PosterREPRINT

Waters

THE USE OF A NANOLOCKSPRAY ELECTROSPRAY INTERFACE FOR EXACT MASS LC/MS/MS STUDIES

Alan Millar, Jim Langridge, Therese McKenna, Steve Pringle, Phil Young, Chris Hughes, Kevin Giles, John Hoyes and Stephanie Dudd

Waters Corporation, Manchester, UK.

Presented at ABRF Colorado, USA, February 10th-13th, 2003

OVERVIEW

- This poster describes the implementation of a dual sprayer NanolockSpray™ source on a Micromass Q-Tof micro™ mass spectrometer
- The source consists of a dual sprayer arrangement; one for the nanolitre/ min flow rate from the analyte, whilst the second is for the reference compound
- This facilitates routine exact mass measurement for nano scale LC/MS and MS/MS studies
- The focus of this poster is to detail the application of this source in proteomic studies.

INTRODUCTION

Due to the complexity encountered in the analysis of proteins obtained from mammalian systems, the primary route for the identification and characterisation of the constituent proteins is electrospray (ESI) LC/MS/MS. The low endogenous levels and large dynamic range of proteins present in these samples dictate that nanoscale LC/MS/MS is often the method of choice due to the concentration dependant nature of the electrospray ionisation technique. This has led to nanoscale LC/MS/MS on a hybrid quadrupole orthogonal acceleration time of flight (Q-Tof) mass spectrometer becoming an established technique for high sensitivity identification and characterisation of proteins. Typically these experiments employ LC columns which have internal diameters of 75 µm, or less, operating at flow rates of approximately 200 nL/min. Whilst this set-up offers the optimum sensitivity it does not allow the post-column addition of an internal reference ion, as this would detrimentally effect the resolution of the LC separation, resulting in peak broadening. The use of an internal reference is required to provide reliable high mass measurement accuracy. Here we report the use of a NanolockSpray interface to routinely provide enhanced mass measurement in the analysis of protein digests.

EXPERIMENTAL

Electrospray LC/MS/MS

- Data were acquired using a Micromass Q-Tof micro™, hybrid quadrupole orthogonal acceleration time-of-flight mass spectrometer (www.waters.com/micromass)
- The analytical system used for the analysis consisted of a Waters Modular CapLC with a ten port valve, the stream select module, attached directly to the **Z** *SPRAY* source of the mass spectrometer, see Figure 1

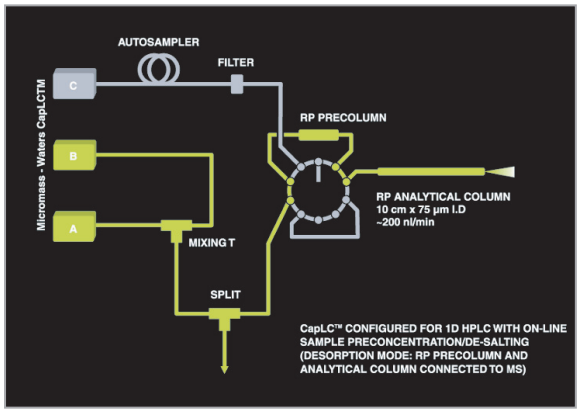

Figure 1. Schematic diagram of the nanoscale HPLC set-up

- The HPLC was configured with a pre-concentration column, to allow large volume sample injection and a nanoscale analytical column. The trapping column was a Symmetry™ 300 C18 OPTI-PAK trap cartridge (www.Waters.com) 300 µm ID x 5 mm, whilst the analytical column was a 150 mmx 75 µM

MICROMASS® MS TECHNOLOGIES

column packed with PepMap C18 material (www.lcpackings.com).

- A splitter was employed to provide a flow through the analytical column of 200 nL/min with the pump programmed to deliver a flow of 2 µL/min. The LC gradient ran from 5% to 60% acetonitrile in 26 minutes.

NanolockSpray

- The nanolockSpray interface consists of a dual sprayer, one for the analyte and one for the reference, see Figure 2. Each spray is sampled individually by the mass spectrometer by means of an electronically controlled baffle plate.

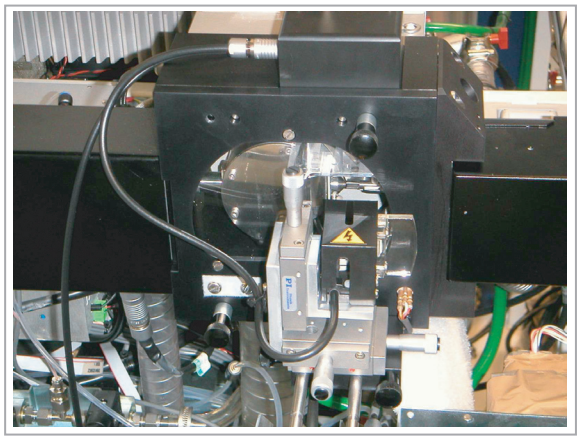

Figure 2. Picture of the NanolockSpray source, consisting of dual sprayers for both analyte and reference spray.

- The software was configured such that the reference spray was sampled for one second in every ten seconds. It is advantageous to separate the reference signal from the analyte signal in order to avoid ion suppression effects and possible interference to the lock mass from ions closely related in m/z. The reference ion used was the doubly charged ion of [Glu1] - Fibrinopeptide B at m/z 785.843.
- The mass spectrometer was operated in a data dependant acquisition (DDA) mode whereby following the interrogation of MS data, ions were selected for MS/MS analysis based on their intensity and charge state. Collision energies were chosen automatically based on the m/z and charge state of the selected precursor ions.

DATA PROCESSING

- All data were processed using ProteinLynx Gobal server version 2.0 (www.waters.com/micromass). The processing consisted of automatically correcting the *m/z* scale of both the MS and MS/MS data utillising the reference ion.
- The MS/MS data was also MaxEnt III processed. The purpose of the MaxEnt processing was to simplify the MS/MS spectra by deconvoluting the data to the single charge state and de-isotoping the data. After processing, the data was searched against SWISS-PROT version 40.

RESULTS

- An initial investigation into the accuracy obtained from the NanoLockSpray source was conducted by analysing 500 fmoles of a tryptic digest of Bovine Serum Albumin by data directed analysis (DDA)
- Results from the analysis are presented in Figure 3, where the search was conducted with a precursor ion tolerance of 20ppm. In this case 13 matching peptides were identified to the BSA sequence. Several other serum albumin proteins from different species were also identified.
- The RMS errors for all the peptides matching to the BSA sequence was 8ppm.
- Analysis of the same data set using a 100ppm window resulted in the identification of BSA as the top hit, with again 13 matching peptides, however an incorrect identification Ribonuclease R was also returned (Figure 4). Despite the poor quality of this identification it would require manual verification to remove it from the protein hit list.

PosterREPRINT

Waters

Figure 3. Databank search results for the Nanolockspray BSA sample, with a 20ppm precursor ion tolerance used in the search

Figure 4. Databank search results for the Nanolockspray BSA sample, with a 100ppm precursor ion tolerance used in the search

- Stability of mass measurement over an extended period of time was investigated through the infusion of two peptides, Glu-fibrinopeptide b (m/z 785.843 2+) and Angiotensin II (m/z 523.775, 2+) over a 16 hour period (Figure 5). Mass measurement errors obtained from the Q-Tof micro equipped with the nanolockspray source were determined, with and without, the external lockmass correction.

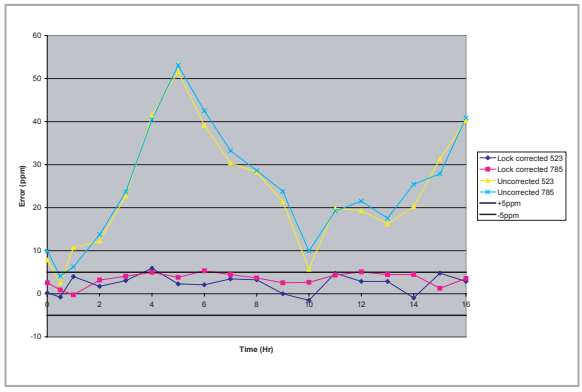

Figure 5. Mass measurement accuracy obtained from the Q-Tof micro equipped with the nanolockspray source. Two peptides, Glu-fibrinopeptide b (m/z 785.843 2+) and Angiotensin II (m/.z 523.775 2+) were measured over a 16 hour period.

- It can be seen that errors, when the single point external calibration was used, were +/- 5ppm or better over the 16 hour period.
- Mass measurements without the correction varied by up to 53ppm.
- Analysis of the BSA sample was also performed by DDA 7 days after calibration. Despite a significant shift in the mass due to temperature fluctuations, the single point external lock mass, from the NanolockSpray source, enabled mass measurement to better than 5ppm. As an example the doubly charged precursor ion for LVNELTEFAK (*m/z* 582.3195) is shown in Figure 6.

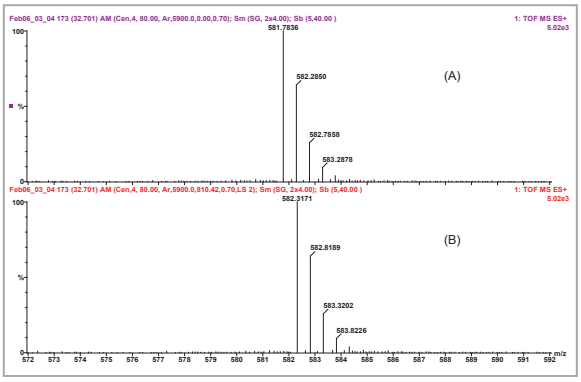

Figure 6. Mass accuracy on the tryptic peptide LVNELTEFAK m/z 582.3195 (2+) from BSA. A) un-corrected, B) Nanolockspray corrected

Waters

- Figure 7 shows the results from a DDA analysis of an *E.coli* sample containing multiple proteins.
- In this case the SWISS-PROT databank was searched with the peptide precursor ion tolerance set to 20ppm
- All of the retrieved proteins originated from *E.coli*. Identification of 17 proteins was made.
- The peptides matching the parent proteins had an RMS error of 10ppm or better.

Figure 7. Databank search results obtained from the E.coli protein mixture

Digital Dead Time Correction (DDTC)

- During a typical nanoflow LC/MS/MS experiment the tryptic peptides present exhibit a wide dynamic range and as such are detected by the mass spectrometer with varying signal intensities. This provides a challenge to achieving routine exact mass measurement as very intense peaks can cause the detector of the mass spectrometer to become saturated or move into 'dead time'
- Once a single ion has been detected there is a 'dead time' during which further ion arrival events will not be detected. The result is a non-linear response between ions detected vs ions arriving at the detector, this manifests itself in a shift to a lower mass being reported for that particular ion.
- An algorithm termed digital dead time correction (DDTC) can be used to correct for this effect. An example of this effect is presented for data obtained from a Q-Tof Ultima API. This is presented in Figure 8 where a doubly charged ion at *m/z* 681.36 is shown both with and without DDTC applied during processing. The ion was assigned the sequence IQTQPGYANTLR during database searching.

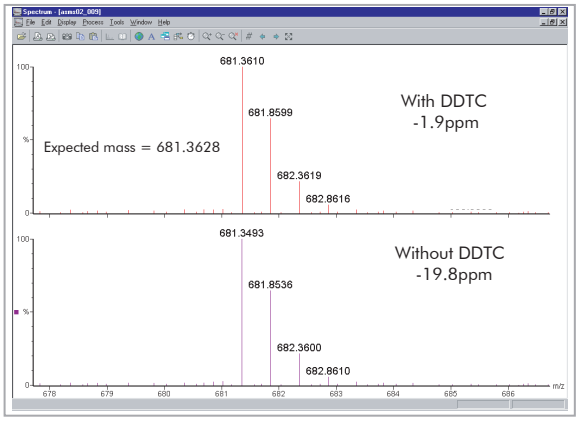

Figure 8. Doubly charged ion at m/z 681.36 processed both with and without the dead time correction algorithm applied

- With DDTC applied the mass measurement for the ion was -1.9 ppm and with out DDTC applied it was measured to be -19.8 ppm

CONCLUSION

- The NanolockSpray source described here provides a routine method for obtaining enhanced mass measurements in LC/MS and MS/MS experiments
- Mass measurement using the NanoLockspray source over a 16 hour period resulted in values +/- 5ppm.
- Use of the NanolockSpray source reduces the false positive identification of proteins even in the case of a BSA standard
- Identification of proteins from both standard digests and E.coli samples resulted in mass measurements better then 10ppm

PosterREPRINT

Waters

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

Author to whom all correspondence should be addressed:

Mark A. Richie

Tel: + 44 (0) 161 946 2400

Fax: + 44 (0) 161 946 2480

e-mail: mark.ritchie@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

Made in the United Kingdom

Waters

MICROMASS® MS TECHNOLOGIES

Opt-In! My Profile www.waters.com

LLOYD'S REGISTER QUALITY ASSURANCE ISO9001

UKAS QUALITY MANAGEMENT 001

IT