

## MS-Based Glycomic and Glycoproteomic Tools to Analyze Glycan and Glycopeptide Isomers in Biological Samples

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Post-translational modifications are vital aspects of functional proteins. Therefore, it is critical to understand their roles in biological processes. Glycosylation is particularly challenging to study among these modifications due to the heterogeneity displayed by the glycans in terms of their isomers. Thus, researchers continue to strive for the development of efficient liquid chromatography techniques for the isomeric separation of glycans. Porous graphitized carbon (PGC) nano column has been one of the most widely used columns for this purpose, but poor stability and lack of reproducibility led to its discontinuation. In our endeavor to find an alternative stationary phase for isomeric glycan separation, we tested the mesoporous graphitized carbon (MGC) material. Unprecedentedly, satisfactory results were obtained with a column only 1 cm long, which was tested on glycans derived from model glycoproteins as well as biological samples. The column was found to be reproducible across months as well as across different column preparations. Additionally, to decrease the dead volume and attain a better resolution, the MGC was utilized to pack a 1 cm length of pulled capillary nanospray emitter and again demonstrated an efficient isomeric separation. Thus, the MGC proved to be a suitable stationary phase to obtain efficient isomeric separation of permethylated glycans with 1 cm long packing length, in both capillary columns and packed nanospray emitters.