Experiment-#

OBJECTIVE

Estimation of Glucose and acetic acid by High Performance Liquid Chromatography (HPLC) method.

INTRODUCTION

HPLC is a separation technique which is based on the principle of distribution or partition coefficient. Partition coefficient is the way in which a compound distributes between two different phases. It is the ratio of concentration of analyte (test compound) in phase A and concentration of analyte in phase B.

All chromatography system is divided into following parts

1. Stationary phase which may be solid, or gel, liquid, or solid liquid mixture packed in column
2. Mobile phase which may be liquid or gaseous acts as a carrier of analytes.
3. High pressure pump which pumps the eluent (mobile phase) from solvent reservoir to the detector through the column coated with stationary phase.
4. Sample injection loop which injects the sample into the mobile phase
5. Detector is the final component of the HPLC system which analyze the separated analytes

The sample is injected through an injector and carried into the column by mobile phase the components are separated on the stationary phase and pass through the detector in succession, a chromatogram is recorded.

Definitions

None

References

Keith Wilson, John Walker Principles and Techniques of Biochemistry and Molecular Biology (2010, Cambridge University Press).

Responsibilities and Guidelines Policies

- It is the responsibility of the person(s) performing this test to be familiar with lab safety procedures and to have basic laboratory skills. The interpretation of results must be done by a person trained in the procedure and familiar with such interpretation.

Materials

1. HPLC equipped with Refractive Index Detector
2. 5mM Sulphuric Acid (2L)
3. Rezex ROA Organic Acid (H+)
4. HPLC vials
5. D- Glucose (Merck, Germany) 5g/L for stock preparations
6. Acetic Acid (Merck, Germany) 5 g/L for stock preparations
7. Deionized water 2L
8. Pipettes 1000ml
9. Centrifuge tubes 50 ml
10. Microcentrifuge tubes 2 ml

Procedure

1. Measure 250 mg D- Glucose in 50 ml Deionized water to make stock concentration of 5g/L.
2. Measure 250 mg (250 ul) of Acetic Acid in 30 ml Deionized water and make up the volume to 50 ml to make stock concentration of 5g/L.
3. Prepare the dilutions from the stock solution (5g/L) in the following manner
4. 1.0 ml of glucose stock + 0.5 ml DI water = 1:1.5 = 6.7 mg/ml (3.35 mg/0.5 ml)
   1.0 ml of glucose stock + 1.0 ml DI water = 1:2 = 5.0 mg/ml (2.5 mg/0.5 ml)
   1.0 ml of glucose stock + 1.5 ml DI water = 1:2.5 = 4.0 mg/ml (2.0 mg/0.5ml)
   1.0 ml of glucose stock + 2.0 ml DI water = 1:3 = 3.3 mg/ml (1.65 mg/0.5 ml)
   1.0 ml of glucose stock + 4.0 ml DI water = 1:5 = 2.0 mg/ml (1.0 mg/0.5 ml)
5. Put 0.5 ml of each dilution into HPLC vials and marked them properly.
6. Set the HPLC following conditions
   Flow rate – 0.6 ml / min
   Column temperature 60°C
   Detector Temperature 36°C
   Injection volume 5 uL
7. Load method on the HPLC software and prepare the sequence table.
8. Load HPLC vials into the HPLC autosampler
9. Start the analysis of samples
10. After completion of the run measure the area of the samples peaks and note it down.

Plot the peak area vs Concentrations of standards to get a straight-line equation.

Standard	**Set1**	**Set2**	**Set3**
Concentration (mg/0.5ml)	**Area 1**	**Area 2**	**Area 3**
1.00			
1.65			
2.00			
2.50			
3.35			

	Average
Concentration(mg/0.5ml)	**Area**
1.00	
1.65	
2.00	
2.50	
3.35	