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Increasing the system peak capacity of LC-MS/MS work-flows for qualitative and quantitative protein profiling by incorporating ion mobility separations

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Over the past decade the complexity of biological samples intended for qualitative and quantitative proteomics analysis has been significantly under estimated. Consequently the peak capacity of typical LC-MS/MS systems used for such analyses has been insufficient.

There is a growing consensus within the proteomics community that, in the analysis of complex digests, exact mass analysis aids the unambiguous matching of tryptic peptide spectra to databanks of known protein structure. Where exact mass measurement of both precursor and product ions contribute significantly to minimizing false discovery in peptide/protein identification.

We will elucidate the challenge of sample complexity in proteomics by summarizing our findings from a one dimensional reversed phase HPLC separation (120 minute gradient) of a complex protein digest (*E. coli*) with Electrospray exact mass MS detection. The data show *Ca* 450,000 unique ions that, following charge state and Isotope deconvolution, reveal *Ca* 40,000 non-redundant precursor ions for identification and quantification. This complexity is further compounded by the fact that the detected ions are not uniformly distributed in time or m/z range. Typically 50% of all ions detected are observed in <25% of the total run time and >60% of all ions detected are observed in the m/z 400-800 range. Moreover 70% of all ions are two or more orders of magnitude less intense than the most abundant ions detected.

We describe a novel approach to address the analytical challenge inherent in such sample complexity embodying the on-line combination three dispersive analytical techniques; HPLC, Ion Mobility Separation (IMS) and Time of Flight MS. Additionally the advantage of collecting LC-IMS-MS data at high mass resolution and mass accuracy will be summarized. Our results illustrate how LC-IMS-MS successfully increases system peak capacity by a factor of 10 thus enabling more components of complex protein digests to be unambiguously identified and quantified per unit time.