Procedure: PGC (Porous Graphitic Carbon) SPE for Released Glycan Samples

Materials:

Hypersep Hypercarb 50mg/1ml SPE cartridge (Thermo 60106-303)

MilliQ Water

1M NaOH – 4g NaOH/100ml MilliQ water

30% Acetic Acid – 30ml glacial acetic acid + 70ml MilliQ water

Elution Solvent – 50% acetonitrile in MilliQ water with 0.1% TFA (v/v)

Wash Solvent – 5% acetonitrile in MilliQ water with 0.1% TFA (v/v)

Notes: Procedure is applicable to released glycans and glycopeptides. TFA should be used. Due to high back pressure during the 30% acetic acid conditioning step, this procedure will not work well with the vacuum manifold. Do not reuse cartridges.

Equilibration: Do not allow air to enter the cartridge bed!

Pass 1ml 1M NaOH through cartridge bed to waste.

Pass 2ml MilliQ water through cartridge bed to waste.

Pass 1ml 30% acetic acid through cartridge bed to waste.

Pass 2ml MilliQ water through cartridge bed to waste.

Pass 1ml Elution Solvent through cartridge bed to waste.

Pass 1ml Wash Solvent through cartridge bed to waste.

Load Sample:
Sample should be in 1ml water with no organic solvent.

Slowly load sample onto cartridge bed at no faster than 1 drop/second, collect the effluent and do not allow air to enter the bed.

Slowly pass 1ml MilliQ water through the bed to complete loading step. Collect the effluent in the same tube as above and do not let air enter the cartridge bed.

**Desalt Sample:**

Pass 1ml Wash Solvent through bed and collect effluent in same tube as above. Do not let air enter the cartridge bed.

**Elute Sample:**

Pass 1ml Elution Solvent through cartridge bed at about 1 drop/second and collect in a clean 1.5ml eppendorf tube. Gently pass air through to elute all solvent into collection tube.

Dry in Speed Vap.

Reconstitute in solvent suitable for MS analysis system.