

For HILIC chromatography, a SeQuant ZIC-pHILIC column (5 µm particle size, 2.1 mm x 150 mm, from Millipore) was used with 10 mM ammonium carbonate in 85:15 acetonitrile to water (Solvent A) and 10 mM ammonium carbonate in 60:40 water to acetonitrile (Solvent B) at a flow rate of 0.15 mL/min.

This column was compared with a Waters UPLC BEH amide and a Millipore cHILIC column;

the pHILIC showed superior reproducibility and peak shapes.

The column was held at 100% A for 2 minutes, ramped to 100% B over 18 minutes, held at 100% B for 5 minutes, and equilibrated at 100% A for 25 minutes (50 minutes total).

The column was maintained at 30 C.

The injection volume was 2 µL for samples and standard mixes.

When starting a batch, the column was equilibrated at the starting conditions for at least 30 minutes.

To improve the performance of the HILIC column, we maintained the same injection volume, kept the instrument running water blanks between samples as necessary, and injected standards in a representative matrix in addition to standards in water.

After each batch, the column was flushed with 10 mM ammonium carbonate in 85:15 water to acetonitrile for 20 to 30 minutes.