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Applikationsbericht

Coupling a Travelling Wave Collision Cell With an oa-Tof Analyzer for Enhanced Detection of Peptide Post-Translational Modifications

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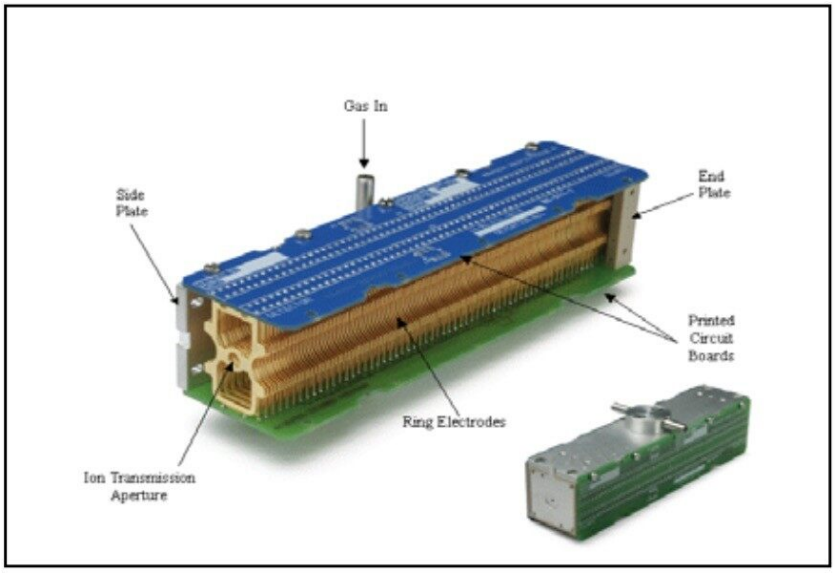
Abstract

- 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 (PID), to characterize glycosylation sites on tryptic peptides

Introduction

Identification and characterization of a post translational modification may be more significant than the identification of the parent protein. It has been common practice for many years to detect post-translationally 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 (T-Wave) 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.



The T-Wave, Stacked Ring Ion Guide collision cell (top left); and the sealed collision cell (bottom right).
The travelling wave device described here is similar to that described by Kirchner in US Patent 5,206,506 (1993).

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 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 C_{18} 180 µm x 20 mm Column. Separation was carried out using an Atlantis dC_{18} 75 µm x 100 mm (3 µm) analytical Column using a 30 minute acetonitrile gradient (0-40%).

In a stacked ring ion guide 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 1). 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 T-Wave cell as discrete ion packets. The flight time of the ions within these packets exiting the collision cell are *m/z* dependent. 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 1).

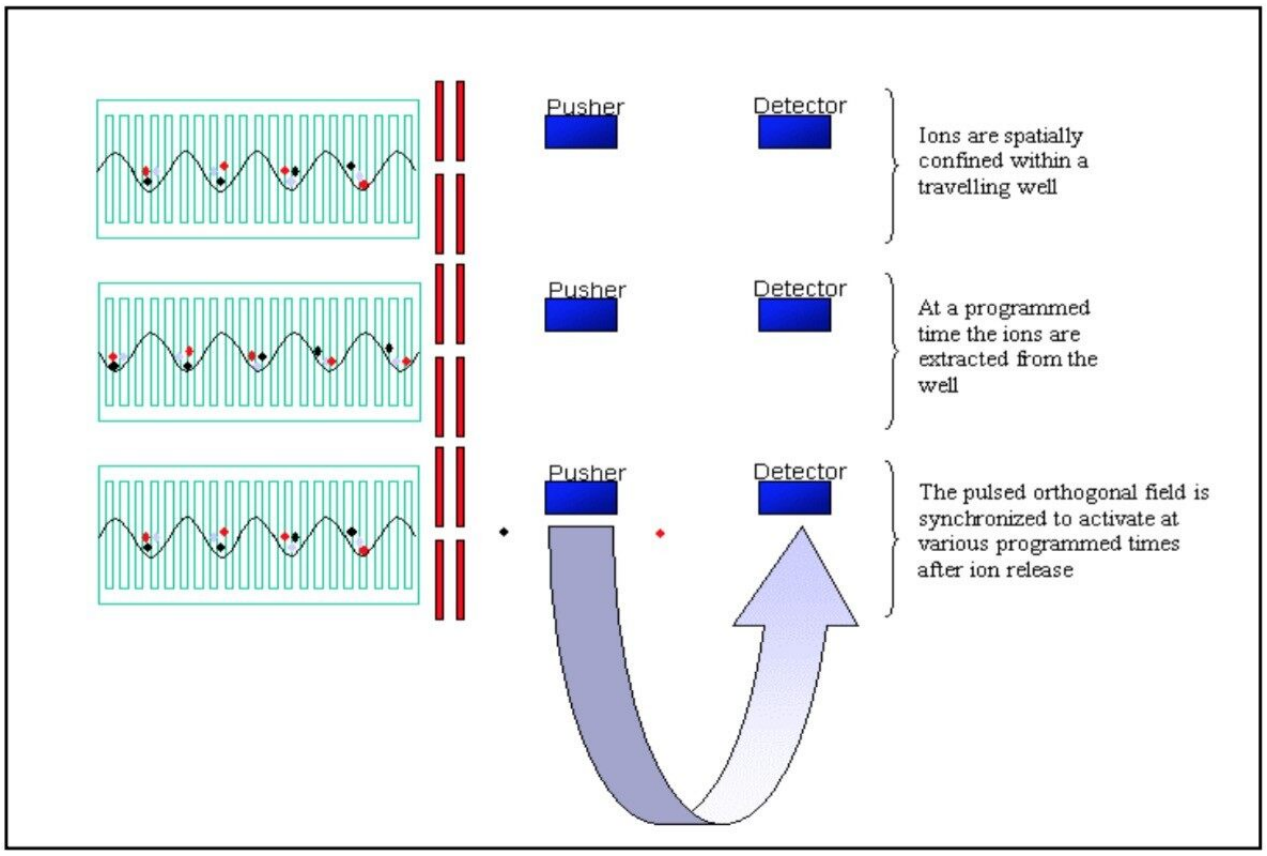


Figure 1. Principle of Enhanced Duty Cycle (EDC) on the Q-Tof Premier.

Tryptic digests of bovine fetuin and recombinant human erythropoietin (EPO) were analysed. A dilution series was analyzed 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 and Discussion

The O-linked glycopeptides from EPO fragmented with a collision energy of 27eV, and upon fragmentation produce a number of signature ions, (Figure 3). The strongest of these is *m/z* 274.093 (dehydrated Nacetylneuraminic acid). In addition to this, good sequence coverage of the glycopeptide structure, both in terms of the glycan and the amino acid sequence was obtained. If we focus upon the low *m/z* oxonium ions from the EPO glycopeptide, and compare a typical experiment, with no synchronization of T-Wave and pusher, to an experiment with EDC activated; there is a clear increase in sensitivity (6.3 fold) on the ion at *m/z* 366.139 when using EDC (Figure 4). PID-EDC was carried out on all of the oxonium ions generated when fragmenting the O-linked EPO glycopeptides.

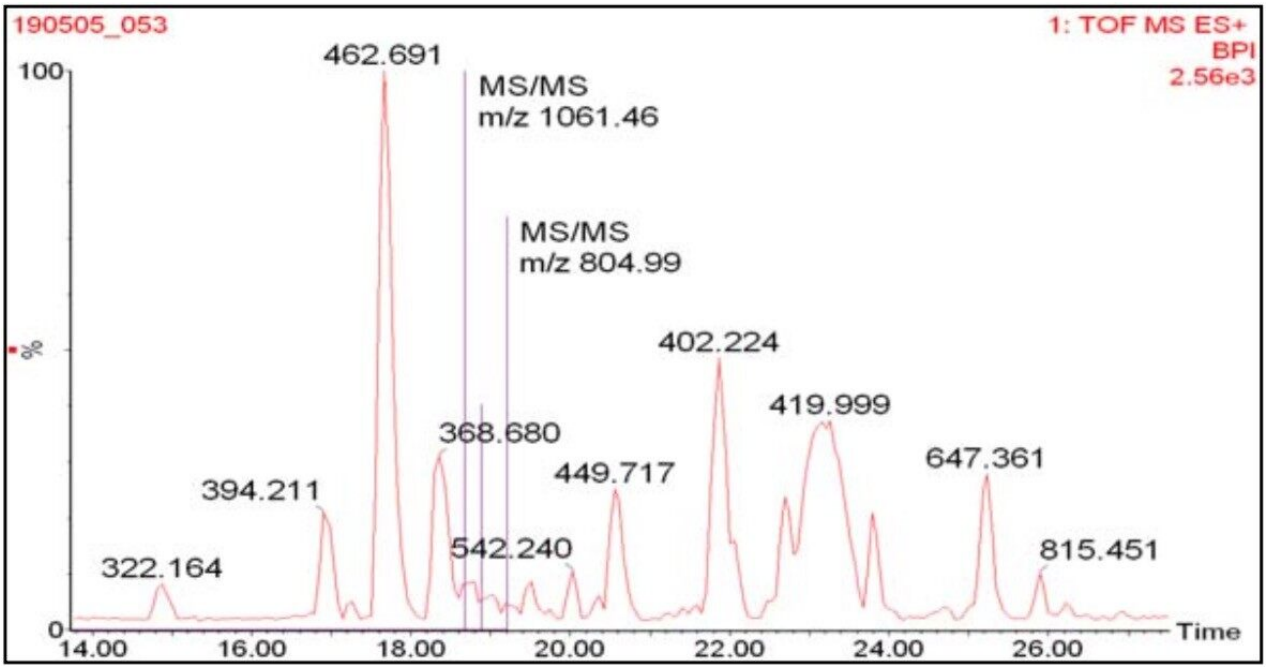


Figure 2. An LC-MS chromatogram of the EPO digest (50 fmol) analyzed using the PID-EDC functionality. The vertical purple lines indicate the point at which product ion MS/MS was performed, as a result of the product ion, m/z 204.087, breaching the threshold in the elevated energy EDC Tof function.

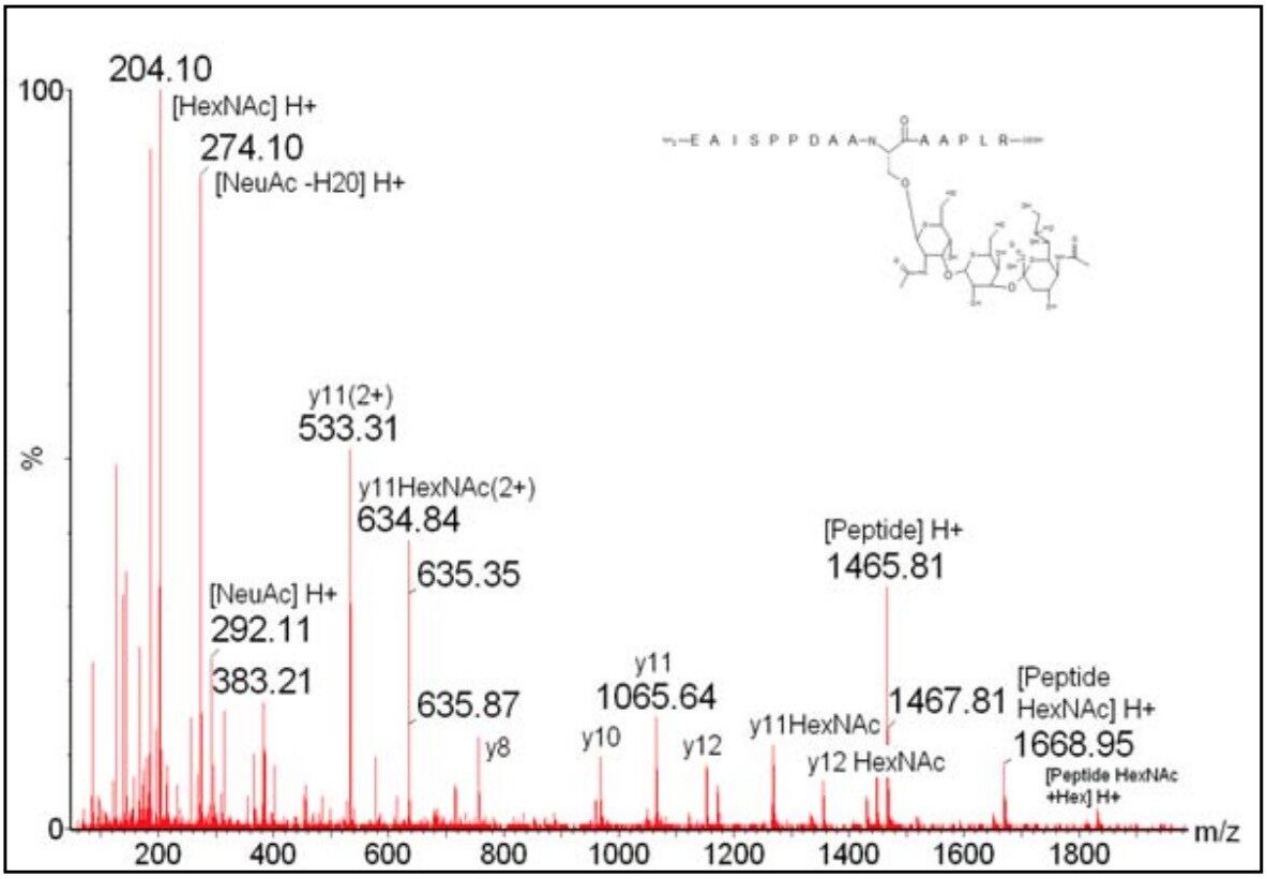


Figure 3. MS/MS spectrum from the doubly charged glycopeptide ion at m/z 1061.61. The spectrum is annotated with the sequence.

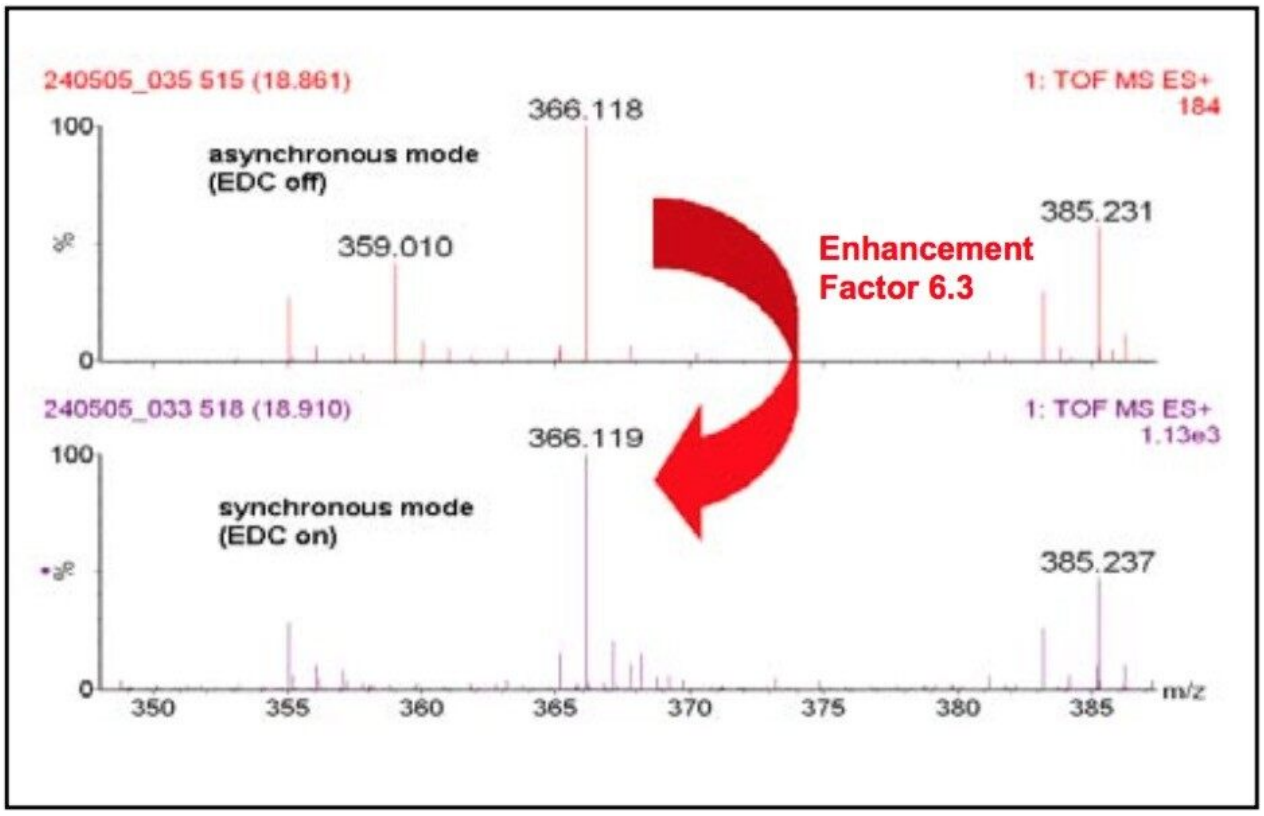


Figure 4. Upper spectrum shows a region of the m/z scale focussed around m/z 366.1 when the collision cell and pusher optics are operating in synchronous mode. The lower spectrum shows the identical m/z region when the collision cell and pusher optics are operating in asynchronous mode. The data was obtained from 50 fmol of EPO tryptic digest injected onto column.

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, Figure 5; 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.

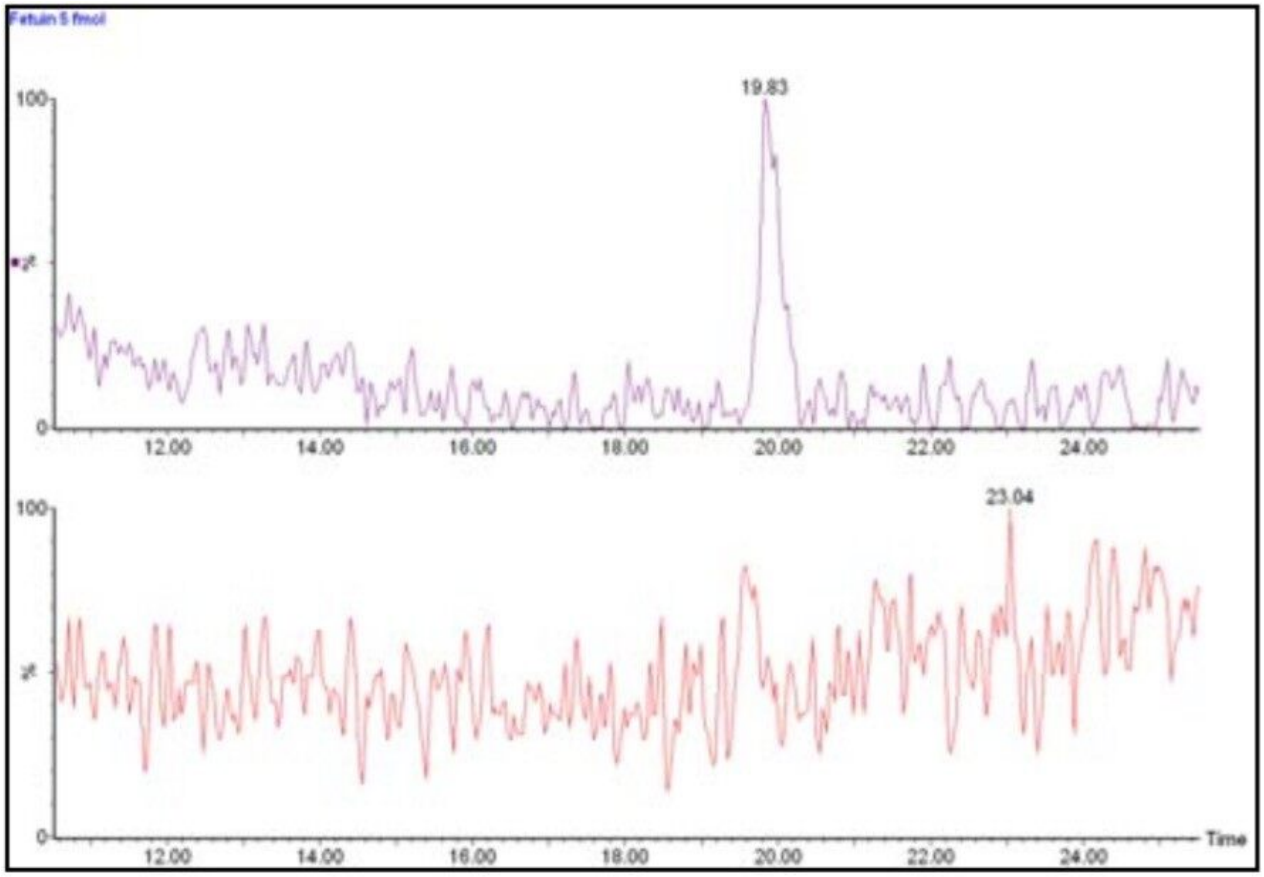


Figure 5. Mass chromatograms for m/z 366.1 [HexHexNAc oxonium ion] from the exact mass LC-MS analysis performed of 5 fmol of a fetuin tryptic digest with elevated collision energy (27eV). Upper trace acquired with EDC enabled, lower trace in the normal, unsynchronized, mode of operation.

Exact mass Precursor Ion Discovery coupled with EDC

Previously we have described an MS-based Precursor Ion Discovery (PID) approach on a Q-Tof mass spectrometer for the specific detection and analysis of modified peptides, such as glycopeptides or phosphopeptides.[1]

The ability to couple PID with EDC for LC-MS experiments is presented in this application note. 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 6). 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.

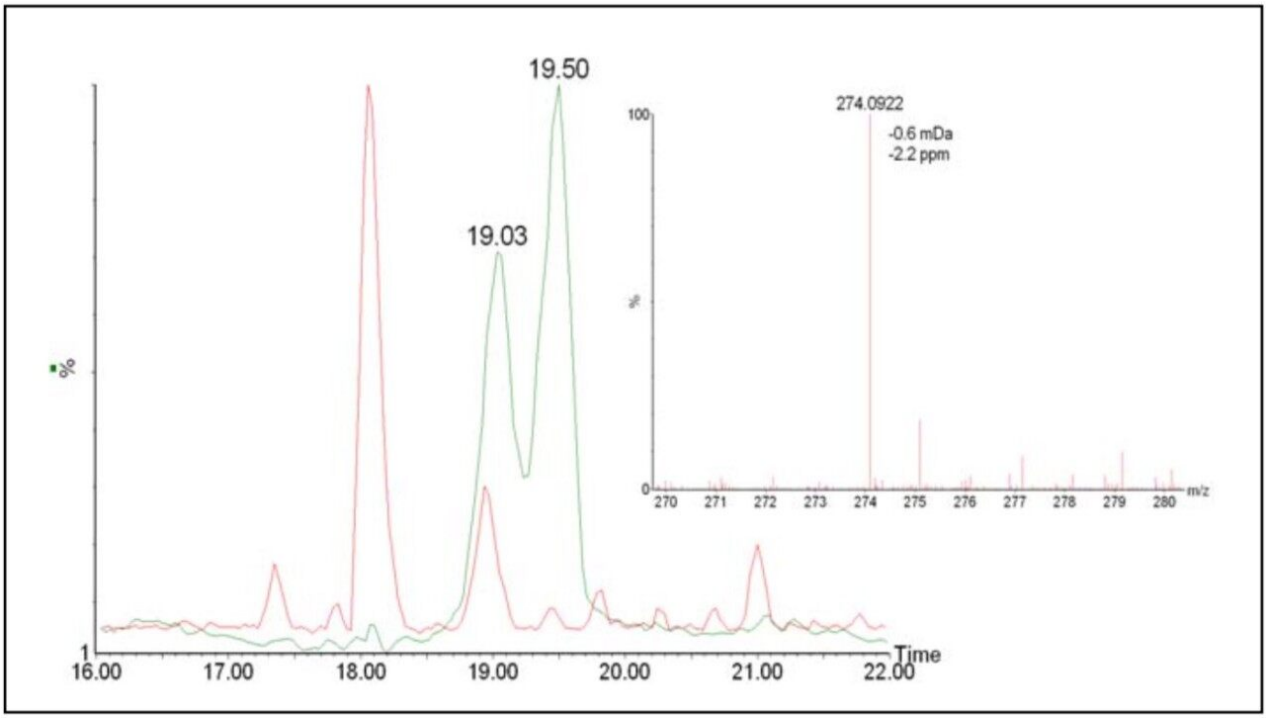


Figure 6. A section of the LC-MS chromatogram from 10 fmoles of EPO injected on-column (16 mins to 22 mins). Red trace represents the low energy BPI plot. Green trace represents mass chromatogram for m/z 274.0928, [NeuAc- H_2O], +/-5mDa. Inset is the targeted product ion.

It can be observed in Figure 6 that the response in the elevated energy function (green trace) for *m/z* 274.0928 shows two distinct signals at 19.03 and 19.50 minutes respectively. However, at this time the low energy BPI plot (Red trace) does not show a significant response, indicating that the glycopeptide ions are of relatively low intensity. The MS/MS, product ion, spectra acquired at both 19.03 and 19.5 minutes were sufficient to allow assignment of both glycopeptide structures.

Conclusion

- In this application note 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.

References

1. Bateman RH, Carruthers R, Hoyes JB, Jones C, Langridge JI, Millar A, Vissers JP.*J Am Soc Mass Spectrom*. 2002 Jul;13(7):792–803.9

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