Evaluation of a nanoLC-IMS-TOF Platform for Broad and Sensitive Quantitative Proteomics Measurements

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Overview

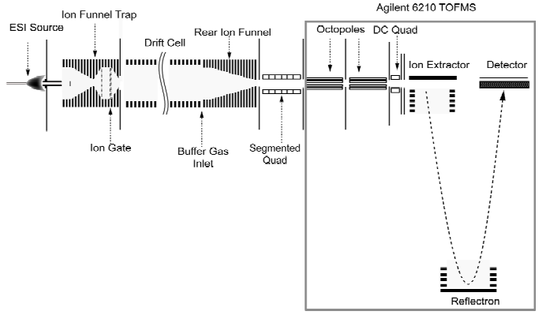
- nanoLC-IMS-TOF platform was evaluated for quantitative measurements
- Peptides from a mixture of five proteins spiked in human plasma were used for evaluation.
- Peptides were identified using IMS-CID-TOF approach
- Good linearity was observed within the concentration range of 30 -300 ng/mL

Introduction

Ion mobility spectrometry (IMS) is increasingly being used in high-throughput proteomics measurements. Uniquely, IMS provides information on the spatial conformation of biomolecules. In addition, IMS complements nanoLC-MS by increasing the experimental throughput, as well as the depth of information obtainable in a time scale that is compatible with both HPLC and MS. It is crucial to evaluate the IMS-MS platform for quantitative measurements in a context that simulates real biological sample complexity and where it is important to maintain the system linearity to the lowest limit of quantitation possible. In this study, we evaluated a recently built sensitive IMS-TOF MS platform for quantitative measurements.

IMS-TOF platform

- 88-cm long drift tube
- 4 Torr pure nitrogen gas
- IMS resolution (for 1+): 75
- O-TOF resolution ~8000
- Acquisition system: 8 bit ADC
- 3 s IMS-TOF frame



Methods

Sample

- 600 ng/mL mixture of five proteins (Bovine Carbonic Anhydrase (BCA),Bovine Cytochrome C (BCC), Chicken Ovalalbumin (CO), E.coli Beta-Galactosidase (EBG), Horse Myoglobin (HM)) was prepared at equal ratios and tryptically digested
- The protein mixture was spiked at different concentrations (18-300 ng/mL) into a tryptically digested human blood plasma sample that was depleted of 12 most abundant proteins

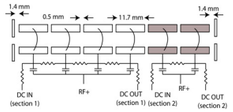
	BCA	BCC	CO	EBG	HM
Sample 1	50 ng	600 ng	400 ng	100 ng	200 ng
Sample 2	150 ng	400 ng	800 ng	600 ng	400 ng

LC system

- 4-column system, 25 min gradient, 10,000 psi, 15 cm, 50 µm i.d., 3 µm C18
- Samples analyzed in triplicate

Fragmentation

- Collision-induced-dissociation (CID) inside a segmented quadrupole
- Pressure ~ 250 mTorr
- CID inside the segmented quadrupole has high efficiency



Results

IMS-CID-TOF

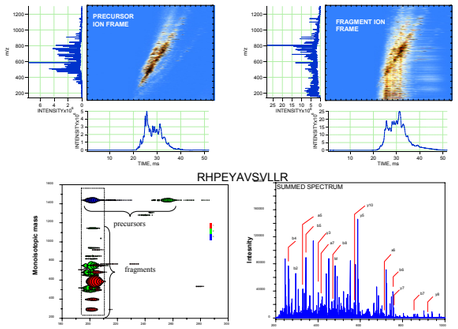


Figure1. Example of peptide identification from IMS-CID-TOF spectra. Note, frame refers to a 2D map of the nested IMS-TOF spectra.

- Peptides from the five proteins were confidently identified
- An algorithm was used to match IMS-CID-TOF spectra to *in silico* digest
- MMA of ±20 ppm and observation of >3 fragments per peptide were used as criteria
- Total of 126 peptides were identified for five proteins with average of 25 peptides identified per protein

LC-IMS-TOF

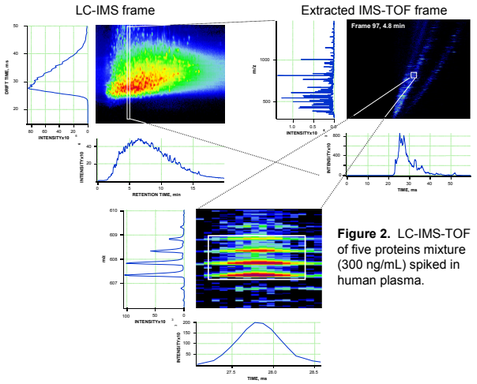


Figure 2. LC-IMS-TOF of five proteins mixture (300 ng/mL) spiked in human plasma.

Alignment of LC retention time

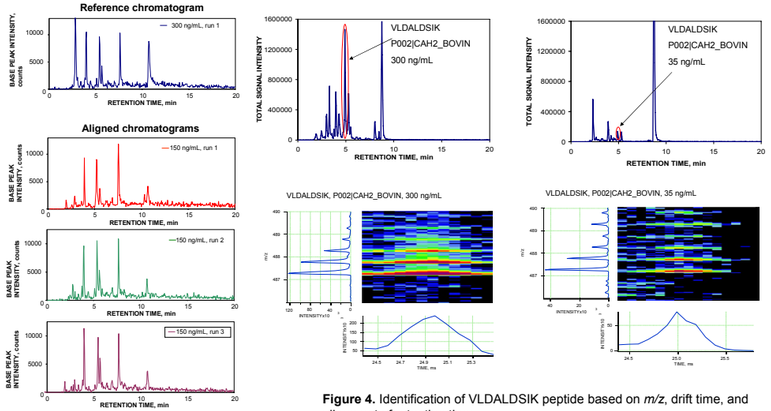


Figure 3. Extracted base peak intensities for three endogenous peptides using drift time information. Alignment of bottom three chromatograms to reference chromatogram was performed using peaks from endogenous human plasma peptides.

Figure 4. Identification of VLDALDSIK peptide based on *m/z*, drift time, and alignment of retention time.

- The alignment parameters were applied to the LC chromatograms obtained for the five proteins-spiked plasma samples.
- This process was automated to calculate the LC peak area for the 8 most abundant peptides from each protein in the mixture.

Linearity of the system

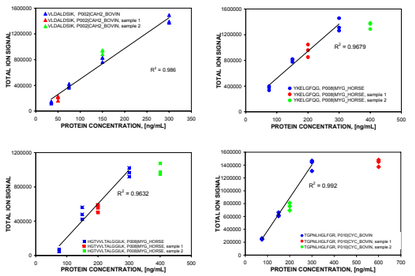


Figure 5. Linearity plots for four different peptides. The system exhibits good linearity within the concentrations range used. The linearity was validated using two samples of different proteins' ratios. At high concentration, the curves deviated from linearity due to saturation of the acquisition system.

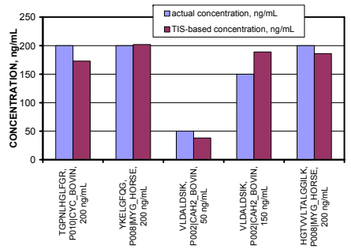


Figure 6. Measured concentrations of selected peptides based on the linearity curves compared to the actual concentration spiked into plasma. Excellent correlation was observed between the measured and the actual concentrations.

Conclusions

- A LC-IMS-TOF platform was evaluated for quantitative measurements.
- The evaluation involved a tryptically digested mixture of five proteins in depleted human plasma.
- Alignment of LC retention time in addition to utilizing drift time helped to correctly identify LC peaks of interest for quantitation in automated fashion.
- Linearity was validated using two samples of different proteins ratios
- The CID efficiency of the IMS-TOF was measured as 39-60%.

Acknowledgements

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Reference

YM Ibrahim, DC Prior, ES Baker, RD Smith, and ME Belov. "Characterization of an ion mobility-multiplexed collision-induced dissociation-tandem time-of-flight mass spectrometry approach," *Int J Mass Spectrom* 293:34-44 (2010).

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