

# Capillary LC–MS/MS Identification of Phosphorylated Peptides from Data Dependent™ Neutral Loss Scans on the Finnigan™ LTQ™ Ion Trap Mass Spectrometer

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## Introduction

Phosphorylation is recognized as one of the most important post-translational modifications of proteins, and is associated with many proteins that have a regulatory function in cells. Phosphoproteins are generally found in low quantities within cells. This low abundance, coupled with the higher acidity of phosphopeptides, increases the complexity of their analysis by mass spectrometry in positive ESI mode. In tandem mass spectrometry, phosphopeptide precursor ions typically exhibit a prominent neutral loss of a phosphate group (98 Da) during fragmentation. However, identification of the phosphoprotein and determination of the exact site of phosphorylation is often limited by inadequate peptide fragmentation and diagnostic sequence ion information.

To overcome the lack of peptide fragmentation information typical in phosphopeptide analysis, a new Data Dependent experiment was created to selectively trigger MS<sup>3</sup> scans on only the MS/MS fragment ions for which a specific, prominent neutral loss ion was detected. This new instrumental method was designed, in part, to take advantage of the superior sensitivity afforded by the design of the new linear ion trap mass spectrometer, the Finnigan LTQ. The increased ion capacity and improved trapping efficiency of the linear ion trap significantly enhances the quality of the MS<sup>2</sup> data, which, in turn, improves the quality and abundance of ions for the MS<sup>3</sup> data that is key to unambiguous identification of phosphorylated proteins.

## Goal

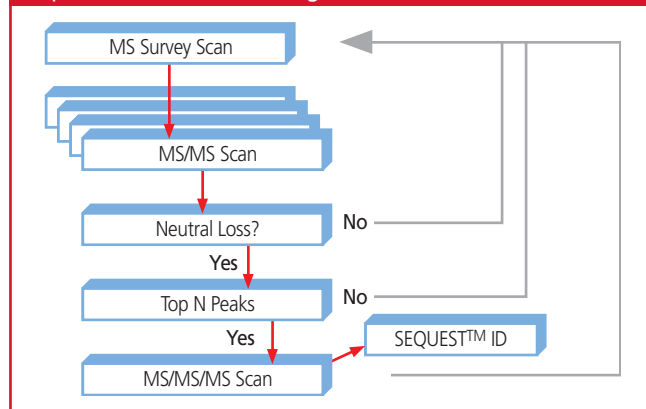
- Demonstrate functionality of the data dependent neutral loss algorithm for detection of phosphorylated peptides.
- Demonstrate exceptional sensitivity of the new Finnigan LTQ linear ion trap mass spectrometer.

## Experimental

### Chromatographic Conditions

HPLC system: Finnigan Surveyor™ MS pump with a flow splitter

**Figure 1: Automated phosphorylation site analysis using Data Dependent MS<sup>3</sup> on the Finnigan LTQ.**



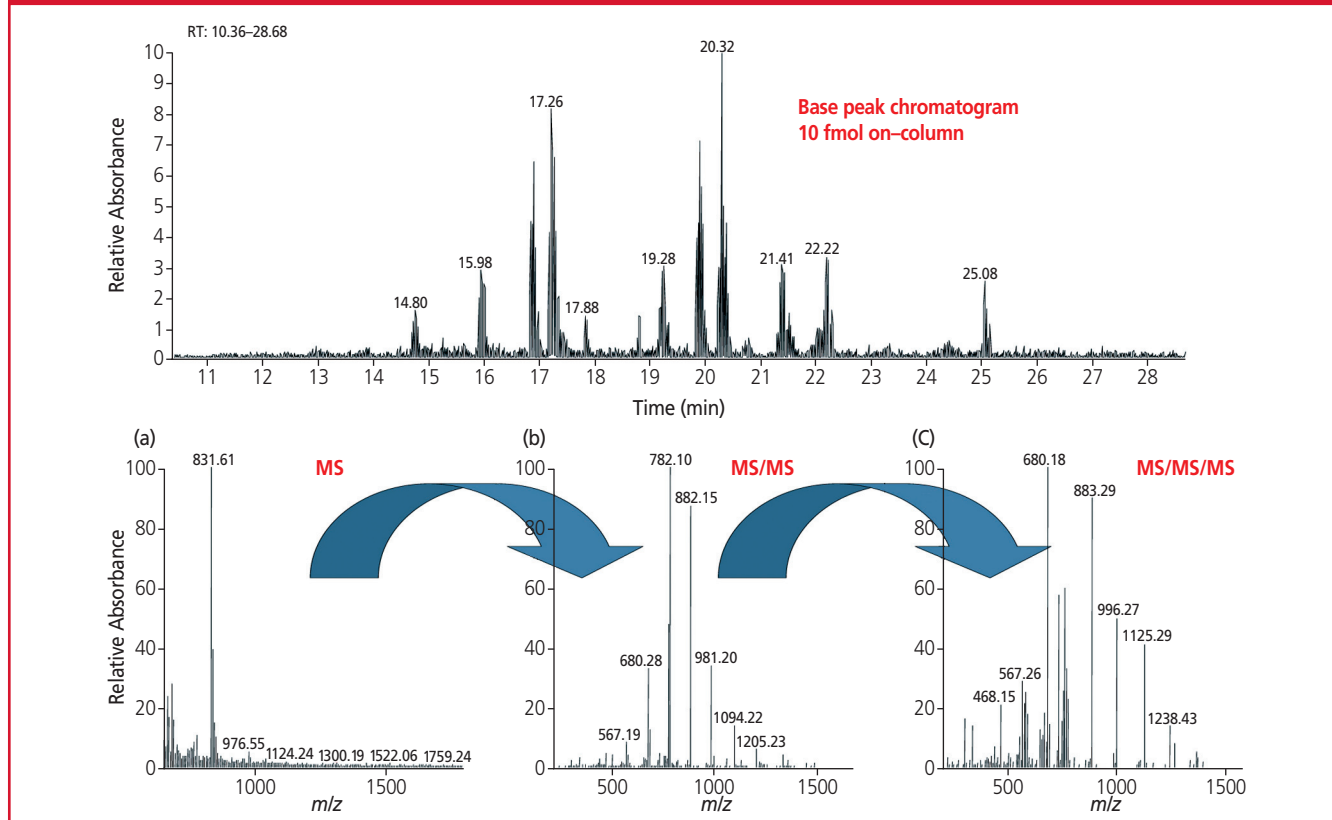
Column: 150  $\mu\text{m}$   $\times$  10 cm column packed with 5  $\mu\text{m}$  C18 stationary phase  
 Injection volume: 2  $\mu\text{L}$   
 Flow-rate: 800 nL/min post split  
 Mobile phase A: Water containing 0.1% formic acid  
 B: Acetonitrile containing 0.1 % formic acid  
 Gradient: 2–60% B in 30 min, 60–80% B in 3 min, and at 80% B for 5 min

### Mass Spectrometric Conditions

Mass spectrometer: Finnigan LTQ linear ion trap mass spectrometer  
 Ion source: Nanospray Ion Source  
 Ion transfer tube temperature: 200  $^{\circ}\text{C}$

## Commercial Applications

**Figure 2:** LC-MS/MS analysis with Data Dependent neutral loss MS<sup>3</sup> scanning of casein digest using the Finnigan LTQ. a) T: + c ESI Full ms [500.00-1800.00] b) T: + c ESI Full ms<sup>2</sup> 831.61@22.00 [215.00-1675.00]; c) T: + c ESI Full ms<sup>3</sup> 831.61@22.00 782.10@22.00 [205.00-1575.00].



**Standards:** Alpha and beta caseins (1 mg/mL in 6 M guanidine hydrochloride) were reduced (DTT), alkylated (IAA) and buffer exchanged with 100 mM ammonium bicarbonate and subsequently enzymatically digested. Appropriate dilutions of the casein digests were separated by capillary liquid chromatography.

### Results and Discussions

The flow chart of scan events for the Neutral Loss Data Dependent experiment is shown in Figure 1. An MS survey scan is initially performed, followed by three MS/MS scan events. If the MS/MS scan event detects a neutral loss ion and this neutral ion intensity is among the top 3 intensities (or top 5, depending on the user's setting), it will trigger the MS/MS/MS scan event. If a neutral loss is not detected, it will repeat with another MS scan and continue the process. Figure 2 shows the base peak chromatogram generated by capillary LC-MS/MS analysis of the casein digest using the Finnigan LTQ linear ion trap mass spectrometer. This data was generated using data dependent acquisition parameters to detect neutral loss ions for 10 fmol casein injected on-column. The full-scan survey detects a peptide at  $m/z$  of 831.6 which upon MS/MS shows a neutral loss of 49 resulting in a peak at  $m/z$  of 782.1. The Data Dependent neutral loss algorithm then triggers an MS/MS/MS event on the ion at  $m/z$  782.1 in the subsequent scan.

### Conclusions

The Finnigan LTQ linear ion trap mass spectrometer shows unparalleled sensitivity for the analysis of a digest of casein, which is evident by the detection of peptides at levels as low as 10 fmol of digest injected on-column. The automated Data Dependent

neutral loss scanning algorithm outlined here is of great utility in identifying sites of phosphorylation on peptides whose fragmentation using only MS/MS would not normally be sufficient for identification. The MS<sup>3</sup> data was searched using TurboSEQUEST™ within BioWorks™ and was successful in identifying the exact site of phosphorylation on the peptide.

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TurboSEQUEST is a trademark and SEQUEST is a registered trademark of the University of Washington, USA.

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