5.0 CONCLUSION

Enalapril during storage and analysis handling in solution undergoes cyclization, isomerization and degradation. Slow cyclization followed by *cis, trans* isomerization of the peptides bonds involving proline of the enalapril is responsible for alternation of the chromatographic behaviour. Thus, several peak were found during the HPLC & GC-MS analysis for enalapril stock solution, even the solution was put in the freezer. As a result, proline is known to play an unique role because its side chain is linked to the α - nitrogen atom. So, *cis* isomers of these peptide bonds are only slightly less stable than trans isomer. Their result clearly show that the slow isomerization of the imido peptide bond is responsible for peak splitting under isocratic condition.

We have developed a method to stop the cyclization, isomerization, degradation of enalapril in methanol by adding pyridine to the freshly prepared enalapril, followed by immediate injection on HPLC. From several experimental of this pyridine adding solution of enalapril, we have seen that there were no more changes of enalapril during our HPLC analysis up to 10ng/ml concentration. While the plasma extracted enalapril and treated pyridine had shown some degradation of the enalapril during the work.

When enalapril derivatised in diazomethane to form corresponding methyl ester, this has prevent to certain degree of cyclization. The enalapril which was treated with the diazomethane and form the methyl ester was quantitative and show to be more stable in methanol/ plasma extractd solution during storage. Further derivatization with acetic acid or trifluoroacetic anhydride was not require since we can detect up to 0.03125ng which was our target level for our enalapril and enalaprilat.

For the analysis of high concentration range of compounds, HPLC promises to become an alternative tool, not only as specific reference method but even more as an alternative for the routine assays. However, the concentration of enalapril in plasma to be detected is up to 6.25ng/mL. As a result, the HPLC technique which is able to satisfyingly quantitate substances in the lower concentration range is not quite reliable.

To analyze this low concentration substances, GC – MS is said to provide higher sensitivity & accurate quantitative results.

The combination of GC – MS has resulted in greater utilization of both because of the compatible manner in which they compliment each other. Both utilize small sample (10^{-6} g) and have god limits of detection (10^{-10} g) where they differ is that GC has good separating ability but is a poor qualitative tool, while simple MS is difficult to use with complex mixtures but is for confirmation of peak identity.

In GC – MS, the mass spectrometer provides a large amount of qualitative information in very small samples. Since any compound that passes through a GC will be converted into ions in the MS, it is safe to say that MS is a universal detector for GC. The sensitivity of total ion current monitoring (MS) is 10^{-8} g and for selected ion monitoring is 10^{-12} g. The MS also provide a multiple ion monitoring where the intensities of two or more ions are records as a function of time.

Both capillary columns DB–1 (dimethylpolysiloxane) and DB–5 (5% phenyl, methylpolysiloxane) are able to achive the desired separation in GC – MS analysis, provided with the suitable chromatographic conditions.

As a conclusion, diazomethane is perfectly satisfactory in the derivatisation of the carboxylic acids in enalapril and without further derivatisation of the amidegroup. Using GC–MS with DB–5 column is a judicious choice for the analysis of enalapril maleate and enalaprilat.

Now, this developed method is being used in pharmacology department of University Malaya to study the pharmacokinetics of the enalapril in the biological fluids.