{N-[1-(ethoxycarbonyl)-3-phenylpropyl]-L-alanyl}-L-proline, (Z)-2-butenedioate (1:1)\(^5\) salt, ENM is a salt of enalapril (EN) and maleic acid (MA). Its empirical formula is C\(_{20}\)H\(_{28}\)N\(_2\)O\(_5\) • C\(_4\)H\(_4\)O\(_4\).

*Structural*

![Structural Figure 1. Enalapril Maleate](image)

Enalapril maleate is a white to off-white, crystalline powder with a molecular weight of 492.53. It is sparingly soluble in water, soluble in ethanol, and freely soluble in methanol. Enalapril is a pro-drug that is activated to the angiotensin-converting enzyme (ACE) inhibitor, enalaprilat (DIAC). This pro-drug inhibits the conversion of angiotensin I to the angiotensin II and exerts an antihypertensive effect by suppressing the renin
angiotensin aldosterone system. This is indicated for the treatment of renivascular hypertension. There are two major potential by products in the substance besides the parent compound: DIAC, the free acid produced by hydrolysis of EN and diketopiperazine (DKP), a cyclization product. The structures of these compounds are shown in Figure 2.

Figure 2. Structural formulae of EN and its by products DIAC and DKP
The configuration of peptide bond can be either *trans* or *cis* (Figure 3). Nearly all amino acid residues in proteins are in the *trans* configuration, in which steric repulsion is minimized. With proline, the *cis* configuration is likely to occur as the *trans* configuration because the amide nitrogen is part of a ring.

![trans and cis configurations](image)

*Figure 3. Configuration around peptides bonds involving proline. The peptide bond interconverts between *trans* and *cis* configurations.*

Recently, elimination of peak splitting in the liquid chromatography of the proline-containing drug enalapril maleate has been studied.

Enalapril maleate contains alanylproline dipeptide and can be split into two or more peaks when separated by reversed - phase liquid chromatography. The optimum separation conditions involve a state where the rotational rates are much higher than that of the separation process, i.e., at a higher temperature and lower pH. At lower pH of the mobile phase, the C – N bond loses its partially double – bond character, which
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restricts free rotation around the peptide bond owing to partial protonation of the imide group on proline. The increase in the relaxation rate of isomerization of proline containing peptides results in a better peak shape in liquid chromatography. Enalapril is eluted as a single peak even at room temperature at higher concentrations of both cetyltrimethylammonium bromide and sodium dodecyl sulphate in the mobile phase. The rate of isomerization rises with increasing temperature, as the activation energy of the peptide bond in the molecule containing alanylproline is relatively high.

Below show a few of established methods that had been developed to determine the enalapril in plasma:

1. Determination of Enalapril and its Active Metabolite Enalaprilat in Plasma and Urine by Gas chromatography/Mass Spectrometry \(^\text{10}\).

Enalapril and enalaprilat in plasma and urine were extracted and cleaned up by using Sep – Pak C18 and silica cartridges. Enalapril and enalaprilat were converted to methyl ester and trifluoroacetyl derivatives and determined by GC/MS using electron capture / NICI. Detection by selected ion monitoring (SIM) was selected to m/z 288 (enalaprilat) and 302 (enalapril). RS-5139 was used as an internal standard. The detection limit of enalapril and enalaprilat was 200pg/mL in plasma and 2ng/mL in urine. This method was applied to the pharmacokinetic analysis of enalapril and enalaprilat in body fluids.

This GC/MS method was used for the quantification of enalapril and enalaprilat in biological fluids with good sensitivity, selectivity and reproducibility.
2. Analysis of ACE inhibitors in Pharmaceutical Dosage Forms by Derivative UV Spectroscopy and Liquid Chromatography (HPLC)\textsuperscript{11}.

Derivative UV spectroscopy and high performance liquid chromatography (HPLC) were applied to the determination of angiotensin converting enzyme (ACE) inhibitors in their pharmaceutical dosage forms. For spectrophotometric determinations, the more appropriate derivative order was selected for each drug: ramipril (third-order), benazepril (second-order), enalapril maleate (second-order), lisinopril (first- and second-order) and quinapril (first-order). Reverse phase HPLC procedures (ODS column) were developed able to provide a single, symmetric peak for each drug; mixtures A-B, where A is 20 mM sodium heptansulphonate (pH 2.5) and B is acetonitrile-THF (95:5 v/v), proved to be suitable mobile phases to obtain selective separations of the cited ACE inhibitors. At ambient temperature, a low pH value (2.5) was found to be critical to avoid peak splitting and band broadening.

3. Simultaneous Determination of Enalapril Maleate and Hydrochlorothiazide by First-Derivative Ultraviolet Spectrophotometry and High-Performance Liquid Chromatography\textsuperscript{12}

Two methods are described for the simultaneous determination of enalapril maleate and hydrochlorothiazide in combined pharmaceutical tablets. The first method depends on first-derivative ultraviolet spectrophotometry, with zero-crossing and peak-to-base measurement methods. The first-derivative amplitudes at 224 and 260 nm were selected
for the assay of enalapril maleate and hydrochlorothiazide, respectively. The second method is based on high-performance liquid chromatography on a reversed-phase column using a mobile phase of acetonitrile-water (20:80, v/v) (pH 3.8) with programmable detection at 215 and 275 nm. Both methods showed good linearity, precision and reproducibility. The proposed methods were successfully applied to the determination of these drugs in laboratory-prepared mixtures and in commercial tablets.
1.3 ENALAPRILAT

Enalaprilat which is an angiotensin converting enzyme inhibitor is chemically described as (S)-1-{(N-[1-(carboxy-3-phenylpropyl)-L-alanyl]-L-proline dihydrate. Its empirical formula is C₁₈H₂₄N₂O₅ • 2H₂O.

Structural

![Enalaprilat structural formula](image)

Figure 4. Enalaprilat

Enalaprilat is a white to off-white, crystalline powder with a molecular weight of 384.43.

It is sparingly soluble in methanol and slightly soluble in water.
1.4 DIAZEPAM

Diazepam is a benzodiazepine derivative developed through original Roche research. Chemically, diazepam is 7-chloro-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one. It is a colorless crystalline compound, insoluble in water and has a molecular weight of 284.74. Its empirical formula is $C_{16}H_{13}ClN_2O$. 

![Figure 5. Diazepam](image-url)