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Ultra-Fast Analysis of Intact Proteins Using SPE-TOF

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Introduction

High-throughput analysis is an essential analytical tool that not only minimizes costs, but also reduces time-to-results and improves laboratory productivity. The use of Solid Phase Extraction Time-of-Flight Liquid Chromatography Mass Spectrometry (SPE-TOF) allows for the rapid analysis of samples without compromising analytical fidelity; the online SPE cleans samples to improve the system's robustness, while TOF analysis eliminates the need for targeted method development. The TOF analysis also offers high mass resolution and sensitivity required to monitor changes on an intact protein level. In this work we explore the use of SPE-TOF on intact proteins.

Experimental

Instrumentation for the SPE-TOF analysis consisted of a RapidFire 365 LC/MS coupled to a Q/TOF LC/MS. Online solid phase extraction (SPE) was performed using various 4ul cartridge containing reverse phase packing material to improve retention and cleanup on intact proteins.

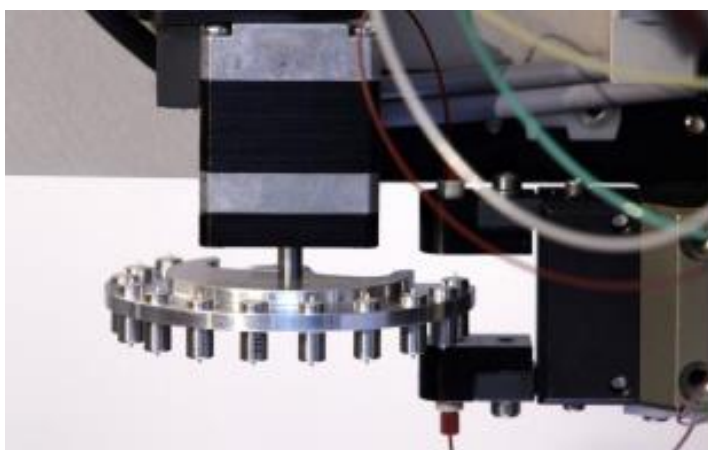


Figure 1. In-line automated cartridge switching

The trap, wash, and elute cycle (Figure 2) was optimized to achieve an analysis time of less than 15 seconds per injection. Initial method development and optimization was performed using the NIST mAb diluted in aqueous solution.

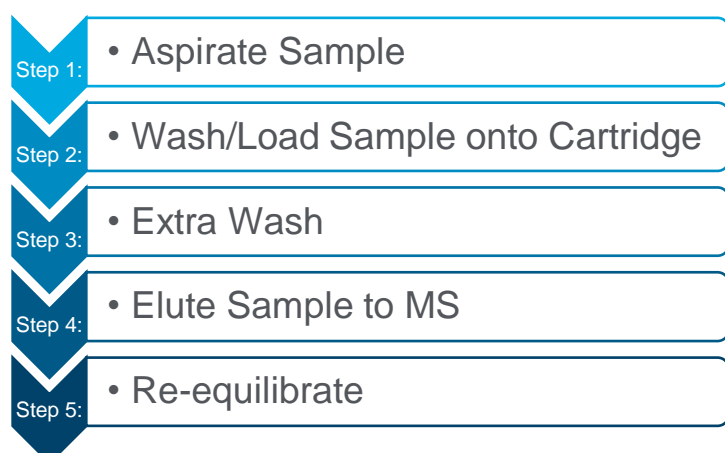


Figure 2. Workflow for RapidFire injection

Experimental



Figure 3. Agilent RapidFire 365

Parameter	Value
Buffer A (Load, Wash 1) Flow Rate = 0.4 mL/min	0.1% Formic Acid, 0.05% TFA, 10% Acetonitrile in Water
Buffer B (Wash 2) Flow Rate = 0.6 mL/min	0.1% Formic Acid, 10% Acetonitrile in Water
Buffer C (Elution) Flow Rate = 0.4 mL/min	0.1% Formic Acid, 60% Acetonitrile in Water
RF State 1 (Aspirate)	300 ms
RF State 2 (Wash 1)	7000 ms
RF State 3 (Wash 2)	0 ms
RF State 4 (Elute)	8000 ms
RF State 5 (Re-equil.)	700 ms

Table 1. RapidFire parameters

Parameter	Value
Ionization mode	Dual AJS ESI
Mass range	100 – 7000 m/z
Spectral acquisition rate	1 spectra/sec
Drying gas flow	13 L/min
Drying gas temperature	350 °C
Sheath gas flow	11 L/min
Sheath gas temperature	350 °C
Nebulizer gas pressure	60 psi
Capillary voltage	3500 V
Fragmentor voltage	275 V
Skimmer voltage	75 V
Reference mass	922.009798
Detection window	100 ppm
Minimum height	1000 counts

Table 2. TOF MS Parameters

Intact Monoclonal Antibody Analysis

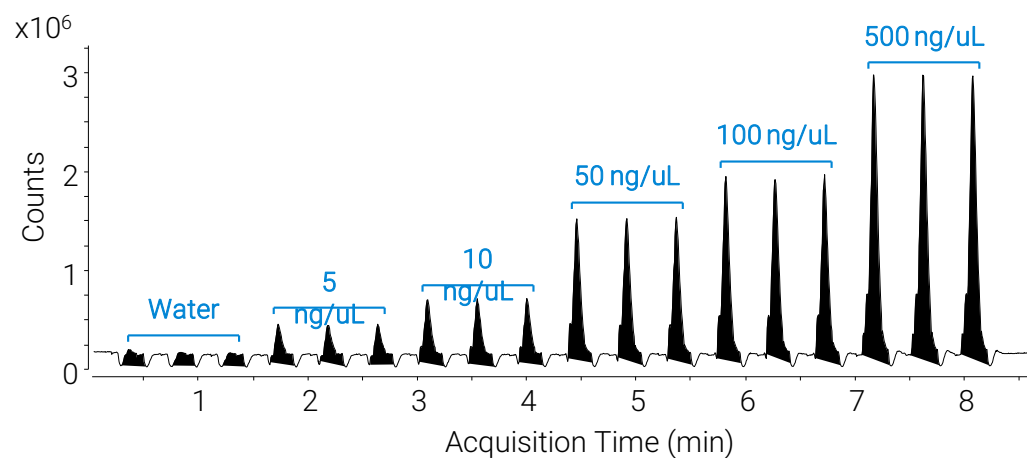


Figure 4. Total ion chromatogram (TIC) of triplicate injections of varying starting concentrations of Intact NIST mAb. Water injections were acquired between injections.

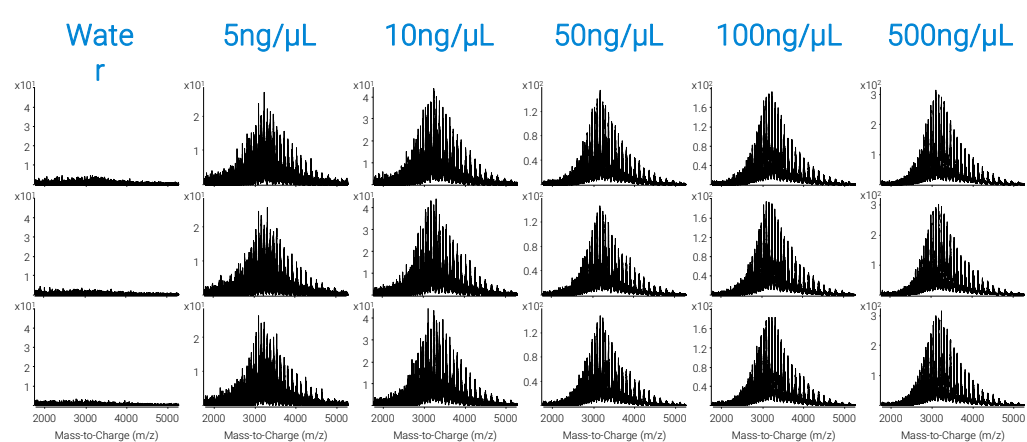


Figure 5. Mass spectra of triplicate injections of varying starting concentrations of the Intact NIST mAb.

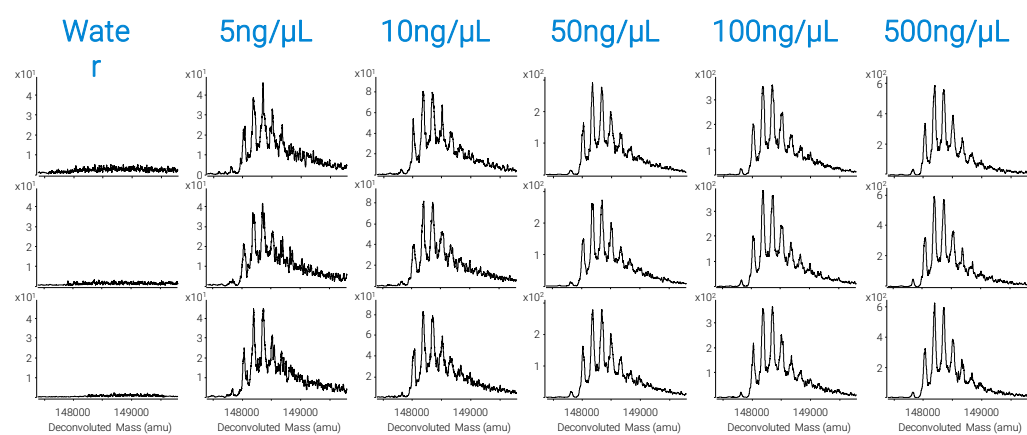


Figure 6. Deconvoluted mass spectra of triplicate injections of varying starting concentrations of the Intact NIST mAb using Maximum Entropy (MaxEnt) from BioConfirm.

Reduced: Light and Heavy Analysis

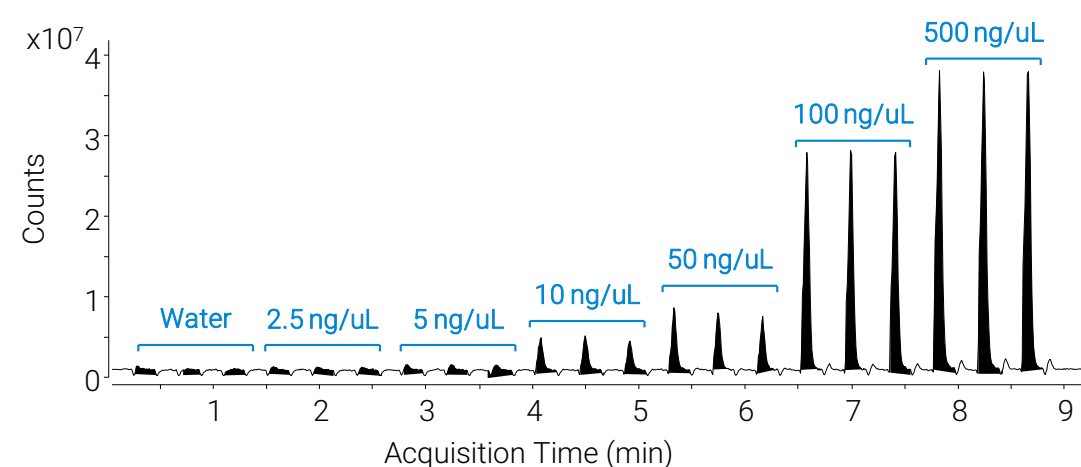


Figure 7. Total ion chromatogram (TIC) of triplicate injections of varying starting concentrations of Reduced NIST mAb. Water injections were acquired between injections.

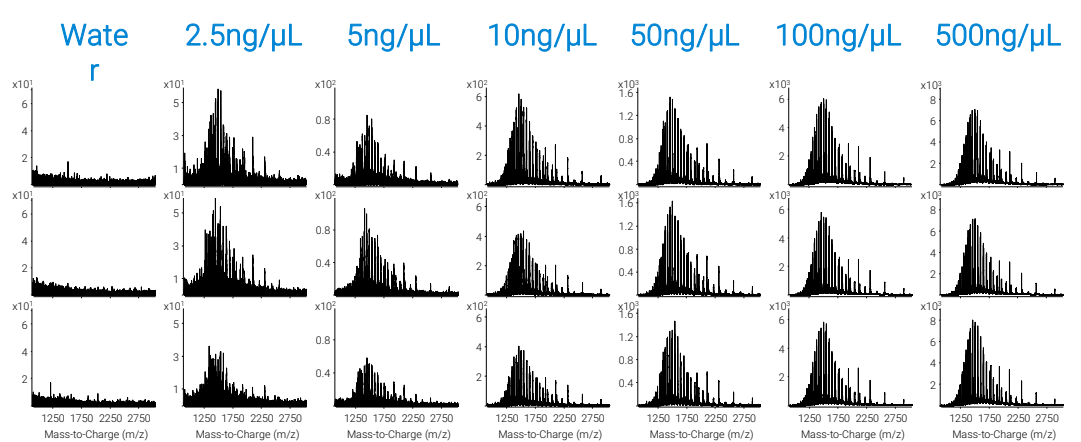


Figure 8. Mass spectra of triplicate injections of varying starting concentrations of the Reduced NIST mAb.

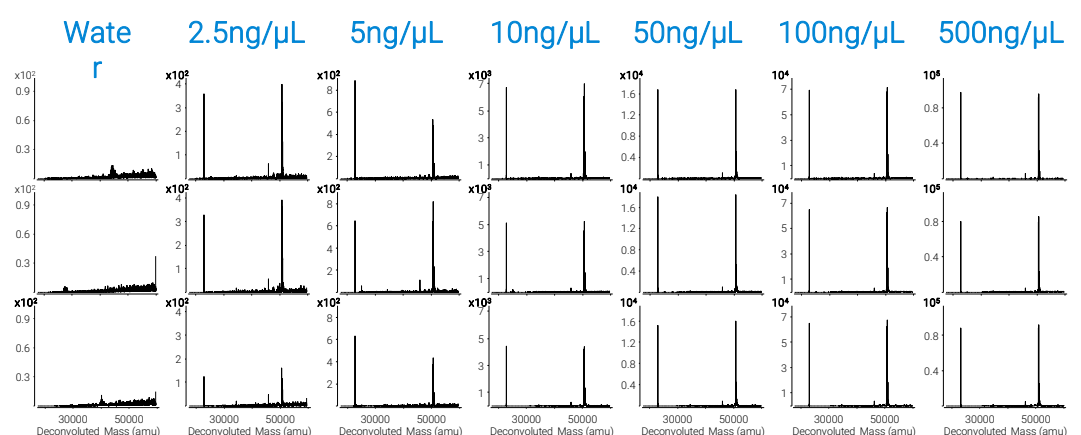


Figure 9. Deconvoluted mass spectra of triplicate injections of varying starting concentrations of the Reduced NIST mAb using Maximum Entropy (MaxEnt) from BioConfirm.

Comparison of C18 and Large-Pore Cartridges

A custom SPE cartridge containing large-pore packing material (1000Å pore, 5µm particle) was compared to a C18 cartridge by analyzing several intact proteins (Figure 10) and a BSA digest (Figure 11). The custom cartridge provided improved peak shape and a significant increase in response for several intact proteins. While the custom cartridge also improved peak shape for the BSA digest, the sequence coverage was better using the C18 cartridge.

Comparison of C18 and Large-Pore Cartridges

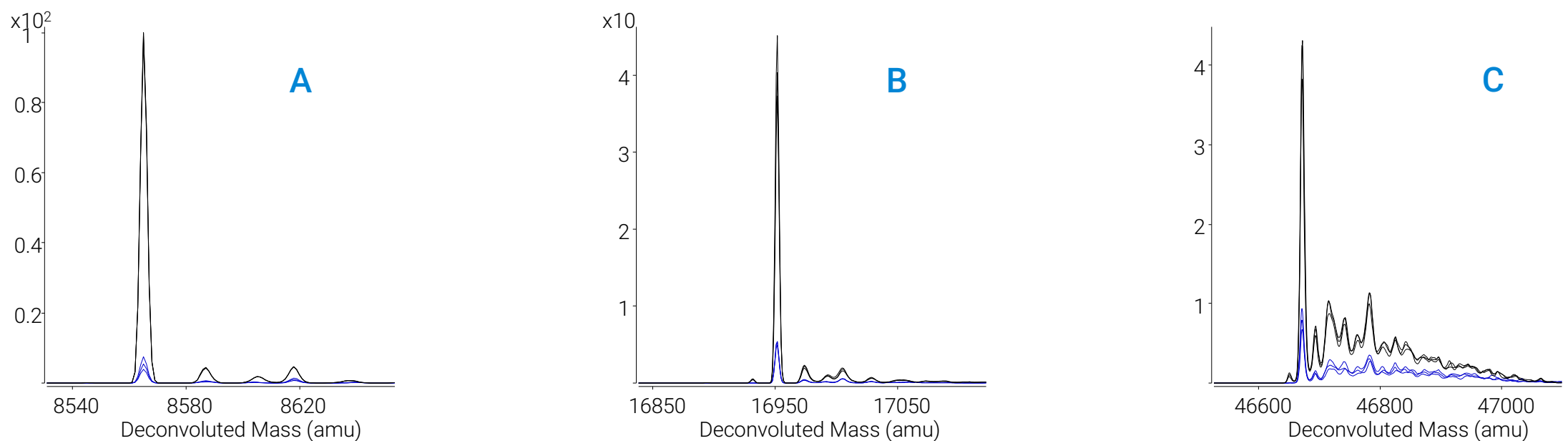


Figure 10. Deconvoluted mass spectra for triplicate injections of ubiquitin (A), myoglobin (B), enolase (C). Blue traces were acquired using a C18 cartridge. Black traces were acquired using a custom cartridge with 1000Å pore size.

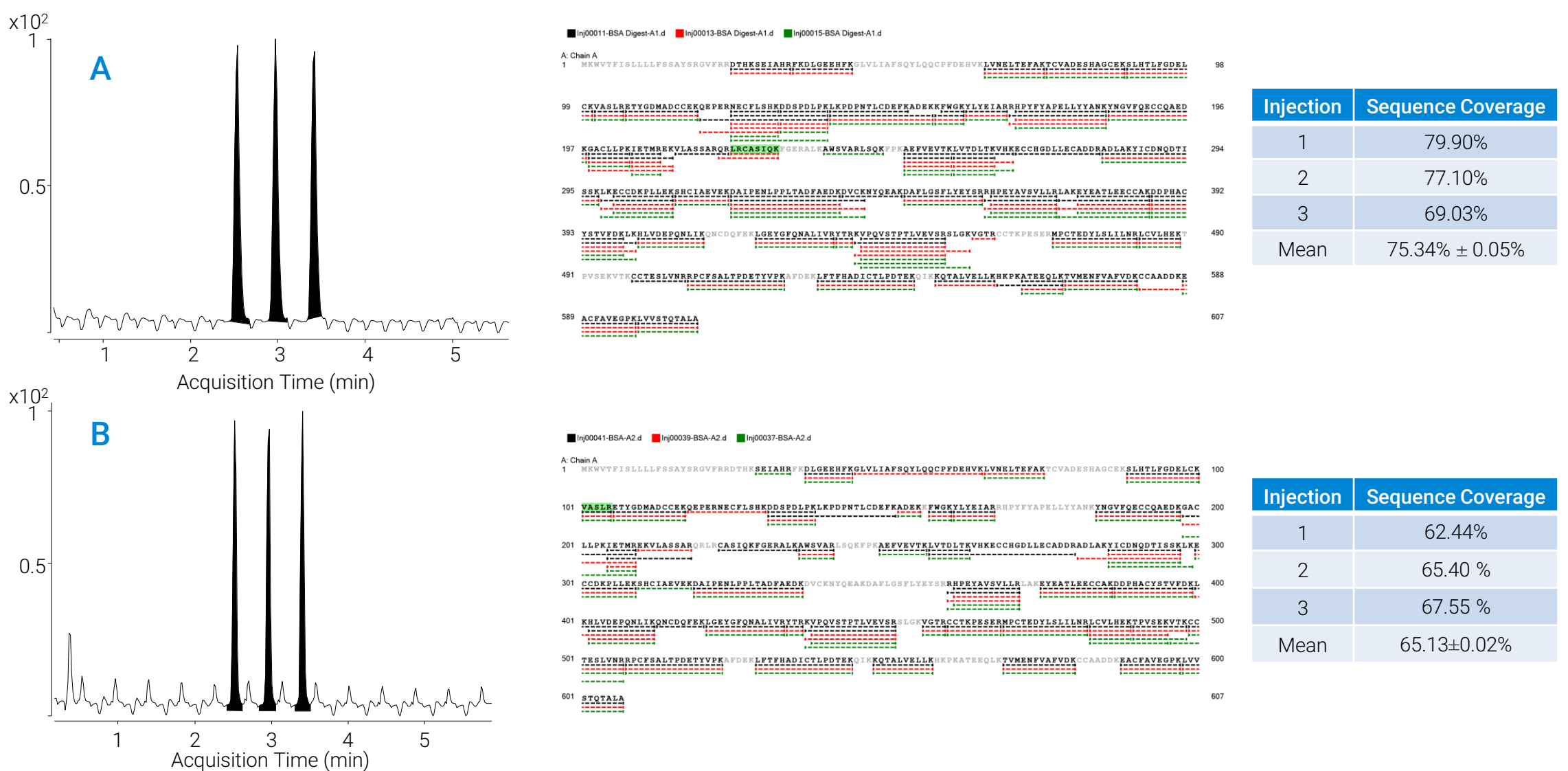


Figure 11. Triplicate injections of a 2 pmol/uL Bovine serum albumin digest (G1990-85001, Trypsin Digest Methylated BSA Standard) using a C18 (A) and a custom 1000Å pore (B) cartridge.

Conclusions

- Agilent's RapidFire coupled to TOF MS provides a robust solution to ultra-fast analysis of intact proteins and peptides.
- A wide variety of SPE cartridges available for the RapidFire allow for development of highly specific and sensitive analytical methods. Here, a custom cartridge with 1000Å pore packing material provided improved peak shape and response for several intact proteins. This additional tool provides for optimized methodologies, leading to simpler and more robust workflows.