Enhanced detection of post-translational modifications on peptides; application of enhanced duty cycle function on a hybrid quadrupole time-of-flight mass spectrometer.

ASMS 2005

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OVERVIEW

- By synchronizing a traveling wave stacked ring ion guide with an oa-Tof analyzer, significant improvements in the duty cycle of the oa-TOF can be achieved
- Sensitivity of detection can therefore be increased over a particular *m/z* range of interest
- The enhanced duty cycle functionality has been used in this work, together with precursor ion discovery, to characterize glycosylation sites on tryptic peptides.

INTRODUCTION

Identification and characterisation of a post translation modification may be more significant than the identification of the parent protein. It has been common practice for many years to detect post-translationaly modified peptides by detecting specific collisionally activated product ions during a mass spectrometric analysis.

A time-of-flight based analyser (Tof) can measure these product ions at very high mass accuracy. By coupling a Travelling Wave Stacked Ring Ion Guide (SRIG) Collision Cell with an oa-Tof detector, the instrument duty cycle can be significantly enhanced for specific ions of interest. As a result the mass spectrometer's sensitivity for detecting specific product ions can be increased, without compromising mass accuracy.

INSTRUMENTAL

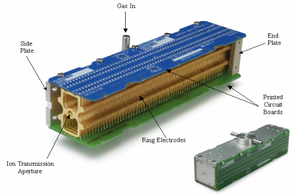

Figure 1. Photograph of the T-Wave, Stacked Ring Ion Guide (SRIG) collision cell; inset is a sealed collision cell.

A stacked ring ion guide is shown in **Figure 1**. Each adjacent lens has an opposite phase RF voltage applied to it. In addition to this RF voltage, there is a superimposed travelling DC voltage wave applied to each individual lens, which then moves to the adjacent lens after a given time, and so on along the ion guide (**Figure 2**). This provides an axial moving DC electric field or "travelling wave" on which the ions can "surf". This application of an axial DC voltage reduces the ion transit time through the collision cell and as a result of the travelling wave, ions are transported through, and released from the SRIG cell as discrete ion packets. The flight time of the ions within these packets exiting the collision cell are m/z dependent. On typical ESI oa-Tof instruments, a continuous ion flux is generated, and therefore, the arrival of ions within the pusher stack is continuous. A particular portion of this continuous ion beam is sampled orthogonally into the flight tube. The remaining ion beam entering the pusher optics are lost, for a period of time. The ratio of pushed ions to ions lost per acquisition is referred to as oa-Tof duty cycle, and is typically around 25% for the highest *m/z* ion equal to the flight time of the highest *m/z* ion being analysed. One can synchronise the pusher pulse with the SRIG ion packet arrival, thus enhancing the oa-Tof duty cycle for a particular *m/z* range (**Figure 3**)..

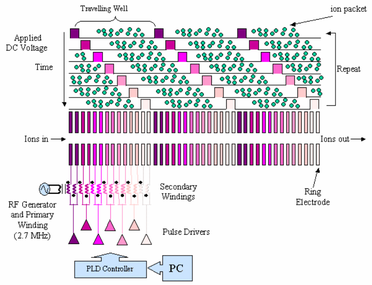

Figure 2. A schematic representation of the Travelling Wave being applied to the SRIG collision cell. Repeating shaded pairs of electrodes indicate their connection using the PCB.

EXPERIMENTAL

All data was acquired on a Q-Tof Premier™ operating in the V-optics, positive ion, continuum mode. The MS was fitted with a NanoLockspray source, with data acquired with an integration time of 0.9 seconds and an inter-scan time of 0.1 seconds. Precursor Ion Discovery (PID) experiments incorporating Enhanced Duty Cycle (EDC) were carried out using LC-MS. An initial Tof-MS scan was carried out at 4eV followed by Tof-MS at an elevated collision energy of 27eV, with EDC enabled, focussing on the oxonium ions *m/z* 204.087, 274.093, 292.103 and 366.139. When the oxonium ion of interest was detected in the elevated collision energy Tof-MS function, product ion MS/MS was automatically performed on ions exceeding the set criteria.Samples were introduced using a nanoACQUITY UPLC™ system. Peptides were trapped on a Symmetry C18 180µm x 20mm column. Separation was carried out using an Atlantis dC18 75µm x 100mm (3µm) analytical column using a 30 minute acetonitrile gradient (0-40%).

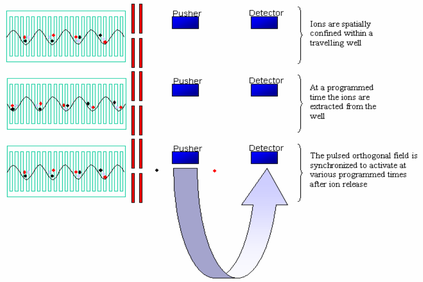

Figure 3. Principle of Enhanced Duty Cycle (EDC) on the Q-Tof Premier.

Tryptic digests of bovine fetuin and recombinant human erythropoietin (EPO) were analysed. A dilution series was analysed using the PID-EDC method from 5 fmol to 50 fmol. Prior to analysis the N-linked glycans were removed from EPO using PNGase F, and as such only O-linked glycopeptides were present on the protein.

RESULTS & DISCUSSION

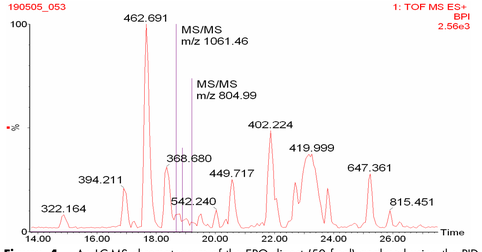

Figure 4. An LC-MS chromatogram of the EPO digest (50 fmol) analysed using the PID-EDC functionality. The vertical purple lines indicate the point at which product ion MS/MS was performed, as a result of the product ion, *m/z* 204.087, breaching the threshold in the elevated energy EDC TOF function.

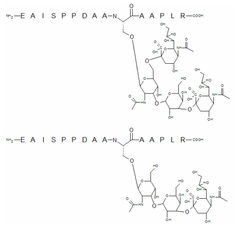

Figure 5. Sequence and the glycan structures of the two O-linked glycosylated tryptic peptides. Upper structure; monoisotopic mass 2120.9849 amu. Lower structure; monoisotopic mass 2412.0803 amu .

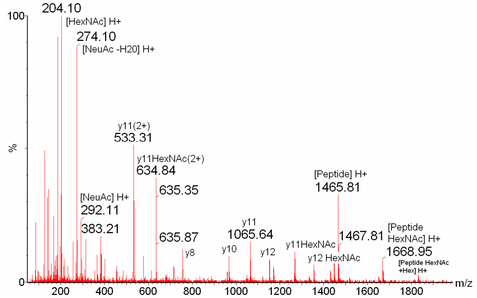

Figure 6. MS/MS spectrum from the doubly charged glycopeptide ion *m/z* 1061.61.

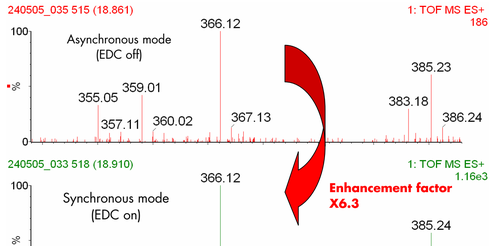

Figure 7. Upper spectrum shows a region of the *m/z* scale focussed around *m/z* 366.1 when the collision cell and pusher optics are operating in synchronous mode. The lower spectrum shows the identical *m/z* region when the collision cell and pusher optics are operating in asynchronous mode. The data was obtained from 50 fmol of EPO tryptic digest injected onto column.

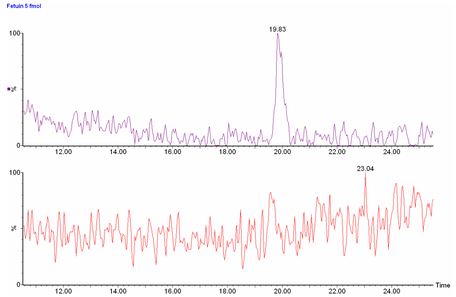

Figure 8. Mass chromatograms for m/z 366.1 [HexHexNAc oxonium ion] from the exact mass LC-MS analysis performed with elevated collision energy (27eV). Upper trace acquired with EDC enabled, lower trace in the normal, unsynchronized, mode of operation.

Ion (*m/z*)	204.087	274.093	292.103	366.139
PID (asynchronous)	459	404	201	184
PID-EDC (synchronous)	3250	3130	1320	1130
Enhancement Factor	7.1	7.8	6.6	6.14

Table 1. Comparison of centroided ion counts between a standard PID and PID-EDC experiment carried out on 50 fmol of an EPO tryptic digest. Also shown is the enhancement factor as a result of acquiring data in synchronous mode (EDC on).

The O-linked glycopeptides from EPO fragmented with a collision energy of 27eV, produce a number of signature ions, **(Figure 6)**. The strongest of these is *m/z* 274.093 (dehydrated N-acetylneuraminic acid). In addition to this, good sequence coverage of the glycopeptide structure **(Figure 5)**, both in terms of the glycan and the amino acid sequence was obtained. **Figure 7** shows the comparison between a typical PID experiment and a PID with EDC activated. When EDC is activated there is a clear increase in sensitivity (9.5 fold) on the ion at m/z 366.139. PID-EDC was carried out on all of the oxonium ions generated when fragmenting the O-linked EPO glycopeptides **(Table 1)**. It can clearly bee seen that using EDC, the duty cycle of such ions can be increased substantially over the conventional mode of operation, and in all cases examined. In certain cases, **Figure8**; showing the analysis of 5fmole of a fetuin tryptic digest by LC-MS; this enhancement can make the difference between an ion breaching the detection threshold, or not.

EXACT MASS PRECURSOR ION DISCOVERY WITH EDC

- The ability to couple PID with EDC in combination for LC-MS experiments is presented. In this case a sample of 10 fmol of the EPO digest was analysed using the LC-MS PID approach.
- TOF-MS was acquired at a collision energy of 4eV. A second sequential TOF-MS function was acquired with the collision energy raised to 27eV, and EDC activated focusing on *m/z* 274.0928 [NeuAc-H_2O] (**Figure 9**). When the ion of *m/z* 274.0928, +/- 5mDa was detected above a threshold of 200 counts/sec, then MS/MS was performed on potential precursor ions.

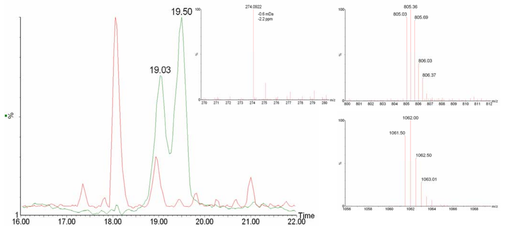

Figure 9. A section of the LC-MS chromatogram from 10fmoles of EPO injected on-column (16mins to 22mins). Red trace represents the low energy BPI plot. Green trace represents mass chromatogram for *m/z* 274.0928, [NeuAc-H_2O], +/-5mDa. The insets show the unfragmented precursor ions m/z 805 rt 19.03 mins and m/z 1061 rt 19.50 mins.

CONCLUSIONS

- In this poster we have shown that by synchronizing a traveling wave, stacked ring, ion guide with an oa-Tof analyzer, significant improvements in the duty cycle of the oa-TOF can be achieved
- Using a combination of PID and EDC, the duty cycle and therefore the sensitivity of detection, for low molecular weight oxonium ions produced by glycopeptides has been enhanced.
- Using exact mass enabled PID-EDC, one can selectively target a specific product ion, such as dehydrated N-acetylneuraminic acid (*m/z* 274.0928) during an LC-MS run, and automatically perform MS/MS on its precursor.

The travelling wave device described here is similar to that described by Kirchner in US Patent 5,206,506 (1993)

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