INTRODUCTION

Yeast Alcohol Dehydrogenase—Normalization Standard

Sample: Human Serum (Digested) was diluted in a solution of ammonium bicarbonate and a surfactant (TopSpec 75). The sample was reduced with dithiothreitol, alkylated with iodoacetamide, and digested with a proteolytic enzyme (trypsin). The resulting peptides were extracted with acid and analyzed by LC-MS/MS. The data was acquired on two LC-MS systems consisting of a nanoACQUITY UPLC system and a Q-TOF Premier mass spectrometer. The data was normalized to the peak intensity of T50, the standard for the normalization of all relative intensity results. The results show that accurate fold-change results may be obtained using data acquired on two LC-MS systems, and that consistent data are obtained across a large number of injections on two instruments, and that consistent fold-change results may be obtained using data acquired on two LC-MS systems.

RESULTS AND DISCUSSION

A standard amount of yeast ADH was added to each sample as a calibrator for the normalization of peptide intensities between injections and between the two systems. The data from all injections was normalized to the peptide intensity of T50, the standard for the normalization of all relative intensity results. The results show that accurate fold-change results may be obtained using data acquired on two LC-MS systems, and that consistent fold-change results may be obtained using data acquired on two LC-MS systems.

CONCLUSIONS

On the basis of the data presented here, we conclude that the data acquired on two LC-MS systems may be combined in a non-protein-specific study approach.

The same model mass spectrometers and liquid chromatography instruments are used.

The same chromatography columns, mobile phases and gradients, and connections are used. A normalization standard or a consistent known concentration is added to each sample for relative quantitation.

Instruments are properly fixed, calibrated, and maintained.

In the future, we hope that accurate fold-change results may be obtained using data acquired on two LC-MS systems and that consistent fold-change results may be obtained using data acquired on two LC-MS systems.

For consistent protein identification, it is important that the elevated collision energy fragmentation data be acquired on a second LC-MS system in order to complete such a study expeditiously, it would be desirable to acquire the data on more than one LC-MS system. The elevated collision energy fragmentation data is intended to demonstrate that, given reasonable controls, data from more than one LC-MS system can indeed be combined in a single experiment.

Finally, armed with the knowledge that peptides are consistently detected on multiple instruments with similar normalized intensity and chromatographic retention time values, we can construct an "intensity" plot of the tryptic peptides of any protein, such as the example for Complement C3 Precursor in the figure shown next. This intensity is indicated by the size and darkness of the spots.

Accordingly, we conclude that data from more than one LC-MS system can indeed be combined in a single experiment. The elevated collision energy fragmentation data is intended to demonstrate that, given reasonable controls, data from more than one LC-MS system can indeed be combined in a single experiment.