

# Robust Micro ESI Technique for Routine High-Throughput Proteomics

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## Overview

A 0.003 inch stainless-steel needle was adapted to the LCQ™ Deca XP orthogonal ESI probe ( $\mu$ ESI) and used for capillary LC/MS/MS protein identification experiments. The modification allowed for the use of nitrogen sheath gas which helped with nebulization of LC flow rates exceeding 500nl/min and eliminated problems caused by the liquid junction. A comparison was made between nanospray ionization (NSI) with a 75 $\mu$ m ID column using a 15 $\mu$ m pulled glass-tip (using a liquid junction), and  $\mu$ ESI with a 150 $\mu$ m ID column. The combination of the 150 $\mu$ m ID column and  $\mu$ ESI gave sensitivity near to that of NSI (250 attomoles horse heart myoglobin tryptic digest on-column) and proved to be more robust than the standard pulled glass-tips of similar ID, allowing for extended, continuous use.

Phosphorylated peptides in a  $\beta$ -casein tryptic digest were also identified using the  $\mu$ ESI interface.

## Introduction

Since the early 1990's, electrospray-tandem mass spectrometry (ESI-MS/MS) has played a pivotal role in the analysis and characterization of proteins. Combined with other protein separation techniques such as 2-D gel electrophoresis and HPLC, data from MS techniques is increasingly being used to correlate protein structure and functionality. Evidence from the human genome sequencing experiments, where protein-encoding genes are being studied, suggests that subtle changes in protein expression and post-translational modifications are responsible for the complexities inherent in human development and disease. The most popular technique for protein characterization assays has been ESI-MS/MS which can deliver the analytical sensitivity (equivalent to 2-D gel techniques) demanded by protein chemists. Protein characterization at femtomole (fmol) levels and protein identification at attomole (amol) levels have been demonstrated. While such levels of performance make ESI-MS/MS appealing for protein identification and characterization, the technique is inherently complicated and not readily amenable to high throughput proteomics applications. What is needed is a robust, reliable ESI-MS/MS system to enable automation for high-throughput proteomics applications without sacrificing sensitivity.  $\mu$ ESI provides such an interface.

## Methods

The mass spectrometer used was an LCQ Deca XP ion trap mass spectrometer. The  $\mu$ ESI interface consists of a 0.003 inch ID stainless-steel needle inserted into the 26 gauge needle housed in the standard orthogonal ESI nozzle. A voltage of +2.8-3.2kV was applied to the tip of the  $\mu$ ESI interface (positive ion mode) and 10au of nitrogen was used as a sheath gas. The ion source conditions were as follows: ion transfer tube temperature was set to 135°C, ion transfer tube voltage was set to +20V, and the "tube" lens offset was set to 0V. The ion transmission settings were as follows: -6.5V on multipole 1, -22V on the inter-pole lens, -9.5V on multipole 2, and the entrance lens was set to -45V. Data acquisition was performed using 2 microscans and 200 millisecond maximum ion injection time in the full MS mode (m/z 400-1400), while 2 microscans and 400 milliseconds maximum ion injection time were used for the data-dependant full scan MS/MS mode. The Data-Dependent™ MS/MS trigger was the most intense ion in the full MS scan with a trigger intensity of 5e6 counts. Normalized Collision Energy™ set to 35% and ion isolation width set to 3 amu. The full scan MS and MS/MS target values were set to 5e7 and 7e7 respectively.

## Results

FIGURE 1. Base peak chromatograms of 100 fmol myoglobin tryptic digest using (a) using  $\mu$ ESI (0.003 in ID metal needle) with a 0.15x100mm C-18 column and (b) 0.075x100 mm packed nanospray tip (15 $\mu$ m tip).

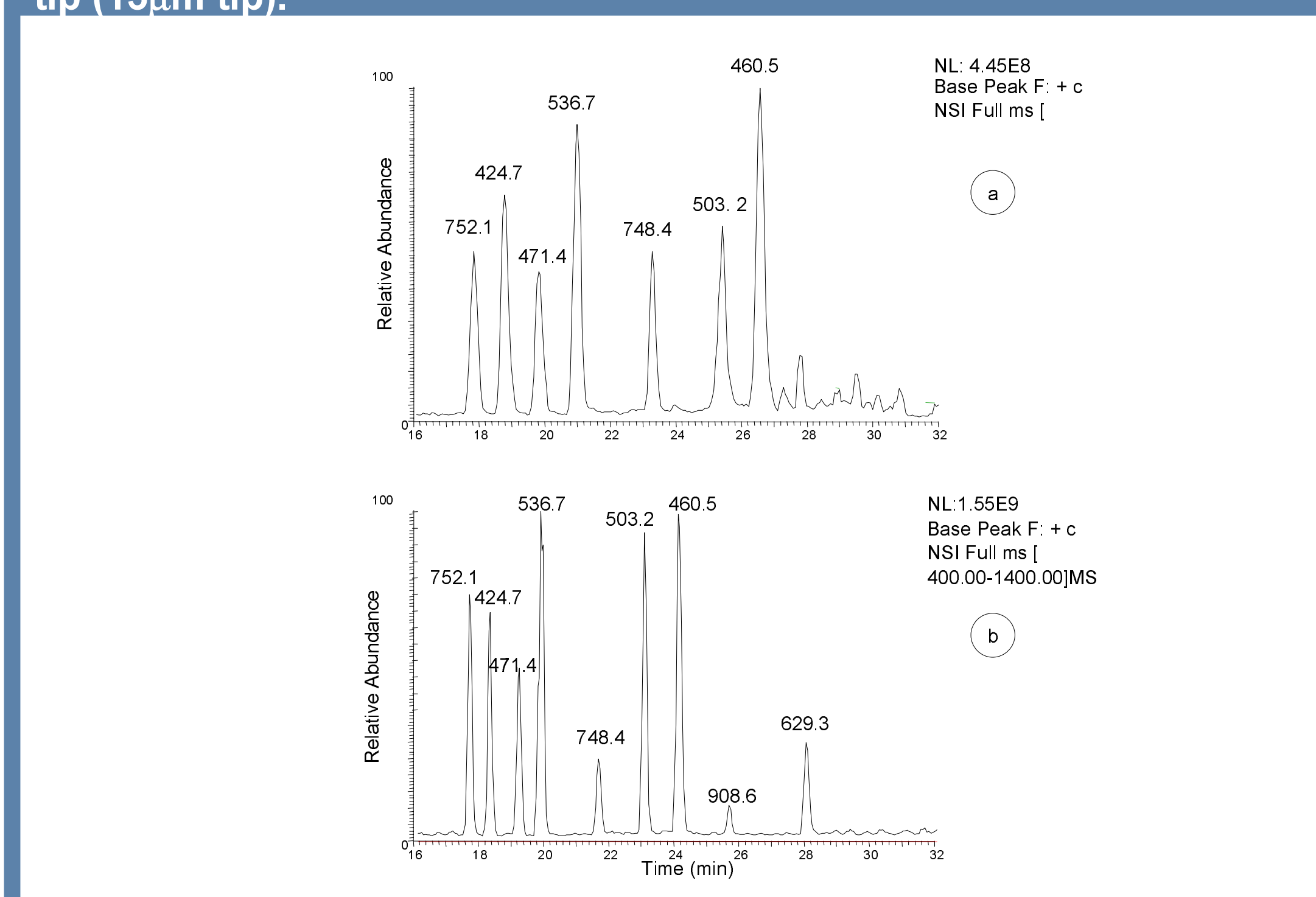


FIGURE 2. Full scan mass spectra (m/z 400-1400) from the LC/MS analysis of 100 fmol injected myoglobin tryptic digest using (a) using  $\mu$ ESI (0.003 in ID metal needle) with a 0.15x100mm C-18 column and (b) 0.075x100 mm packed nanospray tip (15 $\mu$ m tip).

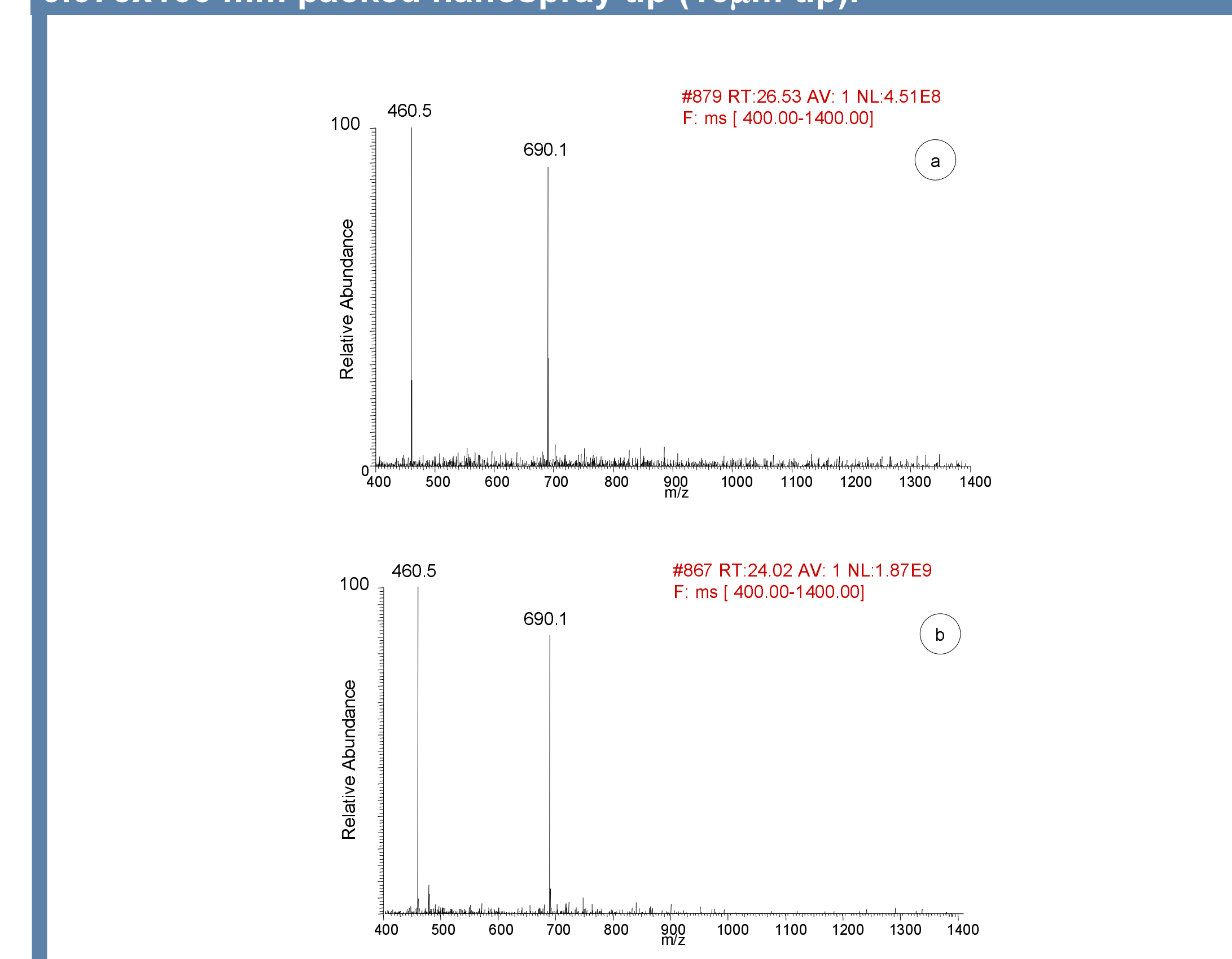


FIGURE 3. (a) 2.5fmol and (b) 250amol myoglobin tryptic digest. Full scan (m/z 400-1400) mass spectrum from (c) 2.5fmol and (d) 250amol myoglobin tryptic digest. Data generated using metal needle  $\mu$ ESI.

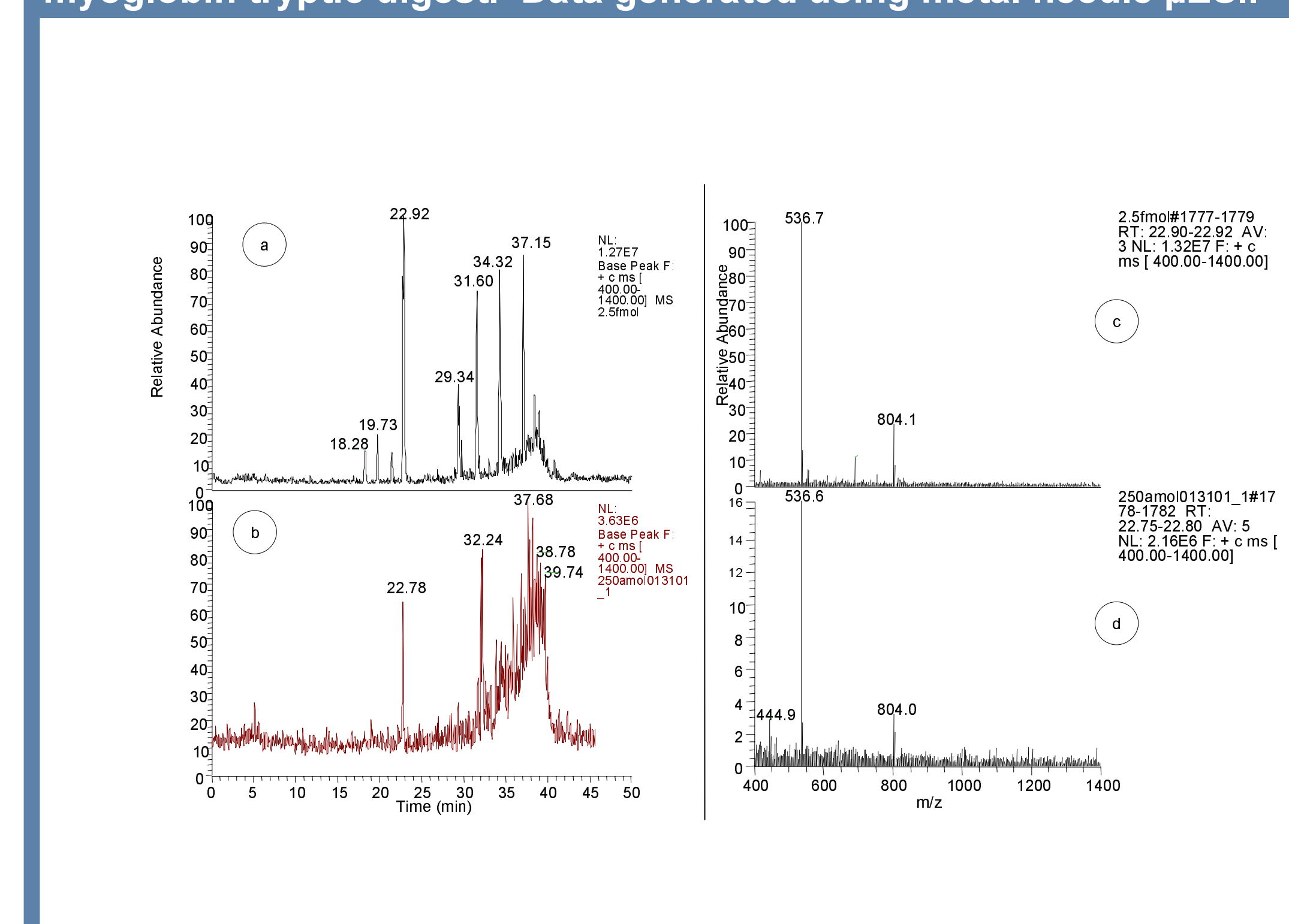
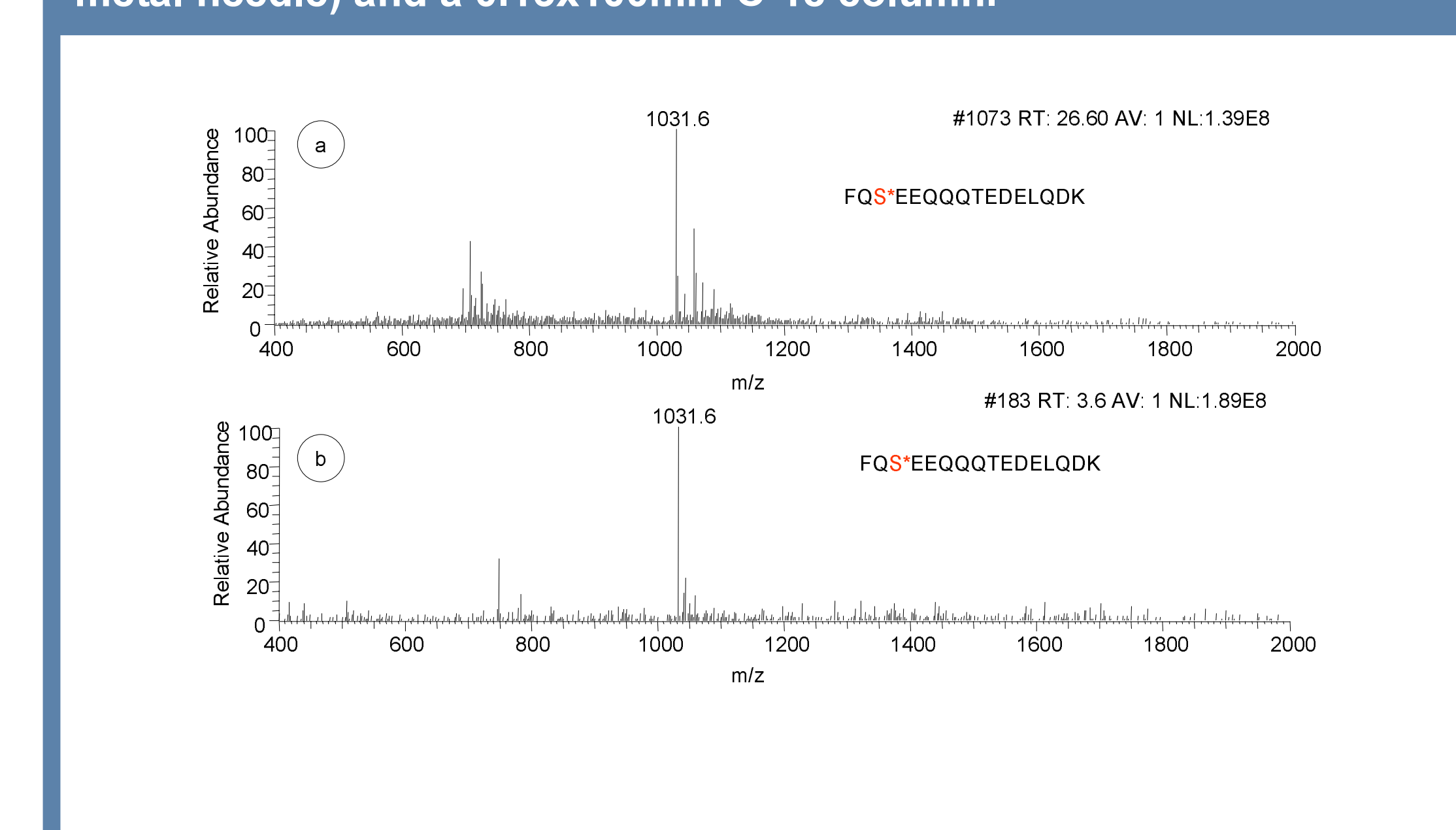


FIGURE 4. Full scan mass spectra of  $\beta$ -casein phosphorylated tryptic peptide FQS\*EEQQQTEDELQDK analyzed on (a) glass tip (30  $\mu$ m) nanospray using liquid junction and (b) using  $\mu$ ESI (0.003 inch ID metal needle) and a 0.15x100mm C-18 column.



## Conclusions

In this study, the 0.003 inch ID metal needle was proven to be a sensitive  $\mu$ ESI emitter routinely enabling protein identification and characterization at fmol levels in LC/MS/MS assays. This  $\mu$ ESI emitter was robust and operated without replacement or adjustment throughout the entire course of the study. It was free from plugging and other issues commonly associated with liquid junction NSI setups, such as trapped air bubbles and electrolysis. Another advantage of the  $\mu$ ESI interface is the ability to handle a broad range of flow rates, typically 500nL/min to 50 $\mu$ L/min, without changing the dimensions of the emitter.  $\mu$ ESI, used with a 150 $\mu$ m ID column, compared well with NSI, using a 75 $\mu$ m fused silica packed tip. Some added benefits of using larger, 150  $\mu$ m ID columns are: extended column life times, increased load capacity and ease of automated capillary HPLC system integration and operation.

$\mu$ ESI is a sensitive and robust alternative to the current state-of-the-art technique of packed tip NSI LC/MS.

## References

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