High performance liquid chromatography (HPLC) of Sugarcane juice

Project: Sequencing
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Introduction

High-performance liquid chromatography (HPLC) is a powerful separation technique used for the analysis of ions, proteins, and organic molecules. HPLC is based on the mechanisms of adsorption, partition and ion exchange, which depend on the type of stationary phase used. We are interested in making auxotrophic bacteria to further strengthen the biosafety of our project. Hence, we are interested to determine the amino acids present in the sugarcane juice using HPLC.

Procedure

Mobile Phase Preparation

1. Add 400 mL of acetonitrile to approximately 1.5 liters of purified deionized water.
2. Add glacial acetic acid (2.4 mL) to this solution.
3. Dilute the solution with purified deionized water to make a total volume of 2.0 L in a volumetric flask. The pH of the resulting solution should be between 2.8 to 3.2.
4. Add 40% sodium hydroxide to adjust the pH to 4.2.
5. Filter the mobile phase under vacuum to remove the gas and the solids that could clog the chromatographic column from the solution.

Sugarcane Sample Preparation

Make the solution of the sugarcane juice as per the requirements into components required.

Making the standard solutions

1. Pipet the appropriate amount of each component in a 50-mL volumetric flask.
2. Dilute the stock solutions to the 50-mL mark on the volumetric flasks with the mobile phase.
3. Pour each standard solution in small vials.
4. Store the samples in a refrigerator, along with the remaining solutions.

Checking the settings of the HPLC system

1. Ensure that the waste line is in the waste container.
2. Set the flow rate of the mobile phase to 0.5 mL/min.
3. Set the minimum and maximum pressure and the flow rate to the correct values on the front panel of the delivery pump.
4. Set the minimum pressure at 250 psi.
5. Set the maximum pressure at 4,000 psi.
6. Press “zero” on the detector’s front panel to set at the pure mobile phase.
7. Rinse a 100-µL syringe with deionized water, then wash it with several volumes of component solutions to be analyzed, and fill the syringe.

Sample injection and data collection

1. Slowly inject 100 µL of the solution with the injector handle in the load position through the septum port.
2. Set the data collection program to collect data for 300 seconds, to get the 3 peaks to elute through the detector.

3. Before starting the trial, rotate the injector handle to the inject position and click “Start Trial” on the computer data collection program immediately.
4. Once 300 seconds have passed, the data collection sends a signal to save the data file.
5. Note the time in seconds for the peak of each trial to identify each component.
6. Remove the syringe from the septum and perform the process for each of the remaining working standards.

Calculations

1. Calculate the concentration of all the components in the sample solution.
2. Determine the peak areas on the chromatograms for each standard and the unknown samples by the triangular method, which equals peak height times the width at ½ height
3. Determine the time taken by the components to reach the peaks.
4. Draw calibration curves of peak area vs. concentration (mg/L) for all components.
5. Calculate the least-squares fit for calibration curves.