Application Note

Quantitative label-free LC-MS using a low resolution ion trap instrument and Progenesis LC-MS

Overview:

- Label-free proteomics is becoming adopted due to the advantages it offers over labelled approaches[1,2]
- Despite the emergence of high-resolution MS instruments as the choice for label-free quantitative proteomics; many labs have low resolution ion trap instruments already in place, which can be applied to this approach.
- Here Progenesis LC-MS was applied to visualise complex MS data and help optimise quantification of a complex protein mixture separated by LC coupled to a low resolution MS instrument.

Introduction & Results:

To produce reliable LC-MS data alterations can be made to either liquid chromatography (LC) or mass spectrometry (MS) resolving power; with the refinement of both key to the acquisition of accurate data. The MS can be operated in different modes and scan rates optimised for quantification and/or identification of peptides and proteins. Progenesis LC-MS quantifies ion intensity of the isotope envelope detected on MS spectra to measure expression differences. Identification results from database searches of MS/MS spectra are linked to the parent ions detected on the MS spectra providing a **quantify then identify approach** with several benefits[3, 4]. Using Progenesis LC-MS we compared analysis of low resolution data at two different scan modes, Ultra and Standard (Enhanced), and determined optimal settings for quantification and identification of a complex protein mixture (Fig 1). In this case the slower scan rate of Standard (Enhanced) mode resolved more features in MS spectra and allowed a greater number of fragmentation ion data to be correctly associated with these features. This improved the number of peptides we could use to confidently quantify and identify proteins of interest.

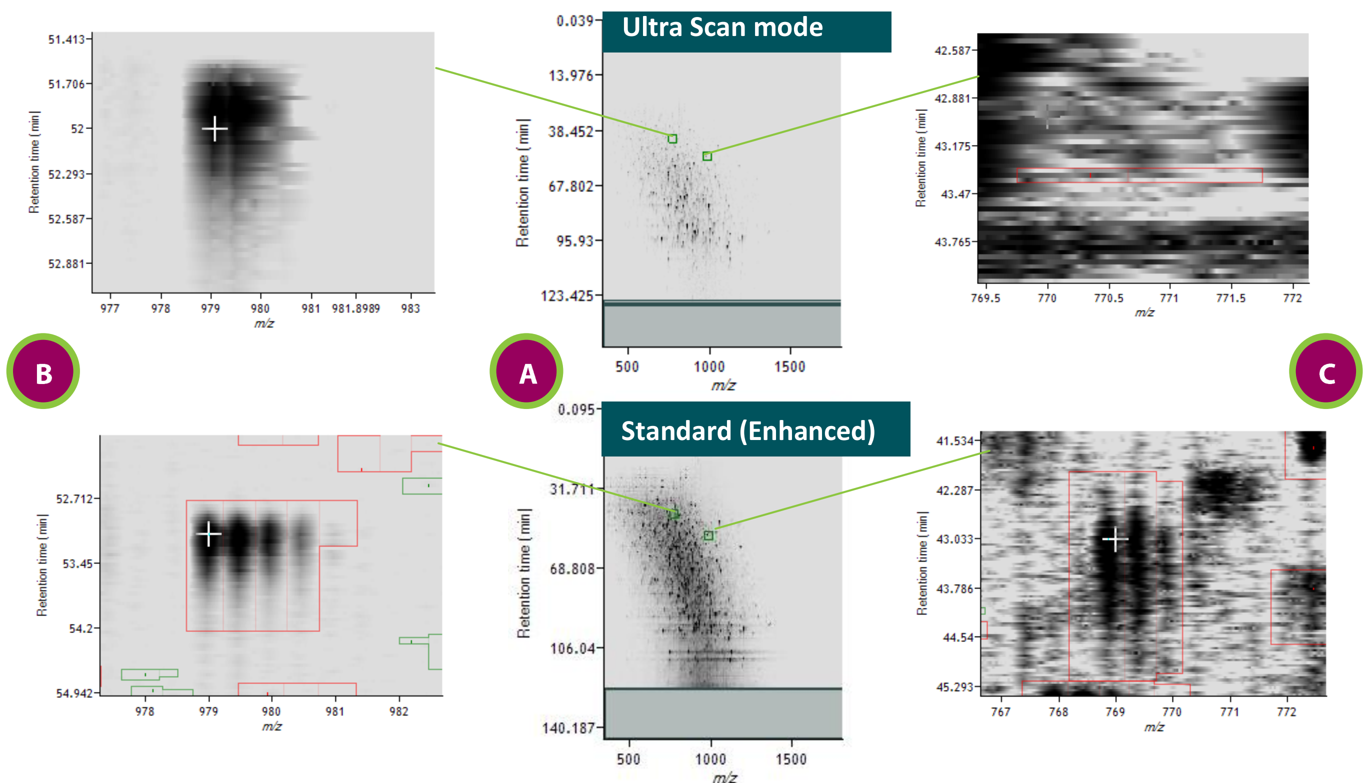

Figure 1: Comparing detection of features in MS spectra between Ultra scan mode vs. Standard (Enhanced) scan mode. (A) Ion intensity map of peptide elution over a whole LC-MS run in each mode with relative ion intensity represented by the grey scale. (B) Zoomed in view of the same high abundance peptide ion quantified in each mode. (C) Zoomed in view of the same low abundance peptide ion quantified in each mode. Standard (Enhanced) mode provided the best resolution of both high and low abundance features.

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Results continued

Standard (Enhanced) mode provided a more effective scan setting for label-free relative quantification based on MS spectra with **8-fold more features detected in Standard (enhanced) mode** compared to Ultra scan mode. Ultra scan mode gave the highest individual peptide score in MASCOT search results but **the number of peptide identifications assigned to parent ions in Standard (Enhanced) mode was 10-fold higher** than Ultra Scan mode (Table 1).

Scan Mode	Number of features detected in MS spectra by Progenesis LC-MS	Number of MS/MS spectra exported for searches	Number of protein identifications in MASCOT search	Number of features with assigned identification in Progenesis LC-MS	Highest Individual peptide score	Highest protein score calculated by Progenesis LC-MS
Standard (Enhanced)	**232,000**	**20,781**	**427**	**2,275**	**96.37**	**758**
Ultra	**29,000**	**3,568**	**119**	**219**	**101.2**	**213**

Table 1: Quantifying differences observed between the different scan modes. Both modes achieved similar highest scores for an individual peptide in both MASCOT search results and within Progenesis LC-MS. But compared to Ultra Scan mode, Standard (Enhanced) detected 8-fold more features in MS spectra with a 3.6-fold increase in protein identifications from MASCOT searches as well as the protein score calculated by Progenesis LC-MS.

In Progenesis LC-MS the protein score is calculated as the sum of scores from positively identified peptides from a specific protein. It is quantifiable due to the detection of peptide ions, which are linked to corresponding MS/MS spectra search results. A higher score in data from Standard (Enhanced) mode is indicative of a larger number of peptides positively identified and related to the parent ion. **This suggests greater protein coverage was achieved in Standard (Enhanced) mode.**

Conclusions:

Analysis of raw data generated in the Standard (Enhanced) mode positively identified more peptides and generated higher protein scores; irrespective of relative natural abundance. In this case it illustrated this scan mode provided greater sequence coverage of the bacterial proteome compared to Ultra scan mode. Even though much current cutting edge research relies on high resolution apparatus, the workhorse machines are still valuable in the production of good quality research data with appropriate steps taken to optimise them for your specific samples, LC system and MS operation.

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Methods:

Sample Preparation: Cell free extract of *Escherichia coli* K12 was prepared, freeze dried and resuspended in 8M Urea, 50mM Ammonium Bicarbonate and 10mM DTT and incubated at 60°C for 1h. 1M Iodoacetic Acid was added to a final concentration of 15mM and the sample incubated in darkness at RT for 30min before an 8-fold dilution with 10mM Ammonium Bicarbonate (final Urea concentration of 1M). A Bradfords Assay determined approximate protein concentration and trypsin was added at a ratio of 20:1 (protein: trypsin). Digests were incubated at 37°C overnight and an SDS gel run to confirm digestion. The digested cell free extract was then freeze dried and re-suspended in LC mobile phase Buffer A. The sample was centrifuged at 10,000xg for 10 min prior to loading on LC-MS to remove insoluble particulates from solution.
LC and MS: Ultimate 3000 (Dionex) coupled to an HCT-Ultra Ion Trap (Bruker). Peptides were separated with a 15 cm C18 column, 3µm particle size, (Dionex) using a 0-30% Acetonitrile gradient over 120min. MS spectra were captured in either Standard (Enhanced) or Ultra scan mode with LC settings kept constant and MS/MS spectra captured in Ultra Scan mode in both cases. 8 technical replicates of the same *E. coli* K12 digest were run with each MS scan setting. Data was analysed using Progenesis LC-MS v2.6 (Nonlinear Dynamics).

References:

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