Interfacing DuoESI Source and Q-TOF Mass Spectrometers for Microflow LC-MS

Booth #507

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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 microflow 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 DuoESI-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

We first developed the Newomics DuoESI source for both microflow and nanoflow LC-MS with plug-and-play and interchangeable interface modules. We then interfaced DuoESI source with Agilent 6545XT Q-TOF mass spectrometer. The detailed conditions for microflow LC-MS analysis are listed below.

Methods	Intact Protein LC-MS	Native LC-MS
Emitter/Sprayer	M3 emitter (20 µm ID, 8-nozzle)	M3 emitter (20 µm ID, 8-nozzle)
Spray Voltage (V)	3,300	3,500
Capillary Temperature	200 °C	250 °C
LC Column	Agilent Poroshell 300SB-C8, 1.0x75 mm, 5 um	PolyLC PolyHydroxyethyla 150x0.30 mm, 3 µm, 1000-Å
Flow rate (ul/min)	10.0	5.0
Solvent system	A: H2O, B: ACN	100 mM Ammonium Acetate
LC system	Agilent 1260 capillary pump(G1376A) with Low Flow HiP Sampler (G1377A)	Agilent 1260 capillary pump(G1376A) with Low Flow HiP Sampler (G1377A)

Newomics platform

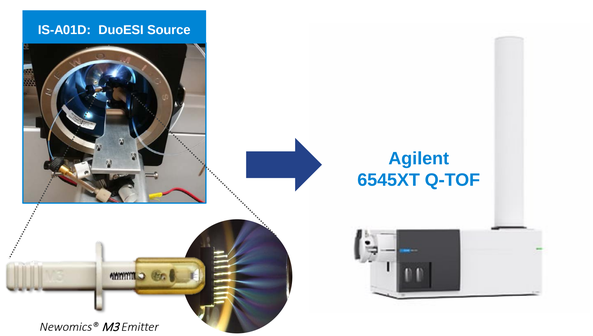
- DuoESI source
- M3 emitters

Agilent LC-MS system

- Agilent 1260 capillary pump(G1376A) with Low Flow HiP Sampler (G1377A)
- Agilent 6545XT Q-TOF mass spectrometer

Samples

- Standard proteins including Cytochrome C and BSA
- Monoclonal antibodies (NIST mAb and Herceptin)

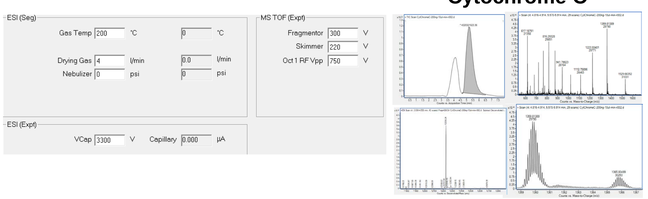


Interface between Newomics DuoESI source with Agilent 6545XT Q-TOF mass spectrometer. The DuoESI source has interchangeable interface modules for microflow LC-MS and nanoflow LC-MS applications, respectively. Shown here is the microflow module only.

RESULTS

(I) Microflow LC-Nanospray MS for intact protein analysis

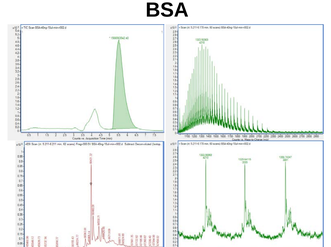
- Left panel: representative Q-TOF settings for intact analysis.
- Right panel (clockwise): LC-chromatogram, m/z, charge state distribution, and deconvoluted MS, for 200 ng of Cytochrome C.



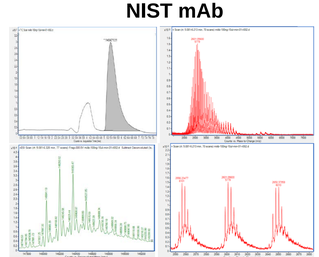
Cytochrome C

- Left panel (clockwise): LC-chromatogram, m/z, charge state distribution, and deconvoluted MS, for 40 ng of BSA.
- Right panel (clockwise): LC-chromatogram, m/z, charge state distribution, and deconvoluted MS, for 100 ng of NIST mAb.

BSA



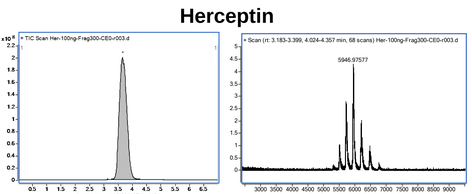
NIST mAb



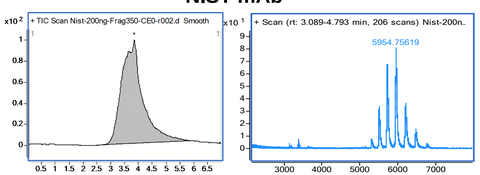
(II) Microflow LC-Nanospray MS for native MS

- **Top**: LC-chromatogram and charge state distribution, for 100 ng of Herceptin under native LC-MS conditions.
- **Bottom**: LC-chromatogram and charge state distribution, for 200 ng of NIST mAb under native LC-MS conditions.

Herceptin



NIST mAb



CONCLUSIONS

1. We have demonstrated for the first time the plug-and-play interface between Newomics DuoESI source and Agilent high-resolution Q-TOF mass spectrometer.
2. We have shown the good performance of the DuoESI-MS platform for microflow LC-MS analysis of intact proteins under both native and denaturing conditions.