Increasing sensitivity in peptide-level studies; Cyclic™ IMS-enabled Wideband Enhancement of CID and ECD product ions

Dale A. Cooper-Shepherd[1], Isabella A. Jones[1], Emma Marsden-Edwards[1], Ramin Rabbani[2]
[1]Waters Corporation, Wilmslow, Cheshire, UK; [2]Waters Corporation, Milford, MA, USA

INTRODUCTION

- Peptide-level analyses of proteins are the gold-standard in both proteomic and characterization workflows
- Performing tandem MS enables identification of the parent protein and the localization of sites of post-translational modifications
- High sensitivity is important for tandem MS to achieve high quality data and confidence in results, particularly for low abundance peptides
- Alternative fragmentation technologies such as electron-capture dissociation (ECD) often have lower fragmentation efficiency than CID but provide unique information content
- Wideband Enhancement (WBE) is employed to significantly increase product ion signal intensity across CID and ECD acquisition modes

METHODS

Liquid Chromatography:
ACQUITY™ Premier Binary UPLC™ system equipped with an ACQUITY Premier Peptide BEH™ C18, 130 Å, 1.7µm 2.1 x 100 mm (P/N 186002352)

Mass Spectrometry:
The Cyclic™ IMS P20 mass spectrometer was equipped with a Waters electron-capture dissociation (ECD) cell in the pre-IMS position.

Samples:
- Glu-fibrinopeptide B (Merck-Sigma) was prepared to 100 fmol/µL.
- Substance P (Merck-Sigma) was prepared to 250 fmol/µL.
- mAb tryptic digest (Waters, P/N 186009126)

Software:
Data was acquired using MassLynx™ v4.2 Software, processed using the peptide mapping and top-down workflows in waters_connect™ UNIFI™ software.

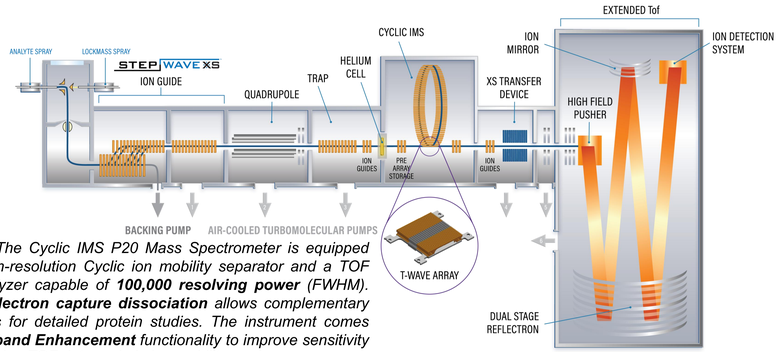

*Figure 1. The Cyclic IMS P20 Mass Spectrometer is equipped with a high-resolution Cyclic ion mobility separator and a TOF mass analyzer capable of **100,000 resolving power** (FWHM). Optional **electron capture dissociation** allows complementary capabilities for detailed protein studies. The instrument comes with **Wideband Enhancement** functionality to improve sensitivity by maximizing TOF duty cycle in ion mobility mode.*

OPTIMIZATION OF CYCLIC IMS AND WIDEBAND ENHANCEMENT

It is possible to enhance the sensitivity of a specified mass range by pulsing ion into the pusher region and synchronizing the pusher pulse with the ion packet's arrival. This has the effect of increasing the duty cycle for the *m/z* in question in what is known as **EDC** (Enhanced Duty Cycle) mode.
On the Cyclic IMS P20 MS ions are separated by their mobilities. Importantly for this work, **ion mobility scales with *m/z***, meaning that in general ions with low *m/z* have higher mobility and hence elute the mobility device first (Figure 2). Those that elute the mobility device first also reach the TOF pusher first.

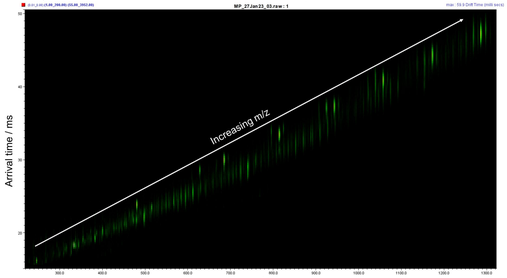

Figure 2. Ion mobility mass spectrum showing the trend of m/z *with arrival time (mobility). Those ions with the lowest* m/z *have the highest mobility and elute from the cyclic ion mobility device first.*

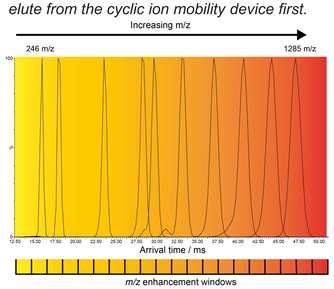

Figure 3. Illustration of the synchronisation of EDC enhancement windows with the elution of ions from the cyclic ion mobility device. High mobility ions reach the pusher and are enhanced first, ions of decreasing mobility are enhanced in turn.

Given the relationship between mobility and *m/z*, the instrument can be operated in a fashion such that the EDC enhancement window, i.e. *m/z*, is scanned concomitantly with the arrival of ions of lower mobilities providing duty cycle and therefore sensitivity enhancement across a broad mass range (Figure 3). We call this mode of operation **Wideband Enhancement (WBE).**

INCREASING SENSITIVITY IN CID AND ECD SPECTRA

Glu-fibrinopeptide B was used to optimize the Cyclic ion mobility separation in single pass mode over the range 200-2000 *m/z* (Figure 4). WBE was then activated to assess the gain in signal intensity across the mass range. ECD product ions of substance P were also investigated (Figure 5).

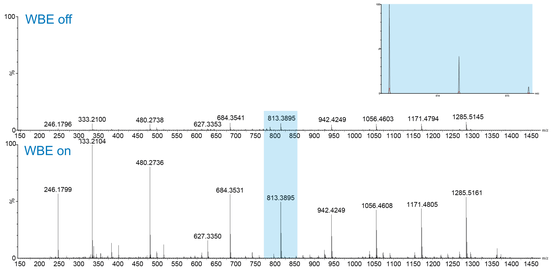

Figure 4. Glu-fibrinopeptide B CID product ion spectra with WBE activated (BOTTOM) and deactivated (TOP). The blue inset clearly highlights the increase in sensitivity for the 813.39 m/z ion. Across the m/z range, the boost in signal intensity was up to 20-fold.

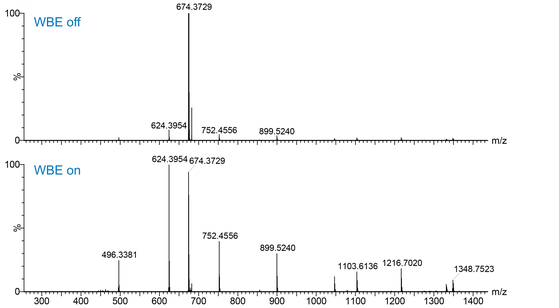

Figure 5. Wideband Enhancement boosts sensitivity of ECD product ions. ECD product ions are enhanced by an average of 10 times over the range 496 – 1348 m/z.

CONFLICT OF INTEREST AND TRADEMARK STATEMENT

Conflict of Interest Statement – Authors are employees of Waters Corporation who manufacture and sell the products highlighted in this work

USES OF WBE IN PEPTIDE MAPPING

Wideband enhancement was used in an MS^E-like acquisition mode to increase sensitivity of the high energy channel. High sequence coverage was obtained when processed in waters_connect.

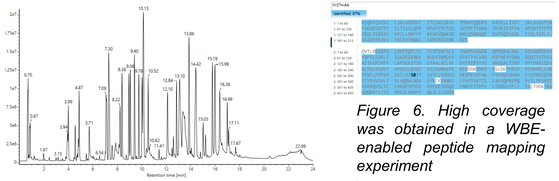

Figure 6. High coverage was obtained in a WBE-enabled peptide mapping experiment

Fragment ion coverage was increased for many peptides but particularly for singly-charged peptides where product ions were lower intensity. For, example, the peptide T35 from the mAb HC showed an intensity increase of >10 times which facilitated confident sequencing (Fig7).

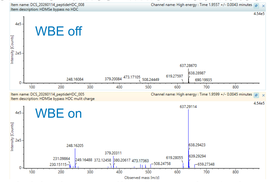

Figure 7. Increase in product ion intensity with WBE for the low-lying product ions of the singly-charge precursor of T35 from the heavy chain of the Waters mAb digest standard.

Applying WBE to ECD-MSMS yielded excellent product ion coverage across multiple peptides and distinction of isomeric amino acids such as leucine and isoleucine (Figure 8).

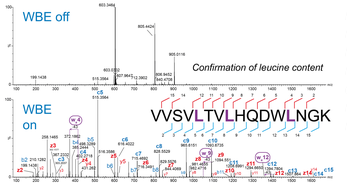

Figure 8. Increase in product ion intensity with WBE for the low intensity characteristic w ions. These w ions enable distinction of leucine and isoleucine by well-defined neutral losses.

CONCLUSIONS

- The Cyclic IMS P20 Mass Spectrometer provides drastically-increased sensitivity for CID and ECD targeted MSMS studies of peptides.
- Sensitivity increases improve fragment ion coverage for low abundance peptide product ions in biotherapeutic peptide mapping workflows.