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PolyGLYCOPLEX™ Columns

Initial Use: PolyGLYCOPLEX A is a silica-based material that operates through hydrophilic interaction chromatography (HILIC). Retention is influenced by the solvent used: ACN~PrOH>EtOH>MeOH. Columns are shipped in methanol. Prepare the column for use in the following manner (200 x 4.6mm ID columns):

Flush with 15 column volumes of water (30 minutes @ 1 mL/min)
Flush with 30 column volumes of conditioning buffer (1 hour @ 1 mL/min)
Flush with 15 column volumes of water (30 minutes @ 1 mL/min)
Flush with 30 column volumes of mobile phase (1 hour @ 1 mL/min)

This can be any buffered solution of high ionic strength between pH 3 – 6.5. i.e. 300mM KH_2PO_4.

It's a good idea to run at least one gradient cycle with a new column before injecting samples. Changes in the topography of the polymeric coating may lead to modest changes in the retention times during the first few runs following exposure to aqueous mobile phases.

New HPLC columns sometimes absorb small quantities of proteins or phosphorylated peptides in a nonspecific manner. The sintered metal frits have been implicated in this. Eluting the column for 20-24 hr. at a low flow rate with 40mM EDTA.2Na usually solves the problem. This passivates all metal surfaces in the HPLC system, as well as the column [CAUTION: This treatment can affect the integrity of the frits in some cases, and should probably be avoided with columns packed with 3-µm material. In some cases this has also caused the collapse of 5-µm, 200-Å column packings].

If a column is to be used in **mass spectroscopy**: Columns of all materials suitable for protein applications will leach coating components when new. This can result in an elevated background in mass spec. To accelerate the loss of leachable coating components, substitute 50 mM formic acid for the salt solution to condition a new column. Elute the column for 24 hours at a low flow rate. Then, flush out the formic acid with water and equilibrate with the HILIC mobile phases.

Routine use: Columns should be used at ambient temperatures. Filter mobile phases and samples before use. Failure to do so may cause the inlet frit to plug. If a gradient is being used, flush the column with 15 column volumes of the low organic solvent before equilibrating with the high organic solvent. At the end of the day, flush the column with 15 column volumes of water and plug the ends.

Loading Capacity: The loading capacity of a 4.6mm ID column is about 1 mg of polar solute, depending on the strength of the solute's binding to the support and the level of organic solvent in the sample solvent. High levels promote binding.

Storage: 1) Overnight: 100% mobile phase A. 2) Several days: Store in water. 3) Longer periods: Store in water in the refrigerator, with the ends plugged. **ACN can be added to the storage solvent (e.g., ACN:Water = 80:20) to retard microbial growth.**

General Operation: Small complex carbohydrates are well retained in mobile phases containing 80% ACN. Larger complex carbohydrates are well retained with 70-75% ACN. Gradient elution is possible using decreasing concentrations of ACN. If the oligosaccharide is an electrolyte, then it is necessary to include an electrolyte in the mobile phase. This will prevent ion exclusion, improve peak shape, and stabilize retention times. 10mM TEAP, pH 4.4 is recommended for a mobile phase transparent at low wavelengths, while ammonium acetate is suitable as a volatile salt.

Factors Influencing Retention: At present, the following seem to promote retention:

1) Residue composition: GlcNAc, GalNAc ≥ sialic acids >>Man, Fuc, Glc, Gal.
For sialic acids, Neu5Gc > Neu5Ac > Neu5Ac2en.

2) Linkage Position: 1→6 , 2→6 > 2→3, 1→4.

3) Anomer separation is observed if the reducing end is a good contact residue (e.g. GlcNAc). This can be prevented by the addition of 0.1% triethylamine to the mobile phase; the elevated pH accelerates mutarotation, collapsing the anomer doublet into a single peak.