Establishing a Rapid, Sensitive QC protocol Utilizing Trapped Ion Mobility Mass Spectrometry and Parallel Accumulation Serial Fragmentation

HUPO 2019

Melvin Gay[1], Michael Krawitzky[2], Chris Adams[2], Pierre-Olivier Schmit[3], Conor Mullens[4], Shourjo Ghose[4], Matthew Willetts[4], Gary Kruppa[2]

[1]Bruker Daltonics, Victoria, AU; [2]Bruker Daltonics, San Jose, CA, USA; [3]Bruker Daltonique S.A France; [4]Bruker Daltonics, Billerica, MA, USA

Introduction

In clinical and multi-omic workflows, the quality control (QC) sample is relied upon to monitor instrument health and performance. Choosing a complex matrice alone does not guarantee both instrument sensitivity and robustness, and the general use of QC samples in all laboratories is still lacking. In this work we assess stable iostope labeled (SIL) peptides of known quantity and spiked them into a human leukemia cell line. We explore low sample concentrations run on short gradients (15 min) to simultaneously optimize qualitative and quantitative qualification in a short period of time. The ultimate goal is data acquisition, data analysis and results in less than 1hr on a mass spectrometer with maximum speed and sensitivity (timsTOF Pro).

Methods

Human myelogenous leukemia cell line (K562 - Promega) was reconstituted to 22.5-180 ng/uL where a series of 35 peptide isotopologues (Pierce 7x5) at 5 orders of magnitude different concentrations, were spiked into the cell lysate. Fifteen SIL yeast peptides (Pierce PRTC) were also spiked into the cell lysate. A 25 cm Ionopticks Aurora column was attached to a Bruker nanoElute HPLC set to 400 nL/min flow rate. Peptides were ionized into the mass spectrometer, which was a timsTOF Pro where data acquisition was performed in a PASEF mode. The tims device was set to accumulate and elute at 100ms, resulting in a 100% duty cycle. Data analysis was performed with PEAKS Studio (PEAKS X+, BSI).

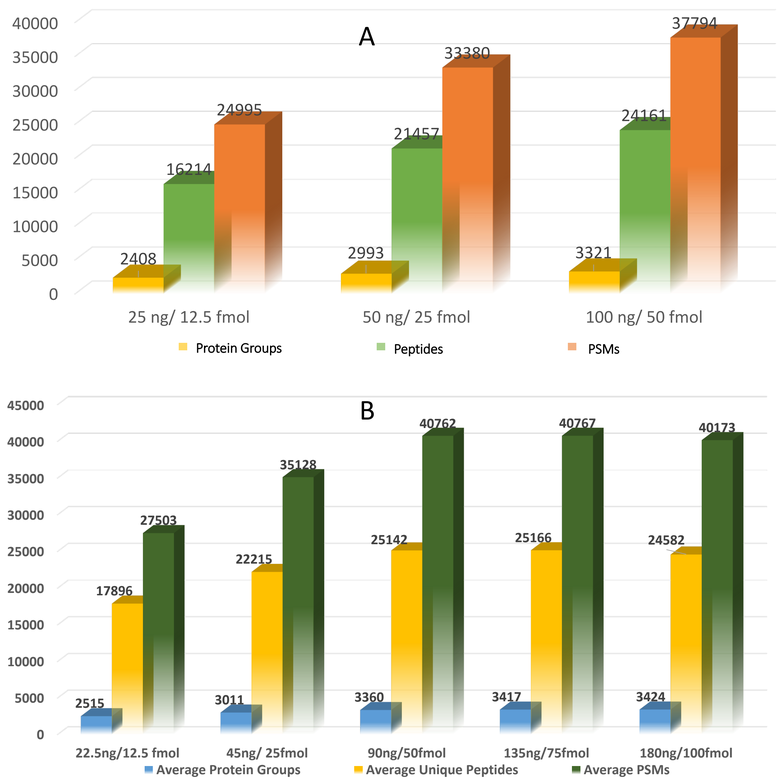

Fig.1 Average protein groups, peptides and PSMs for triplicate injections of (A) K562 spiked with PRTC and (B) K562 spiked with Pierce 7x5 using a **15 min** gradient on the timsTOF Pro. The 7x5 concentration represents the heaviest peptide concentration

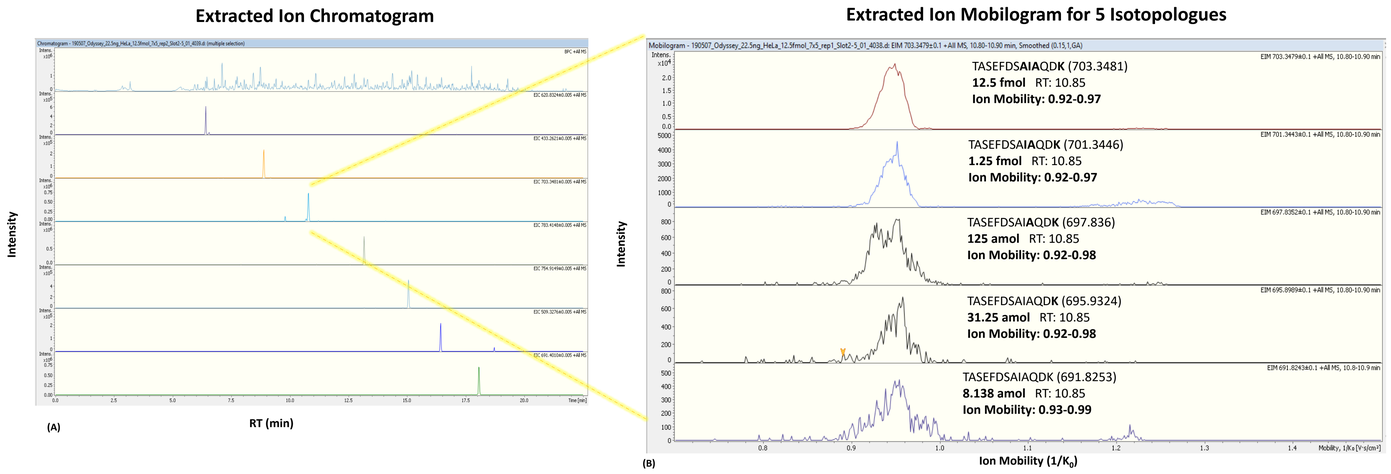

Fig.3 (A) Observed elution of heaviest yeast AQUA peptide at concentration 12.5 fmol (heaviest of the AQUA peptides) using a 15 min gradient on the timsTOF Pro. (B) Extracted ion mobilogram (EIM) for five isotopologue versions containing no heavy, 1 heavy, 2 heavy, 3 heavy, and 4 heavy isotope-labeled amino acids. Isotopologues are chromatographically indistinguishable; however, since they differ in mass, they can be resolved by the EIM.

Table 1. Preliminary Piece Retention Time Calibration Mixture run @ 15 min gradient with added trapped ion mobility values

Peptide Sequence	Mass (M)	Observed *m/z* z = 2	Hydrophobicity Factor	Avg. Peak Width FWHM (Sec)	Avg. RT	RSD %	Avg Ion Mobility
SSAAPPPPPR	985.522	493.7683	7.56	4.27	5.9	0.00	0.75-0.77
GISNEGQNASIK	1224.6189	613.3167	15.5	1.87	6.4	0.00	0.85-0.87
HVLTSIGEK	990.5589	496.2867	15.52	2.20	6.5	0.00	0.80-0.82
DIPVPKPK	900.5524	451.2834	17.65	6.70	8.6	0.67	0.77-0.79
IGDYAGIK	843.4582	422.7363	19.15	2.70	9.0	0.00	0.74-0.76
TASEFDSAIAQDK	1389.6503	695.8324	25.88	2.87	10.9	0.00	0.92-0.96
SAAGAFGPELSR	1171.5861	586.8003	25.24	4.37	11.6	0.50	0.83-0.86
ELGQSGVDTYLQTK	1545.7766	773.8955	28.37	2.47	13.2	0.00	0.99-1.03
GLILVGGYGTR	1114.6374	558.3259	32.18	2.67	15.0	0.00	0.87-0.89
SFANQPLEVVYSK	1488.7704	745.3924	34.96	2.70	15.1	0.38	0.96-0.99
GILFVGSGVSGGEEGAR	1600.8084	801.4115	34.5	2.73	15.4	0.38	0.97-1.02
LTILEELR	995.589	498.8018	37.3	2.47	16.5	0.00	0.81-0.83
NGFILDGFPR	1144.5905	573.3025	40.42	3.70	17.6	0.00	0.81-0.87
ELASGLSFPVGFK	1358.7326	680.3735	41.18	2.27	18.1	0.00	0.90-0.96
LSSEAPALFQFDLK	1572.8279	787.4212	46.66	1.60	18.6	0.00	0.99-1.02

Data

We optimized experimental conditions to achieve peak instrument performance in under an hour (total run time). Sample and instrument parameters were tested to develop a fast, reproducible and robust LC-MS method. Two matrices: K562 spiked with 100 fmol/uL (heaviest peptide) and K562 spiked with PRTC were selected after preliminary studies monitoring protein/peptide/PSM identification values, observed isotopologue sensitivity and chromatographic properties (peak shape, FWHM, tailing, etc). Samples were run on the nanoElute HPLC running at 400 nl/min.

Optimized timsTOF Pro settings: Capillary voltage: 1600V, gas flow 3 l/min, gas temp: 180C, imex values were between 0.7 and 1.50 V • s/cm2 (End set to 2.10 V • s/cm2), accumulation time: 100 ms, cycle time approximately 73.0 ms (with 100% duty cycle enabled), 10 PASEF MS/MS acquired at a rate of **174.3 Hz.** The trapped ion mobility capabilities resulted in determinate collisional cross section (CCS) terms for each of the analytes identified. We have archived the CCS values for all thirty five isotopologues and 15 PRTC (Yeast) peptides, thereby resulting in a third dimension to validate peptide/protein numbers, quantitative response and reproducibility of CCS terms.

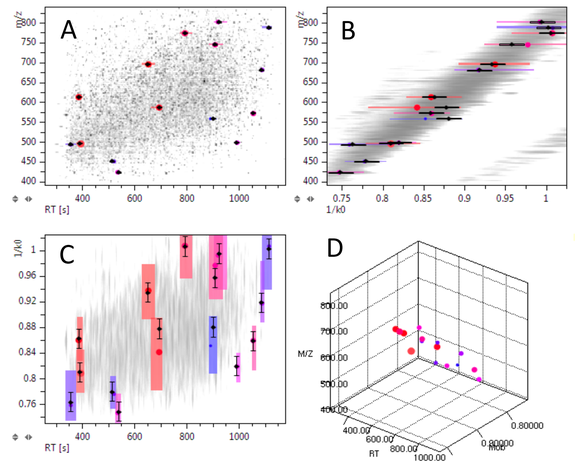

Fig.2 Observed elution of heaviest yeast AQUA peptide at concentration 12.5 fmol (heaviest of the AQUA peptides) using a 15 min gradient on the timsTOF Pro. Figures were created using prototype Feature Finder and Feature Viewer software (A) RT vs m/z (B) Ion Mobility vs m/z (C) RT vs 1/k0 (D) Ion Mobility vs RT vs M/z are displayed

Results

In this study we describe mass offset mobility aligned (MOMA), a phrase defining analytes (i.e. SIL peptides) with mass differences, indistinguishable by retention time or primary sequence yet all having the same collisional cross section (ion mobility)

We established ion mobility values for 50 commonly used Yeast standard peptides. When ion mobility and retention time merge with the complexity of a digested lysate spiked with SIL peptides, the result is the most complete approach to monitor both LC and MS instrument sensitivity and performance.

Conclusions

- More than **3300 Protein groups**, **25000 Unique peptides** were identified from 90 ng using a **15 min** gradient
- Acquisition rate was **>174 Hz**
- To date this is the most complete approach to monitor both LC and MS instrument sensitivity and performance