University of Nebraska Medical Center

WP 210 Multi-nozzle emitters for structure-based glycomics

When: Wednesday, June 8th; 10:30am – 2:30pm CT

Poster presenter: Melinda Wojtkiewicz, Ph.D., University of Nebraska Medical Center

Introduction

Glycans play critical roles in physiology and disease. MS-based methods can reveal both composition and structure of glycans. This knowledge is invaluable in diverse contexts spanning across biologic drugs to novel biomarkers of health and disease. Both biomarker discovery and the characterization of biologics would benefit from robust and sensitive LC-MS/MS methods for structure-based glycomics analyses. While microflow methods are typically more robust and easier to implement for higher throughput analyses, they are less sensitive than nanospray methods. Nanospray methods offer higher sensitivity, but are often less robust. Here, we evaluated the Newomics™ MnESI source and M3 emitters for increased sensitivity of a microflow method for robust, high throughput structure-based glycomics analysis.

Methods

Samples (serum, formalin-fixed paraffin embedded (FFPE) tissue from microscope slides) were solubilized and processed using the glyPAQ kit (ProtiFi). Sample processing was complete within 6-24 hours depending on sample complexity. Reduced native *N*- and *O*-glycan isomers were separated by 200 μm x 10 cm porous graphite carbon (PGC) chromatography and analyzed using an Orbitrap Eclipse equipped with the MnESI source and 20 μm, 5 nozzle M3 emitter. A mixing tee connected the 6 μL/min flow rate to the 20 μL/min post column addition of acetonitrile. Skyline was used for spectral library-based structure assignment of glycans and MSstats was used for statistical analyses of quantitative data.

Preliminary Data

From 2 μL of serum or one FFPE slide, hundreds of *N*- and *O*-linked glycan structures were identified over 5 orders of magnitude of abundance. Compared to the traditional electrospray source, the M3 emitters resulted in a 4-10 fold increase in signal intensity for known glycans. Additionally, from the same amount of starting material, >50 glycan structure isomers were detected only when using the M3 emitters. Coupling the M3 emitters to 200 μm in-house packed PGC columns, glycan

signals from serum samples were stable for >4 weeks of continuous data acquisition (>400 injections). This setup is overall well-suited to high throughput analysis of glycan structures for biomarker studies and analysis of biologics.

Novel Aspect

First use of multi-nozzle emitters in reduced native structure-based glycomics samples.