

How well is your column performing?

Are you obtaining the best results from your chromatography?

No matter what type of chromatography you are using (HILIC-LC for glycan GU, WAX-LC for glycan charge; RP-LC for sialic acid or monosaccharides analysis) it is good practice to run a regular system suitability check.

When you set up a new column it is important to record the peak resolution and shape. These parameters should then be tracked so that you can determine when it is time to clean or change your column. The lifetime of a column is dependent on a number of factors such as the cleanliness of samples and buffers; the number and loading of samples injected; and the effectiveness of post-run wash cycles. Installing an in-line filter and/or filtering all buffers and samples can extend column life-time, as can ensuring that the column is cleaned and stored properly (please refer to the column specific product documents for this information).

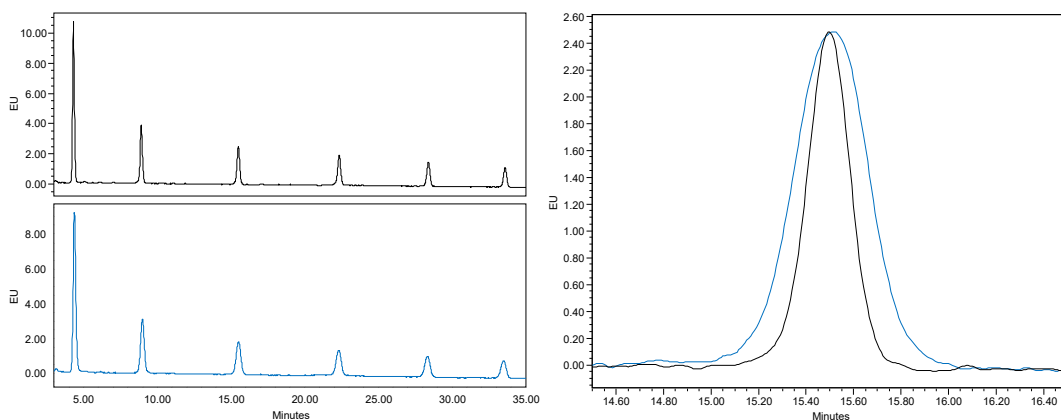


Figure 1: HPLC chromatogram from 2-AB labelled Glucose Homopolymer Ladder [CAB-GHP-30] run on a 4.6 x 150 mm LudgerSepN2 column.

Figure 1 illustrates degradation in performance of a LudgerSepN2 [LS-N2-4.6x150] column which is used for separating fluorescently labelled glycans. The peak width at half height for GU3 has increased from 0.20 min (black trace) to 0.36 min (blue trace), so it is time to change the column.

How often do you check peak resolution and shape?

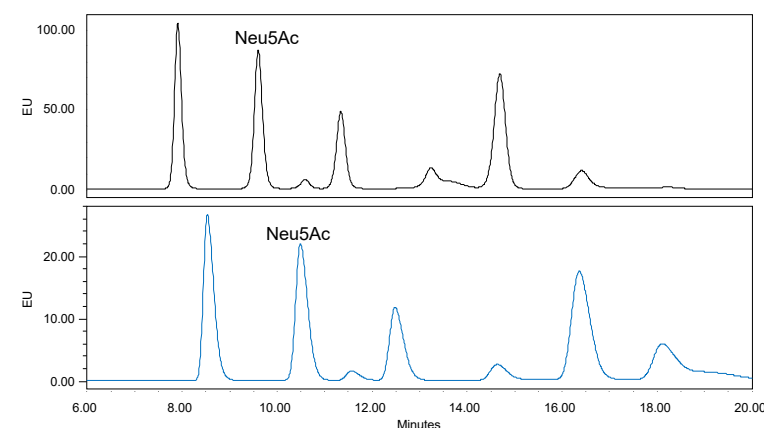


Figure 2: HPLC chromatogram from DMB labelled Sialic Acid Reference Panel [CM-SRP-01] run on a 4.6 x 150 mm LudgerSep-R1 column.

Figure 2 illustrates degradation in performance of a LudgerSepR1 [LS-R1-4.6x150] column which is used for separation of DMB labelled sialic acids. The peak width at half height for Neu5Ac has increased from 0.19 min (black trace) to 0.28 min (blue trace), and the peak is no longer symmetrical, so it is time to change the column.

Continual monitoring of peak width of system suitability standards such as 2-AB Glucose Homopolymer Ladder [CAB-GHP-30], DMB labelled Sialic Acid Reference Panel [CM-SRP-01] or 2AA labelled monomix [CM-MONOMIX-10] will allow you to determine when you will need to clean or replace your columns.