PosterReprint

Waters

AN INVESTIGATION INTO THE DYNAMIC RANGE OF PROTEIN IDENTIFICATION; MALDI VS ELECTROSPRAY ON A Q-TOF MASS SPECTROMETER

John Hoyes[1], Alan Millar[1], Richard Tyldesley[1], Dominic Gostick[1], Jeff Brown[1], Jim Langridge[1], Lu Yu[2], Alicia Ma[2] and Jyoti Choudhary[2]

[1]Micromass UK Ltd, Floats Road, Manchester, M23 9LZ, UK. [2]Cellzome AG, Elstree, Hertfordshire, WD6 3SH, UK.

Presented at 5th Siena Meeting, Italy, 2nd - 5th September 2002

OVERVIEW

- The limit of detection was determined for MS data acquired on a Q-Tof mass spectrometer in both electrospray (ESI) and matrix assisted laser desorption ionisation (MALDI) mode
- The dynamic range of protein identification using ESI and MALDI on unseparated tryptic digest mixtures with a Q-Tof mass spectrometer has been investigated. This has been compared to an ESI-LC-MS/MS approach
- The effect of the HPLC gradient on protein identification by ESI LC-MS/MS is presented
- The effect of protein molecular weight on the dynamic range of identification has been studied
- The MS/MS spectra obtained from low energy CID fragmentation from MALDI and ESI generated ions are compared and contrasted

INTRODUCTION

Mass spectrometry has rapidly become the method of choice for the identification and characterisation of proteins. For organisms whose genomes are completely sequenced peptide mass fingerprinting using MALDI mass spectrometry is useful as a rapid, primary screen.

Electrospray ionisation tandem mass spectrometry (ESI-MS/MS) is an ideal complementary technique to MALDI since it can be used to provide high quality sequence data from individual peptides produced by the enzymatic digestion of protein. However in the case of endogenous complex protein mixtures and individual protein complexes, the dynamic range of proteins present in the sample presented to the mass spectrometer can be large and as such the analytical approach used for the analysis of these samples is of vital importance. Separation of the tryptic peptides by HPLC prior to analysis by on-line ESI-MS/MS has become an established method for the analysis of such samples. This is partly due to its inherent sensitivity, but also the HPLC separation of the tryptic peptides provides a greater coverage of the peptides present in the sample.

In this paper we describe a comparison between MALDI and ESI ionisation techniques on the Q-Tof mass spectrometer. We have compared standard mixtures of tryptic peptides obtained from protein digests over a wide dynamic range. These have been analysed by MALDI and ESI. In both the MALDI and ESI case we have used data dependant analysis to select potential precursor ions for analysis in the product ion MS/MS mode. These MS/MS spectra are then used to identify the parent proteins from a databank search.

METHODS

Sample Preparation

- Phosphorylase 6, Pyruvate kinase (both Rabbit) and Alcohol dehydrogenase (Yeast) were obtained from Sigma Chem Co. (Poole, Dorset, UK)
- Tryptic digestion of the proteins was performed at a concentration of 3mg/mL for 3 hours at 37°C.
- All samples were diluted in aqueous 1% formic acid prior to analysis.

Waters

Mass Spectrometry

MALDI MS and MS/MS

- 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
- 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.

LC-ESI MS/MS

- All data were acquired using a Q-Tof Ultima API (www.micromass.co.uk)
- Samples were introduced using a Micromass CapLC (www.micromass.co.uk) an integrated low flow liquid chromatograph and sample autoinjector system. Full control of the pump operation was performed through MassLynx v4.0.
- The HPLC gradient was programmed from 0% to 60% B between 0 and 30 minutes and for the longer gradient from 0% to 60% over 60 minutes. A= 95:5 H_2O acetonitrile + 0.1% formic acid, whilst B= 5:95 H_2O:acetonitrile +0.1% formic acid
- Automated data directed analysis (DDA) allowed 3 concurrent MS/MS functions to be initiated based on user determined criteria.
- Each MS/MS function was terminated by the data falling below an intensity threshold or after a specified time.

Data Processing

- ProteinLynx Global SERVER v2.0 (www.micromass.co.uk) was used to process the acquired MS/MS data, prior to databank searching. These processing steps consisted of:-
- Quality filtering the dataset to discard those spectra containing insufficient information to represent a peptide
- The remaining MS/MS spectra associated with each precursor ion were combined, transposed to a single charge state, and centroided using the MaxEnt 3 algorithm;
- After conversion to XML, the spectra were searched against the database and returned hits validated using a post-search filter whereby a partial *de novo* sequence, (amino acid tag), is generated for each matching spectrum and compared to the sequence returned from the database; a mismatch invalidates the hit. Corresponding protein identification results were collated into an interactive browser for review.

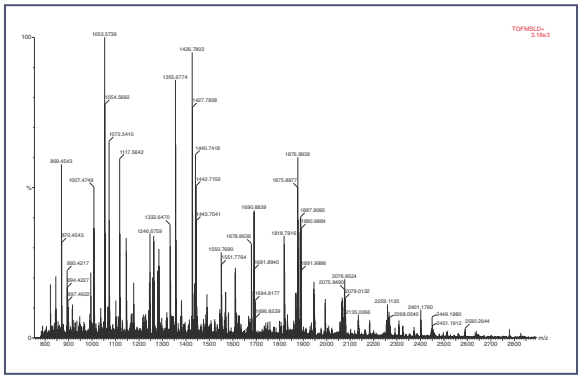

Figure 1a. MALDI MS Phosphorylase 6 5 fmol on Target

Waters

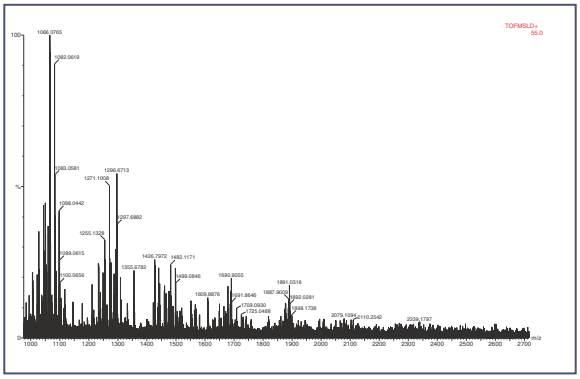

Figure 1b. MALDI MS Phosphorylase 6 0.5 fmol on Target

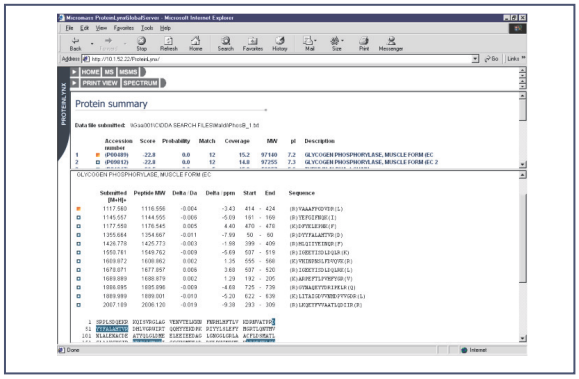

Figure 2. Databank Search Result from Phosphorylase 6 0.5fmol

RESULTS

Sensitivity of detection- MALDI vs ESI

- MS spectra were recorded from 5 and 0.5 fmol loading of Phosphorylase 6 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.
- The recorded spectrum from 0.5 fmol appears 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 2**).
- This unambiguous result was primarily due to the high mass accuracy obtained from the orthogonal TOF analyser. This high mass accuracy allows a 10 ppm mass tolerance in the databank search which removes the matrix ions from interfering with the protein identification.
- Interestingly the result obtained in ESI mode produced a very similar limit of detection (data not shown).

Dynamic range of protein identification

Analysis of unseparated tryptic peptides by ESI and MALDI

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 6) 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 1µl deposition (**Figures 4a &b**). The resulting spectra were databank searched and positive identifications were obtained from all diluted protein digest samples (**Figure 5**). **Table 1** summarises the databank search results for phosphorylase B.

Waters

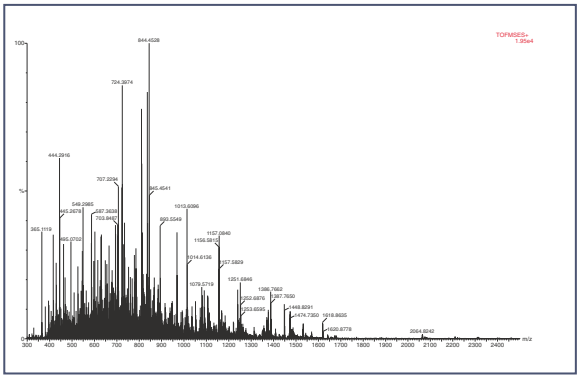

Figure 3a. ESI MS Alcohol Dehydrogenase 1 pmol and Phosphorylase 6 100 fmol

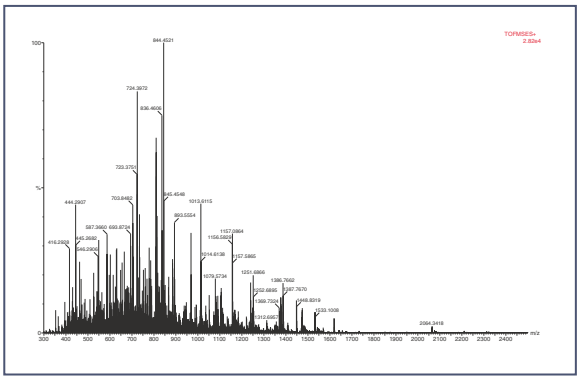

Figure 3b. ESI MS Alcohol Dehydrogenase 1 pmol and Phosphorylase 6 10 fmol

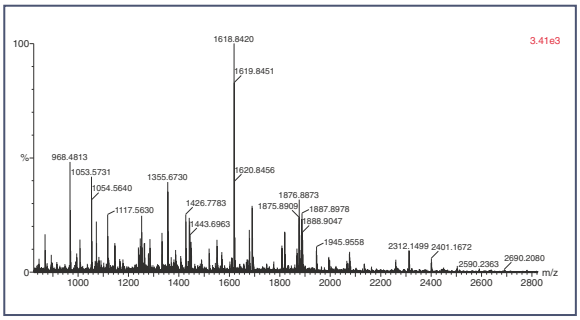

Figure 4a. MALDI MS Alcohol Dehydrogenase 1 pmol and Phosphorylase 6 100 fmol on Target

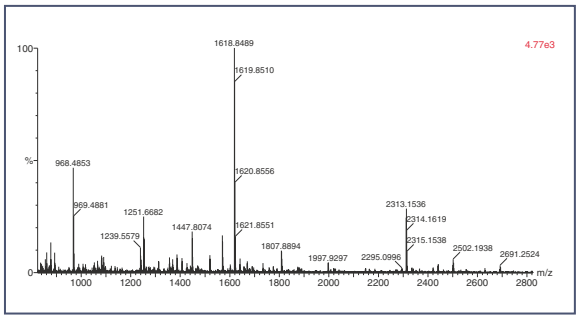

Figure 4b. MALDI MS Alcohol Dehydrogenase 1 pmol and Phosphorylase 6 10 fmol on Target

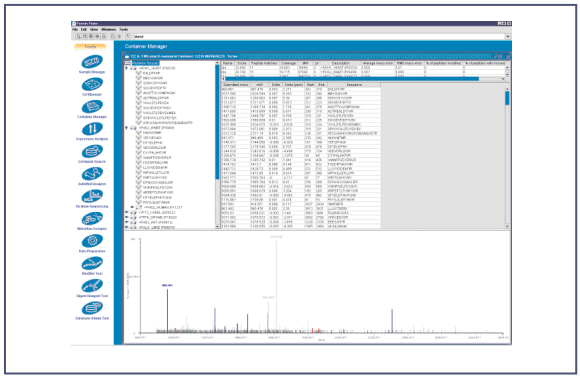

Figure 5. MALDI Databank Search Result from Alcohol Dehydrogenase with 1% Phosphorylase 6

	Databank search result of the 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 dynamic range experiments from the analysis of unseparated tryptic digests by MALDI and ESI

Waters

ESI LC-MS/MS

- ESI LC-MS/MS analysis was performed on the two-protein mixture (see above) to assess the effective dynamic range for protein identification using this technique.
- A mixture of Alcohol dehydrogenase and Phosphorylase b were analysed in varying ratios. The results from this are presented in **Table 2**.
- The analysis of proteins with varying molecular mass (**Table 2**) was performed to establish the effect that protein molecular weight had on the dynamic range of identification.
- Results from the ESI LC-MS/MS experiments indicate that a low level of a small protein (few tryptic peptides) in the presence of a large protein (numerous tryptic peptides) precludes a large effective dynamic range (~ 40:1).
- The reverse is true that the presence of a low level of a large protein gives a high effective dynamic range, for protein identification, in the presence of a high level of a small protein (200:1).
- The effect of the HPLC gradient on the dynamic range of protein identification was investigated. **Table 3** highlights the effect of using a 30 minute gradient from 5% to 60% acetonitrile, in comparison to a 60 minute gradient.
- It can be seen from **Table 3** that the longer gradient over 60 minutes results in an improved dynamic range for protein identification. This is due to the higher chromatographic resolution, that results in simplified mixtures of peptides being presented to the ESI source of the mass spectrometer.

Phosphorylase b fmoles injected	Alcohol dehydrogenase fmoles injected	Databank search result from the LC-MS/MS datasets	
		No. Peptides	No. Peptides
1000	5	19	-
1000	25	17	1
1000	100	19	3
25	1000	5	13
5	1000	1	13

Table 2. Summary of databank search results from the ESI LC-MS/MS dynamic range experiments

Pyruvate kinase fmoles injected	Alcohol dehydrogenase fmoles injected	Reverse phase grd. 5-60% B	Databank search result from the LC-MS/MS datasets	
			No. Peptides	No. Peptides
250	1000	30 mins	8	10
100	1000	30 mins	4	12
25	1000	30 mins	-	12
25	1000	60 mins	3	12
10	1000	60 mins	2	14

Table 3. Effect of the reverse phase gradient on the dynamic range of protein identification

Comparison of MS/MS product ion spectra

- MS/MS fragmentation on Q-Tof instruments is performed using low energy collision induced dissociation (CID). MALDI produces primarily singly charged ions, where as in the case of ESI multiply charged ions are observed, especially for tryptic 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, whereas the peptide with monoisotopic mass of 1617.84 produce singly charged ions by MALDI and doubly charged ions by ESI.
- The resulting MALDI and ESI MS/MS spectra of $(M+H)^+$ 968 and 1448 are shown in **Figures 6 and 8**, respectively. The fragmentation observed for both modes of ionisation are virtually identical on these singly charged ions.

Waters

- It can be seen from the spectral annotation obtained from databank search result that the information content in both the MALDI and ESI spectra are identical (**Figures 7 and 9**).
- In contrast the MS/MS spectra produced from the singly charged MALDI $[M+H]^+$ at m/z 1618 and doubly charged ESI $[M+2H]^{2+}$ m/z 809 show significant differences (**Figure 10**).
- Noticeably the MALDI spectra lack intense ions at m/z 1236 and 1406.
- 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 11 a & b**).

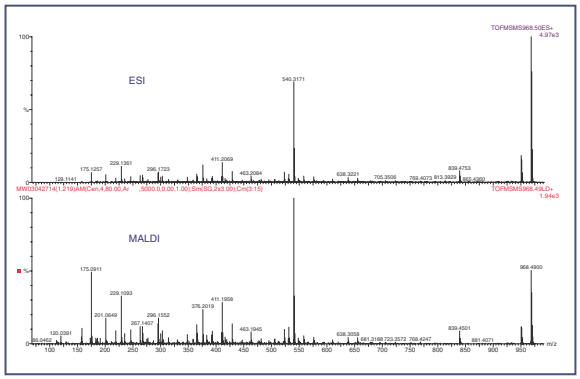

Figure 6. Comparison of MALDI and ESI MS/MS peptide 968

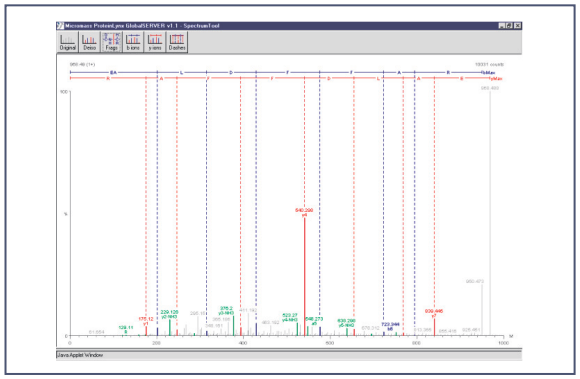

Figure 7a. Annotated spectrum from ESI MS/MS m/z 968

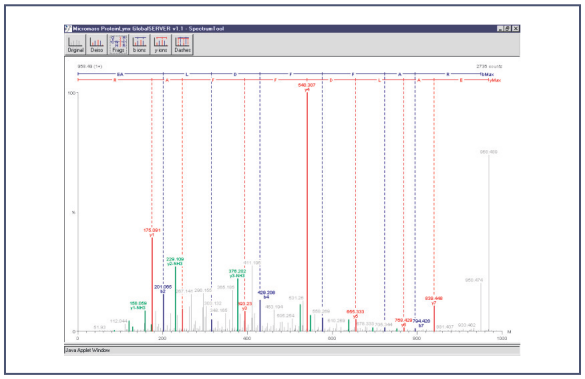

Figure 7b. Annotated spectrum from MALDI MS/MS m/z 968

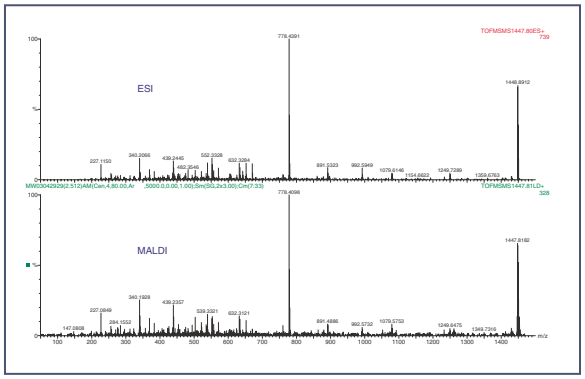

Figure 8. Comparison of MALDI and ESI MS/MS peptide 1446

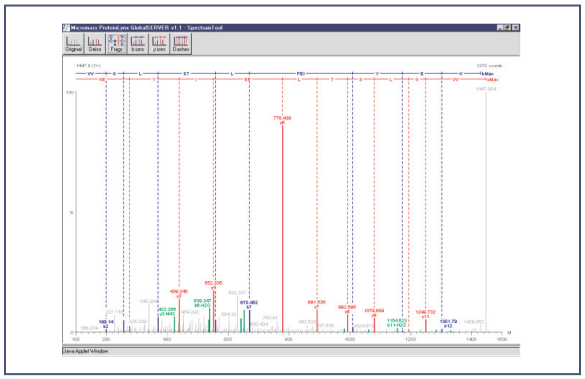

Figure 9a. Annotated spectrum from ESI MS/MS m/z 1447

Waters

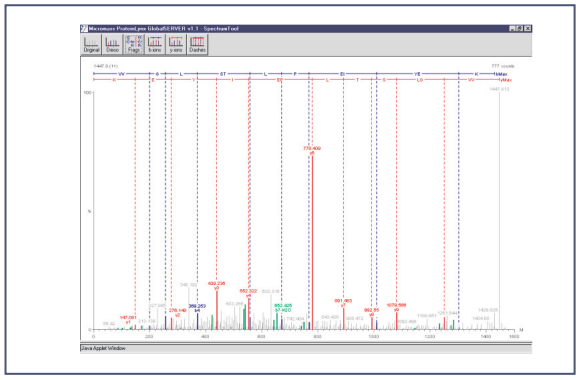

Figure 9b. Annotated spectrum from MALDI MS/MS m/z 1447

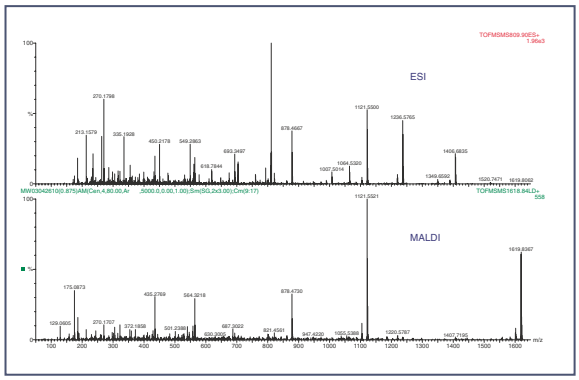

Figure 10. Comparison of MALDI and ESI MS/MS peptide 1617

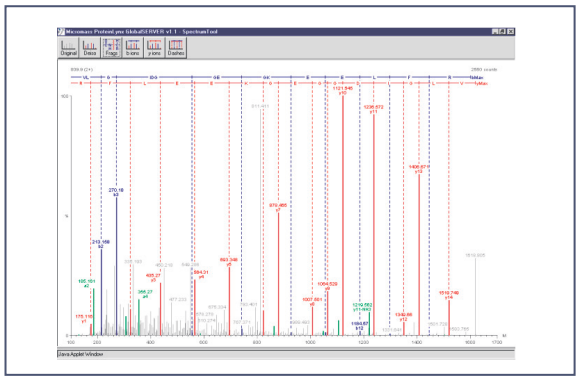

Figure 11a. Annotated spectrum from ESI MS/MS m/z 809

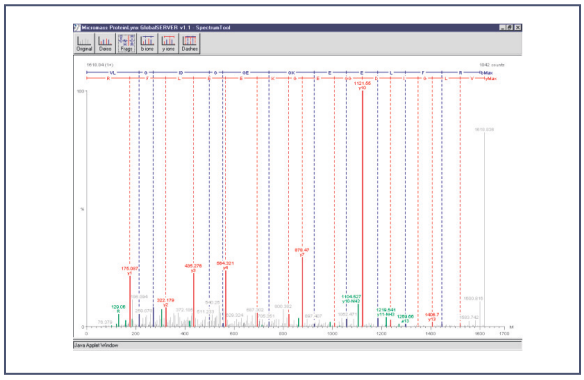

Figure 11b. Annotated spectrum from MALDI MS/MS m/z 1618

CONCLUSION

- The limit of detection for MALDI MS on a Q-Tof hybrid quadruple orthogonal acceleration time-of-flight mass spectrometer is 500 attomole of a tryptic digest of Phosphorylase b.
- Preliminary 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 dynamic range of 1:100.
- ESI LC-MS/MS has the highest dynamic range for protein identification (>250:1), with the choice of HPLC gradient being crucial to the effective dynamic range obtained
- The low energy CID fragmentation spectra were shown to be identical for singly charged ions produced by either MALDI or ESI modes of ionisation.
- The MS/MS fragment ion information showed several distinct differences for singly charged MALDI ions and doubly charged ESI ions. Whilst the quality and sequence coverage of the ESI MS/MS data was superior, databank searching resulted in identification of the parent protein from either the MALDI or ESI product ion spectra.

PosterReprint

MS Sales Offices:

BELGIUM 02-2534550

CANADA 514 694-1200

DENMARK 4657 4101

EUROPE +31 (0) 36-540 6000

FINLAND 02 284 56 11

FRANCE 0800-907016

GERMANY 0800-1817249

ITALY 02 2159 1415

NETHERLANDS 036-540 6160

NORDIC +46 (0) 8 555 115 10

SPAIN 93 440 71 30

SWEDEN 08 555 115 10

SWITZERLAND (FRENCH) 0800-558334

SWITZERLAND (GERMAN) 0800-556190

UK 0161 435 4125

USA 978 524-8200

To whom all correspondence should be addressed:
Tel: + 44 (0) 161 435 4100
Fax: + 44 (0) 161 435 4444
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

Waters
RIGHT ON TIME.

MICROMASS®
MS TECHNOLOGIES

Renewable Fuels Association, www.ethanolrfa.org
California Energy Commission Report, P600-01-017, August 2001.

Certificate No: 951387