Bruker Daltonics

Application Note # ET-16

Improved Peptide Identification with an Ultra High Resolution Quadrupole Time of Flight MS

Introduction

Analysis of biological samples frequently involves the identification of peptides from low amounts of complex samples. Confident identification of these peptides requires rapid generation of high quality, high sensitivity MS and MSMS data. The maXis™ ultra high resolution quadropole TOF incorporates novel technical innovations, which together produce an unprecedented level of data quality, resulting in a significant increase in the number of peptides identified from challenging samples.

The goal for the analysis of complex and low abundance biological samples is to confidently and uniquely identify as many peptides as possible. Achieving this goal is dependent upon the quality of the mass spectrometric data. MS & MSMS mass accuracy, resolution and speed of acquisition are all critical parameters for success.

The maXis is the first mass spectrometer to deliver a no compromise solution for proteomics, delivering high MS and MSMS mass accuracy with high resolution at speed. High MS and MSMS mass accuracy are fundamental to successful proteomics analysis. Increased mass accuracy allows a more specific and stringent database search, ultimately resulting in a reduction of false positive hits and increased certainty for identified peptides. High MSMS mass accuracy facilitates the interpretation of spectra which contain only a few fragment ions or those with modified amino acids, both of which add to the number of identified peptides.

For complex samples, resolution is required from both the LC and the mass spec. Modern day LC systems exploit rapid LC gradients which offer increased speed of analysis. Very narrow peak widths are produced, commonly only 1 sec wide compared to 30 sec with a standard LC. This effectively increases the peptide concentration reaching the mass spec and therefore results in increased sensitivity. It is however, essential that the mass spec duty cycle is fast enough to detect these peaks as they rapidly emerge from the LC system and that enough data points are acquired from each peak profile, particularly if quantitation is desired. For complex samples many peptides elute from the column simultaneously. High MS resolving power is necessary to separate potentially overlapping signals from parent ions. This permits correct assignment of the mono-isotopic peak and ensures the correct mass is utilized for fragmentation, and database searching. The maXis incorporates several patented technical innovations which together deliver enhanced performance. Fundamental for the delivery of very

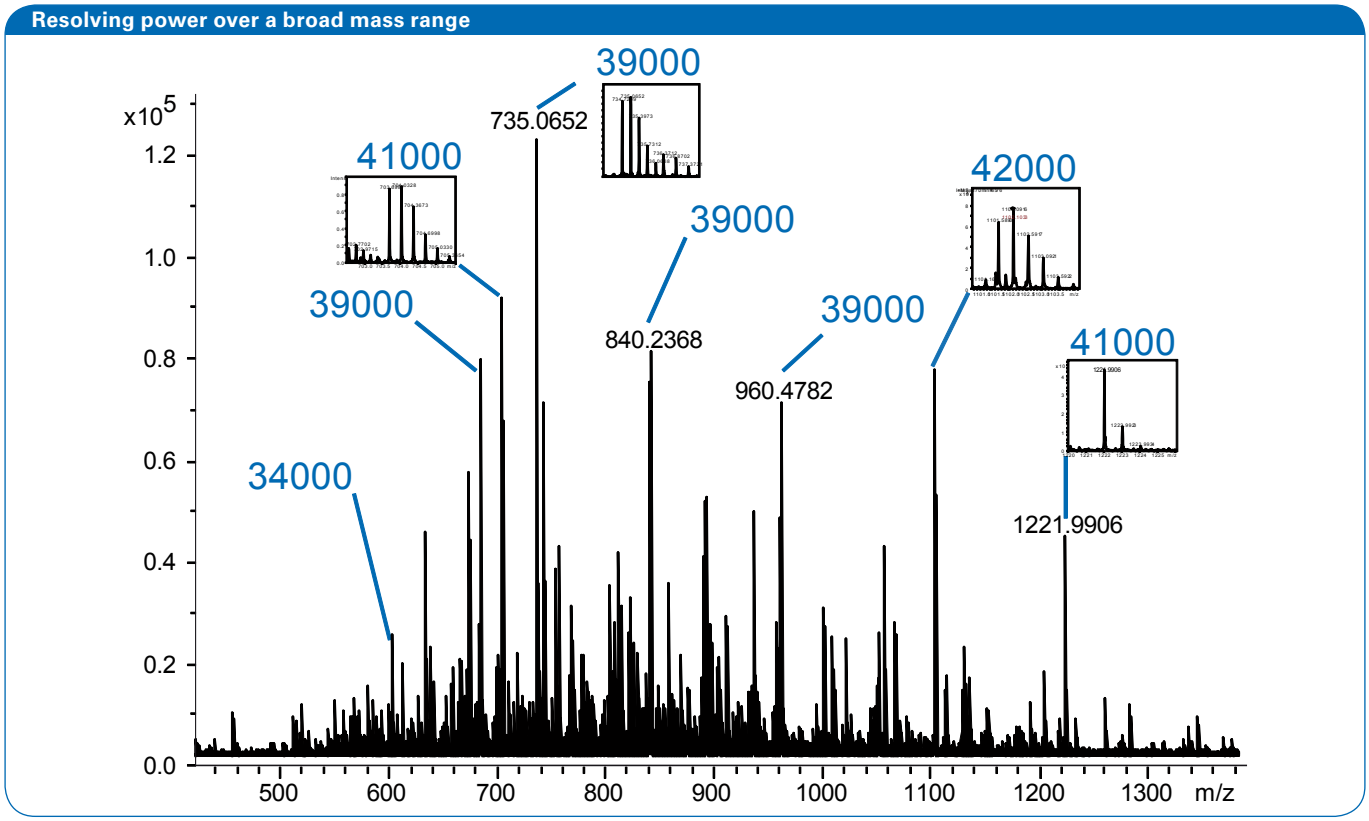

Fig. 1: Resolution in an MS spectrum from an LC run of a tryptic digest of *E.coli* cells.

high sensitivity, high quality information rich spectra has been the novel addition of an ion cooler cell after the collision cell. The ion cooler captures and makes available many high energy fragment products that are lost in conventional mass spectrometers. The collision cell and ion cooler together provide efficient fragmentation and efficient transfer of the fragment ions of a broad mass range into the orthogonal accelerator. Technical strategies have also been employed to reduce the energy distribution in the direction of flight and to ensure a precise starting point for orthogonal deflection. These features serve to increase both the resolution and mass accuracy. Figure 1, shows a resolving power of around 40,000 over a broad mass range. The resolving power of the maXis is unaffected by either peptide mass or speed of analysis. The choice of a long tube, "V" flight path minimizes ion losses in flight and allows use of the full UHR-TOF resolution at highest sensitivity, avoiding the severe ion losses associated with multireflectron, folded pathway TOF geometries.

Experimental

To demonstrate the technological advantages of the maXis, samples were selected to represent either complex or low abundance samples. The complex sample consisted of 100 ng of *E. coli* cells, whilst 500 amol Bovine serum albumin (BSA) represents a low abundance sample. Both samples were digested with trypsin. LC Systems and gradients: Ultimate 3000 (Dionex) and EASY-nLC (Bruker). Gradient: from 5 to 40% ACN, 0.1% formic acid, in 90min (*E. coli*.) or 10 min (BSA), flow rate 300 nl/min. Analytical column: 75 µm × 150 mm, trapping column, 100 µm × 10 mm; Acclaim PepMap100 C18(Dionex). MS System: Bruker Daltonics maXis ultra high resolution TOF.

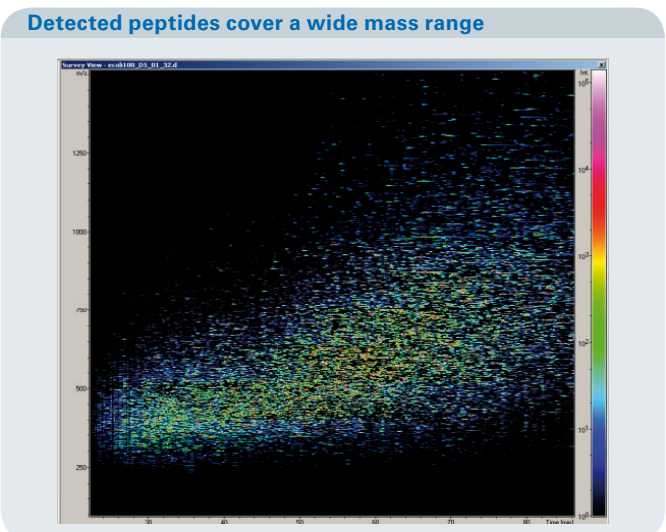

Fig. 2: Peptides detected from 100ng *E. coli*, showing retention time (x axis) and m/z (y axis).

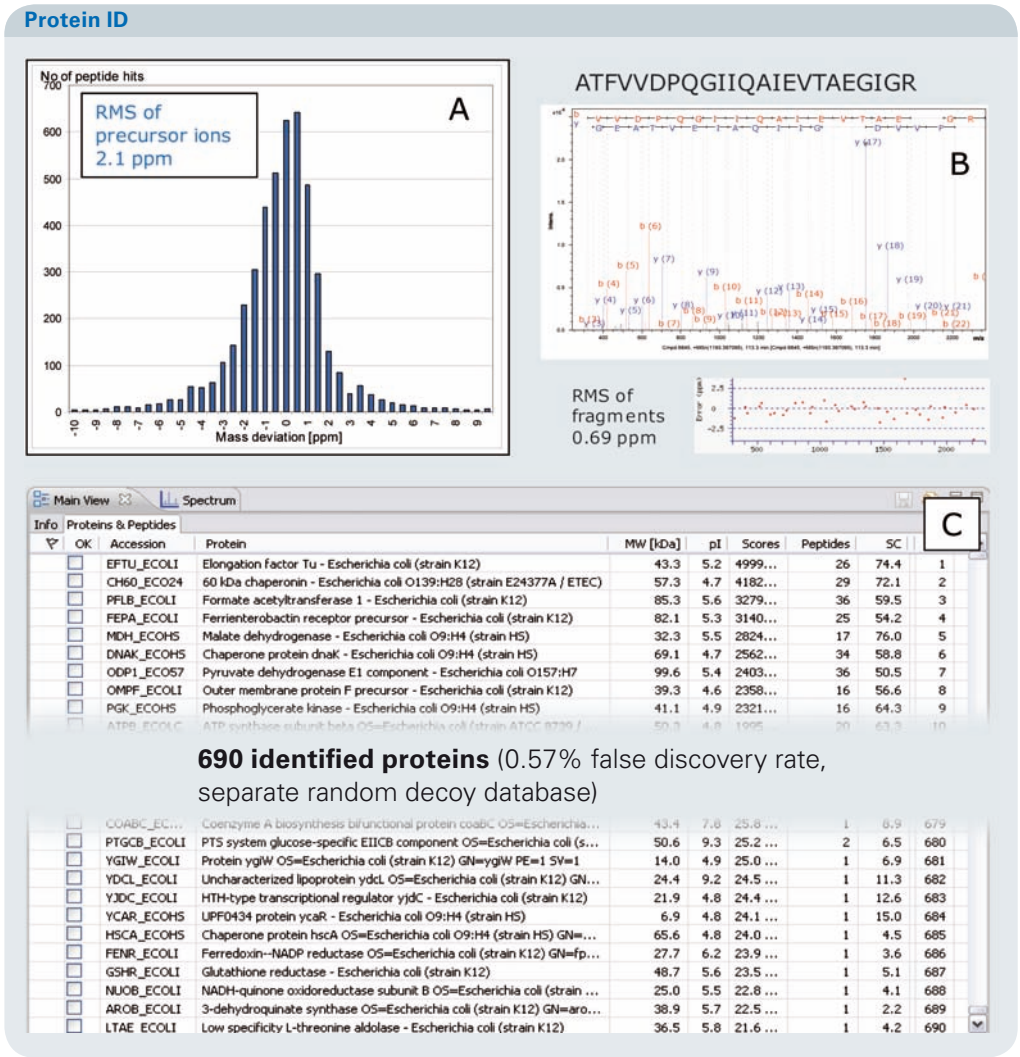

Fig. 3: Protein ID results from an *E. coli* digest, 100 ng on column. Error distribution of precursor masses in 0.5 ppm steps (A), example of fragment spectra (B), start and end of protein list (C).

Results

Complex Sample

Figure 2 provides a visual representation of the peptides detected from 100ng of *E. coli.* It can be observed that the detected peptides cover a wide mass range which will ultimately lead to increased sequence coverage. In addition larger peptides tend to produce unique hits from the protein database, which increases the certainty of the identified proteins. From 100ng of digested *E. coli.* cells 690 proteins were identified with a stringent false positive detection rate of 0.57% (Fig.3). 8507 MSMS spectra were acquired. These spectra were searched by Mascot version 2.2, using the Swissprot 56.1 database, taxonomy was restricted to *E coli.*, modifications included carbamidomethyl Cys (fixed) and oxidized Met (variable) Of these spectra 4917 peptides matched above the identity threshold (28 decoy hits, FDR = 0.57%), with an average Mascot ion score of 58.8.

Even with minimum modifications considered, over 58% of MSMS spectra generated were identified as significant. This demonstrates the high quality of the MSMS data produced, an example of the spectra quality can be seen in Fig. 3.

Low abundance Samples

The results from 500 amol BSA digest separated with a 10 min gradient are shown in Fig. 4. Spectra were searched by Mascot version 2.2, using the Swissprot 56.1 database, taxonomy was restricted to mammals, modifications included carbamidomethyl Cys (fixed) and oxidized Met (variable). 13 unique peptides were identified representing a sequence coverage of 23% and an overall Mascot score of 412 (using standard scoring). Previous blank injections showed no BSA peptides. The quality of the MSMS data can be observed in Fig. 4. Many y and b type ions have been assigned to each peptide sequence, resulting in confident identification of these peptides.

Low abundance Samples ID

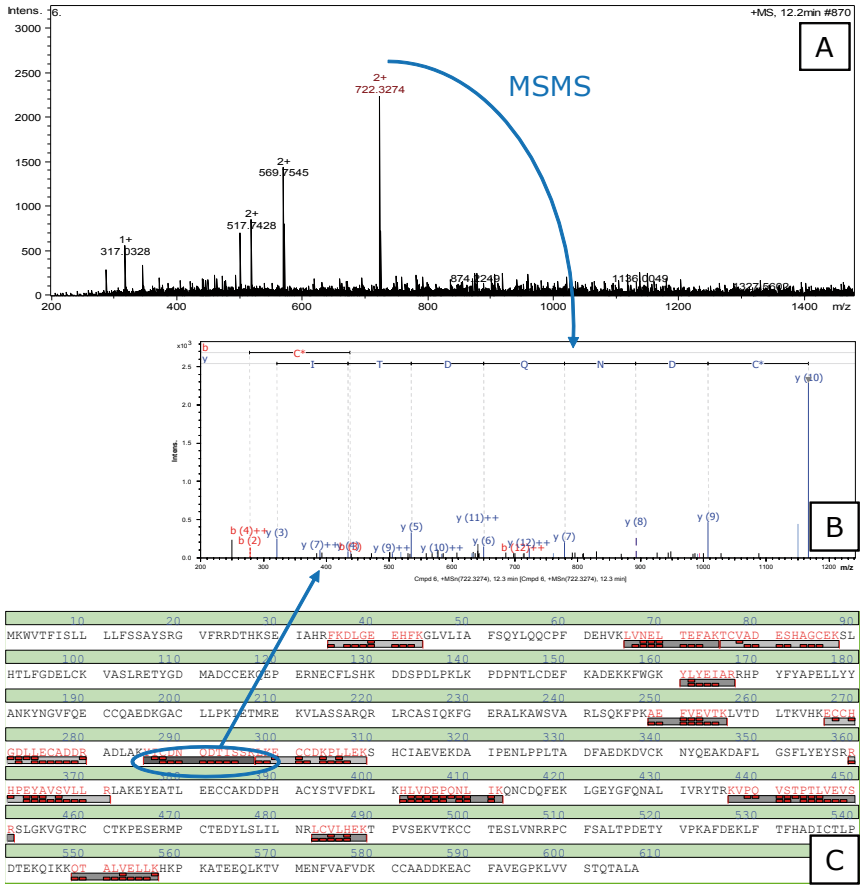

Fig. 4: Protein ID results from 500 amol BSA digest (10 min nanoLC gradient). MS Spectrum of precursor YICDNQDTISSK (A), fragment spectrum (B), sequence coverage (C).

Conclusions

The maXis ultra high resolution TOF brings together novel design technology to achieve the key proteomics goals of more peptides identified with more certainty. The technological advances include a hexapole collision cell together with an ion cooler which provide; Efficient fragmentation of ions from a broad mass range and efficient transfer of fragment ions from the collision cell into orthogonal acceleration. In addition techniques have been employed to reduce energy distribution in the direction of flight and to ensure a precise starting position for orthogonal deflection. In combination, these technical developments result in high quality, information rich spectra at high resolution (40,000) with incredible mass accuracy (low to sub ppm) at the MS and MSMS level along with fast acquisition speeds. We have demonstrated a substantial increase in the number of peptides identified from both complex and low abundance samples with an increase in significance and certainty for the peptides identified.

Authors

Laura C. Main; Bruker Daltonics Ltd, Coventry, UK;
Markus Lubeck, Marina Behrens, Andrea Schneider, Carsten Baessmann, Oliver Raether; Bruker Daltonik, Bremen, Germany

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www.bdal.com

Bruker Daltonics Inc.

Billerica, MA · USA
Phone +1 (978) 663-3660
Fax +1 (978) 667-5993
ms-sales@bdal.com

Bruker Daltonik GmbH

Bremen · Germany
Phone +49 (0)421-2205-0
Fax +49 (0)421-2205-103
sales@bdal.de