

Improved Coverage in Global Proteomics Survey Experiments by Decreasing Cycle Time on a Linear Ion Trap Mass Spectrometer

Julian Phillips, Julie A. Horner, Tonya Second, Justin Blethrow,

Thermo Fisher Scientific, San Jose, CA, USA

Overview

Purpose: Improve protein coverage for a complex mixture by decreasing instrument cycle time

Methods: Data dependent MS² of top ten most intense peaks from the full scan

Results: Protein coverage improvement of up to 6X with decreased cycle time of 50%

Introduction

A major goal of proteomics research is to develop the biochemical, instrumental and software technologies to increase the coverage and identification of proteins with greater confidence. Linear ion traps, due to their high full scan sensitivity, wide dynamic range, ease of use and superior ability to perform multiple MSⁿ experiments, are currently the instrument of choice for this type of analysis. The time taken to complete a series of events starting from injection of ions into the trap to ion detection is referred to as the *cycle time*. In order to maximize the number of proteins identified and increase the sample coverage, the cycle time must be minimized without adversely affecting the data quality.

Methods

Sample

A suite of eight proteins were individually alkylated using iodoacetamide and enzymatically digested according to protocol. A mixture of the digested proteins was prepared (see Table 1) to span slightly more than two orders of magnitude in concentration. The most abundant was chosen to be Bovine serum albumin to act as a simulant for complex mixtures such as plasma.

Chromatography

Two μ L of the 8-protein mixture was loaded using a Thermo Scientific Accela Autosampler onto a 150 μ m x 100 mm 5 μ m Vydac® C18 column (Micro-Tech Scientific, Sunnyvale, CA). Peptides were eluted using a 5%→60% gradient over 3, 6 or 12 minutes at a flow rate of 1 μ L/min (standard), 2 μ L/min (compressed) post split and introduced into the mass spectrometer via nanospray.

Mass Spectrometry

The top ten data-dependent MS/MS spectra were acquired on either a Thermo Scientific LTQ XL linear ion trap mass spectrometer or a modified linear ion trap with intelligent AGC (Automatic Gain Control) and fast isolation and activation. Dynamic exclusion was employed with a repeat count of three.

Table 1. Concentrations in, and amounts on column, for an eight-protein digest mixture

Protein	Concentration (fmol/ μ L)	Amount on Column (fmol)
Equine Apomyoglobin	60	120
Chicken Lysozyme c	40	80
Human α -Lactalbumin	4.4	8.8
Chicken Conalbumin	100	200
Bovine Serum Albumin	500	1000
Carbonic Anhydrase	7.5	15
Yeast Glucose-6-Phosphate Dehydrogenase	120	240
Equine Cytochrome c	20	40

Results

Space charge is a phenomenon that occurs when too many like charges are stored in a confined space. Space charge in an ion trap adversely affects the resolution and mass assignment of the ions being scanned out. Space charge is minimized by carefully regulating the ion current into the ion trap by making an estimate of the number ions just prior to the analytical scan (the prescan). Via the prescan, AGC (Automatic Gain Control) ensures all scans contain the optimum number of charges and maintains mass accuracy and resolution across the entire mass range.

In this experiment, a linear ion trap was modified to use an alternate method of ion population regulation. The traditional AGC method determines ion injection time by acquiring a ~40 ms prescan prior to every scan. In this new predictive approach, the injection time for the data-dependent MS/MS precursor ion is estimated from the intensity in the full MS scan preceding it. For example, an N=5 double play experiment using the traditional AGC method would acquire 6 prescans during one complete scan sequence. With predictive AGC, only one prescan is performed, resulting in a saving of about 200 ms – the approximate time for acquisition of a single MS/MS scan.

In addition to the modified AGC, two other means of reducing the total scan sequence cycle time have been implemented (Table 2). First, the activation time has been reduced three-fold from 30 ms to 10 ms. The reduction in activation time affects no change in the MS/MS spectral data acquired. Second, the isolation method has been modified resulting in a four-fold decrease in isolation time.

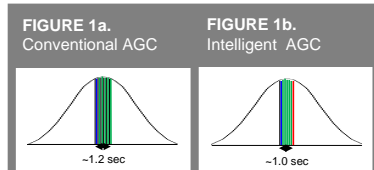


Table 2. Collision-induced dissociation method individual step times

	Activation Time	Isolation Time	Prescan Time	Scans /sec.
LTQ XL	30 ms	16 ms	40 ms	3.71
Modified LTQ	10 ms	4 ms	--	6.32

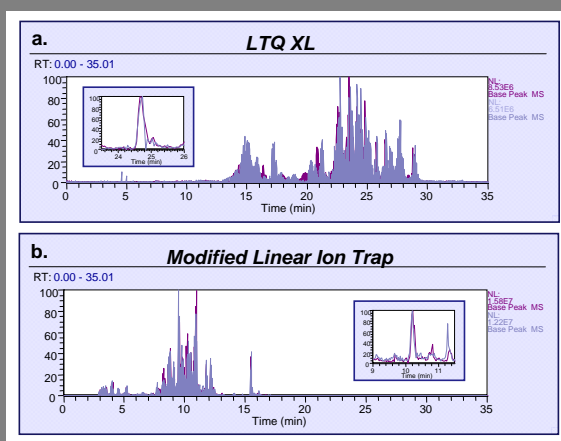
The total time for a CID event (excluding ion trap fill time and mass analysis) of 86 ms for a standard LTQ XL™ is reduced to 14 ms for the modified ion trap.

The summed reduction of each of the three parameters results in an overall decrease in cycle time of 72 ms for a data-dependent MS/MS scan. The observed improvement in scans acquired during the densest portion of the chromatography on each instrument is listed in the rightmost column of Table 2. We note nearly a two-fold increase in the number of spectra acquired per unit time - indicating a potential for improved coverage in global proteomics survey experiments.

A mixture of eight enzymatically digested proteins with two orders of magnitude dynamic range is used to simulate a complex mixture. The sample is separated using a linear gradient (either 6 or 12 min.) at either the standard flow mode (1 μ L/min) or the compressed flow mode (2 μ L/min) which halves the total time for analysis.

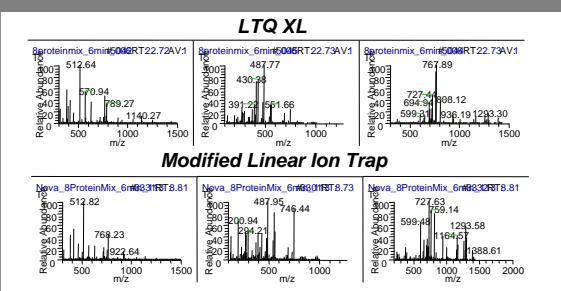
Base peak chromatograms are shown in Figure 2 for duplicate runs for a) LTQ XL using a 6-min. gradient with standard flow and b) modified linear ion trap using a 6-min. gradient with compressed flow. Based solely on the appearance, it may seem that the chromatograms in top panel offer more spectral information; however, the two are surprisingly comparable. In the standard LTQ XL, 3,713 scans are obtained during 17 minutes in the densest portion of the elution profile (t_R =13-30 min.), whereas 3,783 scans are obtained during 10 minutes in the densest portion of the elution profile (t_R =3-13 min.) for the modified linear ion trap.

Figure 2. Base peak chromatograms for LTQ XL and modified linear ion trap with insets showing chromatographic peak width



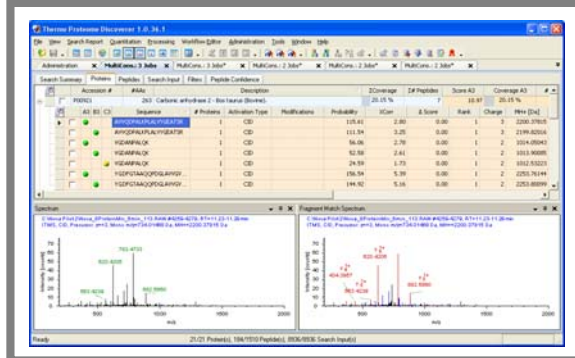
Sample full scan MS and MS/MS spectral data for the standard LTQ XL (top) and modified linear ion trap (bottom) from the same point in the elution profile are shown in Figure 3. The qualitative feature of both the full scan MS (left panel) and MS/MS (middle and right panels) and signal to noise ratios are comparable. We infer that spectral quality is not compromised by prediction of ion injection times.

Figure 3. Filtered search results obtained using Proteome Discoverer 1.0 and the SEQUEST algorithm



Sample search results, filtered by Xcorr vs charge state, obtained using the SEQUEST™ algorithm in the Thermo Scientific Proteome Discoverer 1.0.0 software are shown in Figure 4. The top panel displays peptide hits; the bottom left the MS/MS spectrum and the bottom right show the matched fragments. A summary of relevant search results is shown in Figure 5. Results for the 6-min. gradient for LTQ XL are compared with those for the modified linear ion trap in Figure 5a; results for the 12-min. gradient for LTQ XL are compared with those for the modified linear ion trap in Figure 5b. Proteins are listed from top to bottom in order of increasing concentration in the mixture, so carbonic anhydrase is the least abundant and BSA is the most abundant protein.

Figure 4. Filtered search results obtained using Proteome Discoverer 1.0.0 and the SEQUEST algorithm



Comparing the results for the same length gradient, twelve minutes, but a compressed time scale on the modified linear ion trap (Figure 5b), we see that coverage is comparable for all proteins except carbonic anhydrase where a significant difference in coverage (six-fold) is observed.

Figure 5a. Multiconsensus results for 8-protein digest mixture on an LTQ XL using a 6-minute gradient and on a modified linear ion trap using a 6-minute gradient and accelerated chromatography

Description	Conc	6-minute gradient				Ratio
		LTQ XL		Modified linear trap		
		ZCov	Z#	ZCov	Z#	
Carbonic anhydrase 2 - Bos taurus (Bovine).	7.5	0.0380	2	0.2015	7	5.30
Cytochrome c - Equus caballus (Horse).	20	0.1238	2	0.3714	9	3.00
Lysozyme C - Gallus gallus (Chicken).	40	0.0541	5	0.1554	7	2.87
Myoglobin - Equus caballus (Horse).	60	0.7032	22	0.6194	26	0.88
Ovotransferrin - Gallus gallus (Chicken).	100	0.1357	26	0.1189	27	0.88
Glucose-6-phosphate 1-dehydrogenase - Saccharomyces cerevisiae (strain YJM789)	120	0.3209	32	0.4384	55	1.37
Serum albumin - Bos taurus (Bovine).	500	0.2760	41	0.2955	53	1.07

Figure 5b. Multiconsensus results for 8-protein digest mixture on an LTQ XL using a 12-minute gradient and on a modified linear ion trap using a 12-minute gradient and accelerated chromatography

Description	Conc	12 minute gradient				Ratio
		LTQ XL		Modified linear trap		
		ZCov	Z#	ZCov	Z#	
Carbonic anhydrase 2 - Bos taurus (Bovine).	7.5	0.0380	1	0.2357	7	6.20
Cytochrome c - Equus caballus (Horse).	20	0.3810	8	0.3714	13	0.97
Lysozyme C - Gallus gallus (Chicken).	40	0.1622	7	0.2162	10	1.33
Myoglobin - Equus caballus (Horse).	60	0.7613	23	0.6774	30	0.89
Ovotransferrin - Gallus gallus (Chicken).	100	0.1231	32	0.1329	37	1.08
Glucose-6-phosphate 1-dehydrogenase - Saccharomyces cerevisiae (strain YJM789)	120	0.3816	38	0.4951	69	1.30
Serum albumin - Bos taurus (Bovine).	500	0.2711	41	0.3084	63	1.14

Thus, **the higher-abundance proteins show comparable coverage and the low-abundance proteins show up to a six-fold increase in coverage in half the time when the scan sequence cycle time is reduced.** As the gradient length decreases, protein coverage tends to decrease on both the LTQ XL and the modified linear ion trap, but the decreased cycle time of the modified linear trap serves to mitigate this effect and shows improved results over the standard configuration.

Conclusions

A modified linear ion trap with decreased cycle time has been compared with a standard LTQ XL under different chromatographic conditions for global proteomics survey experiments. As the gradient length is reduced on a given instrument, protein coverage decreases. However, the modified linear ion trap shows superior coverage for shorter gradients and compressed chromatographic timescales over the standard LTQ XL.

Acknowledgements

The author would like to thank Jae Schwartz, and Philip Remes for assistance with instrumentation.

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