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Complex Peptide Mixture Analysis on the Finnigan LTQ Linear Ion Trap Mass Spectrometer

Key Words

- Peptide coverage
- Protein identification
- Triple play
- Human plasma
- Finnigan™ LTQ™

Chromatography and Mass Spectrometry Application Note

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Introduction

Rapid identification of low abundance proteins in complex mixtures is one of the key objectives for the use of mass spectrometry in proteomics research. Traditionally, this approach has utilized 3-dimensional quadrupole ion trap systems, such as the Finnigan LCQ™ Deca XP Plus and the Finnigan ProteomeX.™ Recent developments in ion trap mass spectrometry, and the introduction of the Finnigan LTQ linear ion trap mass spectrometer provide further tools to boost sample throughput, and produce higher peptide coverage with a higher degree of confidence for protein identification.

Goal

This study demonstrates how the fast cycle time and unparalleled MS/MS sensitivity of the high performance Finnigan LTQ linear ion trap mass spectrometer result in increased coverage, and faster and more confident protein identification.

Experimental

LC/MS/MS analysis of enzymatically modified human plasma samples was performed using NanoSpray ionization. The TurboSEQUEST™ algorithm was used for data analysis and protein identification.

Sample Preparation

A whole human plasma sample (5 mg, Sigma) was reduced, alkylated, and enzymatically digested. A total of 3 uL of the digested mixture (1 ug/uL) was loaded onto a reversed-phase (RP) C18 column for LC/MS analysis.

LC Separation and MS Analysis

HPLC

HPLC system:	Finnigan Surveyor® MS pump with a flow splitter.
Column:	0.15×100 mm C18 (Thermo Electron)
Flow rate:	600 nL/min
Mobile phase	A: Water with 0.1% Formic Acid B: Acetonitrile with 0.1% Formic Acid
Gradients:	
Normal gradient:	2-65% B in 180 min., 65-80% B in 5 min and hold for 5 min, 80-2% B in 0.5 min
Fast gradient:	2-65% B in 120 min, 65-80% B in 5 min and hold for 5 min, 80-2% B in 0.5 min.

Mass Spectrometers

Finnigan LTQ, and Finnigan LCQ Deca XP Plus

Ionization mode:	NanoSpray, positive ion
Scan sequence:	Full-scan MS, Zoom scan, MS/MS scan
Acquisition modes:	Normal, Data Dependent™ and Dynamic Exclusion™

The zoom scan was used to determine the charge state of each peptide, therefore shortening the time required for data searching analysis. Results were compared to those obtained from the same experiment performed using a conventional 3D ion trap.

Results and Discussions

Data Analysis

Protein identification was performed using the TurboSEQUEST algorithm in the BioWorks™ 3.1 software package (Thermo Electron) and the Swiss-Prot human database (Swiss Institute of Bioinformatics, Geneva, Switzerland). The identified peptides were further evaluated using charge state versus cross-correlation number (XCorr). The criteria for positive identification of peptides was XCorr > 1.5 for singly charged ions, XCorr > 2.0 for doubly charged ions, and XCorr > 2.5 for triply charged ions.

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Results

The plasma peptide mixture was analyzed under two different LC gradients on the Finnigan LTQ and Finnigan LCQ Deca XP Plus. Figure 1 shows a four-fold increase in the number of scans acquired on the Finnigan LTQ in comparison to the Finnigan LCQ Deca XP Plus. This fast scan speed capability, in combination with the unparalleled high sensitivity on the Finnigan LTQ, produces extremely high quality MS/MS spectra, which in turn results in increased confidence of analyte identification. Figures 2 and 3 compare the MS/MS spectra obtained on the two instruments.

Moreover, with the rapid duty cycle on the Finnigan LTQ, more experiments and thus more information is obtained from each sample, and in less time. Figure 4 illustrates the Triple Play™ feature, with a sequence setup to perform one full-scan, one zoom scan, and one MS/MS scan in less than 750 milliseconds.

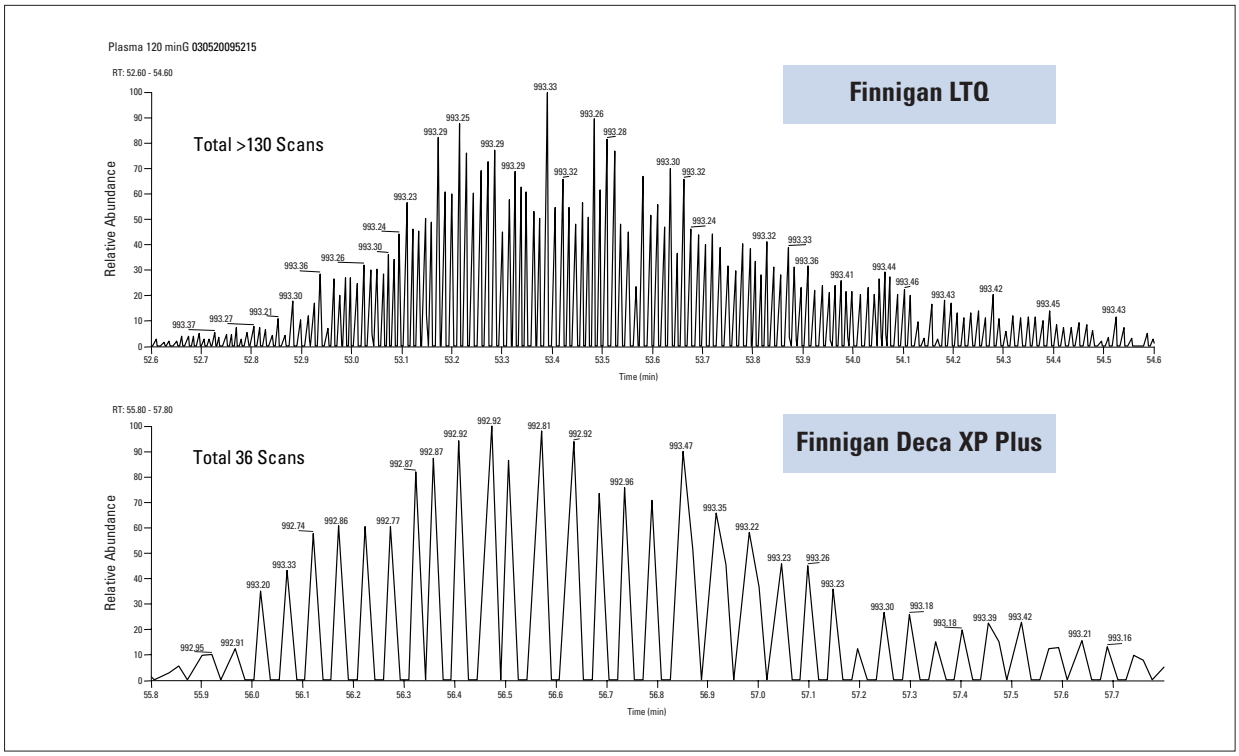
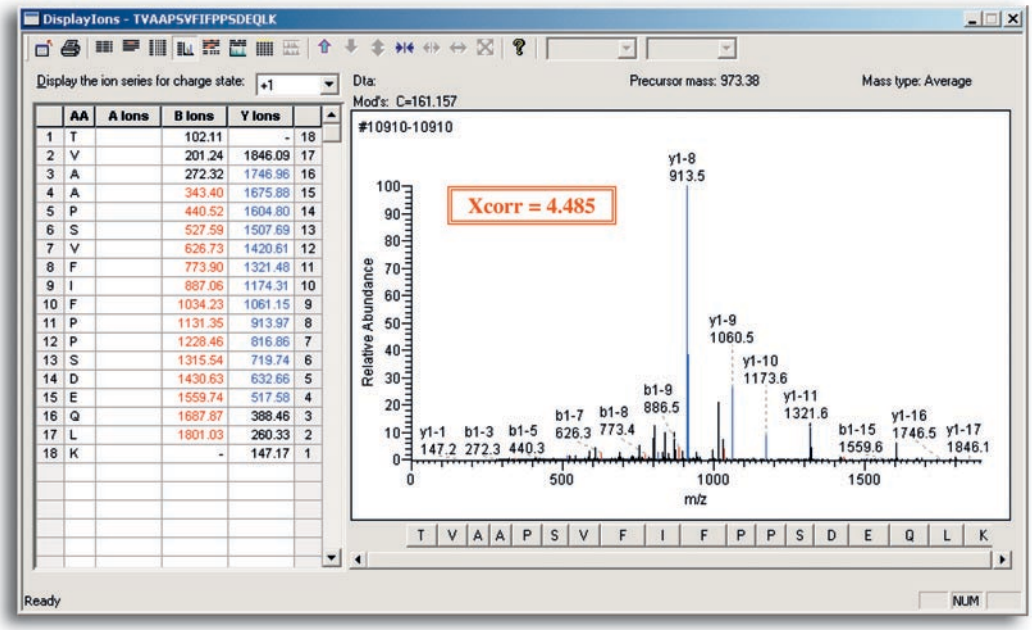


Figure 1. Number of scans across peak 993 with one MS, one zoom scan, and one MS/MS.



	AA	A Ions	B Ions	Y Ions	
1	T		102.11	-	18
2	V		201.24	1846.09	17
3	A		272.32	1746.96	16
4	A		343.40	1675.88	15
5	P		440.52	1604.80	14
6	S		527.59	1507.69	13
7	V		626.73	1420.61	12
8	F		773.90	1321.48	11
9	I		887.06	1174.31	10
10	F		1034.23	1061.15	9
11	P		1131.35	913.97	8
12	P		1228.46	816.86	7
13	S		1315.54	719.74	6
14	D		1430.63	632.66	5
15	E		1559.74	517.58	4
16	Q		1687.87	388.46	3
17	L		1801.03	260.33	2
18	K		-	147.17	1

Figure 2. Finnigan LTQ MS^2 of peptide 973 from human IG Kappa Chain C Region.

The zoom scan capability (Figure 4B) is used to determine the charge state of each peptide in order to improve data analysis speed. For example, the zoom scan of the m/z 418 peptide determined that this peptide is doubly charged based on the mass difference of 0.5 for each sequential peak from the zoom scan. This figure also demonstrates the high quality MS/MS spectra (Figure 4C) produced from a low abundant ion in the MS spectrum (Figure 4A). This added performance provides additional structural information and added confidence in the identification of minor components in a complex mixture.

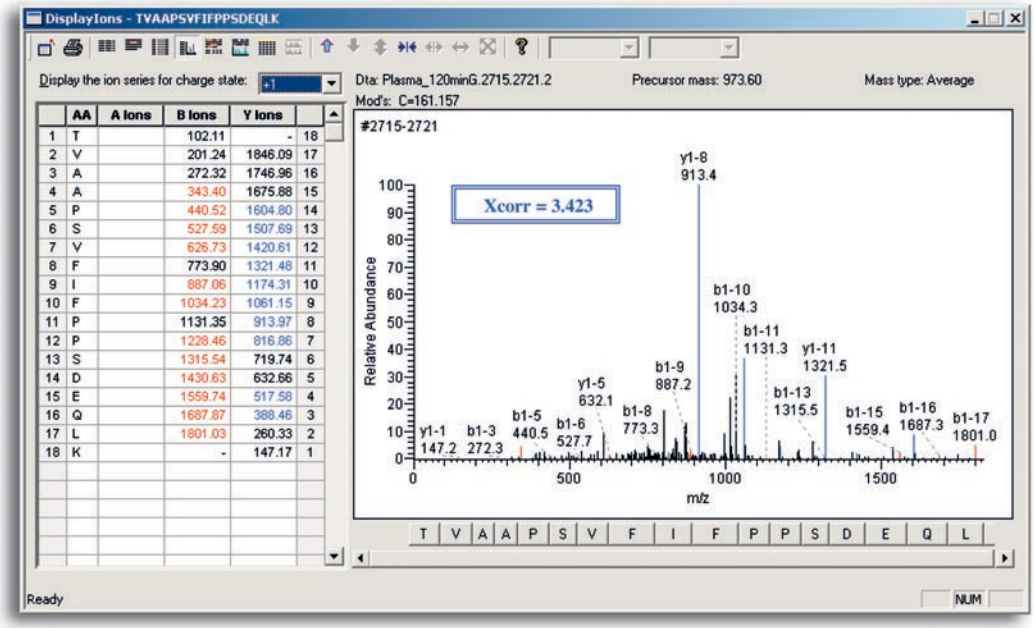


Figure 3. Finnigan Deca XP Plus MS^2 of peptide 973 from human IG Kappa Chain C Region.

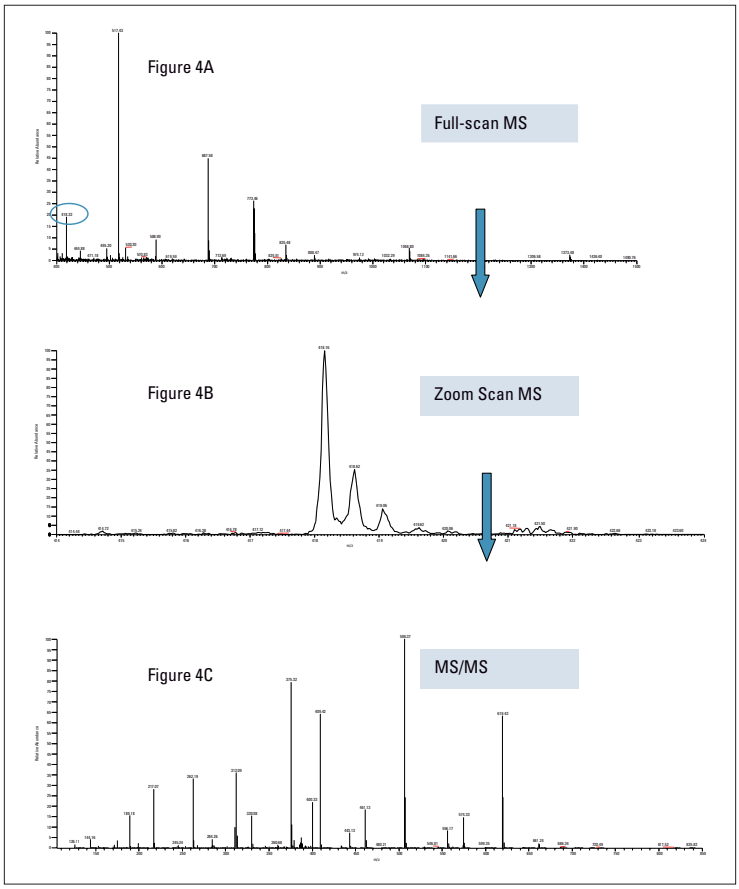


Figure 4. Triple play—one full-scan MS, one zoom scan, and one MS/MS m/z 418.

Increased peptide coverage is also achieved with this higher performance. An example is shown in Table 1, illustrating a two-fold increase in coverage for four proteins. Percentage is based on the number of amino acids matched in this protein.

A dramatic increase (>5 times) in protein identification is summarized in Table 2. A comparison of results from the two different gradients also illustrates that use of a faster gradient on the LTQ still yields a significantly greater number of protein identifications.

	Finnigan Instrument	
Protein	**LCQ Deca XP Plus**	**LTQ**
Serotransferrin Precursor	27.2%	62.3%
Apoliprotein A-I Precursor	24.3%	50.2%
IG Gamma 2 Chain C region	18.1%	35.6%
Complement Factor B Precursor	4.1%	13.7%

Table 1. Peptide Coverage Comparison (180 min gradient)

	Finnigan Instrument	
Gradient	**LCQ Deca XP Plus**	**LTQ**
120 min	26	176
180 min	43	243

Table 2. Protein Identification Comparison

Conclusions

Speed to results, and increased confidence in the results are important factors in advancing an investigation or research project.

These examples illustrate the benefits resulting from some of the key performance attributes of the Finnigan LTQ. Increased peptide coverage and protein identification, along with increased confidence in results are achieved in less time. The rapid scanning and unmatched fast cycle time, in combination with unprecedented high sensitivity of the Finnigan LTQ, yield high quality MS/MS spectra and greater number of scans across an LC peak. This helps to maximize the amount of information obtained from each analysis. Coupled with the BioWorks software package, the Finnigan LTQ enables faster decisions on proteomics research samples.

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