**OVERVIEW**

- Software tools were developed for application to peptide maps and chromatograms.
- Two novel algorithms were developed to detect and deconvolute peptide signals in LC/MS maps.
- A matching algorithm relates the observed peptides to the protein structure, including modifications.

**INTRODUCTION**

The primary structure of a protein can be characterized by peptide maps, and the same analysis procedure can be used to identify modifications to the protein structure, highly resolving chromatographic methods, accurate mass LC/MS, and software tools have been combined to more efficiently correlate peptide maps with protein structure. One optimized methodology can be used to enhance fragmentation of peptides, which is highly desirable to help resolve the separation of the exact mass measurements possible with on-off mass spectrometry. Peptides can be identified based on molecular weight, and co-identifications can be detected. This additional information links the peptides to the protein sequence.

Additionally, the amount of trace degradation or contamination can be assessed. In particular, batches can be compared for the amounts of degradation.

Complete interpretation of LC/MS chromatograms with accurate mass measurements is time-consuming and labor-intensive. New dedicated software has been developed for these large data sets. The peaks are detected by the Apex3D algorithm to deconvolute multiply-charged ions and combine isotopes. The combination of UPLC, oa-ToF MS, and advanced software architecture dramatically improve the interpretation of peptide maps.

**METHODS**

**Analysis of LC/MS Peptide Map**

Forced degradation, such as oxidation, produces highly modified peptides. Multiplication combined with de 

**RESULTS**

**Figure 2** - Analysis of LC/MS Peptide Map

- Source conditions can be altered to enhance fragmentation of peptides. In a typical example of fragmentation is performed using increasing energy (Gorenstein, Plumb Stumpf, patent pending.) At the apex, the fil-

**Figure 3** - Comparing LC/MS Peptide Maps

- The method is used in all cases where relative retention times fall within restricted ranges. As an example, consider a peptide that is not at the apex in the control. All its ions must also elute at the same retention time. Variations from this are due to only minor and measurement errors. The algorithm then has the same retention time to within measurement error, the algorithm amplifies spectra. Such amplified spectra can more clearly reveal the unique, mass-ion signature of peptides.

**Figure 4** - Fragment Contaminant Detection

- Source conditions can be altered to enhance fragmentation of peptides. In a typical example of fragmentation is performed using increasing energy (Gorenstein, Plumb Stumpf, patent pending.) The use of this method can vary between different instruments.

**Figure 5** - Trace Contaminant Detection

- The method is used in all cases where relative retention times fall within restricted ranges. As an example, consider a peptide that is not at the apex in the control. All its ions must also elute at the same retention time. Variations from this are due to only minor and measurement errors. The algorithm then has the same retention time to within measurement error, the algorithm amplifies spectra. Such amplified spectra can more clearly reveal the unique, mass-ion signature of peptides.

Software

- MassPREP™ Phosphorylase b Digestion Standard
- MassPREP™ Hemoglobin Digestion Standard
- MassPREP™ Peptide Digestion Standard
- MassPREP™ Peptide Digestion Kit
- Add 250 µL water to each vial. Vortex. Final Concentration of digest is 1 µg/mL.
- Add 20µL of 0.1% hydroxyl peroxide to 1 µL digest.
- Incubate at room temperature for 2 hours.

Instruments

- Waters ACQUITY UPLC™ System including: ACQUITY UPLC™ I-Class System, ACQUITY UPLC™ 1290 Binary Column Waters Micromass LCT Premier Mass Specrometer or Waters Micromass Q-ToF Premier Mass Spectrometer
- Software
- MassLynx™ B.0.8, Data Analysis: B.0.8, BioPharm Lyza 1.1, Data

**CONCLUSIONS**

- Detection time alignment and intensity normalization allow accurate comparison of chromatograms obtained with different instruments.
- The protein coverage of peptide maps can be readily measured and compared using software tools.
- Less than 0.5% of trace contaminants and modifications can be detected, with confidence.
- MS source conditions can be set to take advantage of ion-source fragmentation for sequence confirmation.
- Disulfide bond linkages can be confirmed.

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