ENGINEERING/TECHNOLOGY

Using Centrifugal Partition Chromatography to Separate Xylose from Glucose: SOP Development

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Efficient methods of extracting natural compounds from their native source are essential to the medical, biological, and food industries. Xylose and glucose are two major sugars used in a variety of food, bioenergy, and environmental industries and are found in hemicellulose, a compound present in all biomass. The aim of this project was to generate protocols for an effective separation of xylose from glucose using centrifugal partition chromatography (CPC). CPC, commonly used for the separation of natural products, operates on the combined concepts of rotary motion and biphasic liquid separation. It is preferred to other chromatographic techniques for natural product recovery because the liquid stationary phase allows for complete recovery of compounds as compared to conventional chromatography using a solid stationary phase. Our specific goal was to develop standard operating procedures (SOPs) to separate xylose from glucose. The two liquid phases present in the CPC rotor—generally termed the solvent-system—separate compounds depending on their affinity for each liquid

phase. The suitability of a solvent-system is based on two different chromatography measures, partition coefficient (K) and separation factor (α), which were determined using the shake-flask method. We tested three solvent-systems mixed in various ratios: butanol-ethanol-water (BEW), butanol-ethyl acetate-water (BEAW), and hexane-ethyl acetate-methanol-water (HEMWat). The concentration of each compound in each phase of the three solvent-systems was determined using high performance liquid chromatography (HPLC). The BEW system, mixed in a 3:1:4 ratio by volume, was chosen as most appropriate as it produced K and α values within acceptable ranges. This solvent-system was then used in the CPC to purify xylose from glucose. Protocols based on these findings were documented and will be helpful to add proficiency in creating bioproducts in future.

Research advisor Abigail Engelberth notes, “The use of centrifugal partition chromatography to purify hemicellulose oligomers is gaining momentum within the renewable energy sector. The components that make up hemicellulose could prove to be valuable as building blocks for commodity chemicals from biomass. The work performed by Srishti was essential as a starting point to use this new-to-us instrument to further our research.”

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