PROTEIN CHARACTERIZATION FROM ACCURATE MASS LC/MS PEPTIDE MAPS: SOFTWARE TOOLS

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INTRODUCTION

Complete interpretation of complex LC/MS chromatograms with accurate mass measurement is time-consuming and labor intensive. To automate most of this process, we have developed software to address the large data sets found in protein characterization laboratories. The goal of these experiments is to validate quantitative measures of protein variants. Forced degradation and spike studies were used to test accuracy and the limits of detection of the software tools. The peaks are detected by the Apex3D/Peptide3D algorithms to deconvolute multiply-charged ions and combine isotopes. Apex3D and Peptide3D are specifically designed to interpret the resolved isotope clusters produced by oa-Tof mass spectrometers. The results can be viewed as a table, as a combined spectrum, or as a processed chromatogram.

METHODS

Samples:

Solutions of trypsin digests of rabbit phosphorylase b
Sample "analyte" was treated with 0.01% H2O2 for 24 hours and then acidified with TFA
Sample "control" was dissolved in water and held for 24 hours and then acidified with TFA

Chromatography:

Injection:	10 µL of 4 pmol/µL digest solution
Columns:	Peptide Separation Technology ACQUITY UPLC™ BEH 300 C_{18} 1.7 µm 2.1 x 100mm
Temperature:	40°C
Flow Rate:	200 µL/min
Solvent A:	0.1% TFA in water
Solvent B:	0.08% TFA in acetonitrile
Mixer:	High Sensitivity Peptide Analysis mixer (P/N 205000403)

Gradient Table:

Time (min)	%A	%B
Init	100	0
2	100	0
118	50	50
120	25	75
122	25	75
125	100	0

UV Detection:	214 nm
Detection Rate:	10 scans/sec

MS Conditions

LCT Premier™	oa-Tof mass spectrometer
Source Temperature:	100°C
Scan Range:	400-3000 *m/z*
Scan Rate:	2 scans/sec

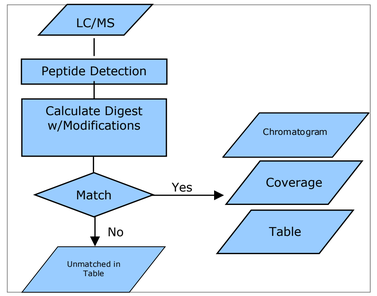


Figure 1. High resolution LC/MS data from protein digests are processed to automatically determine the molecular weights of peptides, match them to sequence and identify modifiers.

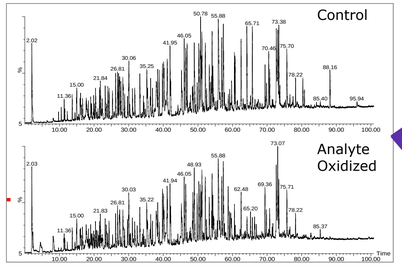
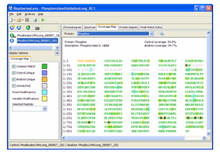


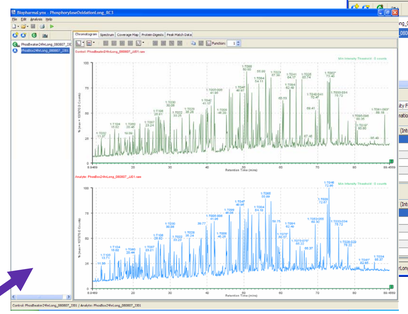
Figure 1. Forty picomoles of a 97 kDa protein, phosphorylase b, were analyzed by LC/MS. The analyte sample was treated to forced degradation with peroxide with the goal to determine the oxidation state of the protein before and after treatment.

To test a software tool, two samples differing in oxidation state were processed. One sample was subjected to forced degradation with peroxide. The data were processed to give chromatographic, molecular weight, coverage map, and percent oxidation outputs.

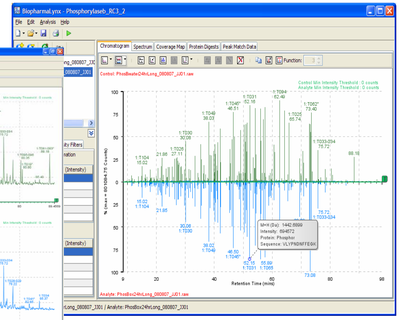


RESULTS

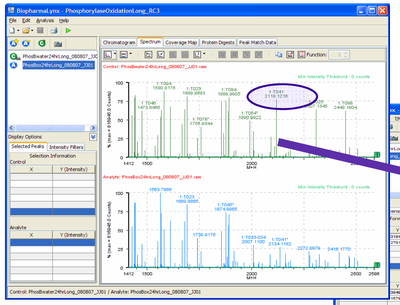
Annotated Total Ion Chromatograms
Stacked View



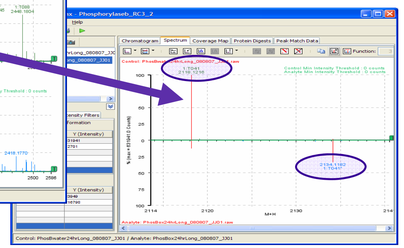
Annotated Retention Times
Mirror View



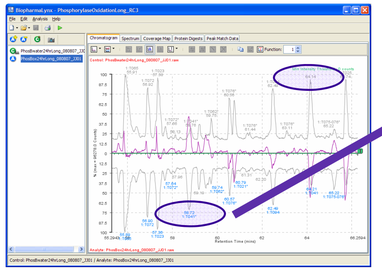
Annotated Molecular Weights
Stacked View



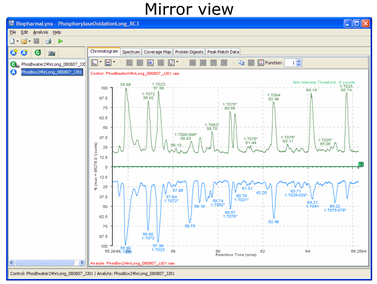
Annotated Molecular Weights
Mirror View



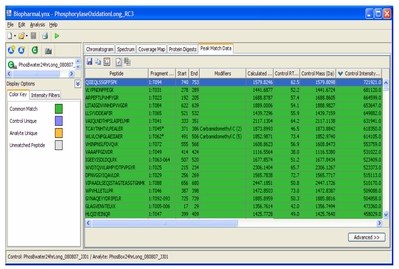
Annotated UV Chromatograms
Difference view



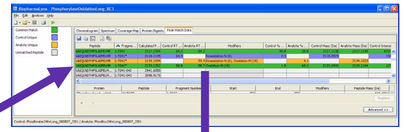
Annotated UV Chromatograms
Mirror view



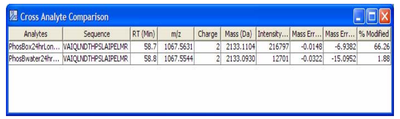
Peak Match Results
Ordered by Descending Intensity



Peak Match Table
Control and Analyte T41



Cross Analyte Comparison
Oxidized T41



CONCLUSION

- Automates time consuming and tedious manual data analysis.
- Produces annotated mass spectrometry and UV chromatograms.
- Produces coverage maps.
- Facilitates comparison between a reference standard and any number of batches or variant samples, including the calculation of percent modification.
- Outputs include formal reports with tabular data including comparison of intensity, retention time and mass.

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