ADVANCING THE ISOMERIC SEPARATION OF GLYCAN AND GLYCOPEPTIDE ISOMERS BY LC-MS/MSTITLE IN CAPITAL LETTERS

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Mesoporous graphitized carbon (MGC) has emerged as a versatile and robust stationary phase, enabling high-resolution separations of structurally diverse glycan and glycopeptide mixtures—a challenge central to glycochemistry and glycobiology. This work analyzed permethylated glycans derived from model glycoproteins (e.g., bovine fetuin, RNase B) and biological specimens (human blood serum and cancer cell lines) using a custom-packed, 1 cm MGC nano-column. By fine-tuning mobile-phase composition—combining isopropanol and acetonitrile—and operating at elevated temperature (75 °C), highly branched and sialylated N-glycan isomers were baseline-resolved, underscoring the utility of MGC in addressing critical chemical and structural questions in carbohydrate research.

In a complementary approach, the same MGC platform was used to separate N- and O-glycopeptides from bovine fetuin, asialofetuin, alpha-1 acid glycoprotein, and human serum, effectively highlighting the interplay between glycan linkage microheterogeneity and protein context. Subtly different glycoforms—such as α2,3- versus α2,6-linked sialic acids—were resolved and reliably detected in a single run. Moreover, O-glycopeptide isomers were separated with minimal carryover, reflecting both the strong chromatographic performance and the method's adaptability to a range of glycoproteins. Notably, robust reproducibility across three months of continuous use reaffirms the potential of MGC-based separations for collaborative research efforts that bring together multiple facets of glycan-focused studies and applied technologies.

These findings emphasize MGC's effectiveness in supporting high-throughput glycomic and glycoproteomic investigations. By integrating advanced chromatographic selectivity, reliable quantitation, and detailed structural analysis, MGC columns can drive innovation in disease biomarker identification, therapeutic development, and fundamental glycoscience research.

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