Purification of simple carbohydrates with flash chromatography

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Chromatography Application Note AN140

Abstract

Carbohydrate compounds are easily purified using RediSep Gold Amine columns with Evaporative Light Scattering Detection (ELSD). The column was run using a Hydrophilic Interaction LIquid Chromatography (HILIC) gradient method using acetonitrile or acetone and water gradients. Dissolving the sample to be purified in DMSO allows a large sample load while allowing good resolution.

Background

Carbohydrates are commonly analyzed using an amine column with good resolution. This method generally uses acetonitrile and water, with the sample commonly dissolved in water. As the sample injection volume is small, the sample has an opportunity to adsorb on the stationary phase. In preparative chromatography, the sample load and injection volumes are much larger relative to the columns size, so injecting the sample in water prevents the carbohydrates from adsorbing on the column, causing them to elute at the void. Dry-loading the sample on solid load cartridges is commonly used for flash chromatography, but a user would need to pack their cartridges with amine media. The sample is still dissolved in water to load the sample, which requires a long time to evaporate before running the sample.

Dimethyl sulfoxide (DMSO) is commonly used to dissolve samples for reverse phase chromatography because it solubilizes most compounds. DMSO dissolves carbohydrates, yet it is a weak solvent in HILIC, so it allows the sample to adsorb on the column. DMSO elutes at the early in the elution when using amine columns; however, it may elute late in the gradient during other HILIC runs using media other than amine.

Results and discussion

Although HILIC is normal phase, it employs the solvents generally used for reverse phase chromatography, so the ELSD settings need to be changed using the settings in Table 1 to allow baseline stability while maintaining sensitivity.

ELSD control	Setting value
Spray Chamber	20 °C
Drift Tube	60 °C
Gain	1
Sensitivity	High

Table 1. ELSD settings for purifying carbohydrates.

The samples were all dissolved in DMSO. When needed, the sample was heated in a hot water bath to facilitate dissolution. The PeakTrak Flash Focus Gradient Generator was used to develop the method on the system. A scouting gradient was run to verify that the sample would elute, and to show that there was sufficient resolution between compounds to allow a successful purification. The retention of the desired compound is used to calculate the solvent composition for the focused gradient. Redi*Sep* Gold® Amine columns were used for all runs. After the runs were complete, the columns were washed and stored in 2-propanol, which is miscible with organic solvents, allowing rapid purification of less polar compounds.

The first example uses ribose and glucose. Both the scout and focused gradient used acetonitrile as the weak solvent. Only a few milligrams were used for the scout run, and the ELSD gain was increased to 3 to improve sensitivity for this small sample load. The second eluting peak was used to focus the gradient; after the gradient was calculated, the ELSD gain was reset to 1 to keep the ELSD response on scale. The total sample load was 100 mg on a 50 g Redi*Sep* Gold Amine column.

Fructose and sucrose are commonly found together in samples. The purification of fructose from a glucose impurity is shown in Figure 2.

The mixture was run in a similar fashion as the ribose-glucose sample, with the gradient focused on glucose. The peak appearing at ~1.8 column volumes (CV) is the DMSO used to dissolve the sample.

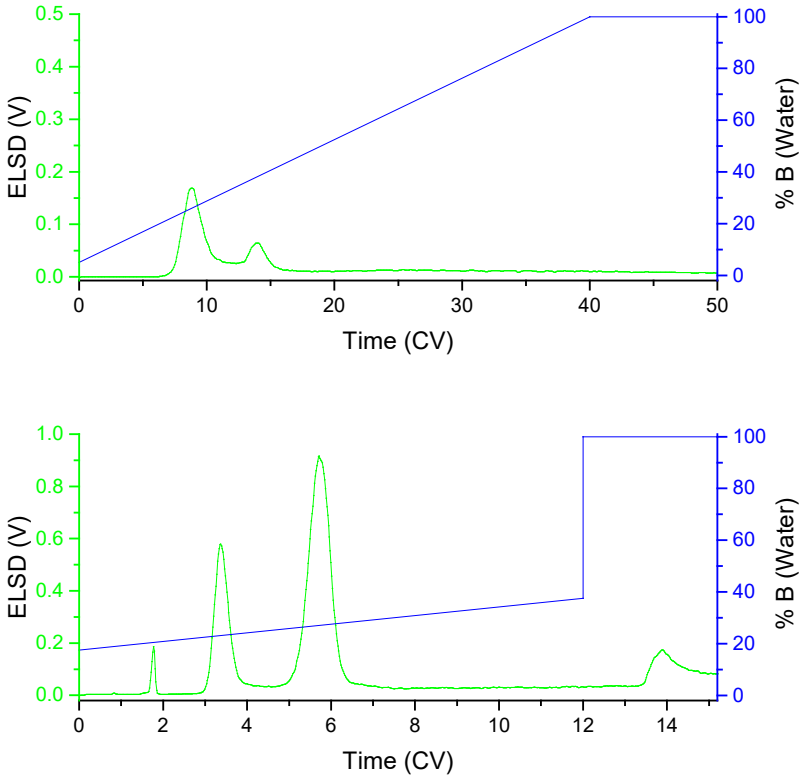

Figure 1. Ribose and glucose run in a scouting method on a 5.5 g RediSep Gold Amine column (top) and focused to a 50 g RediSep Gold amine column. The total sample load was 50 mg each of ribose and glucose. The small peak at ~1.8 column volumes in the focused gradient is DMSO.

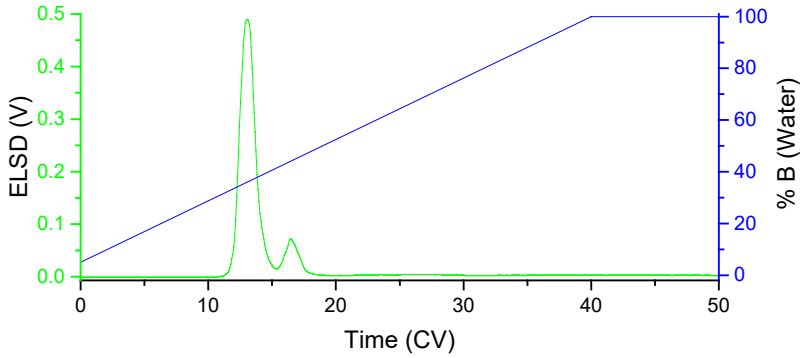

Figure 2. Purification of impure fructose from sucrose using RediSep Gold Amine columns and an acetonitrile/water gradient.

Acetone as a weak solvent

Acetone is also a weak solvent for HILIC and can be used instead of acetonitrile. Although alcohols can be used for HILIC, those solvents are too strong to purify carbohydrates on an amine column. A sample of fructose and glucose was purified using acetone.

The mixture was purified like the earlier examples, except that a 15.5 g RediSep Gold Amine column was used for the scout gradient, as PeakTrak allows any size Teledyne ISCO column to be used for scout runs. The focused gradient used a 50 g RediSep Gold Amine column, but the calculated gradient needed a lower concentration of water to purify the glucose indicating that acetone is a stronger solvent compared to acetonitrile for these compounds.

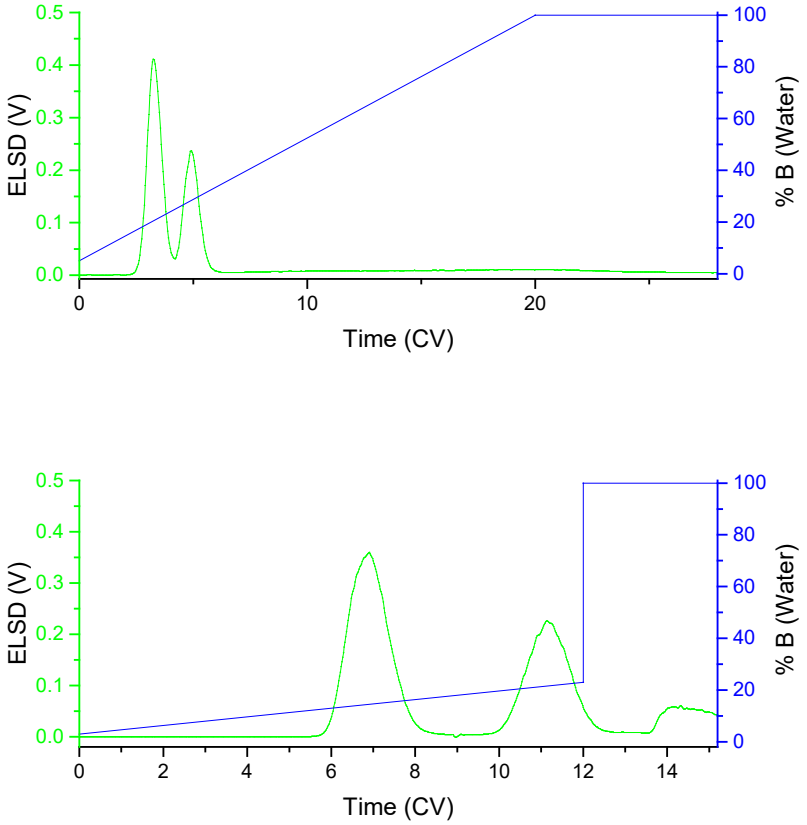

Figure 3. Fructose and sucrose purified using an acetone/water gradient. A 15.5 g RediSep Gold amine was used for the scouting run.

Conclusion

Carbohydrates can be efficiently purified with the NextGen 300+ with ELSD using RediSep Gold amine columns using a HILIC gradient method. Using DMSO to dissolve the samples allows for both high sample load and good resolution. The PeakTrak Flash Focus Gradient Generator allows for rapid method development and scale-up for all columns manufactured by Teledyne ISCO.

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