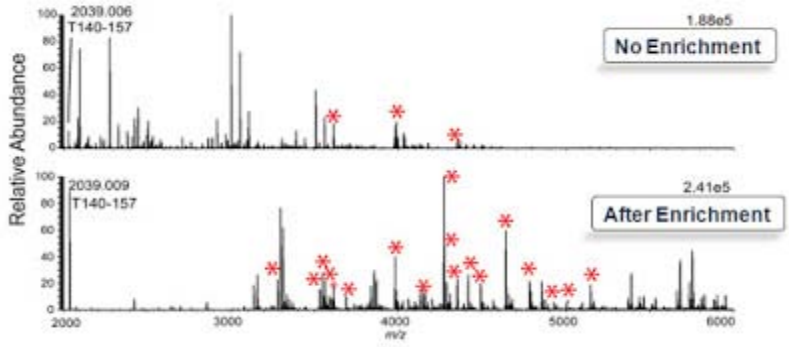
SAX for quick and simple enrichment of glycopeptides

Note that the following is not an official product offering for glycopeptides enrichment by Thermo Fisher Scientific. The following protocol is meant to be helpful as it has worked well in our hand but we haven't tested thoroughly with different sample sources.

Glycopeptides similar to phosphopeptides requires enrichment prior to mass spectrometry (MS) analysis. Without some sort of enrichment it will be very difficult to detect *N*-linked glycopeptides in the presence of non-glycopeptides. O-linked glycopeptides are even tougher to see than N-linked glycopeptides due to their low occupancy and structural heterogeneity. Below is an example that illustrates this issue. 1:1 mixtures of bovine and human alpha 1 acid glycoprotein have been tryptically digested and analyzed by LC-MS/MS. The LC region where the glycopeptides elute have been deconvoluted to show M+H ions (similar to what you would see in a MALDI spectrum). The red stars indicate glycopeptides. The top spectrum shows the analysis prior to enrichment, while the bottom spectrum shows after enrichment (limited numbers are highlighted due to availability of space in the figure, but 64 glycopeptides were detected). Even in a simple 2 glycoproteins mixture like this you can see how much difference enrichment can make.



The enrichment protocol described below is quick (less than hour), simple to do, and keeps the glycan on the peptide backbone. The approach involves the use of strong anion exchange (HyperSep Retain-AX 60mg 3mL SPE Column 50Pk; PN:60107-40) for enrichment. Besides the column, a vacuum manifold is required. One can use gravity for elution (hook it up to a ring stand) but we find that a vacuum manifold gives a nice control of the flow rate.

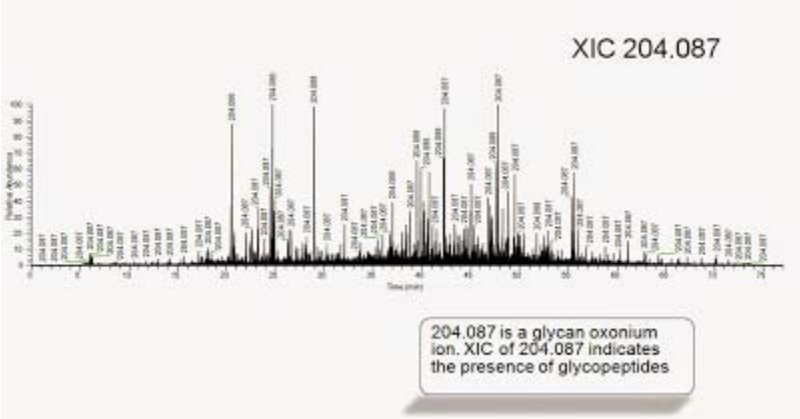
1. Equilibrate the column:
    - Wash with 1 mL ACN
    - then with 1 mL of 100 mM triethylammonium acetate
    - followed by 3 mL of water
    - and finally 1 mL of 95% ACN with 1% TFA

2. Fill the column with 3 mL of 95% ACN 1% TFA and add 200 uL of sample (50% ACN 0.1% TFA) into the column.
3. Allow the contents to flow through.
4. Wash with 3 mL of 95% acetonitrile with 1% TFA.

5. Elute bound glycopeptides with 2-3mL of 50% ACN with 0.1% TFA

   Tip: Keep the flow rate nice and steady, approximately 1 mL/min. Do not let the stationary phase dry up.

   The figure below shows enrichment of tryptically digested human serum using this protocol. LC-MS/MS chromatogram is shown for a 75 min run. The glycan oxonium ion 204.087 (HexNAc) is extracted from the chromatogram. The XIC of 204.087 indicates the presence of glycopeptides. You can see that the entire run is full of glycopeptides.



   The great thing about this protocol is that it is non-specific. Both neutral and acidic *N*-linked glycopeptides are enriched.