SUMMARY

Chromatography is a widely used analytical tool that is often at the forefront of new discoveries in chemistry, biology, medicine, pharmacy, clinical chemistry, and the environmental sciences. As the technique is represented by many literatures there has been a trend in recent years to divide its subject matter into a series of subsections or specialities.

Liquid chromatography (LC) is frequently reffered to as the single most successful method in modern analytical chemistry, because LC allows separation at room temperature and in most cases does not need a derivatization before detection. Therefore, the list of application of LC has become increasingly impressive during the last few years. Using selective columns and mobile phase compositions, LC has become an extremely valuable method for separation, identification, and quantitation of the various compounds.

Recently, miniaturization of LC has become very important, because the features of microcolumn LC has been utilized in many fields and numerous applications have been developed. The amount of sample required for analysis using microcolumn LC is small, which favours biological applications. Therefore, microcolumn LC is applicable for biological fluids, foods, drugs and environmental analysis.

This dissertation describes works focused on the development of microcolumn LC for separation and optimization of fat soluble vitamins and also isomers of tocopherols.

In this dissertation, microcolumn LC has been used for separation of fat soluble vitamins on Vydac 201TPB5 (polymeric C18) column using isocratic ternary mobile phase consisted of acetonitrile, ethanol and methanol (**Chapter 3**). The proposed method was used to analyze fat soluble vitamins in margarine, butter and multivitamin tablets. A simple method for extraction of the vitamins from margarine and butter was used.

Furthermore, elution behaviours of fat soluble vitamins on various chemical bonded materials in non-aqueous reversed-phase microcolumn LC have been investigated (**Chapter 4**). As the results, the best performance was found on polymer based octadecyl bonded phase. Optimization of separation for vitamins D2, D3, E and E acetate has been tried using this polymer based column and has been found that binary mobile phase consisted of acetonitrile and methanol was enough to obtain complete separation of the fat soluble vitamins.

Then the method has been used for quantitative analysis of fat soluble vitamins in commercial pharmaceutical formulations (**Chapter 5**), in which the fat soluble vitamins in them were able to be determined without any special treatment.

From the result of optimization of fat soluble vitamins separation in **Chapter 4**, it has been found that polymer based octadecyl column offers the best separation. Recent much interests in packing materials, however, are focusing on polymer coated silica packings (**Chapter 6**). It has been confirmed that polymer coated ODS-bonded silica packing acts like polymer based octadecyl bonded phases in the separation

of fat soluble vitamins.

Since separation of isomers of tocopherols is important for determination of "total" tocopherols in foods, vegetable materials, and oils, the work has been focused for this object. The separation of the four isomers of tocopherols were evaluated with both normal-phase and reversed-phase microcolumn LC (**Chapter 7**). In normal-phase system, naphthylethyl bonded phase was the best for the separation of the isomers with 0.1% hexafluoroisopropanol in n-hexane as the mobile phase. The tocopherols were well separated on reversed-phase system using polymeric octadecyl bonded phase with the mobile phase consisted of acetonitrile and n-hexane modifier.

Furthermore, in **Chapter 8** simultaneous separation of fat soluble vitamins and tocopherols has been investigated as extention of the previous Chapters. The best performance of the separation has been achieved using the polymeric octadecyl bonded phase column and the mobile phase composition of acetonitrile, ethanol, methanol and n-hexane, 72.67%, 15%, 6.67% and 5.66%, respectively.