

**A SYSTEMATIC STUDY INTO THE REPRODUCIBILITY OF HPLC AND MASS SPECTROMETRY FOR THE ANALYSIS OF COMPLEX PROTEIN TRYPTIC DIGESTS BY LC-MS**

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The coupling of liquid chromatography with mass spectrometry is now firmly established as a routine method for the identification of proteins that have been subjected to enzymatic digestion. In an on-line LC-MS experiment, the column eluent is coupled to the electrospray source via an emitter and any tryptic peptides present in the mixture are mass analysed as they elute from the HPLC column. Should there be any co-eluting species in the eluent, these will be separated in the mass analyser by their mass-to-charge ratio.

It has become increasingly clear that relative quantification of protein expression changes is important in modern biology and medicine. Several current approaches have been developed that utilise stable isotope labelling of samples in combination with separation and subsequent analysis by mass spectrometry. However, we have recently described an LC-MS strategy where quantification is achieved via normalisation of the MS datasets and comparison of the peptide intensities across samples is performed. In this case, it is desirable to perform replicate injections and hence reduce statistical errors. This approach places a requirement upon good chromatography, especially in terms of retention time reproducibility. In addition exact mass measurement of the eluting ions is required as well as the ability to generate reproducible and reliable peak intensity, or area, calculations for the eluting tryptic peptides. The ability to measure the mass to charge ratios of ions accurately, across injections and across samples, increases confidence that the same ions have been matched from each sample injection.

In this study, we have systematically investigated the reproducibility of an LC-MS system to determine the levels that are required, and those that may be expected. In particular we have investigated chromatographic reproducibility (at the 75uM scale), and determined the clustering of known peptide ions, from run-to-run using an automated algorithm. We have investigated the effect of changing HPLC column, and also the complete analytical system (LC and MS) on the number of ions and the intensity of the ions that can be identified and matched. In addition we have studied the effect of mass spectrometer resolution on the mass measurement accuracy obtained and the number of peptide species which may be confidently assigned.