I. INTRODUCTION

Antihypertensives are a class of drugs that are used to treat hypertension (high blood pressure). Antihypertensive therapy seeks to prevent the complications of high blood pressure, such as stroke and myocardial infarction. Evidence suggests that reduction of the blood pressure by 5 mmHg can decrease the risk of stroke by 34%, of ischaemic heart disease by 21%, and reduce the likelihood of dementia, heart failure, and mortality from cardiovascular disease. There are many classes of antihypertensives, which lower blood pressure by different means. Among the most important and most widely used drugs are thiazide diuretics, calcium channel blockers, ACE inhibitors, angiotensin II receptor antagonists (ARBs), and beta blockers.

Which type of medication to use initially for hypertension has been the subject of several large studies and resulting national guidelines. The fundamental goal of treatment should be the prevention of the important endpoints of hypertension, such as heart attack, stroke and heart failure. Patient age, associated clinical conditions and end-organ damage also play a part in determining dosage and type of medication administered. The several classes of antihypertensives differ in side effect profiles, ability to prevent endpoints, and cost. The choice of more expensive agents, where cheaper ones would be equally effective, may have negative impacts on national healthcare budgets. As of 2009, the best available evidence favors the thiazide diuretics as the first-line treatment of choice for high blood pressure when drugs are necessary. Although clinical evidence shows calcium channel blockers and thiazide-type diuretics are preferred first-line treatments for most people (from both efficacy and cost points of view), an ACE inhibitor is recommended by NICE in the UK for those under 55 years old.

Calcium channel blocker

Calcium channel blockers (CCB), calcium channel antagonists or calcium antagonists are several medications that disrupt the movement of calcium (Ca2+) through calcium channels. Calcium channel blockers are used as antihypertensive drugs, i.e., as medications to decrease blood pressure in patients with hypertension. CCBs are particularly effective against large vessel stiffness, one of the common causes of elevated systolic blood pressure in elderly patients. Calcium channel blockers are also frequently used to alter heart rate, to prevent cerebral vasospasm, and to reduce chest pain caused by angina pectoris. N-type, L-type, and T-type
voltage-dependent calcium channels are present in the zona glomerulosa of the human adrenal, and CCBs can directly influence the biosynthesis of aldosterone in adrenocortical cells, with consequent impact on the clinical treatment of hypertension with these agents. CCBs have been shown to be slightly more effective than beta blockers at lowering cardiovascular mortality, but they are associated with more side effects. Potential major risks however were mainly found to be associated with short-acting CCBs.

**ACE Inhibitors**

An angiotensin-converting-enzyme inhibitor (ACE inhibitor) is a pharmaceutical drug used primarily for the treatment of hypertension (elevated blood pressure) and congestive heart failure.

This group of drugs cause relaxation of blood vessels, as well as a decreased blood volume, which leads to lower blood pressure and decreased oxygen demand from the heart. They inhibit the angiotensin-converting enzyme, an important component of the renin–angiotensin–aldosterone system. Frequently prescribed ACE inhibitors include perindopril, trandolapril, captopril, enalapril, lisinopril, and ramipril.

ACE inhibitors inhibit the activity of angiotensin-converting enzyme (ACE), an enzyme responsible for the conversion of angiotensin I into angiotensin II, a potent vasoconstrictor. Captopril, enalapril, fosinopril, lisinopril, perindopril, quinapril, ramipril, trandolapril, benazepril

A systematic review of 63 trials with over 35,000 participants indicated ACE inhibitors significantly reduced doubling of serum creatinine levels compared to other drugs (ARBs, α blockers, β blockers, etc.), and the authors suggested this as a first line of defense. The AASK trial showed that ACE inhibitors are more effective at slowing down the decline of kidney function compared to calcium channel blockers and beta blockers. As such, ACE inhibitors should be the drug treatment of choice for patients with chronic kidney disease regardless of race or diabetic status.

However, ACE inhibitors (and angiotensin II receptor antagonists) should not be a first-line treatment for black hypertensives without chronic kidney disease. Results from the ALLHAT trial showed that thiazide-type diuretics and calcium channel blockers were both more effective as monotherapy in improving cardiovascular outcomes compared to ACE inhibitors for
this subgroup. Furthermore, ACE inhibitors were less effective in reducing blood pressure and had a 51% higher risk of stroke in black hypertensives when used as initial therapy compared to a calcium channel blocker. There are fixed-dose combination drugs, such as ACE inhibitor and thiazide combinations.

**Lercanidipine:**

Lercanidipine (trade name Zanidip, among others) is an antihypertensive (blood pressure lowering) drug. It belongs to the dihydropyridine class of calcium channel blockers, which work by relaxing and opening the blood vessels allowing the blood to circulate more freely around the body. This lowers the blood pressure and allows the heart to work more efficiently.

The drug acts more slowly than older dihydropyridines. It probably has fewer adverse effects, but a comparatively high potential for drug interactions. Lercanidipine was first marketed in 1997.

Like other dihydropyridine class calcium channel blockers, lercanidipine blocks L-type calcium channels in the smooth muscle cells of blood vessels, relaxing them and thus lowering blood pressure. In contrast to the non-dihydropyridine calcium channel blockers verapamil and diltiazem, it does not significantly act on calcium channels in the atrioventricular node, and therefore does not decrease heart rate, in usual therapeutic doses.

Lercanidipine is slowly but completely absorbed from the gut. It has a total bioavailability of 10% due to an extensive first-pass effect, or up to 40% if taken after a fatty meal. Highest blood plasma levels are reached after 1.5 to 3 hours. The substance is quickly distributed into the tissues and bound to lipid membranes, where it forms a depot. The circulating fraction is almost completely (>98%) bound to plasma proteins.

It is completely metabolized in the liver, mainly via CYP3A4. Elimination half-life is 8 to 10 hours, and the drug does not accumulate. Because of the depot effect, the antihypertensive action lasts for at least 24 hours. 50% are excreted via the urine.

The lercanidipine molecule has got one asymmetric carbon atom. While the S-enantiomer is more effective than the R-enantiomer, marketed formulations contain a 1:1 mixture of both (i.e., the racemate).
Enalapril:

Enalapril (marketed as Vasotec in the US, Enadex and Renitec in some other countries, and Enacard for veterinary use) is an angiotensin-converting-enzyme (ACE) inhibitor used in the treatment of hypertension, diabetic nephropathy, and some types of chronic heart failure.

ACE converts the peptide hormone angiotensin I to angiotensin II. One of the actions of angiotensin II is the vasoconstriction of blood vessels, resulting in an increase in blood pressure. ACE inhibitors such as enalapril prevent this effect. Enalapril has been shown to lower the death rate in systolic heart failure.

It is on the World Health Organization's List of Essential Medicines, the most important medications needed in a basic health system. Enalapril is used to treat hypertension, symptomatic heart failure, and asymptomatic left ventricular dysfunction. It has been proven to protect the function of the kidneys in hypertension, heart failure, and diabetes, and may be used in the absence of hypertension for its kidney protective effects. It is widely used in chronic kidney failure.

Normally, angiotensin I is converted to angiotensin II by an angiotensin-converting enzyme (ACE). Angiotensin II constricts blood vessels, increasing blood pressure. By inhibiting the ACE, enalaprilat, the active metabolite of enalapril, decreases levels of angiotensin II leading to less vasoconstriction and decreased blood pressure.
Enalaprilat is the active metabolite of enalapril. It is the first dicarboxylate-containing ACE inhibitor and was developed partly to overcome these limitations of captopril. The sulfhydryl-moiety was replaced by a carboxylate-moiety, but additional modifications were required in its structure-based design to achieve a potency similar to captopril.

Enalaprilat, however, had a problem of its own. The consequence of the structural modifications was to give it ionisation characteristics that do not allow sufficient GI absorption for oral administration (in tablets). Thus, enalaprilat was only suitable for intravenous administration. This was overcome by the esterification of enalaprilat with ethanol to produce enalapril.

As a prodrug, enalapril is metabolized in vivo to the active form enalaprilat by various esterases. Peak plasma enalaprilat concentrations occur 2 to 4 hours after oral enalapril administration. Elimination thereafter is biphasic, with an initial phase which reflects renal filtration (elimination half-life 2 to 6 hours) and a subsequent prolonged phase (elimination half-life 36 hours), the latter representing equilibration of drug from tissue distribution sites.

The prolonged phase does not contribute to drug accumulation on repeated administration but is thought to be of pharmacological significance in mediating drug effects. Renal impairment [particularly creatinine clearance < 20 ml/min (< 1.2 L/h)] results in significant accumulation of enalaprilat and necessitates dosage reduction. Accumulation is probably the cause of reduced elimination in healthy elderly individuals and in patients with concomitant diabetes, hypertension and heart failure.
Accurate quantification of drug is of utmost importance for determination and correlation of pharmacokinetic (PK) and pharmacodynamic (PD) evaluation. Several reports have been published with emphasis on exact correlation of these factors. Several regulatory agencies have issued guidance to industry/researchers on deciding the critical parameters to prove the suitability of quantification method for intended application. Selection of appropriate extraction technique and detection method is very important to achieve accurate and reproducible method for quantification of xenobiotics. Drug analysis plays important role in the development, manufacture and therapeutic use of drugs. Drug analysis means identification, characterization, and quantification of drugs. It is also useful in assuring quality during the manufacture of drug formulations. Bioanalytical methods play essential roles in in-vitro and pharmacokinetic studies i.e., studies of the absorption, distribution, metabolism and elimination of drugs on animals and humans.

Analysis of drug in biological fluids generally involve two steps viz., extraction from complex biomatrices (blood, plasma, serum, liver microsomes, hepatocytes, Caco-2 cells, bile, urine, feces, tissues etc.) and measurement of compound of interest in extracted fluids by chromatographic method coupled to detection module. There is a growing need for development of bioanalytical method/s in drug discovery research and drug therapy (post market surveillance and clinical).

Development and validation of simple, selective, accurate and reproducible bioanalytical method/s is difficult as quantification of drugs has to be suitable for determination of drug at very low concentration levels (e.g. ng/mL levels). Assessment of various pharmacokinetic parameters (such as AUC, tmax, Cmax, Kel, Vd , t1/2) in discovery and/or clinical studies is important for decision making on treatment regime.