

I. STATEMENT OF PURPOSE

The objective of this study is to measure the effects of Column Temperature, Solvent Flow Rate, Feed Concentration and Injection Volume on retention time of carbohydrates in the HPLC system. Each of these variables must be optimized to achieve the best possible separation and improved resolution for the unknown compounds to be detected using HPLC analytical technique.

II. EXECUTIVE SUMMARY

High Performance Liquid Chromatography (HPLC) is widely used analytical technique for Chemical Separations, Purification, Detection and Quantification of unknown compound. Our research involves the use of this technique to detect unknown compounds formed during the reactions that can possibly be carbohydrates molecules. Thus it is important to study the effects of important factors like on temperature, flow rates, concentration, pH and various other factors on these compounds so as to a chieve good separation, detection and quantification of each unknown compound in the reaction mixture.

A 2^4 factorial experiment was designed to study the effects of Column Temperature, Solvent Flow Rate, Feed Concentration and Injection Volume on the retention time of Glucose, a representative compound of the carbohydrate group. The runs were randomized and experiments were conducted with 2 replicates data sets. After studying the effects of these factors on one compound or a mixture of 2 known compounds, the knowledge can be applied to get effective results for a complex problem.

After the statistical analysis it was found that flow rate and column temperature has pronounced effects on the retention time of the glucose. Even though the effect of flow rate is much larger, temperature effect cannot be neglected specially when we are dealing with unknown compounds and there is distinct possibility of components have retention times very close to each other. The results obtained can be further studied with other factors like eluants ionic strength, pH of the solvent etc. to better understand the complex system. Each of these variables should be optimized to achieve the best possible separation.