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COMPLEMENTARY IDENTIFICATION OF PROTEIN DIGESTS USING A COMBINED MALDI AND ESI DUAL SOURCE Q-TOF MASS SPECTROMETER

Richard Tyldesley[1], Keith Compson[1], Andreas Wattenberg[2], Klaus Schneider[2], Dominic Gostick[1], James Langridge[1], Matthew Willetts[1] and John Hoyes[1]

[1] Waters Corporation, Floats road, Wythenshawe, Manchester, M23 9LZ. UK

[2] Bioanalytical Sciences, CASS, GlaxoSmithKline, Harlow, Essex CM19 5AW, UK

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Overview

- The limit of detection was determined for MS data acquired on a Q-Tof mass spectrometer in both electrospray ionisation (ESI) and matrix assisted laser desorption ionisation (MALDI)
- The dynamic range of both ESI and MALDI ionisation modes were investigated with unseparated mixtures of typtic peptides
- The resulting MS/MS spectra from low energy CID fragmentation are compared and contrasted for both MALDI and ESI

Introduction

Protein identification by mass spectrometry is now a routine procedure in many biochemistry laboratories. There are several alternative methods for producing an identification of an unknown protein, but these nearly always require the mass measurement of peptides or its associated fragment ions in the MS/MS mode. Analysis of the peptides produced from enzymatic digestion of the protein is typically performed by either ESI or MALDI. These two ionisation techniques have until now always been performed on different types of mass spectrometers. In this poster we compare the MS and MS/MS spectra produced from the analysis of protein digests using ESI and MALDI on the same instrument. This is now possible using the dual source available on the Q-Tof mass spectrometer.

The two ionisation techniques are known to produce different MS and MS/MS spectra from the analysis of the same sample due to the differential ionisation of tryptic peptides. In this poster we investigate these differences and illustrate their complementary nature for protein identification using database searching and bioinformatics.

Materials and Methods

Sample Preparation

Phosphorylase B from Rabbit muscle and Yeast Alcohol dehydrogenase tryptic digest samples (Michrom Bioresources Inc.) were diluted in aqueous 1% formic acid prior to analysis. Samples analysed by MALDI were diluted 1:1 in matrix (α-CHCA, 5mg/ml; ACN 50% (v/v); TFA 0.1% (v/v). One microlitre of this solution was spotted onto the MALDI sample stage. Samples analysed by ESI were diluted 1:1 in ACN.

Mass Spectrometry

All MS and MS/MS data were acquired using a Q-Tof Ultima Global (Micromass UK Ltd.) hybrid quadrupole orthogonal acceleration time-of-flight mass spectrometer. This mass spectrometer is fitted with a combined source to allow MALDI and ESI to be performed on the same instrument. ESI was performed by infusion of the appropriate sample using the nano flow source at 200 nl/min.

Data bank searching

MS and MS/MS spectra were processed, post acquisition and databank searched using the search programme ProteinLynx Global Server v1.1 and v2.0 (Micromass UK Ltd). The spectra were processed using a proprietary algorithm to produce monoisoptic singly charged spectra. These were then searched against a SwissProt v1.0 databank to produce protein identification.

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Results

MS sensitivity

MS spectra were recorded from 5 and 0.5 fmol loading of Phosphylase B on the Q-Tof Mass spectrometer in MALDI mode (**Figures 1a and b**). Databank searching of the resultant spectrum at 5 fmol produced identification of the expected protein with the majority of the intense peptide species mapped to the protein sequence (**Figure 2a**). The intense signals from the analyte is suppressing any signal from the MALDI matrix. However, the recorded spectrum from 0.5 fmol appear very different from the 5 fmol spectrum. This is due to the presence of intense matrix ion clusters. Despite the presence of these species it was still possible to obtain a positive identification of the associated protein from the mass spectrum through databank searching (**Figure 2b**). This unambiguous result was mainly due to the high mass accuracy obtained from the orthogonal MALDI analyser. This high mass accuracy ment it was possible to have a 10 ppm mass tolerance on the databank search parameters which avoided the matrix ions from interfering with the protein identification.

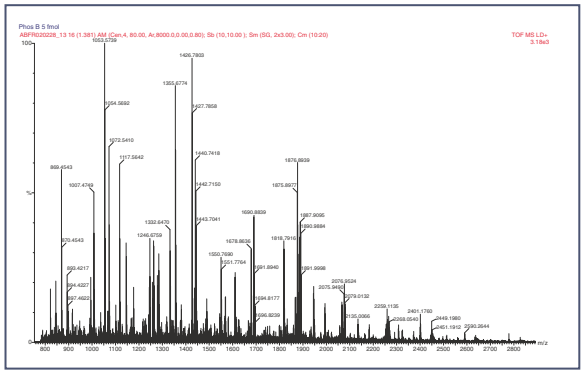

Figure 1a. MALDI MS Phosphorylase B 5 fmol on Target

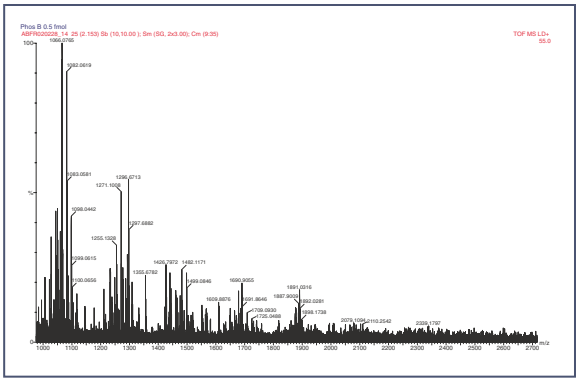

Figure 1b. MALDI MS Phosphorylase B 0.5 fmol on Target

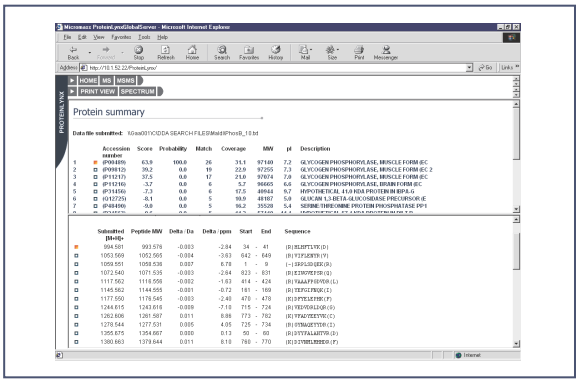

Figure 2a. Databank Search Result of Phosphorylase B 5 fmol

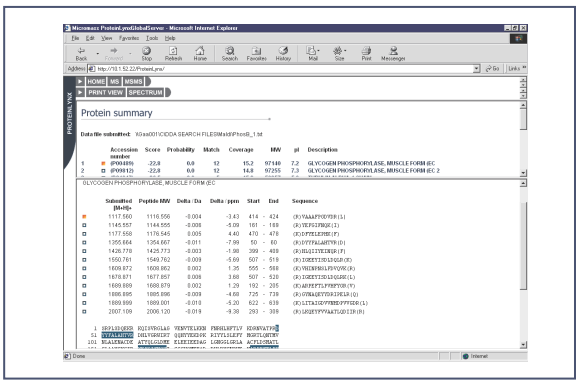

Figure 2b. Databank Search Result from Phosphorylase B 0.5 fmol

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Interestingly the result obtained in ESI mode by direct infusion produced a very similar limit of detection (data not shown). However, typically with ESI analysis the detection limit is lower because samples which are separated by liquid chromatograph where individual peptides are eluted sequentially from the stationary phase and are presented to the mass spectrometer in a low volume with a high sample concentration.

Dynamic range

The dynamic range of protein identification was determined by the analysis of a mixture of two protein digests at different concentration by both ionisation techniques (MALDI and ESI). The concentration of one of the protein digests (Alcohol dehydrogenase) was kept constant at 1 pmol throughout the analysis, while the second protein (Phosphorylase B) was added at a concentration of 10% (100 fmol) and 1% (10 fmol) of the first protein. Spectra were obtained by ESI using direct infusion of the solutions at 200 nl/min (Figures 3a & b) and by MALDI from less than 25% of a 1 µL deposition (**Figures 4a & b**). The resulting spectra were databank search and positive identifications were obtained from all diluted protein digest samples (**Figure 5**). It is possible to consider the effective dynamic range by the number of peptides seen from the lower concentrations of protein.

Table 1 summarises the databank search results for phosphorylase B and it can be seen that by MALDI the number of peptides observer in the MS mode at 10 fmol were 15, this gives a coverage of almost 20%. However, by ESI this reduces to 9 peptides which only gave a coverage of approximately 10%.

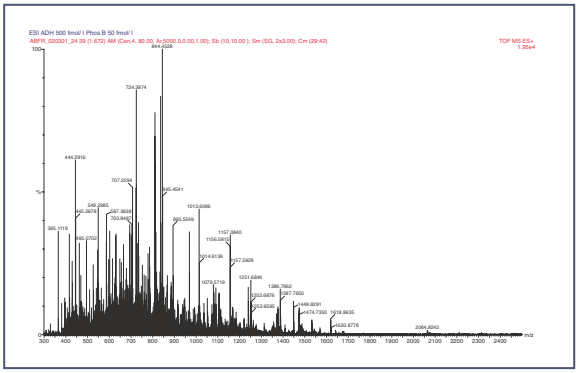

Figure 3a. ESI MS Alcohol Dehydrogenase 1 pmol and Phosphorylase B 100 fmol

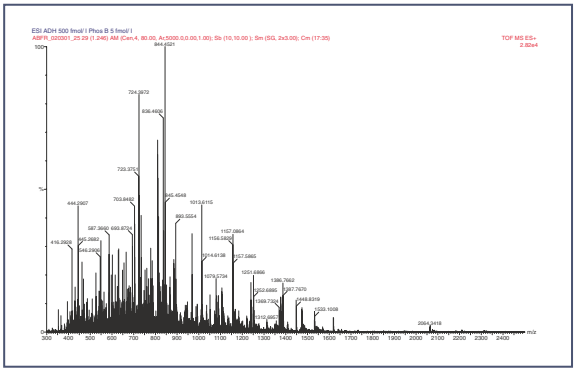

Figure 3b. ESI MS Alcohol Dehydrogenase 1 pmol and Phosphorylase B 10 fmol

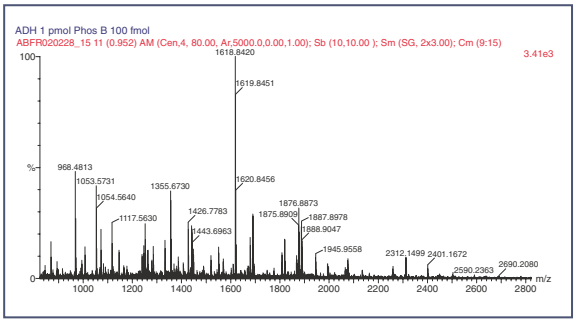

Figure 4a. MALDI MS Alcohol Dehydrogenase 1 pmol and Phosphorylase B 100 fmol on Target

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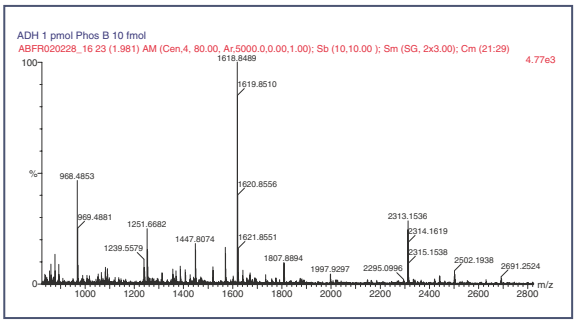

Figure 4b. MALDI MS Alcohol Dehydrogenase 1 pmol and Phosphorylase B 10 fmol on Target

	Databank search results from minor component	
	No. Peptides	Coverage (%)
MALDI 10%	17	19.3
MALDI 1%	15	19.7
ESI 10%	15	19.6
ESI 1%	9	9.6

Table 1. Summary of Databank search results from dynamic range experiment

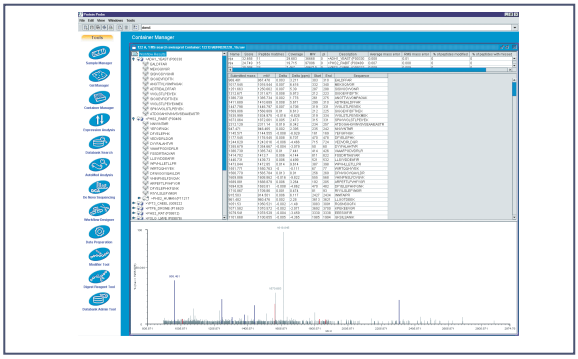

Figure 5. MALDI Databank Search Result from Alcohol Dehydrogenase with 1% Phosphorylase B

Comparison of MS/MS product ion spectra

On Q-Tof type instruments MS/MS fragmentation is performed by low energy collision induced dissociation (CID). MALDI only produces singly charged ions where as ESI often produce multiply charged ions especially for typtic peptides. Presented here is a study of the fragmentation patterns observed from four peptide ions from the Alcohol dehydrogenase tryptic digest which have been generated by both MALDI and ESI.

The peptides with monoisotopic masses of 967.48 and 1446.80 generate singly charged ions in both MALDI and ESI, where as the peptides with monoisotopic masses of 1617.84 and 2311.14 produce singly charged ions by MALDI and doubly charged ions by ESI.

The resulting MALDI and ESI MS/MS spectra of MH^+ 968 and 1448 are shown in **Figures 6 and 8**, respectively. The fragmentation observed for both modes of ionisation are similar on these singly charged ions. It can be seen from the spectral annotation obtained from databank search result that the information content both the MALDI and ESI spectra are identical (**Figures 7 and 9**).
In contrast the MS/MS spectra produced from the singly charge MALDI MH^+ 1618 and doubly charged ESI MH^{2+} 809 show significant differences (**Figure 10**). Noticeably the MALDI spectra lack intense ions at m/z 1236 and 1406. However, the spectral annotation from the databank search result, after the spectra are de-convoluted and de-isotoped, shows the presence of minor ions in the MALDI spectrum at those masses resulting from the y"12 and y"13 ions (**Figures 11a and b**).

Likewise the MS/MS spectra resulting from the MH^+ 2312 and MH^{2+} 1156 appear to have gross differences (**Figure 12**). Once again the spectral annotation from the databank search result show that most of the fragment ion information is present in both the ESI and MALDI spectra. These data show that although some of the spectra obtained by MALDI and ESI have significant differences both MALDI and ESI MS/MS information is suitable to provide identification of the parent proteins, however, by databank searching.

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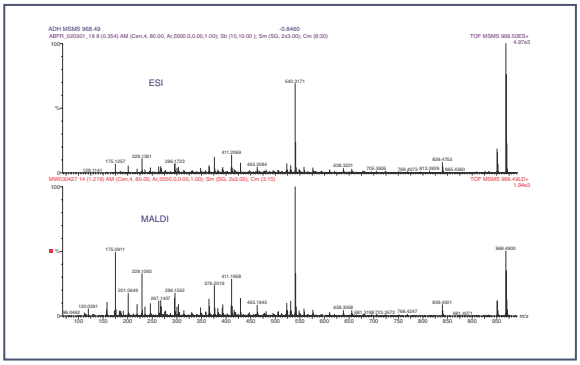

Figure 6. Comparison of singly charged MALDI and ESI MS/MS peptide 967

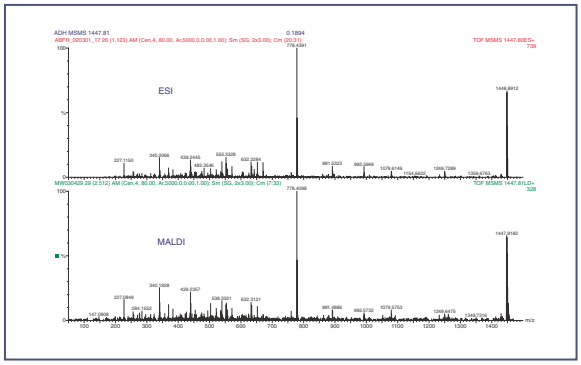

Figure 8. Comparison of singly charged MALDI and ESI MS/MS peptide 1446

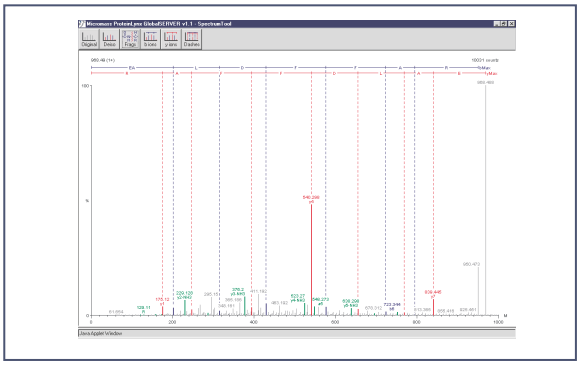

Figure 7a. Annotated spectrum from ESI MS/MS m/z 968

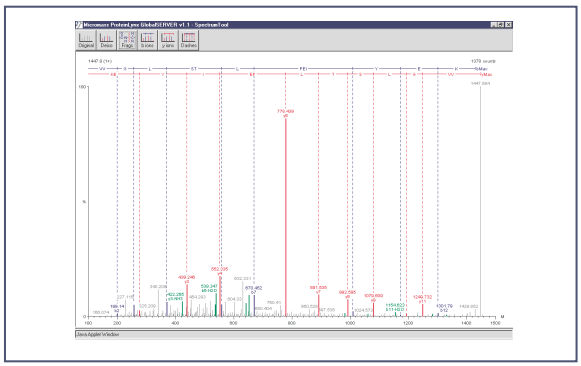

Figure 9a. Annotated spectrum from ESI MS/MS m/z 1447

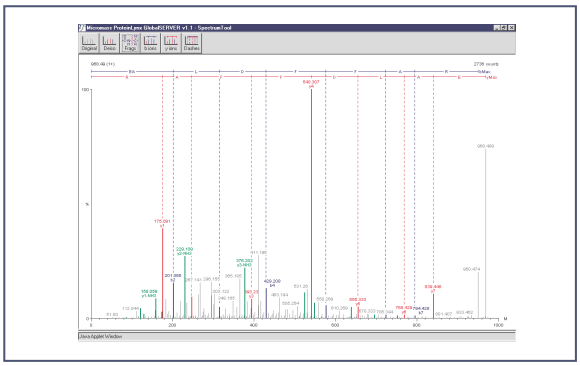

Figure 7b. Annotated spectrum from MALDI MS/MS m/z 968

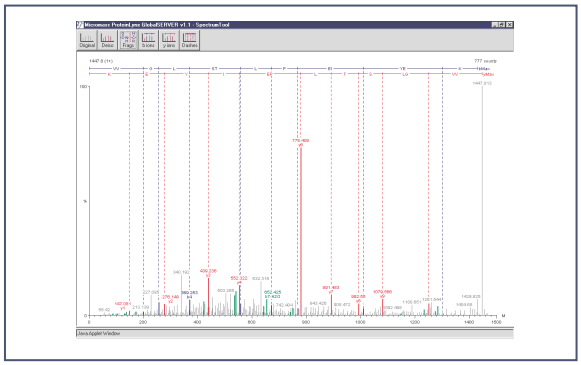

Figure 9b. Annotated spectrum from MALDI MS/MS m/z 1447

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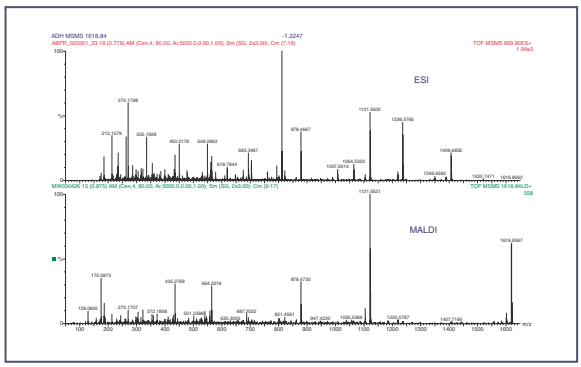

Figure 10. Comparison of singly charged MALDI and doubly charged ESI MS/MS peptide 1617

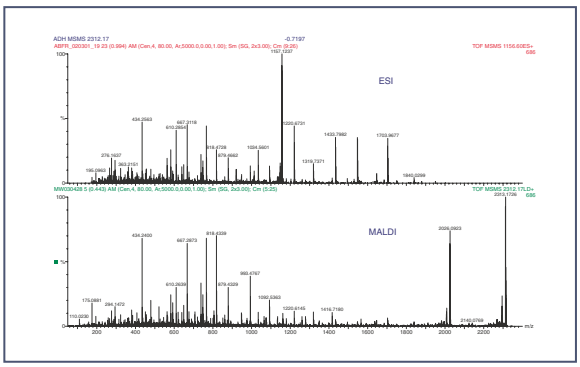

Figure 12. Comparison of singly charged MALDI and doubly charged ESI MS/MS peptide 2312

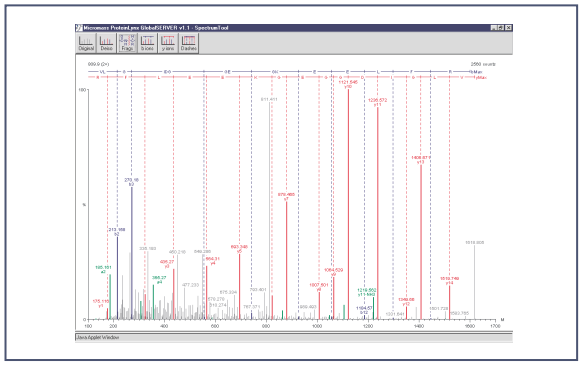

Figure 11a. Annotated spectrum from ESI MS/MS m/z 809

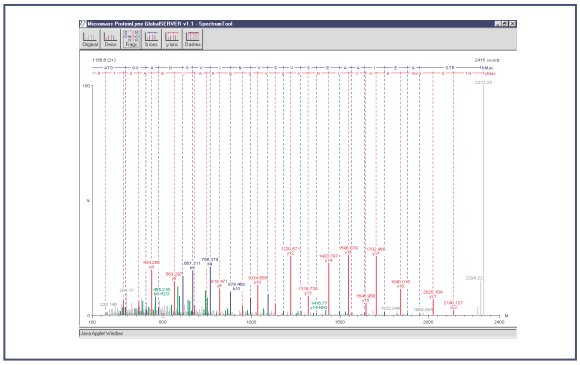

Figure 13a. Annotated spectrum from ESI MS/MS m/z 1156

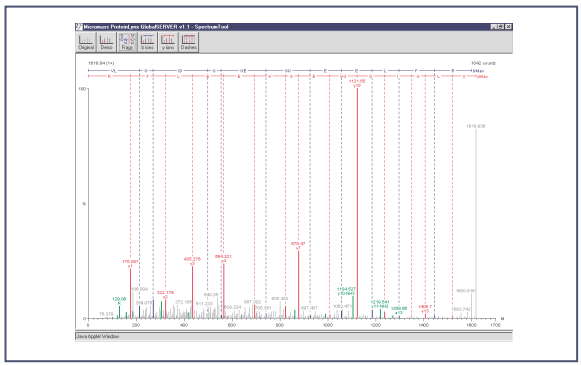

Figure 11b. Annotated spectrum from MALDI MS/MS m/z 1618

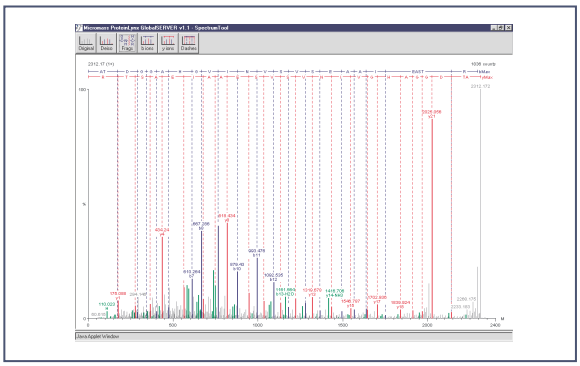

Figure 13b. Annotated spectrum from MALDI MS/MS m/z 2312

Conclusion

- The limit of detection for MALDI MS on a Q-Tof hybrid quadruple orthogonal acceleration time-of-flight mass spectrometer is 500 amol of a tryptic digest of Phosphorylase B.
- Initial results suggest that the dynamic range for MALDI is comparable with the dynamic range for ESI on an unseparated mixture of tryptic peptides. A protein digest could be positively identified at a concentration of 1% of a second protein digest.
- The low MS-MS energy CID fragmentation spectra were shown to be identical for singly charged ion produced by either MALDI and ESI modes of ionisation.
- The MS/MS fragment ion information showed several distinct differences between singly charged MALDI ions and doubly charged ESI ions. Databank searching resulted in identification of the parent protein from either the MALDI or ESI product ion spectra.

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Author to whom all correspondence should be addressed:
Dominic Gostick
Waters Corporation
(Micromass UK Limited)
Floats Road, Wythenshawe
Manchester, M23 9LZ
Tel: + 44 (0) 161 946 2400
Fax: + 44 (0) 161 946 2480
e-mail: dominic.gostick@micromass.co.uk

WATERS CORPORATION
34 Maple St.
Milford, MA 01757 U.S.A.
T: 508 478 2000
F: 508 872 1990
www.waters.com

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