NEWOMICS
Enabling Precision Medicine

TP 269 Interfacing MnESI and Q-TOF Mass Spectrometers for Microflow LC-MS

When: Tuesday, June 7th; 10:30am – 2:30pm CT

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Introduction

Recent advances in sample preparation techniques and high-resolution MS instruments have led to the increased applications of quantitative bioanalysis of large protein therapeutics and protein complexes using top-down approach. Currently mainly high-flow LC-MS has been used for analysis of intact proteins and protein complexes. Due to the low ionization efficiency at high flow rates, the sensitivity is poor. The Newomics silicon multinozzle emitters (M3 emitters) split the incoming microfl-ow eluent into multiple nanoflows at each nozzle, thereby significantly enhancing the ionization efficiency and reducing the matrix effects for ESI-MS. Herein we demonstrate a new microflow-nanospray ESI-MS platform by interfacing M3 emitters with Agilent Q-TOF mass spectrometers for sensitive and robust quantification of intact proteins under both native and denatured conditions.

Methods

Intact monoclonal antibody from NIST (SRM 8671), cytochrome C and bovine serum albumin (from SIGMA) were used for the analysis. LC-MS system included Agilent 1260 capillary pump(G1376A) with Low Flow HiPSampler (G1377A) interfaced to Agilent 6545XT Q-TOF. For microflow LC-MS, samples were separated on an Agilent Poroshell300SB-C8 column (1.0x75mm, 5um beads), at a flow rate of 2-10 uL/min, before introduced to a Newomics MnESI source and sprayed via a M3 multinozzle emitter (20 um id, 8-nozzle). For high-flow LC-MS, samples were separated on a 2.1mm ID C8 column before introduced to the Q-TOF. Intact mAb and other proteins were detected in the full-scan mode and the data were processed to obtain peak area intensity.

Preliminary Data

We first interfaced Newomics MnESI with Agilent LC-MS and optimized the position and spray voltage of the M3 emitters for diverse flow rates and analytes. We then performed a three-way comparison to assess the sensitivity performance of the MnESI-MS platform for intact mAb analysis. Compared to the conventional ESI Jet Stream source at the high flow (250 μl/min), we obtained over 10-fold improvement in both peak area intensity and Limit of Quantification (LOQ) with MnESI-MS platform at 5 μl/min flow rate. A LOQ of 10 ng/mL and a linear dynamic range of more than 3 orders of magnitudes were achieved using MnESI for intact NIST mAb. Compared to other microflow platform with the same column and flow rate, we obtained an average of 2.5-fold sensitivity gain.The assay robustness of the MnESI-MS platform was assessed using 100 ng intact mAb on-column injection. We achieved a CV of less than 5% with 100 consecutive injections, which outperformed the high-flow LC-MS method with the same amount of injection material.We also evaluated the performance of coupling MnESI with a high-flow LC system using a Newomics® post-column splitting kit. A T-splitter was used to split the analytical flow of 200 μl/min down to 20 μl/min that is delivered to a M3 emitter. Compared to the conventional high-flow method without splitting, this

new method by flow splitting to M3 emitters achieved slightly better sensitivity, with only 10% of IgG delivered to MS. Our MnESI-MS workflow with high-flow post-column splitting allows simultaneous detection of mAb by UV and MS without the need to change the LC system. In addition, majority of the IgG injected was diverted to UV detector or waste, minimizing the contamination of MS.

Novel Aspect

First demonstration of interfacing Newomics MnESI with Agilent QTOF-MS for significantly improving sensitivity and robustness of intact protein analysis.