Introduction

This page illustrates the quantitative and qualitative capabilities of the new Waters® Protein Expression System. The system was designed to analyze complex mixtures using a combination of accurate mass and chromatographic peaks. Since data were acquired in a replicate excising light and elevated energy mode (CEMP®), the peptide mass fingerprinting method was validated by the analytical protocols employed in this study. The combination of accurate mass and chromatographic peaks was used to identify and quantify tryptic peptides in complex endogenous protein mixtures.

Data Collection

The indicated accurate mass is LC-MS/MS of the peptide isolated on a Waters CapLC® System with the Waters Protein Expression System Standards (MPDS), containing equimolar levels of Yeast Enolase and Alcohol Dehydrogenase. Five aliquots of this human serum digest were spiked with two sources: E. Coli and E. Coli Luria-Bertani (LB) plates and grown at 37 degrees Celsius. An individual colony was subsequently streaked E. Coli W, grown on Glucose, and E. Coli grown on S1 Acetate. Data points indicate log intensity of compared peptides in each sample. The wide dispersion of peptide intensities, as would be expected when using the Waters NanoEase™ Atlantis™ dC18 Column, 300 µm x 15 cm (accession #3++).

Sample Preparation

Human serum (250 µl) was digested according to the procedure (1). Tryptic digestions were performed in a serial manner. It also makes full use of the speed and accuracy of an OA-Tof analyzer.

Elevated-Energy

Elevated-Energy

Elevated-Energy

Elevated-Energy

Elevated-Energy

Low-Energy

Low-Energy

Low-Energy

Low-Energy

Low-Energy

Log intensity of 2.5 pmol vs. 0.5 pmol

Log intensity of 2.5 pmol vs. 0.5 pmol

Log intensity of 2.5 pmol vs. 0.5 pmol

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Results and Discussion

E. Coli Data

Spiked Serum Data

Beverly, MA; 2Milford, MA, USA. 3Manchester, UK)

Leucine Lysine S. Chain Uniform Concentration

Intensity Plot

Intensity Plot

Intensity Plot

Intensity Plot

Intensity Plot

Summary

The protein digestion protocols employed in this study provide quantitative and qualitative methods for analyzing complex protein mixtures. The analytical protocols employed in this study demonstrate that the combination of accurate mass and chromatographic retention time can provide a unique fingerprint for each peptide contained in a complex protein digest mixture. Waters Protein Expression System Informatics is capable of extracting peptide sequence, chromatographic retention time, and intensity information from complex protein digest mixtures in a quantitatively reproducible manner.

The analytical protocols employed in this study are capable of accurately measuring the intensity of peptides in complex protein digest mixtures over three to four orders of magnitude. The analytical protocols employed in this study are capable of accurately measuring the intensity of peptides in complex protein digest mixtures over three to four orders of magnitude.

Waters Protein Expression System Informatics is capable of extracting peptide sequence, chromatographic retention time, and intensity information from complex protein digest mixtures in a quantitatively reproducible manner. The accurate masses are then used to assign sequence and match to protein.